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Oleksandra Liudvytska

Aktywność biologiczna metabolitów wtórnych rzewieni (*Rheum* L.) - ocena wpływu ekstraktów z *Rheum rhabarbarum* oraz *Rheum rhaponticum* na wybrane osoczowe oraz komórkowe składniki układu hemostazy *in vitro*

Biological activity of (*Rheum* L.) secondary metabolites - assessment of the effect of *Rheum rhabarbarum* and *Rheum rhaponticum* extracts on selected plasma and cell components of the haemostatic system *in vitro*

Praca doktorska
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pod kierunkiem
dr hab. Joanna Kołodziejczyk-Czepas,
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OMÓWIENIE CELU NAUKOWEGO I UZYSKANYCH WYNIKÓW

Wprowadzenie

Choroby układu krążenia, są najczęstszą przyczyną zgonów w krajach rozwiniętych. Mimo coraz lepszej diagnostyki i coraz nowocześniejszych metod leczenia, w Polsce, co roku z powodu chorób układu krążenia umiera około 200 tysięcy osób. Główny Urząd Statystyczny (GUS) podaje, że odpowiadają one za 46% wszystkich zgonów (https://stat.gov.pl). W przeciwdziałaniu rozwojowi chorób układu sercowo-naczyniowego kluczowe znaczenie ma styl życia i dieta, które w odróżnieniu od predyspozycji genetycznych, są czynnikami modyfikowalnymi. Wiele badań potwierdziło, że dieta bogata w składniki roślinne stanowi ważny element profilaktyki chorób cywilizacyjnych (Salas-Salvadó i wsp., 2018; Yu i wsp., 2018; Szczepańska i wsp., 2022). Z kolei surowce zielarskie są ważnym elementem współczesnych terapii, a ich zastosowanie może stanowić główną formę leczenia lub mieć charakter pomocniczy. W kontekście profilaktyki pierwotnej, wtórnej oraz leczenia różnorodnych zaburzeń funkcjonowania układu sercowo-naczyniowego, prowadzone są liczne badania nad efektami fizjologicznymi i farmakologicznymi ekstraktów lub pojedynczych związków, pochodzących z różnych gatunków roślin (Shang i wsp., 2021).

Ze względu na istotną rolę stresu oksydacyjnego w patofizjologii różnych schorzeń (w tym chorób cywilizacyjnych), aktywność przeciwutleniająca pozostaje od wielu lat jednym z głównych kierunków w badaniach dotyczących prozdrowotnych właściwości substancji pochodzenia naturalnego. Liczne badania potwierdzają, że dieta bogata w naturalne przeciwutleniacze może odgrywać istotną rolę w profilaktyce wielu schorzeń, w tym chorób układu sercowo-naczyniowego (Dal i Sigrist, 2016; Varadharaj i wsp., 2017). Jednak wyniki z ostatnich dwóch dekad wyraźnie wskazują, że samo działanie antyoksydacyjne stanowi tylko jeden z wielu możliwych aspektów korzystnego działania substancji naturalnych na układ sercowo-naczyniowy. Zwraca się uwagę zarówno na złożoność fizjologii człowieka, jak i możliwość plejotropowej aktywności wielu substancji naturalnych, która przekłada się finalnie na działanie pro-zdrowotne, czy lecznicze. Wśród aktywności o kluczowym znaczeniu, poza wspomnianym działaniem antyoksydacyjnym, wskazuje się właściwości przeciwzapalne, przeciwzakrzepowe, przeciwcukrzycowe oraz hipolipidemiczne (Bijak i wsp., 2016; Tressera-Rimbau i wsp., 2017; Keihanian i wsp., 2018; Adefegha, 2018). Obserwacje te wynikają między innymi z lepszego zrozumienia wieloczynnikowych patomechanizmów etiologii i patofizjologii chorób układu sercowo-naczyniowego. Molekularne podłoże tych schorzeń

obejmuje nie tylko procesy zapalne i związany z nimi stres oksydacyjny, ale także pobudzenie płytek krwi oraz osłabienie mechanizmów przeciwzakrzepowych osocza krwi i śródbłonka naczyń krwionośnych (dysfunkcja śródbłonka). Dlatego też poszukuje się substancji o wielokierunkowym prozdrowotnym działaniu, mogących modulować funkcjonowanie różnych elementów układu sercowo-naczyniowego - zarówno komponentów ściany naczyniowej, jak i składników krwi. Szczególną uwagę w tych badaniach zwraca się na substancje pochodzenia roślinnego (Weseler i Bast, 2012; Adegbola i wsp., 2017; Marmitt i wsp., 2022; Bachneti i wsp., 2022).

Rzewień/rabarbar (*Rheum* L.) to rodzaj obejmujący około 60 gatunków roślin wieloletnich, należących do rodziny *Polygonaceae* i cenionych jako surowiec jadalny i/lub leczniczy. Rabarbar znany jest przede wszystkim od tysiącleci w tradycyjnej medycynie Azji, ale część gatunków uprawiana jest również w celach spożywczych i jako surowiec zielarski na innych kontynentach (Agarwal i wsp., 2001; Cao i wsp., 2017; He i wsp., 1992; Pourjabali i wsp., 2017; Rehman i wsp., 2014). Badane w niniejszej pracy gatunki rzewienia, tj. *Rheum rhaponticum* (rabarbar ogrodowy) oraz *R. rhabarbarum* (rabarbar kędzierzawy) obecne są również od stuleci w tradycyjnej medycynie terenów Polski (*Herbarz Polski*, Marcin z Urzędowa, 1595).

Pomimo powszechnego zastosowania rzewienia do celów kulinarnych i etnomedycznych, biologiczne i fizjologiczne działanie ekstraktów pozyskiwanych z większości gatunków nadal pozostaje jedynie częściowo poznane. Chociaż w literaturze można znaleźć wiele danych na temat profilu chemicznego i właściwości biologicznych ekstraktów z różnych rzewieni, takich jak *R. palmatum* L., *R. officinale* Baill., *R. tanguticum* Maxim. Ex Balf. lub *R. emodi* L. (Komatsu i wsp., 2006; Rashid i wsp., 2014; Rokaya i wsp., 2012; Wang i wsp., 2013; Zhang i wsp., 2015a), brak jest kompleksowego spojrzenia na działanie tych roślin, zwłaszcza pod kątem wpływu na układ sercowo-naczyniowy. Co więcej, mechanizmy większości typów aktywności biologicznej niektórych gatunków, jak na przykład *R. rhaponticum* i *R. rhabarbarum*, są tylko częściowo lub słabo opisane. W przypadku roślin badanych w niniejszej pracy, znaczna część dostępnych danych dotyczy aktywności estrogenowej preparatów z części podziemnych *R. rhaponticum* oraz innych właściwości związanych z łagodzeniem objawów menopauzy. Jest to jedyny kierunek aktywności farmakologicznej tego surowca, który został dobrze poznany, opisany i potwierdzony w badaniach przedklinicznych oraz klinicznych (Kaszkin-Bettag i wsp., 2007; Chang i wsp., 2016; Wilson i wsp., 2021). Inne kierunki działania badanych gatunków rzewienia zostały poznane w znacznie mniejszym zakresie (głównie w doświadczeniach *in vitro*), ale dostępne

dane sugerują znaczący potencjał prozdrowotny tych roślin, między innymi w kontekście przeciwdziałania otyłości i cukrzycy (Liudvytska i Kolodziejczyk-Czepas, 2022).

Cel pracy

Celem prowadzonych badań była ocena wpływu ekstraktów z dwóch gatunków rzewienia - *Rheum rhaponticum* L. oraz *Rheum rhabarbarum* L. oraz typowych dla tych surowców stilbenów (rapontygeniny i rapontycyny) na funkcjonowanie wybranych osoczowych i komórkowych składników układu hemostazy.

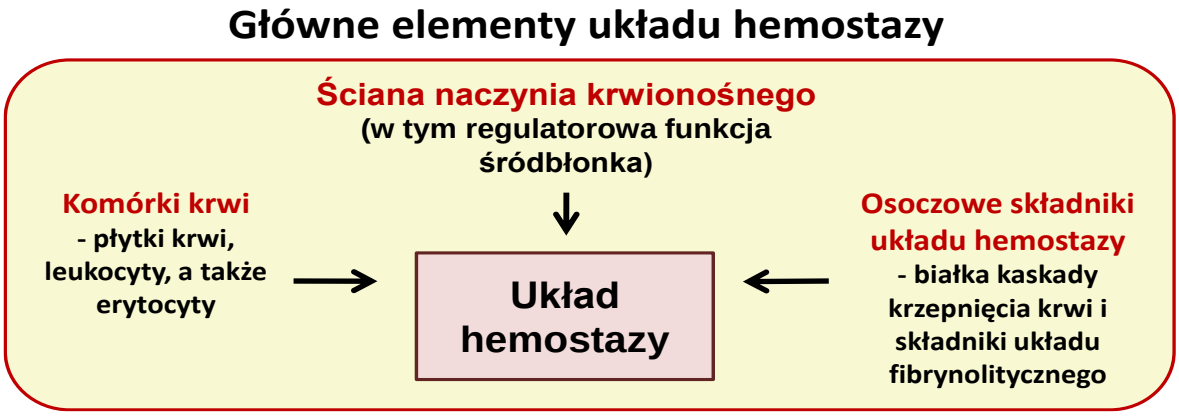
Przedmiotem prac badawczych były aktywności biologiczne wskazywane jako jedne z kluczowych dla kardioprotekcyjnego działania substancji naturalnych, to jest: działanie przeciwzapalne, antykoagulacyjne/przeciwzakrzepowe oraz przeciwutleniające.

Koncepcja badań i plan pracy

Rzewień kojarzony jest przede wszystkim z kulinarnym zastosowaniem jego ogonków liściowych. Części podziemne stanowią z kolei materiał zielarski wykorzystywany od stuleci w etnomedycynie, a także we współczesnej fitoterapii. Standaryzowany ekstrakt z *R. rhaponticum* - ERr731® stanowi produkt leczniczy, stosowany w łagodzeniu objawów menopauzy (Chang i wsp., 2016). Ponadto, ekstrakty z tego gatunku rzewienia są składnikiem różnych suplementów diety adresowanych do kobiet w wieku okołomenopauzalnym. Natomiast aktywność *R. rhabarbarum* została przebadana w znacznie węższym zakresie i większość danych na temat właściwości tego gatunku pochodzi z badań *in vitro*. Jednak zarówno dostępne dane fitochemiczne, jak i literatura dotycząca ich aktywności biologicznej (zebrane w **pracy I**) sugerują, że oba gatunki i rodzaje surowca (ogonki liściowe i korzeń) mogą wykazywać korzystne właściwości dla zdrowia człowieka. Stąd też, w prezentowanej pracy przeprowadzono badania ekstraktów pozyskanych zarówno z części nadziemnych (ogonków liściowych), jak i podziemnych (karpa korzeniowa).

Prace badawcze będące przedmiotem prezentowanej dysertacji opierały się na ocenie *in vitro* aktywności biologicznej ekstraktów roślinnych z dwóch gatunków rzewienia (*R. rhaponticum* oraz *R. rhabarbarum*) oraz typowych dla tych roślin pochodnych stilbenu: rapontygeniny (RHPG) oraz jej glikozydu - rapontycyny (RHPT), w układach doświadczalnych związanych z hemostazą i fizjologią układu sercowo-naczyniowego. Prawidłowe funkcjonowanie układu hemostazy (ryc. 1) stanowi ważny składnik homeostazy organizmu, a zaburzenia w jego obrębie (takie jak na przykład zmiany w strukturze powstającego włóknika

(fibryny), zwiększenie potencjału prokoagulacyjnego krwi, czy dysfunkcja śródbłonka) stanowią istotny element patofizjologii różnorodnych chorób układu sercowo-naczyniowego (Margetic, 2012; Koch i Zernecke, 2014; Valente-Acosta i wsp., 2016; Larsen i Hvas, 2021).



Ryc. 1. Schematyczne ujęcie głównych elementów układu hemostazy. Funkcjonowanie układu hemostazy opiera się na złożonym zespole mechanizmów zapobiegających utracie krwi w przypadku uszkodzenia naczynia krwionośnego i utrzymujących jej płynność oraz naturalne funkcje w warunkach fizjologicznych. Składniki układu hemostazy umownie klasyfikuje się na trzy grupy, a są to: (1) ściana naczynia krwionośnego, w której kluczowe znaczenie dla hemostazy ma aktywność komórek śródbłonka, (2) składniki morfotyczne krwi oraz (3) osoczowe składniki układu hemostazy (białka krzepnięcia i fibrynolizy).

Plan badań został opracowany w kontekście oceny możliwego kardioprotekcyjnego działania ekstraktów z rzewienia oraz stilbenów, a w prowadzonych doświadczeniach skupiono się na ich wpływie komórki śródbłonka ściany naczyniowej, leukocyty oraz na osoczowe składniki układu hemostazy (ze szczególnym uwzględnieniem kluczowych białek kaskady krzepnięcia krwi oraz fibrynolizy).

Prace badawcze podzielono na trzy moduły eksperymentalne, zawierające panele doświadczeń, dotyczące wyżej wspomnianych typów aktywności:

(1) moduł przeciwzapalny: badania właściwości przeciwzapalnych ekstraktów z rzewienia i stilbenów, prowadzone w celu oceny zdolności badanych substancji modulowania odpowiedzi zapalnej komórek śródbłonka i leukocytów, w tym regulacji ekspresji genów i hamowania aktywności enzymów prozapalnych - cyklooksygenazy-2 (COX-2) i 5-lipooksygenazy (5- LOX);

(2) moduł hemostatyczny: ocena wpływu ekstraktów z rzewienia i stilbenów na aktywność osoczowych elementów układu hemostazy (białek kaskady krzepnięcia krwi i układu fibrynolitycznego) oraz odpowiedź hemostatyczną komórek śródbłonka;

(3) moduł antyoksydacyjny: ocena działania przeciwutleniającego badanych ekstraktów i stilbenów w aspekcie ich zdolności do ochrony składników osocza krwi przed uszkodzeniami, w warunkach stresu oksydacyjnego indukowanego nadtlenoazotynem.

Badane ekstrakty i związki

Materiał roślinny z *Rheum rhaponticum* L. otrzymano z Ogrodu Botanicznego Uniwersytetu Marii Curie-Skłodowskiej w Lublinie, natomiast surowiec z *Rheum rhabarbarum* L. pochodził z Ogrodu Botanicznego w Łodzi. Zbiór materiału przeprowadzono w dwóch turach, zgodnie z sezonem wegetacyjnym badanych roślin: części nadziemne zebrano w czerwcu 2019 roku, natomiast części podziemne pozyskano w październiku tego samego roku. Badane pochodne stilbenu: rapontygeninę (RHPG) i rapontycynę (RHPT) zakupiono w firmie Sigma-Aldrich (Grupa Merck).

W prezentowanej pracy oceniano aktywność biologiczną frakcji butanolowych otrzymanych z części podziemnych oraz ogonków liściowych obu gatunków rzewienia. Ekstrakcję oraz analizy jakościowe i ilościowe ekstraktów wykonano w Instytucie Uprawy, Nawożenia i Gleboznawstwa - Państwowym Instytucie Badawczym. Charakterystykę fitochemiczną badanych ekstraktów określono metodą HR-QTOF-MS/MS.

Modele doświadczalne i główne metody badawcze

(1) Moduł przeciwzapalny (praca II i część pracy IV)

W ocenie wpływu badanych substancji na odpowiedź zapalną śródbłonka **(praca II)** jako model badawczy zastosowano komórki śródbłonka żyły pępowinowej (HUVECs, ang. *human umbilical vein endothelial cells*), niestymulowane lub poddane stymulacji prozapalnej przy użyciu lipopolisacharydu (LPS). W doświadczeniach dotyczących oceny zdolności hamowania aktywności COX-2 i 5-LOX przez badane ekstrakty i stilbeny stosowano komercyjne kity, zawierające oczyszczone białka enzymatyczne. Zastosowano następujące metody analityczne:

- test redukcji resazuryny - ocena żywotności komórek (badania cytotoksyczności),
- qPCR - ocena ekspresji genów *COX2* i *ALOX5,*
- mikropłytkowy spektrofotometryczny test skriningowy - ocena zdolności inhibicji COX-2 i 5-LOX,

- profilowanie metodą blottingu z użyciem komercyjnego zestawu *Proteome Profiler Human Cytokine Array* - oznaczenia uwalnianych cytokin,
- mikroskopia fluorescencyjna - ocena cytotoksyczności badanych substancji roślinnych (barwienie kalceiną) oraz badania adhezji makrofagów linii U-937 do komórek śródbłonka.

Drugim modelem badawczym, użytym w tym module **(część pracy IV)** były ludzkie jednojądrzaste komórki krwi obwodowej (PBMCs, ang. *peripheral blood mononuclear cells*), które zostały poddane stymulacji prozapalnej przy użyciu konkanawaliny A (ConA).

W ocenie wpływu badanych substancji roślinnych na tworzenie inflammasomów, jako model badawczy zastosowano natomiast komórki THP1-ASC-GFP (ang. *human THP-1 monocytes - ASC speck reporter cells*), poddane stymulacji prozapalnej przy użyciu LPS.
Odpowiedź zapalną leukocytów badano następującymi metodami:

- test z zastosowaniem błękitu trypanu oraz test metaboliczny (pomiar zdolności redukcji resazuryny) - ocena cytotoksyczności wobec PBMCs,
- mikropłytkowe enzymatyczne testy immunosorpcyjne ELISA (ang. *enzyme-linked immunosorbent assay*) - pomiary poziomu cytokin (TNF-α oraz IL-2), prostaglandyny E_2 (PGE_2) i metaloproteinazy 9 (MMP-9),
- mikroskopia fluorescencyjna - badanie uwalniania adaptorowego białka ASC z komórek THP1-ASC-GFP.

(2) *Moduł hemostatyczny* (praca III)

Jako model badawczy w tym module zastosowano ludzkie osocze krwi (pozyskiwane z kożuszków leukocytarno-płytkowych), wybrane białka hemostazy (tj. trombinę - czynnik krzepnięcia IIa, czynnik krzepnięcia Xa, fibrynogen i plazminogen) oraz komórki śródbłonka (HUVECs).

Badano następujące aspekty: (1) wpływ ekstraktów z rzewienia i stilbenów na proces krzepnięcia osocza krwi i aktywność hemostatyczną kluczowych proteaz osoczowej kaskady krzepnięcia krwi: trombiny i czynnika Xa; (2) aktywność generowanej plazminy - głównego enzymu układu fibrynolitycznego oraz (3) hemostatyczną odpowiedź komórek śródbłonka. W oznaczeniach zastosowano następujące metody:

- kinetyczne pomiary aktywności enzymatycznej trombiny - w tym oznaczenia amidolityczne (w oparciu o zastosowanie komercyjnego substratu chromogennego) i proteolityczne (z użyciem fizjologicznego substratu trombiny - fibrynogenu);

- kinetyczne monitorowanie procesu wykrzepiania osocza - ocena wpływu badanych substancji na aktywację zewnątrzpochodnego szlaku krzepnięcia krwi;
- oznaczenia fluorymetryczne - ocena wpływu na aktywność czynnika krzepnięcia Xa;
- amidolityczne oznaczenia aktywności plazminy - ocena wpływu na proces generowania plazminy po aktywacji streptokinazą i t-PA,
- mikropłytkowe testy immunosorpcyjne ELISA - określenie wpływu na odpowiedź prokoagulacyjną (uwalnianie czynnika von Willebranda) oraz potencjał fibrynolityczny komórek śródbłonka (uwalnianie t-PA oraz poziom kompleksów t-PA+PAI-1).

(3) Moduł antyoksydacyjny **(część pracy IV)**

Do oznaczeń aktywności przeciwutleniającej, jako układy doświadczalne zastosowano osocze ludzkie, izolowane z kożuszków leukocytarno-płytkowych oraz ludzki fibrynogen. Stosowano następujące metody analityczne:

- mikropłytkowy test immunosorpcyjny ELISA - ocena poziomu 3-nitrotyrozyny w białkach osocza;
- pomiary spektrofotometryczne - oznaczenie poziomu białkowych grup -SH, substancji reagujących z kwasem tiobarbiturowym (TBARS, ang. *thiobarbituric acid-reactive substances*) oraz pomiar pojemności antyoksydacyjnej osocza (metodą opartą na zdolności do redukcji jonów żelaza - FRAP, ang. *ferric reducing ability of plasma*),
- elektroforezę 1D (SDS-PAGE) w połączeniu z analizami densytometrycznymi - ocena zmian (tworzenia agregatów białkowych) w strukturze fibrynogenu ludzkiego, eksponowanego na działanie nadtlenoazotynu,
- oznaczenia fluorymetryczne - ocena utleniania tryptofanu w cząsteczce fibrynogenu.

OMÓWIENIE PRAC WCHODZĄCYCH W ZAKRES ROZPRAWY DOKTORSKIEJ

Rozprawa doktorska składa się z czterech publikacji (jedna praca przeglądowa oraz trzy prace oryginalne), które stanowią spójny tematycznie cykl artykułów, obejmujących analizy właściwości biologicznych badanych substancji roślinnych, będące przedmiotem prezentowanej dysertacji. Trzy artykuły zostały opublikowane w indeksowanych czasopismach naukowych, znajdujących się w bazie *Journal Citation Reports*. Manuskrypt czwartej pracy został złożony do redakcji.

Praca I (artykuł poglądowy, pt. „*Rheum rhaponticum and Rheum rhabarbarum: a review of phytochemistry, biological activities and therapeutic potential*”) stanowi przegląd dostępnych danych na temat profilu fitochemicznego, zastosowań etnomedycznych oraz aktywności biologicznej (a także właściwości leczniczych) badanych gatunków rzewienia. Powstała jako pierwsza z cyklu publikacji objętych rozprawą, a informacje w niej zawarte odzwierciedlają stan wiedzy w momencie podjęcia prac badawczych związanych z prezentowaną dysertacją. W artykule przedstawiono dostępne w tym czasie (rok 2019 i 2020) informacje na temat substancji biologicznie czynnych obecnych w *R. rhaponticum* i *R. rhabarbarum* (m.in. przedstawicieli hydroksystilbenów, antrachinononów i flawonoidów). Przygotowując pracę poglądową przeanalizowano dane etnomedyczne (z włączeniem źródeł historycznych) i informacje na temat aktywności biologicznej *R. rhaponticum* i *R. rhabarbarum.* Omówiono aktualny stan wiedzy na temat właściwości rzewieni i krytycznie przeanalizowano stan zaawansowania badań różnych typów aktywności wyżej wspominanych roślin, w oparciu o informacje pochodzące głównie z czasopism indeksowanych w międzynarodowych bazach danych (Medline/Pubmed, Scopus, Science Direct/Elsevier, Springer Link/ICM). Szczególną uwagę zwrócono na nieliczne badania dotyczące działania przeciwutleniającego i przeciwzapalnego, a także przesłanki sugerujące, że opisywane gatunki rzewienia mogą wykazywać działanie kardioprotekcyjne. Zauważono, że jedynie działanie ekstraktu z *R. rhaponticum* (ERr 731®) zostało udokumentowane w badaniach klinicznych (pod kątem łagodzenia dolegliwości związanych z menopauzą), a wiele danych dotyczących innych kierunków działania obu gatunków rzewienia pochodzi jedynie z badań wstępnych.

Przedstawione **prace oryginalne II**, **III** i **IV** są odzwierciedleniem założonego i zrealizowanego planu badań. Powstały we współpracy z Zakładem Biochemii i Jakości

Plonów - Państwowym Instytutem Badawczym w Puławach (IUNG-PIB). Zespół Badaczy z IUNG-PIB zajmował się wyizolowaniem badanych ekstraktów oraz analizą ich profilu fitochemicznego. Szczegółowe dane dotyczące profilowania badanych ekstraktów zawarto jako *Supplementary materials 1* oraz *Supplementary_Table 1, 2, 3* w pracy **II**, a także w tekście pracy **III**. W załącznikach do pracy **II** przedstawiono wyniki profilowania metabolitów obecnych w ekstraktach z ogonków liściowych (*Supplementary_Table 1*) oraz korzeni (*Supplementary_Table 2*) obu gatunków rzewienia. W osobnej tabeli (*Supplementary_Table 3*) zestawiono różnice w ich profilu.

Ponadto, w pracach **II i III** zawarto wyniki mechanistycznych analiz *in silico*, obejmujących interakcje głównych składników badanych ekstraktów z enzymami prozapalnymi (COX-2 oraz 5-LOX) oraz wybranymi białkami hemostazy (trombiną, czynnikiem krzepnięcia Xa oraz plazminą). Podobnie, jak wyniki profilowania fitochemicznego, również analizy *in silico* nie wchodzą bezpośrednio w zakres badawczy pracy doktorskiej, ale stanowią element realizowanego projektu i wsparcie dla badań *in vitro*.

Publikacje II i III to prace oryginalne, których kluczowym elementem jest analiza odpowiedzi zapalnej i hemostatycznej ludzkich komórek śródbłonka. Obie powstały we współpracy z Katedrą Biologii Nowotworów i Epigenetyki, Wydziału Biologii i Ochrony Środowiska, Uniwersytetu Łódzkiego. W pierwszym etapie prac badawczych dokonano izolacji ludzkich komórek śródbłonka żyły pępowinowej (HUVECs) oraz optymalizacji warunków hodowli na potrzeby prowadzonych doświadczeń. Etap wstępny obejmował między innymi ustalenie warunków stymulacji komórek LPSem lub trombiną.

Praca II, pt. ***„Rheum rhaponticum and Rheum rhabarbarum extracts as modulators of endothelial cell inflammatory response*”** obejmuje doświadczenia związane z oceną aktywności przeciwzapalnej badanych substancji roślinnych w aspekcie ich zdolności do modulowania odpowiedzi zapalnej śródbłonka. Komórki śródbłonka są nie tylko integralną częścią fizjologii naczyń krwionośnych i układu hemostazy, ale także są zaangażowane w wiele innych procesów fizjologicznych i patologicznych, w tym w patofizjologię chorób układu krążenia. Dysfunkcja śródbłonka oraz utrata jego przeciwzakrzepowych i regulatorowych funkcji są zmianami o charakterze krytycznym dla rozwoju miażdżycy i jej następstw (Gimbrone i wsp., 2016; Medina-Leyte i wsp., 2021; Bockus i Kim, 2022).

W pierwszym etapie badań wykonano oznaczenia wpływu badanych ekstraktów oraz stilbenów na żywotność komórek HUVECs, w celu wykluczenia ich cytotoksyczności i wyboru optymalnych, bezpiecznych dla komórek stężeń. W przypadku ekstraktów z korzeni

R. rhabarbarum i jednego ze stilbenów (RHPG), bezpieczny zakres stężeń był niższy od pierwotnie założonego (1-50 μg/ml) i wynosił odpowiednio 1-30 oraz 1-25 μg/ml, natomiast dla pozostałych badanych substancji założony wstępnie zakres stężeniowy został utrzymany. Zastosowanie badanych ekstraktów z rzewienia w wymienionych stężeniach odpowiada nano- lub mikromolowym stężeniom ich składników bioaktywnych w układach doświadczalnych. Według danych literaturowych, fizjologicznie osiągalne poziomy naturalnych związków pochodzenia roślinnego lub ich metabolitów w osoczu krwi wynoszą od nanomoli do kilku mikromoli na litr (Manach i wsp., 2005).

W pracy zastosowano panel doświadczeń umożliwiający ocenę wpływu badanych ekstraktów i stilbenów na różne elementy i etapy odpowiedzi zapalnej komórek śródbłonka. Wykazano, że badane substancje roślinne posiadają właściwości przeciwzapalne i mogą modulować odpowiedź prozapalną komórek śródbłonka na różnych poziomach: ekspresji genów dla enzymów prozapalnych, uwalniania cytokin i interakcji z leukocytami. Ze względu na to, że substancje zawarte w ekstraktach są związkami egzogennymi (ksenobiotykami) i mogą same wpływać na niepobudzone komórki, w prowadzonych badaniach zastosowano dwa układy badawcze: komórki śródbłonka niestymulowane oraz komórki stymulowane LPS. W oparciu o wyniki oznaczeń ekspresji genów dla enzymów prozapalnych (*COX2* oraz *ALOX5*), stwierdzono różnice w działaniu preparatów z ogonków liściowych i korzeni oraz stilbenów w obu układach doświadczalnych. W przypadku genu *COX2*, w niestymulowanych komórkach odnotowano częściowe zahamowanie ekspresji przez ekstrakty z ogonków liściowych obu gatunków, z korzeni *R. rhabarbarum* oraz RHPG, natomiast RHPT nie wpływała na poziom ekspresji tego genu. Z kolei ekstrakty z korzeni *R. rhaponticum* częściowo zwiększały ekspresję *COX2*. Natomiast w komórkach śródbłonka stymulowanych do odpowiedzi zapalnej, ekstrakty z ogonków liściowych oraz oba stilbeny wyraźnie zmniejszały poziom ekspresji genu *COX2*, podczas gdy ekstrakty z korzeni zwiększały ekspresję genu *COX2*. Ocena poziomu ekspresji *ALOX5* w komórkach niestymulowanych wykazała efekt hamujący ekstraktów z ogonków liściowych *R. rhaponticum*, a pozostałe badane substancje nie wykazywały istotnego statystycznie wpływu lub był on niewielki. W komórkach stymulowanych LPS, spadek ekspresji *ALOX5* odnotowano dla stilbenów oraz ekstraktów z ogonków liściowych, natomiast ekstrakty z korzeni działały znacznie słabiej lub nie wykazywały efektu.

Aby sprawdzić, czy oprócz modulowania ekspresji genów, badane substancje mogą wpływać na COX-2 i 5-LOX także na poziomie już powstałego białka enzymatycznego, w kolejnym etapie badań zastosowano komercyjne testy skriningowe i wykonano oznaczenia

zdolności hamowania aktywności tych enzymów. Badane substancje hamowały aktywność COX-2 z różną skutecznością, ale wyraźnie dało się zauważyć silniejszy efekt ekstraktów z korzeni obu gatunków (ok. 80% inhibicji, przy stężeniu 50 μg/ml). Natomiast efektywność inhibicyjnego działania RHPG przy tym samym stężeniu wynosiła ok. 40%. Obserwacje z przeprowadzonych badań znalazły potwierdzenie w analizach *in silico*, które stanowią mechanistyczne uzupełnienie wykonanych badań. Dane uzyskane z analiz metodami chemii obliczeniowej wykazały, że składniki, takie jak m.in. galoiloglukozyd piceatannolu, winiferyna, emodyna, rapontycyna, deoksyrapontycyna, 3-*O*-beta-glukopiranozyd resweratrolu, piceid, 8-glukozyd chryzofanolu, czy astringina mogą się wiązać w centrum aktywnym COX-2, z powinowactwem bliskim indometacynie (lek przeciwzapalny, zastosowany jako związek referencyjny). Zdolność hamowania aktywności 5-LOX przez badane substancje była niższa i w większości nie przekraczała 20% (z wyjątkiem RHPG, której maksymalny efekt wynosił ok. 40% zahamowania aktywności 5-LOX). Analizy *in silico*, wskazujące, że większość związków wykazuje niskie powinowactwo do centrum aktywnego 5-LOX, potwierdziły wspomniany słabszy efekt hamowania 5-LOX odnotowany w badaniach *in vitro*.

Przeprowadzono także ocenę wpływu badanych substancji roślinnych na proces sekrecji czynników prozapalnych z komórek śródbłonka do środowiska, stosując panelową metodę profilowania *Proteome Profiler Human Cytokine Array*. Wyniki profilowania cytokin uwolnionych z komórek HUVECs do medium hodowlanego wykazały, że większość badanych substancji (m.in. RHPT, ekstrakty z ogonków liściowych *R. rhaponticum* i *R. rhabarbarum* oraz ekstrakt z korzenia *R. rhaponticum*) hamuje uwalnianie kluczowych czynników prozapalnych, takich jak na przykład CCL5/RANTES, CXCL10/IP-10, CXCL12/SDF-1 i IL- 18/IL-IF4.

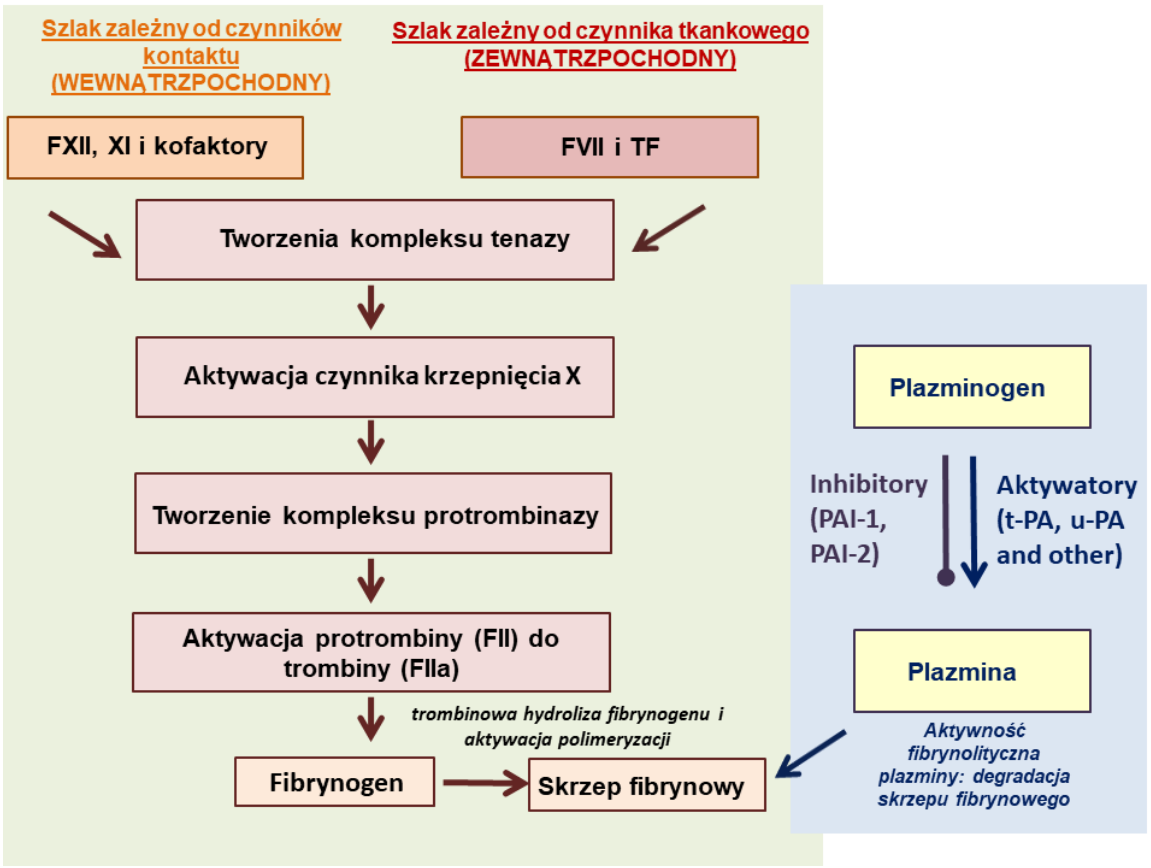
Ze względu na fakt, że w naczyniowej odpowiedzi zapalnej ważną rolę pełni zmiana antyadhezyjnego fenotypu komórek śródbłonka na aktywność proadhezyjną, w kolejnych doświadczeniach zbadano wpływ ekstraktów z rzewieni oraz stilbenów na interakcje komórek HUVECs z leukocytami. Ekstrakty z korzeni i ekstrakty z ogonków liściowych obu gatunków rzewienia oraz stilbeny (RHPG i RHPT) wykazywały podobny efekt w zmniejszaniu adhezji monocytów do komórek śródbłonka. W większości przypadków odnotowano spadek adhezji na poziomie ≥50% (w stosunku do kontroli stymulowanej LPS).

Praca III, pt. ***„Effects of Rheum rhaponticum and Rheum rhabarbarum extracts on haemostatic activity of blood plasma components and endothelial cells in vitro”*** zawiera

wyniki doświadczeń dotyczących wpływu badanych ekstraktów i stilbenów na odpowiedź hemostatyczną komórek śródbłonka oraz aktywność hemostatyczną wybranych składników osocza krwi. Analiza danych etnomedycznych wskazuje, że preparaty z obu gatunków rzewienia stosowane były tradycyjnie m.in. do leczenia zaburzeń związanych z układem krążenia. Dostępne informacje są jednak niejednoznaczne - niektóre wskazania sugerują prokoagulacyjne (przeciwkrwotoczne) działanie tego surowca roślinnego, ale był on również rekomendowany do usprawniania przepływu krwi.

Założeniem pracy było uzyskanie odpowiedzi na 2 główne pytania: (1) *czy badane substancje mogą wpływać na hemostatyczne właściwości białek osocza krwi i komórek śródbłonka* oraz (2) *czy mają właściwości przeciwzakrzepowe, czy raczej prokoagulacyjne*? Aby uzyskać jak najszerszy obraz działania badanych ekstraktów z rzewieni i stilbenów, doświadczenia objęte publikacją zostały podzielone na trzy bloki eksperymentalne, obejmujące aktywność osoczowych białek krzepnięcia krwi, działanie układu fibrynolitycznego oraz odpowiedź hemostatyczną komórek śródbłonka.

W pierwszej części doświadczeń zbadano proces wykrzepiania osocza, monitorując wpływ ekstraktów z rzewienia i stilbenów na szlak krzepnięcia zależny od czynnika tkankowego (tzw. szlak zewnątrzpochodny), będący głównym fizjologicznym torem aktywacji krzepnięcia krwi (ryc. 2). Przeprowadzone testy wykazały, że wszystkie badane ekstrakty z rzewienia wpływały hamująco na zewnątrzpochodny szlak krzepnięcia krwi. Aby wskazać najbardziej prawdopodobne mechanizmy i/lub cele molekularne dla badanych substancji, kolejne etapy badania obejmowały analizy ich wpływu na dwie kluczowe proteazy serynowe krzepnięcia: czynnik krzepnięcia Xa i trombinę (czynnik krzepnięcia IIa). W osoczowej kaskadzie krzepnięcia, trombina pełni funkcję proteazy wykonawczej - bezpośrednio inicjuje polimeryzację fibrynogenu i tworzenie skrzepu fibrynowego. Z kolei czynnik Xa jest kluczowym enzymem kompleksu protrombinazy i odpowiada za generowanie aktywnej enzymatycznie trombiny z jej proenzymu - protrombiny (ryc. 2). Zarówno czynnik Xa, jak i trombina uważane są za tzw. punkty kontrolne w procesie krzepnięcia krwi i stanowią główny cel dla obecnych i opracowywanych terapii przeciwzakrzepowych (Ansell, 2007; Al-Amer, 2022).



Ryc. 2. Schematyczna prezentacja głównych etapów kaskady krzepnięcia krwi oraz działania układu fibrynolitycznego (Fig. 1, praca III). Proces krzepnięcia krwi może być inicjowany przez dwa różne mechanizmy, tj. szlak zewnątrzpochodny i szlak wewnątrzpochodny. Szlak zewnątrzpochodny (zależny od czynnika tkankowego) jest główną fizjologiczną drogą aktywacji krzepnięcia krwi. Czynnik tkankowy (TF) ulega ekspresji w tkankach podśródbłonkowych, jednak po urazie naczynia dochodzi do odsłonięcia warstwy podśródbłonkowej i jego kontaktu z osoczowymi składnikami kaskady krzepnięcia krwi. Natomiast wewnątrzpochodny szlak krzepnięcia krwi (szlak zależny od tzw. czynników kontaktu) jest inicjowany poprzez autoaktywację czynnika krzepnięcia XII na ujemnie naładowanych powierzchniach (aktywacja kontaktowa). Oba szlaki inicjacji kaskady krzepnięcia łączą się i prowadzą do powstania kompleksów enzymatycznych: tenazy i protrombinazy. Kompleks tenazy jest odpowiedzialny za aktywację proenzymu czynnika krzepnięcia X (FX) do aktywnej enzymatycznie proteazy serynowej - czynnika FXa. Wytworzony FXa jest z kolei kluczowym enzymem kompleksu protrombinazy, aktywującego zymogen - protrombinę, do trombiny (FIIa). Katalizowana przez trombinę hydroliza fibrynogenu w osoczu krwi umożliwia jego polimeryzację i tworzenie skrzepu fibrynowego. Natomiast układ fibrynolityczny odpowiada za degradację fibryny i kontrolowanie wielkości powstającego skrzepu. Jego funkcjonowanie opiera się na aktywności plazminy, powstającej w wyniku aktywacji plazminogenu przez jego aktywatory (głównie przez tkankowy aktywator plazminogenu (t-PA) i aktywator plazminogenu typu urokinazowego (u-PA)). Na poziomie aktywacji plazminogenu do plazminy, funkcjonowanie układu fibrynolitycznego jest kontrolowane przez inhibitory aktywatorów plazminogenu (głównie przez inhibitor aktywatora plazminogenu-1 (PAI-1)). Aktywność plazminy jest regulowana głównie przez α_2-antyplazminę.

Wykazano, że badane substancje roślinne hamowały aktywność obu badanych enzymów kaskady krzepnięcia. W badaniach zdolności hamowania aktywności czynnika Xa, zaobserwowano ok. 40-50% inhibicji w przypadku stilbenów oraz ekstraktu z ogonków

liściowych *R. rhaponticum* w stężeniu 50 μg/ml, natomiast w tym samym stężeniu, ekstrakty z korzeni *R. rhaponticum* i *R. rhabarbarum* oraz ekstrakt z ogonków liściowych *R. rhabarbarum* wykazywały efektywność na poziomie >70% inhibicji. Badane ekstrakty roślinne hamowały również aktywność amidolityczną i proteolityczną trombiny (zwłaszcza frakcje uzyskane z korzeni), natomiast stilbeny wykazywały niewielką zdolność hamowania hydrolitycznej aktywności trombiny.

Kolejną ważną obserwacją wynikającą z badań jest znikomy wpływ badanych ekstraktów na aktywność generowanej plazminy, centralnej proteazy serynowej układu fibrynolitycznego. Jest to szczególnie ważne w kontekście poszukiwania selektywnych, naturalnych inhibitorów kaskady krzepnięcia, niezaburzających aktywności białek fibrynolitycznych. Ze względu na znaczącą homologię strukturalną i funkcjonalną proteaz chymotrypsynopodobnych (w tym enzymów układu krzepnięcia i fibrynolizy), brak selektywności jest dużym problemem w poszukiwaniach ich inhibitorów. Wiele potencjalnych inhibitorów enzymów kaskady krzepnięcia hamuje również białka fibrynolizy. I odwrotnie, potencjalne inhibitory fibrynolizy również mogą obniżać aktywność czynników krzepnięcia (Kołodziejczyk-Czepas i Czepas, 2023). Uzyskane w niniejszej pracy wyniki wskazują natomiast, że badane ekstrakty mogą być rozpatrywane i badane dalej, jako potencjalne selektywne inhibitory białek kaskady krzepnięcia, pod kątem działania przeciwzakrzepowego.

Badane substancje pochodzące z rzewienia zmniejszały reaktywność stymulowanych trombiną komórek śródbłonka. Spadek sekrecji czynnika von Willebranda wskazuje, że część z nich (tj. RHPG, RPT, ekstrakt z ogonków liściowych *R. rhaponticum* i ekstrakt z korzeni *R. rhabarbarum*) zmniejszają aktywność prokoagulacyjną komórek śródbłonka. Odnotowano także nieznaczny spadek poziomu kompleksów PAI-1+t-PA w przypadku HUVECs traktowanych ekstraktem z ogonków liściowych i korzeni *R. rhaponticum,* korzeni *R. rhabarbarum* (w stężeniu 1 μg/ml) oraz stilbenami. Natomiast, ekstrakt z korzeni *R. rhabarbarum* w stężeniu 30 μg/ml wyraźnie zwiększał poziom kompleksów PAI-1+t-PA. Zaobserwowano również spadek uwalniania t-PA. Uzyskane wyniki sugerują, że badane substancje modulują aktywność profibrynolityczną i ograniczają procesy sekrecyjne komórek śródbłonka.

Praca pt. **"*Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro*"** (**praca IV**) dotyczy właściwości przeciwutleniających i przeciwzapalnych badanych ekstraktów oraz stilbenów. Doświadczenia prowadzono w aspekcie oceny zdolności badanych substancji do

ochrony składników osocza krwi przed uszkodzeniami oksydacyjnymi, a także hamowania odpowiedzi zapalnej leukocytów. Manuskrypt **pracy IV** powstał częściowo we współpracy z Zakładem Immunologii Translacyjnej i Eksperymentalnej Intensywnej Terapii, Centrum Medycznego Kształcenia Podyplomowego.

Ocenę efektywności działania przeciwzapalnego w stosunku do leukocytów prowadzono stosując 2 modele badawcze - ludzkie jednojądrzaste komórki krwi obwodowej (PBMCs) oraz inflammasomalne komórki reporterowe THP1-ASC-GFP.

Zaobserwowano, że preinkubacja PBMCs z badanymi substancjami znacznie ogranicza nasilenie odpowiedzi zapalnej tych komórek. Wyniki pomiaru generowania prostaglandyny E2, jako markera aktywacji kaskady kwasu arachidonowego wskazują na zdolność badanych substancji do ograniczania jego metabolizmu. W PBMCs traktowanych ekstraktami i stilbenami zaobserwowano nie tylko częściowe zahamowanie kaskady przemian kwasu arachidonowego (przejawiające się spadkiem ilości generowanej i uwalnianej do środowiska prostaglandyny E2), ale także poziomu uwalnianych cytokin (IL-2 i TNF-α) oraz metaloproteinazy-9. Monitorowanie procesu uwalniania inflammasomów, jako mediatorów odpowiedzi immunologicznej i zapalnej komórek THP1-ASC-GFP, również potwierdziło przeciwzapalne właściwości badanych substancji roślinnych. Zaobserwowano wyraźnie zmniejszenie poziomu uwalnianego z nich białka adaptorowego ASC.

W ramach modułu badawczego obejmującego ocenę właściwości antyoksydacyjnych, przeprowadzono badania efektywności działania badanych substancji w ochronie składników osocza krwi przed uszkodzeniami wywołanymi stresem oksydacyjnym. Stres oksydacyjny wywoływano działaniem nadtlenoazotynu ($ONOO^-$), który jest jednym z głównych oksydantów generowanych w układnie krążenia (Lalu i wsp., 2002; Zou i wsp., 2003; Szabó i wsp., 2007). Przeprowadzone doświadczenia stanowią pierwsze badania dotyczące właściwości ochronnych ekstraktów i stilbenów z rzewienia w stosunku do składników osocza. Badane substancje roślinne zmniejszały zakres oksydacyjnych i nitracyjnych modyfikacji białek oraz peroksydacji lipidów osocza krwi, wywołanych działaniem $ONOO^-$. Ponadto, badane substancje roślinne normalizowały, a nawet wzmacniały pojemność antyoksydacyjną osocza krwi.

W prowadzonych doświadczeniach szczególną uwagę zwrócono na ocenę zdolności badanych ekstraktów z rzewienia i stilbenów do ograniczania oksydacyjnych uszkodzeń fibrynogenu. Jest to białko odgrywające kluczową rolę nie tylko w hemostazie, ale także w innych procesach fizjologicznych. Jednocześnie należy podkreślić, że dane literaturowe wyraźnie wskazują, że już niewielki zakres modyfikacji/uszkodzeń oksydacyjnych przekłada się na zaburzenia

fizjologicznych funkcji fibrynogenu. Na przykład, nitrowanie reszt tyrozyny w fibrynogenie w na poziomie mikromolowym (~45-65 μmol nitrotyrozyny/mol tyrozyny) znacznie nasila jego polimeryzację i tworzenie fibryny, a także zmienia strukturę powstającego skrzepu (Vadseth i wsp., 2004). W badaniach przeprowadzonych w ramach prezentowanej pracy doktorskiej wykazano ochronne działanie badanych substancji, obejmujące częściowe lub większościowe zmniejszenie zakresu uszkodzeń oksydacyjnych cząsteczek fibrynogenu, takich jak modyfikacja reszt tyrozyny i tryptofanu oraz tworzenie agregatów białkowych.

PODSUMOWANIE

Po raz pierwszy podjęto badania obejmujące aktywność biologiczną ekstraktów z różnych organów dwóch gatunków rzewienia (*R. rhaponticum* i *R. rhabarbarum*) oraz dwóch stilbenów (RHPG i RHPT), występujących w tych roślinach, w kontekście ich wielokierunkowego działania ochronnego na składniki układu hemostazy. Prace badawcze dotyczyły oceny właściwości przeciwzapalnych, antykoagulacyjnych oraz przeciwutleniających. Maksymalny efekt działania badanych substancji, zaobserwowany w głównych doświadczeniach zestawiono w tabeli 1.

Wykazano, że badane ekstrakty i stilbeny posiadają właściwości przeciwzapalne **(praca II i IV**) i mogą modulować odpowiedź zapalną komórek śródbłonka oraz badanych leukocytów, a także interakcje komórek śródbłonka z makrofagami, stanowiące ważny element naczyniowej odpowiedzi zapalnej. Badane substancje roślinne zmniejszały odpowiedź prozapalną komórek śródbłonka (HUVECs) na różnych poziomach molekularnych, obejmujących ekspresję genów, procesy uwalniania czynników prozapalnych oraz wzrost adhezyjności. Uzyskane wyniki wskazują, że obserwowany efekt jest między innymi wynikiem supresji genów kontrolujących syntezę enzymów prozapalnych (głównie COX-2), a także częściowo zdolności badanych substancji do bezpośredniego hamowania aktywności już zsyntetyzowanego białka enzymatycznego. Pomiary poziomu prostaglandyny E_2 wyraźnie wskazały na hamujący wpływ badanych substancji na przemiany kwasu arachidonowego w komórkach.

Badania objęte modułem hemostatycznym (**praca III**) dotyczyły oceny zdolności badanych ekstraktów i stilbenów do modulowania odpowiedzi hemostatycznej komórek śródbłonka oraz aktywności wybranych osoczowych składników układu hemostazy. Dotychczas dostępne dane etnomedyczne są niejednorodne i obejmują tradycyjne zastosowanie preparatów opartych na ekstraktach z badanych gatunków rzewienia zarówno w celach

przeciwkrwotocznych, jak i usprawniających przepływ krwi. Badania przeprowadzone w prezentowanej pracy wskazują, że badane ekstrakty posiadają właściwości antykoagluacyjne, co może przekładać się na efekt przeciwzakrzepowy (którego potwierdzenie wymaga jednak dalszych badań, w tym doświadczeń *in vivo*). Ekstrakty z rzewienia hamowały proces krzepnięcia krwi indukowany czynnikiem tkankowym, a analizy ich bezpośredniego wpływu na kluczowe proteazy serynowe kaskady krzepnięcia: trombinę i czynnik Xa wskazują, że obserwowany efekt może być wynikiem inhibicji tych czynników.

Doświadczenia wchodzące w skład modułu antyoksydacyjnego **(praca IV)** wykazały działanie ochronne ekstraktów *R. rhaponticum* i *R. rhabarbarum* oraz stilbenów przed uszkodzeniami składników osocza krwi wywołanymi nadtlenoazotynem. Zaobserwowano ponadto, że badane substancje ograniczają oksydacyjne i nitracyjne uszkodzenia fibrynogenu, co może mieć istotne znaczenie w utrzymaniu fizjologicznej funkcjonalności tego białka.

Tabela 1. Podsumowanie efektywności działania ekstraktów z badanych gatunków rzewienia oraz stilbenów w przeprowadzonych doświadczeniach. Zestawienie obejmuje maksymalny efekt hamujący stwierdzony w oznaczeniach głównych parametrów/markerów, monitorowanych w kontekście oceny zdolności badanych substancji roślinnych do ograniczania odpowiedzi zapalnej i hemostatycznej, a także przeciwdziałania uszkodzeniom składników osocza, wywołanym stresem oksydacyjnym.

Oznaczenie i model badawczy/układ doświadczalny			***Maksymalny efekt hamujący***					
			RHPG (rapontygenina)	RHPT (rapontycyna)	Ekstrakt z ogonków liściowych *R. rhaponticum*	Ekstrakt z ogonków liściowych *R. rhabarbarum*	Ekstrakt z korzeni *R. rhaponticum*	Ekstrakt z korzeni *R. rhabarbarum*
Właściwości przeciwzapalne	***COX2* (gen)/ (HUVECs)**		78%	69%	59%	61%	brak efektu hamujacego	brak efektu hamującego
	***ALOX5* (gen)/(HUVECS)**		22%	28%	48%	52%	52%	brak efektu hamującego
	COX-2/(enzym)		38%	14%	23%	26%	81%	81%
	5-LOX/(enzym)		39%	20%	10%	18%	15%	15%
	TNF-α/(PBMCs)		89%	21%	68%	79%	33%	brak efektu hamującego
	IL-2/(PBMCs)		99%	53%	68%	76%	69%	70%
	Profilowanie cytokin/ chemokin/(HUVECs)	**CCL2/MCP-1**	19%	brak efektu hamującego	11%	brak efektu hamującego	brak efektu hamującego	31%
		CCL5/RANTES	80%	100%	100%	100%	100%	11%
		CXCL1/GROα	brak efektu hamującego	brak efektu hamującego	17%	brak efektu hamującego	brak efektu hamującego	brak efektu hamującego
		CXCL10/IP-10	13%	100%	100%	100%	100%	15%
		G-CSF	brak efektu hamującego	71%	83%	90%	62%	brak efektu hamującego
		GM-CSF	brak efektu hamującego	69%	73%	79%	42%	11%
		ICAM-1/CD54	25%	brak efektu hamującego	87%	81%	73%	25%
		IL-6	brak efektu hamującego	40%	22%	22%	28%	21%
		MIF	25%	59%	61%	70%	36%	29%

		Serpina E1/PAI-1	40%	16%	brak efektu hamującego	brak efektu hamującego	brak efektu hamującego	73%
	PGE_2/(PBMCs)		61%	29%	28%	63%	35%	22%
	MMP-9/(PBMCs)		72%	42%	59%	74%	43%	39%
	Adhezja monocytów do HUVECs		86%	85%	59%	72%	81%	88%
	Uwalnianie białka ASC/(THP1-ASC-GFPs)		83%	60%	43%	46%	47%	58%
Wpływ na aktywność hemostatyczną	**Krzepnięcie osocza krwi aktywowane czynnikiem tkankowym**		brak efektu hamującego	brak efektu hamującego	38%	27%	29%	22%
	Amidolityczna aktywność trombiny		18%	15%	52%	71%	80%	90%
	Proteolityczna aktywność trombiny		brak efektu hamującego	brak efektu hamującego	29%	69%	89%	88%
	Aktywność czynnika Xa		45%	59%	54%	91%	89%	99%
	Generowanie plazminy (indukowane t-PA)		18%	brak efektu hamującego	brak efektu hamującego	brak efektu hamującego	brak efektu hamującego	11%
	Wydzielanie czynnika vW/(HUVECs)		50%	38%	20%	brak efektu hamującego	brak efektu hamującego	27%
	Uwalnianie t-PA/(HUVECs)		53%	50%	59%	50%	54%	57%
	Poziom kompleksów PAI-I + t-PA/(HUVECs)		15%	14%	27%	brak efektu hamującego	10%	22%
Właściwości antyoksydacyjne	**TBARS/(osocze)**		35%	18%	20%	30%	43%	14%
	Powstawanie 3-NT w białkach osocza		58%	54%	52%	44%	65%	52%
	Tworzenie 3-NT w fibrynogenie		99%	98%	55%	75%	98%	98%
	Powstawanie agregatów białkowych z fibrynogenu		76%	76%	brak efektu hamującego	34%	47%	75%

WNIOSKI

Badane ekstrakty z rzewienia i stilbeny:

- wykazują właściwości przeciwzapalne, a ich działanie obejmuje między innymi supresję genu *COX2*, bezpośrednie hamowanie enzymu COX-2 i metabolizmu kwasu arachidonowego, a także ograniczenie uwalniania substancji o działaniu prozapalnym z komórek śródbłonka i leukocytów;

- modulują aktywność hemostatyczną komórek śródbłonka i działają antykoagulacyjnie. Mechanizmy obserwowanego efektu antykoagulacyjnego obejmują hamowanie czynnika Xa krzepnięcia oraz trombiny;

- wykazują selektywność w kierunku hamowania badanych osoczowych czynników krzepnięcia, natomiast ich wpływ na aktywność powstającej plazminy, głównego enzymu układu fibrynolizy, jest znikomy;

- posiadają właściwości przeciwutleniające i działają ochronnie na składniki osocza krwi w warunkach stresu oksydacyjnego.

STRESZCZENIE

Choroby układu sercowo-naczyniowego są główną przyczyną śmiertelności w krajach rozwiniętych. Stąd też rośnie zainteresowanie opracowaniem nowych terapii profilaktycznych i leczniczych, pomocnych w zwalczaniu tych schorzeń. W grupie środków leczniczych przydatnych w działaniach profilaktycznych i terapii chorób cywilizacyjnych, coraz większą rolę odgrywają preparaty pochodzenia roślinnego.

Rodzaj *Rheum* L. (rzewień, rabarbar) od dawna jest znany w różnych regionach świata, nie tylko jako źródło gatunków jadalnych, ale także jako ceniony surowiec zielarski. Wiele aspektów działania tych roślin na organizm człowieka pozostaje jednak słabo opisanych, a ich stosowanie w wielu przypadkach opiera się jedynie na danych pochodzących z medycyny tradycyjnej. Celem prowadzonych badań była ocena wpływu ekstraktów z dwóch gatunków rzewienia - *Rheum rhaponticum* L. oraz *Rheum rhabarbarum* L. oraz typowych dla tych roślin stilbenów na funkcjonowanie wybranych osoczowych i komórkowych składników układu hemostazy. Badania skupiały się na aktywnościach biologicznych wskazywanych jako jedne z kluczowych dla kardioprotekcyjnego działania substancji naturalnych i obejmowały działanie przeciwzapalne, antykoagulacyjne/przeciwzakrzepowe oraz przeciwutleniające. Oceniano efektywność działania ekstraktów (frakcje butanolowe) z korzeni oraz ogonków liściowych wyżej wspomnianych gatunków rzewienia oraz obecnych tych roślinach pochodnych stilbenu: rapontygeniny i rapontycyny.

Plan badań obejmował zastosowanie modeli eksperymentalnych ściśle związanych z fizjologią układu sercowo-naczyniowego i hemostazą, tj. komórek śródbłonka ściany naczyniowej, leukocytów oraz osoczowych składników układu hemostazy (w tym kluczowych białek kaskady krzepnięcia krwi oraz układu fibrynolitycznego). W celu uzyskania jak najszerszego obrazu aktywności biologicznej badanych substancji, zastosowano zróżnicowany panel metod analitycznych: badania ekspresji genów, testy skriningowe inhibitorów, oznaczenia spektrofotometryczne i fluorymetryczne, kinetyczną analizę aktywności enzymów, monitorowanie procesu krzepnięcia osocza krwi (oznaczenia turbidymetryczne), testy ELISA, mikroskopię fluorescencyjną, elektroforezę 1D oraz profilowanie wielocytokinowe oparte na technice blottingu.

Badane substancje roślinne zmniejszały odpowiedź zapalną komórek śródbłonka (HUVECs), działając na różnych poziomach molekularnych (modulacji ekspresji genów, hamowania uwalniania czynników prozapalnych oraz redukcji adhezyjności komórek). Odnotowano istotny spadek uwalniania markerów/białek odpowiedzi zapalnej (np. TNF-α,

IL- 2, MMP-9). Wykazano również właściwości antykoagluacyjne badanych ekstraktów, co może przekładać się na efekt przeciwzakrzepowy. Ekstrakty z rzewienia hamowały proces krzepnięcia krwi indukowany czynnikiem tkankowym, stanowiącym główny fizjologiczny szlak aktywacji hemostazy osoczowej. Analizy bezpośredniego wpływu badanych substancji roślinnych na kluczowe proteazy serynowe kaskady krzepnięcia: trombinę i czynnik Xa wskazują, że obserwowany efekt antykoagulacyjny może być wynikiem inhibicji tych czynników. Ponadto, po raz pierwszy wykazano również zdolność badanych ekstraktów z rzewienia i stilbenów do ograniczania uszkodzeń składników osocza, wywołanych stresem oksydacyjnym.

Przeprowadzone badania dostarczyły nowych informacji na temat właściwości biologicznych metabolitów rzewienia w kontekście kardioprotekcyjnym, a uzyskane wyniki mogą stanowić podstawę do dalszych prac badawczych.

ABSTRACT

Cardiovascular diseases are the leading cause of mortality in developed countries. Hence, there is a growing interest in the development of new preventive and therapeutic therapies to help combat these diseases. In the group of agents useful in prophylaxis and therapy of civilization diseases, preparations of plant origin play an increasingly important role.

The *Rheum* L. (rhubarb) genus has long been known in various regions of the world, not only as a source of edible species, but also as a valuable herbal material. However, many aspects of the effects of these plants on the human body remain poorly described, and in numerous cases, their use is still based only on data derived from traditional medicine. The aim of this study was to assess the effect of extracts from two species of rhubarb - *Rheum rhaponticum* L. and *Rheum rhabarbarum* L. and stilbenes typical for these plants on functions of selected plasma and cellular components of the haemostatic system. The work was focused on biological activities indicated as the key to the cardioprotective effect of natural substances, and included anti-inflammatory, anticoagulant/antithrombotic and antioxidant effects. Effectiveness of extracts (butanol fractions) from the roots and petioles of the above-mentioned rhubarb species and the stilbene derivatives that are present in these plants, *i.e.* rhapontigenin and rhaponticin, was evaluated.

The study design included the use of experimental models closely related to the physiology of the cardiovascular system and haemostasis, *i.e.* endothelial cells, leukocytes and plasma components of the haemostasis system (including key proteins of the blood coagulation cascade and the fibrinolytic system). To obtain the most comprehensive insight into the biological activity of the examined substances, a diverse panel of analytical methods was used: gene expression studies, inhibitor screening tests, spectrophotometric and fluorimetric assays, kinetic analysis of enzyme activity, monitoring of the blood plasma coagulation process (turbidimetric assays), ELISA, fluorescence microscopy, 1D-electrophoresis and the blotting-based multicytokine profiling.

The examined plant substances reduced the inflammatory response of endothelial cells (HUVECs), acting at various molecular levels (modulation of gene expression, inhibition of the release of pro-inflammatory factors and reduction of cell adhesion). There was a significant decrease in the release of inflammatory response markers/proteins (*e.g.* TNF-α, IL-2, MMP-9). The anticoagulant properties of the examined extracts were also demonstrated, which may result in an antithrombotic effect. Rhubarb extracts inhibited the blood plasma coagulation process, induced by the tissue factor, which is the main physiological pathway of

the blood plasma hemostasis activation. Analyzes of direct effects of the examined plant substances on the key serine proteases of the coagulation cascade: thrombin and factor Xa indicated that the observed anticoagulant effect might be a result of inhibition of these factors. Furthermore, for the first time, the ability of the investigated rhubarb extracts and stilbenes to reduce the oxidative stress-induced damage to plasma components was demonstrated as well.

The executed experiments provided new information on the biological properties of rhubarb metabolites in the cardioprotective context, and the obtained results may be a basis for further research.

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DOROBEK NAUKOWY I ORGANIZACYJNY

PUBLIKACJE JCR

1. Kołodziejczyk-Czepas, J., **Liudvytska, O.*** *Rheum rhaponticum and Rheum rhabarbarum: a review of phytochemistry, biological activities and therapeutic potential*. Phytochemistry Reviews (SPRINGER), 2021, 20, 589-607. DOI: 10.1007/s11101-020-09715-3. Punkty MEiN$_{2021}$: **100;** Impact Factor$_{2021}$ **7,741**

2. Liudvytska O.*, Ponczek M.B., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kołodziejczyk-Czepas J. *Rheum rhaponticum and Rheum rhabarbarum extracts as modulators of endothelial cell inflammatory response*. Nutrients (MDPI), 2023; DOI: 10.3390/nu15040949. Punkty MEiN$_{2021}$: **140,** Impact Factor$_{2021}$ **6,706**

3. Liudvytska O.*, Ponczek M.B., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kołodziejczyk-Czepas J. *Effects of Rheum rhaponticum and Rheum rhabarbarum extracts on haemostatic activity of blood plasma components and endothelial cells in vitro.* Journal of Ethnopharmacology (ELSEVIER), 2023, DOI: 10.1016/j.jep.2023.116562; Punkty MEiN$_{2021}$: **140,** Impact Factor$_{2021}$ **5,195**

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5. Machała P.*, **Liudvytska O.**, Kicel A., Dziedzic A., Olszewska MA., Żbikowska HM. *Valorization of the photo-protective potential of the phytochemically standardized olive (Olea europaea L.) leaf extract in UVA-irradiated human skin fibroblasts*. Molecules (MDPI). 2022, DOI: 10.3390/molecules27165144. Punkty MEiN$_{2021}$: **140,** Impact Factor$_{2021}$: **4,927**

Sumaryczny IF: 31,275 (660 pkt MEiN)

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2. Janicka M., Machała P., **Liudvytska O**., Forma E. *Polimorfizm genu TopBP1 a ryzyko rozwoju raka piersi kobiet.* Dokonania naukowe doktorantów. Monografia pokonferencyjna, ISBN: 978-83-63058-66-1.

3. Janicka M., Machała P., **Liudvytska O.**, Forma E. *Udział białka TopBP1 w regulacji naprawy DNA i nowotworzeniu.* Dokonania naukowe doktorantów. Monografia pokonferencyjna, ISBN: 978-83-63058-66-1.

DONIESIENIA KONFERENCYJNE

Prezentacje ustne *(w języku polskim i angielskim)*

1. Liudvytska O.*, Kołodziejczyk-Czepas J. *„The presence of rhubarb in the diet may support the cardiovascular health"* - National Scientific Conference Knowledge – Key to Success 4rd edition. Toruń, 18.01.2020.

2. Liudvytska O.*, Krzyżanowska-Kowalczyk J., Kołodziejczyk-Czepas J. *„Ekstrakty z Rheum rhaponticum L. i Rheum rhabarbarum L. jako potencjalne inhibitory trombiny: wyniki wstępne"* III Ogólnopolska Konferencja Naukowa Rośliny w naukach medycznych i przyrodniczych. On-line, 05.09.2020.

3. Liudvytska O.*, Kołodziejczyk-Czepas J. *„Antrachinony gatunków rodzaju Rheum L. (Polygonaceae) i ich aktywność biologiczna"* - III Ogólnopolska Konferencja Naukowa Pierwotne i wtórne metabolity roślin i grzybów. On-line, 15.12.2020.

4. Liudvytska O.*, Krzyżanowska-Kowalczyk J., Kołodziejczyk-Czepas J. *„Ocena właściwości przeciwzapalnych ekstraktów z Rheum rhabarbarum oraz Rheum rhaponticum in vitro"* - VI Ogólnopolska konferencja Doktorantów Nauk o Życiu BioOpen. On-line, 15-16.04.2021.

5. Liudvytska O.*, Kołodziejczyk-Czepas J. *„Właściwości przeciwcukrzycowe antrachinonów obecnych w różnych gatunkach rzewiena (Rheum L.)"* - IV Ogólnopolska Konferencja Naukowa Pierwotne i wtórne metabolity roślin i grzybów. On-line, 15.07.2021.

6. Liudvytska O.*„ *Evaluation of anti-inflammatory properties of Rheum rhabarbarum and Rheum rhaponticum extracts and stilbenes in vitro"* – Multidysyplinarna Konferencja Doktorantów Uniwersytetu Szczecińskiego 2.0. On-line, 22.06.2022.

7. Liudvytska O.*, Krzyżanowska-Kowalczyk J., Kołodziejczyk-Czepas J. *„Ocena wpływu ekstraktów z Rheum rhaponticum L. i Rheum rhabarbarum L. na właściwości hemostatyczne osocza krwi i komórek śródbłonka (HUVECs)"* - IV Ogólnopolska Konferencja Naukowa „Perspektywy wykorzystania roślin w nauce i przemyśle". On-line, 26.11.2022

Postery

1. Liudvytska O.*, Machała P. *„Genisteina i daidzeina – izoflawony soi o zróżnicowanym machanizmie działania”*. I Ogólnopolska Konferencja Naukowa “Naukowo o Żywieniu”. Gdańsk, 18.05.2019.

2. Liudvytska O.*, Kołodziejczyk-Czepas J., Krzyżanowska-Kowalczyk J. *„Rheum rhaponticum w walce z dolegliwościami w okresie okołomenopauzalnym”* - X Ogólnokrajowa Konferencja Naukowa Młodzi Naukowcy w Polsce – Badania i Rozwój. Lublin, 20.11.2019.

3. Liudvytska O.*, Krzyżanowska-Kowalczyk J., Kołodziejczyk-Czepas J. *„Przeciwzapalne i przeciwutleniające właściwości rzewienia (Rheum sp.) – wyniki analiz in vitro”* - VI Ogólnopolskie Sympozjum Biomedyczne Eskulap. Lublin, 30.11.2019.

4. Michaś K.*, **Liudvytska O**., Krzyżanowska-Kowalczyk J., Kowalczyk M., Kołodziejczyk-Czepas J. *„Ocena wpływu ekstraktów wybranych gatunków rzewienia (Rheum L.) na odpowiedź zapalną komórek śródbłonka”* - VIII Ogólnopolska Konferencja Doktorantów Nauk o Życiu BioOpen. On-line, 13-14.04.2023

PROJEKTY

- **Wykonawca** w projekcie badawczym, finansowanym ze środków Narodowego Centrum Nauki „*Aktywność biologiczna ekstraktów z wybranych gatunków rzewienia (Rheum L.) - badania właściwości surowca w aspekcie oceny potencjału kardioprotekcyjnego*” (2018/31/B/NZ9/01238)

- **Uczestnik** międzynarodowego konsorcyjnego *projektu RiEcoLab Responsible Innovation Led Entrepreneurial University Transformation Centres (Ecosystem Integration Labs)* (Konsorcjum Innowacyjnych Uczelni Wyższych - Centra Transformacji Przedsiębiorczych Uniwersytetów na Rzecz Odpowiedzialnych Innowacji (Laboratoria Transformacji Ekosystemu); projekt współfinansowany ze środków programu ramowego Unii Europejskiej w zakresie badań naukowych i innowacji „Horyzont Europa”

KURSY PRAKTYCZNE ZAKOŃCZONE CERTYFIKATEM

1. *Immunocytochemia* (01-708/0-00-024-2020) – CMKP, Warszawa,05-07.02.2020
2. *Charakterystyka białek: elektroforeza SDS PAGE i western blotting* (01-700/0-00-001-2020) – CMKP , Warszawa, 04-06.03.2020
3. *Elektroforeza dwukierunkowa 2-DE metoda i zastosowanie* (01-700/0-00-004-2021) – CMKP, Warszawa, 6-8.10.2021
4. *Hodowla komórkowa* (01-708/0-00-052-2021) – CMKP, Warszawa, 26-29.10.2021
5. *Podstawy techniki immunoenzymatycznej ELISA* (01-708/0-00-028-2021) – CMKP, Warszawa, 26.11.2021
6. *Technika blotingu w diagnostyce autoimmunizacyjnych chorób układu pokarmowe*go (01-708/0-00-022-2021) – CMKP, Warszawa, 14.12.2021
7. *Podstawy cytometrii przepływowej - zastosowanie w diagnostyce i badaniach naukowych* (01-708/0-00-027-2022) – CMKP, Warszawa, 11-14.01.2022

8. *Technika cytometrii przepływowej - obsługa cytometru przepływowego oraz analiza fenotypu, proliferacji i żywotności komórek* (01-708/0-00-025-2022) – CMPK, Warszawa, 20-21.10.2022
9. N*owoczesne metody analizy sekwencji i ekspresji genów - medycyna molekularna* (01-700/0-00-009-2022) – CMPK, Warszawa, 24-28.10.2023
10. *Badanie aktywacji komórek układu odpornościowego z wykorzystaniem mikroskopii w czasie rzeczywistym* (01-700/0-00-009-2023) – CMPK, Warszawa, 9-10.03.2023

SZKOLENIA ZAWODOWE ZAKOŃCZONE UZYSKANIEM CERTYFIKATÓW

1. Świadectwo kwalifikacyjne № E/1788/186/22 – uprawniające do zajmowania się eksploatacją urządzeń, instalacji i sieci na stanowisku: eksploatacji (GRUPA 2)
2. Świadectwo kwalifikacyjne № E/1821/186/22 – uprawniające do zajmowania się eksploatacją urządzeń, instalacji i sieci na stanowisku: eksploatacji (GRUPA 3)
3. Świadectwo kwalifikacyjne № E/100/861/23 – uprawniające do zajmowania się eksploatacją urządzeń, instalacji i sieci na stanowisku: eksploatacji (GRUPA 2)

DZIAŁALNOŚĆ ORGANIZACYJNA I PROMOCYJNA

1. Udział w przeprowadzeniu wyborów uzupełniających na Przedstawiciela do Rady Wydziału Biologii i Ochrony Środowiska, jako członek Komisji rekrutacyjnej (2019).
2. W ramach akcji „*Uniwersytet Zawsze Otwarty*" współprowadzenie zajęć dla uczniów szkoły podstawowej: „*Wykrywanie aktywności enzymatycznej w materiale biologicznym*" (2019)
3. Pomoc w organizacji III Krajowej Naukowo-Szkoleniowej Konferencji Biobanków Polskich (2020).
4. Udział w promocji Wydziału BiOŚ poprzez udział w Nocy Biologów (2020).
5. Pomoc w organizacji VI, VII oraz VIII Ogólnopolskiej Konferencji Doktorantów Nauk o Życiu BioOpen (2021, 2022, 2023)
6. Członkostwo w Sekcji Genetycznej SKNB – czynny udział w jego funkcjonowaniu (2019 – 2022)
7. Członkostwo w Polskim Towarzystwie Biochemicznym (PTBioch) (2022-obecnie)

KOPIE PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

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Rheum rhaponticum and *Rheum rhabarbarum*: a review of phytochemistry, biological activities and therapeutic potential

Joanna Kolodziejczyk-Czepas · Oleksandra Liudvytska

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Abstract The *Rheum* genus (Polygonaceae) covers about 60 species of rhubarbs, including specimens with a long ethnomedicinal history in Asia, Europe and other regions of the world. The work reviews available literature (until March, 2020) on phytochemical profile, ethnomedicinal recommendations, biological activities, pharmacological uses and future prospects for therapeutic applications of *Rheum rhabarbarum* L. (garden rhubarb) and *Rheum rhaponticum* L. (rhapontic rhubarb). Although the above species are well-known vegetables, scientific interest in these plants is a relatively new issue; most of evidence of their biological activities and therapeutic potential derives from the last 15 years. Rhubarbs contain numerous bioactive substances, belonging to diverse groups of phytochemicals, e.g. stilbenes, anthraquinones and flavonoids. The registered special extract of *R. rhaponticum* (ERr731®) is administered to alleviate the menopause-related complaints. Furthermore, both ethnomedicinal surveys and recent studies on bioactive substances from rhubarbs indicate that these plants may have significantly broader range of beneficial effects such as antioxidant, anti-inflammatory, antimicrobial and cardioprotective activities.

Keywords Rhubarb · Ethnomedicine · Biological activity · Bioactive substances

Introduction

The genus *Rheum* L. (rhubarb) covers about 60 species of perennial herbs, belonging to the Polygonaceae family. Rhubarbs have been known worldwide (in Asia, in particular) as medicinal and/or edible plants (Agarwal et al. 2001; Cao et al. 2017; He et al. 1992; Pourjabali et al. 2017; Rehman et al. 2014). Scientific interest in chemical composition and biological properties of two of the most popular rhubarb species, i.e. garden rhubarb (*Rheum rhabarbarum* L./syn. *R. undulatum* L.—according to www.theplantlist.org and http://www.worldfloraonline.org) and rhapontic rhubarb (*Rheum rhaponticum* L.; also called Siberian rhubarb) has significantly increased for the last 15 years. Despite the popularity of these species as foods, their phytochemical profiles, bioactive components and pharmacological relevance still remain partly elucidated. Their petioles (stalks) are commonly used for culinary purposes, and the leaves are toxic (Slaughter et al. 2012) due to a high content of oxalic

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J. Kolodziejczyk-Czepas · O. Liudvytska (✉)
Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska, 141/143, 90-236 Lodz, Poland
e-mail: oleksandra.liudvytska@unilodz.eu

acid. However, contrary to the roots of *Rheum officinale* Baill., which are predominantly used as a laxative, underground parts of both the garden and rhapontic rhubarb have a broader range of applicability in traditional medicine and contemporary herbal therapies.

While the chemical profile and biological properties of other rhubarb species such as *R. australe* D. Don, *R. palmatum* L., *R. ribes* L., *R. officinale* Baill., *R. tanguticum* Maxim. ex Balf. or *R. emodi* L. have been extensively described and/or reviewed in the literature (Komatsu et al. 2006; Rashid et al. 2014; Rokaya et al. 2012; Wang et al. 2013; Zhang et al. 2015a), there is a lack of a comprehensive look at *R. rhabarbarum* and *R. rhaponticum*. The present work is based on a review of available literature dealing with ethnomedicinal data, phytochemical composition, biological activities and prospects for future pharmacological use of these rhubarb species. Since the evaluation of some of biological activities or pharmacological significance of rhubarb-derived compounds and extracts partly remain at the preliminary stage or available data are inconsistent, disputable issues have been critically presented in this work. Our review covers data (until March, 2020) originating from journals indexed in international databases such as Medline/Pubmed, Scopus, Science Direct/Elsevier and Springer Link/ICM. Additionally, local scientific publications, not indexed in the mentioned databases were also taken into consideration.

Rheum rhaponticum and *Rheum rhabarbarum* in traditional medicine

Rhubarb is known in traditional medicine of Asian, European and other cultures (Agarwal et al. 2001; Pourjabali et al. 2017; Rehman et al. 2014), but a detailed appraisal of ethnomedicinal relevance of individual species or their biological actions is limited by the fact that in some cases, no precise botanical identification (including Latin names) of the used/described species is given. Even in recent literature, rhubarbs are frequently described generally, as "*Rheum* sp." or just "rhubarb(s)". Nevertheless, available data from ethnomedicinal and ethnobotanical surveys confirm the presence of *R. rhaponticum* and *R. rhabarbarum* in medicine from the Middle Ages (Nedelcheva 2009). According to data from one of the oldest Polish books devoted to medicinal plants, i.e. *Herbarz Polski''* (Marcin z Urzędowa/Marcin of Urzędów 1595) roots of both these species were used in medicine of that time to cure a wide range of disorders. The author suggested using 1–2 or more "dragma" units (1 dragma corresponded to 1/8 of one ounce). Internally, *R. rhaponticum* (in wine, beer or mead) was used in therapy of gastrointestinal pain, gastritis, liver and spleen disorders, heartache and pain in pericardium, pulmonary system dysfunctions as well as disorders related to the reproductive system, including uterine and breast pains. Drinking of the *R. rhaponticum*-based mixtures or chewing its root was believed to alleviate indigestion. Externally, a vinegar macerate from this plant was applied to cure skin disorders such as itching and scratches; a water macerate was recommended in the therapy of ulcers. *R. rhabarbarum* was mainly administered as a purgative agent. Other recommendations for its use included liver, spleen and stomach dysfunctions as well as blood purification. Some fragments of this historical source indicated that this plant was also used to stop bleeding and to alleviate fever as well as to cure injuries, trauma after falls from a height and vein disorders.

Current literature devoted to ethnomedicinal issues also confirms that the roots of *R. rhaponticum* were an ingredient of different mixtures, administered to cure fever (a sugar syrup), to improve voice (roots boiled in red wine and sweetened with honey), to alleviate heart problems or stomachache (a brandy-based balsam), and a honey-based herbal paste was used in jaundice and distress (Nedelcheva 2012). Furthermore, underground parts of this rhubarb are an ingredient of Turkish folk food and the remedy, i.e. *Mesir* paste, having over 500 years history of use (Oskay et al. 2010). In Brazilian folk medicine, *R. rhaponticum* is used to treat gastrointestinal disorders (Cogo et al. 2010), while in Iran, it is one of the herbs traditionally used to treat hyperpigmentation (Ghafari et al. 2017). This rhubarb species has been also mentioned in different types of studies as one of medicinal plants in European and Asian countries (Debnath et al. 2006; Lachumy et al. 2013).

Similarly to *R. rhaponticum*, also the position of *R. rhabarbarum* in ethnomedicine of different cultures has been built for centuries. Both species are known in Central Asia and Russia as a natural demulcent, helpful in wound healing and relieving skin problems

(e.g. irritations, allergy and dermatitis) (Mamedov et al. 2008). *R. rhabarbarum* is known in Ayurvedic medicine (Dandekar and Deshpande 2016). In Chinese medicine, this plant has been administered to heal ulcers as well as to treat constipation, jaundice and gastrointestinal hemorrhage (State Pharmacopoeia Committee 2010), or as a natural anti-inflammatory ingredient of different formulations, useful in the therapy of appendicitis, cholecystitis and rheumatoid arthritis (Ma et al. 2009). Furthermore, *R. rhabarbarum* is a component of Yīn–Chén–Hāo decoction, a traditional Chinese herbal medicine used to treat damp-heat jaundice and hepatic disorders (Li et al. 2017). This rhubarb species was also listed in the Chilandar Medical Codex, a medieval Serbian manuscript, covering documents on European medical knowledge from the 12th to 15th century. Recommendations for the use of *R. rhabarbarum* included blood detoxification, gastrointestinal tract disorders, hydrops, melancholy as well as its administration as a mucolytic, purgative, sedative and antispasmodic preparation (Jarić et al. 2014). It is described as one of ethnomedicinal plants in Hungary (Babulka 1993), depurative herbal medicine in South-Western Germany (Pieroni and Gray 2008) and traditional Korean remedy for gastrointestinal disorders (Kim et al. 2014).

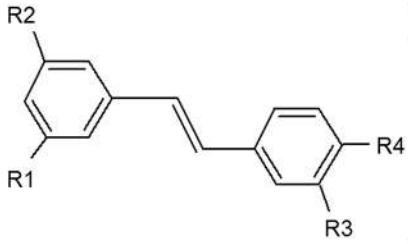
Phytochemical profile of *R. rhaponticum* and *R. rhabarbarum*

Studies on the phytochemical composition of different species of rhubarb were undertaken as early as at the beginning of the 20th century, and provided information on the presence of a variety of inorganic and organic acids (including tartaric, oxalic, citric, malic and ascorbic acid), anthraquinones (e.g. emodin, aloe-emodin and rhein) and stilbenes (Pucher et al. 1938; Tutin and Clewer 1911; Viehoever 1933). The last two decades have provided more detailed qualitative analyses of the chemical profile of *R. rhaponticum,* covering examinations of phenolic and non-phenolic components of this plant. Results of these studies, with names of the identified compounds, are summarized in the Table 1. Some information on methodological aspects of isolation of the individual components, e.g. rhaponticin and desoxyrhaponticin are also available (Smolarz et al. 2013a). For the first time, the composition and content of flavonoids was investigated by German scientists who discovered rutin in leaves of *R. rhabarbarum* (0.7%) and *R. rhaponticum* (0.61%) (Hörhammer and Müller 1954). Later, glycosides of quercetin and kaempferol were found in the leaves and petioles of *R. rhabarbarum*, while in the flower petals, quercetin 3-rhamnoside and quercetin 3-rutinoside were identified (Vysochina 2012). In raw stalks of the rhapontic rhubarb, 42 components belonging to different groups of flavonoids as well as stilbenes, antraquinones and naphthalene derivatives (for details, see the Table 1) were found. In the roots, derivatives of *trans*-piceatannol, *trans*-resveratrol, *trans*-rhapontigenin and *trans*-desoxyrhapontigenin, were identified (Fig. 1). Root samples contained pterostilbene acetylglucosides as well as hydroxyanthraquinones and their glycosides (Püssa et al. 2009). The presence of numerous organic compounds, representing various classes of (phyto)-chemicals and their distribution in *R. rhabarbarum* and hybrids of *R. rhabarbarum* and *R. rhaponticum* were described as well (Dregus and Engel 2003; Ha et al. 2020; Ko 2000; Krafczyk et al. 2008; Nizioł et al. 2017; Will and Dietrich 2013) (data collected in Table 2).

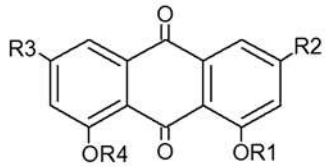
Different cooking regimes influence the content of polyphenols in rhubarb. Compared to raw petioles, both fast and slow stewing and baking were found to enhance the total content of polyphenolic compounds and overall antioxidant capacity of the examined rhubarb samples. This increase was attributed to thermal degradation of plant material, leading to a release of bioactive substances (McDougall et al. 2010). The literature provides reports dealing with quantitative data on different groups of metabolites in *R. rhaponticum* and *R. rhabarbarum*. A comparative examination of crude ethanol (96%; v/v) extracts, derived from the rhizomes and roots of *R. rhaponticum*, *R. palmatum* L. and *R. rhabarbarum* revealed that the rhapontic rhubarb had the lowest contents of total polyphenols, total anthracene derivatives, total anthraquinones and overall tanin concentration, attaining 46.11 ± 0.81 mg/g, 19.8 ± 0.60 mg/g, 16.6 ± 0.50, mg/g and 7.07 ± 0.25 mg/g of dry material, respectively (Kosikowska et al. 2010). The total concentrations of phenolic acids in *R. rhaponticum* rhizome amounted to 195.5 µg/g, with concentrations of individual acids ranging between 2.2 µg/g (*p*-hydroxybenzoic acid) and 77.7 µg/g (ferulic acid)

Table 1 Phytochemical composition of *R. rhaponticum*

Plant part/solvent	Identified substances	References
Air-dried roots/methanol (> 99.9%)	*Stilbene compounds*: piceatannol *O*-glucoside 1, piceatannol *O*-glucoside 2, resveratrol *O*-glucoside 1, resveratrol *O*-glucoside 2 (piceid), piceatannol *O*-galloylglucoside 1, piceatannol, piceatannol *O*-galloylglucoside 2, rhapontigenin *O*-glucoside (rhapontin), resveratrol *O*-galloylglucoside, rhapontigenin *O*-glucoside 2, *trans*-resveratrol, rhapontigenin *O*-galloylglucoside, rhapontigenin *O*-acetylglucoside 1, rhapontigenin, rhapontigenin *O*-acetylglucoside 2, desoxyrhapontigenin *O*-glucoside (desoxyrhapontin), desoxyrhapontigenin *O*-galloylglucoside, desoxyrhapontigenin *O*-acetylglucoside, pterostilbene *O*-acetylglucoside 1, resveratrol dimers 1-3, pterostilbene *O*-acetylglucoside 2, desoxyrhapontigenin	Püssa et al. (2009)
	Hydroxyanthraquinones: emodin, aloe-emodin *O*-glucoside, chrysophanol *O*-glucoside, emodin *O*-glucoside, emodin *O*-malonylglucoside, chrysophanol *O*-acetylglucoside 1, rhein *O*-glucoside, chrysophanol *O*-acetylglucoside 2	
	Hydroxynaphthalenes: torachrysone *O*-glucoside, torachrysone *O*-acetylglucoside	
Air-dried petioles/methanol (> 99.9%)	Composition similar to the phytochemical profile of roots, with a few additional flavonoids, i.e. myricetin-*O*-rhamnoside, rutin, quercetin-*O*-glucuronide, quercetin-*O*-glucoside, quercetin-*O*-rhamnoside, quercetin	
Air-dried roots and rhizomes/80% methanol	*Phenolic acids*: ellagic acid, chlorogenic acid, gallic acid, protocatechuic acid, protocatechuic acid, caffeic acid, α-resorcyclic acid, *p*-hydroxyphenylactetic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, syringic acid, vanillic acid, ferulic acid	Smolarz and Medyńska (2005)
Air-dried petioles/80% methanol	*Phenolic acids*: ellagic, gallic, protocatechuic, homoprotocatechuic, caffeic, *p*-hydroxybenzoic, *p*-coumaric, syringic, vanillic, ferulic, α-resorcylic and *p*-hydroxyphenylacetic acid	Smolarz et al. (2005)
	Hydroxyanthraquinone: emodin	
Fresh petioles/50% acetonitrile with 0.1% formic acid	*Flavonoids*: cyanidin rutinoside, cyanidin hexose, proanthocyanidins, rutin, quercetin hexose/quercetin glucuronide, quercetin-rhamnose	McDougall et al. (2010)
	Stilbene compounds: rhapontigenin glycoside	
	Anthraquinones: aloe-emodin, emodin glycosides and dimers, chrysophanol and its derivatives	
	Hydroxynaphthalenes: torachyrosone and its derivatives	
Fresh petioles/hexane	*Alcohols*: 3-methyl-3-pentanol, 3-hexanol, 2-hexanol, cyclopentanol, pentanol, 1-methylcyclopentanol and 2-methylcyclopentanol, 3-methylcyclopentanol, 1-octen-3-ol, octanol	Wu et al. (2017)
	Aldehydes and ketones: acetone, butanal, pentanal, 3-methyl-2-pentanone, 3-hexanone, 2-hexanone, hexanal, 2-methylcyclopentanone, 3-methylcyclopentanone, octanal, nonanal, 3-octen-2-one, (E,E)-2,4-nonadienal	
	Acids and esters: butyl acetate, pentanoic acid, hexanoic acid, 3-hydroxy-2,2,4-trimethypentyl isobutanoate, 2,2,4-trimetyl-1,3-pentanediol diisobutanoate, heptanoic acid, octanoic acid, nonanoic acid	
	Aromatic compounds: benzaldehyde, acetophenone, benzyl alcohol, phenetyl alcohol, *p*-anisaldehyde; 2,5-dimethyl-tetra-hydrofuran, 5-methylfurfural, 2-acetylpyrrole, 2-(hydroxyacetyl)furan, pyrrole-2-carboxaldehyde, dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone, 5-pentyl-2(5H)-furanone, 3-methyl-1H-pyrrole-2-carboxaldehyde, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	
	Hydrocarbons: cyclohexane, methylcyclohexane, tetradecane	



Stilbene compounds	R1	R2	R3	R4
Desoxyrhapontigenin	OH	OH	H	OCH_3
Isorhapontigenin	OH	OH	OCH_3	OH
Piceatannol	OH	OH	OH	OH
Pterostilbene	OCH_3	OCH_3	H	OH
Rhapontigenin	OH	OH	OH	OCH_3
Resveratrol	OH	OH	H	OH



Antraquinone compounds	R1	R2	R3	R4
Aloe-emodin	H	CH_2OH	H	H
Emodin	H	CH_3	OH	H
Chrysophanol	H	CH_3	H	H
Physcion	H	CH_3	OCH_3	H
Rhein	H	COOH	H	H

Fig. 1 Main antraquinone and stilbene compounds identified in *Rheum rhaponticum* and *Rheum rhabarbarum*

in the air-dried plant material (Smolarz and Medyńska 2005). Phenolic acids (at the total concentration of 198.16 μg/g) and emodin (at a concentration of 5.8 μg/g of dry weight) were also found in the petioles (Smolarz et al. 2005). Recent work on phytochemical profiles of two varieties of *R. rhabarbarum* demonstrated that the content of flavan-3-ols in rhubarb stalks was ranging between 86.6 and 196.0 mg/100 g (of dry mass), the flavonol content was 49.8–73.5 mg/100 g (d.m.), anthocyanins were present at the level of 4.3–96.2 mg/100 g (d.m.), and gallotannin content was 6.3–13.6 mg/100 g (d.m.). In general, the total phenolic content (TPC) was higher in samples collected in spring, than in those from autumn; in the "*Red Malinowy*" variety, TPC amounted up to 1270.3 mg/100 g d.m. (Kalisz et al. 2020).

Biological activities and pharmacological actions of *R. rhapoticum* and *R. rhabarbarum*

Menopausal complaints

To date, the terms "phytoestrogens" and "phytoestrogenic activity" have been mostly associated with isoflavones from the soya bean (*Glycine max* L.). On the other hand, the last two decades have brought better understanding of the hormone-like effects of different classes of phytochemicals and identification of various sources of phytoestrogens. Although the soya bean is still considered the main source of phytoestrogens, it has been partly replaced by red clover (*Trifolium pratense* L.). Furthermore, following the discovery that not only isoflavones display estrogenic properties, the area of studies on hormonal effects of plant metabolites has been significantly extended (Dietz et al. 2016; Franco et al. 2016; Sirotkin and Harrath 2014; Schloss and Steel 2016). Scientific and medical interest in herbal preparations with estrogenic effects are additionally enhanced by data suggesting a beneficial role of phytoestrogens in prophylaxis of different women diseases, including osteoporosis and breast cancer (Obi et al. 2009).

Since the phytochemical profile of *R. rhaponticum* contains substances with estrogenic activities, this plant has gained scientific attention as a source of natural medicines, useful in the therapy of menopausal complaints or other hormone-related disorders (Chang et al. 2016; Hasper et al. 2009; Heger et al. 2006; Kaszkin-Bettag et al. 2008a, b, 2009; Keiler et al. 2012; Möller et al. 2007; Papke et al. 2009; Vollmer et al. 2010; Wober et al. 2007). The most important studies on molecular mechanisms of its biological action, pharmacological relevance and safety were summarized in the Table 3. The estrogenic action of *R. rhaponticum* is primarily attributed to the presence of hydroxystilbene compounds such as rhaponticin, desoxyrhaponticin, rhapontigenin, desoxyrhapontigenin, resveratrol and piceatannol. A preparation containing the *Rheum rhaponticum extract* ERr731® was

Table 2 Phytochemical composition of *R. rhabarbarum* and hybrids of *R. rhabarbarum* and *R. rhaponticum*

Plant part/solvent	Identified substances	References
R. rhabarbarum rhizome/hot water extract	piceatannol-3, 4'-*O*-β-D-diglucopyranoside, desoxyrhaponticin	Ko (2000)
	Anthraquinone compounds: emodin-l-*O*-β-D-glucopyranoside, physcion-8-*O*-β-D-glucopyranoside	
R. rhabarbarum stalks/volatiles	*Alcohols*: 1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-pentanol, 4-methyl-1-pentanol, 1-pentanol, 2-pentanol, 3-pentanol, 1-penten-3-ol, (Z)-2-penten-1-ol, 4-methyl-3-penten-1-ol, hexanol, (E)-2-hexenol, (Z)-2-hexenol, (E)-3-hexen-1-ol, (Z)-3-hexen-1-ol, cyclohexanol, 4-methyl-1-hexanol, 2-ethyl-1-hexanol, hexadecanol, benzyl alcohol, 2-phenylethanol	Dregus and Engel (2003)
	Aldehydes: (E)-2-pentenal, hexanal, (E)-2-hexenal, (Z)-2-hexenal, (Z)-3-hexenal, (E)-2-nonenal, (E,Z)-2,6-nonadienal, decanal	
	Esters: ethyl formate, ethyl acetate, hexyl acetate, 2-methylbutyl 2-methylbutanoate, methyl (E)-2-hexenoate, ethyl (E)-2-hexenoate, (E)-2-hexenyl acetate, (E)-2-hexenyl butanoate, (E)-2-hexenyl hexanoate, (E)-3-hexenyl butanoate, (E)-2-hexenyl (E)-2-hexenoate, isopropyl myristate, diisobutyl phthalate, dibutyl phthalate, di(2-ethylhexyl) phthalate	
	Ketones: 3-pentanone, 6-methyl-5-hepten-2-one, (E)-geranylacetone, 2,6,6-trimethyl-2-vinyltetrahydropyran-3-one	
	Acids: acetic acid, 2-methylbutanoic acid, 4-methylpentanoic acid, hexanoic acid, 4-methylhexanoic acid, (E)-2-hexenoic acid, (E)-3-hexenoic acid, octanoic acid, nonanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, 9-hexadecenoic acid, octadecanoic acid	
	Other compounds: limonene, linalool, sesquiterpenes, squalene, β-ionone, anethole, *p*-allylphenol, methyleugenol, 1,2-dimethoxybenzene, 1,4-dimethoxybenzene, indole	
R. rhabarbarum roots and rhizomes/80% methanol	Ellagic, chlorogenic, gallic, protocatechuic, protocatechuic, caffeic (3,4-dihydroxycinnamic), α-resorcyclic, *p*-hydroxyphenylactetic, *p*-hydroxybenzoic, *p*-coumaric, syringic, vanillic and ferulic acid	Smolarz and Medyńska (2005)
R. rhabarbarum stalks fresh juice	Catechin, cyanidin-3-glucoside, cyanidin-3-rutinoside, 5-carboxypyrano-cyanidin-3-rutinoside, delphinidin-3-glucoside, delphinidin-3-rutinoside, myrecitin-3-rutinoside, procyanidin B1, procyanidin B2, quercetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-glucuronide, quercetin-3-glucoside, kaempferol-3-rutinoside, quercetin-3-rhamnoside	Will and Dietrich (2013)
	Acids: ascorbic acid, citric acid, fumaric acid, lactic acid, mailc acid, oxalic acid	
R. rhabarbarum rhizome/methanol	Rheundulin A	Ha et al. (2020)
	Stilbene compounds: rheundulins B-D, δ-viniferin, rhapontigenin, rhaponticin, piceid	
	Anthraquinones: chrysophanol-1-*O*-β-*D*-glucopyranoside, chrysophanol-8-*O*-β-*D*-glucopyranoside	
	*Hydroxynaphthalene*s: torachrysone-8-*O*-β-*D*-glucoside	
	Naphthoquinone derivative: 6-methoxy-2-acetyl-3-methyl-1,4-naphthoquinone-8-*O*-β-*D*-glucopyranoside	

Table 2 continued

Plant part/solvent	Identified substances	References
R. rhabarbarum stalks/methanol acidified with 1% acetic acid	*Anthocyanins:* delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside	Kalisz et al. (2020)
	Flavan-3-ols: procyanidin B1, (+)-catechin, procyanidin B2, (−)-epicatechin, procyanidin B dimer	
	Flavonols: myreticin-3-*O*-glucuronide, myreticin-3-*O*-rutinoside, myreticin-3-*O*-ramnoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucuronide, quercetin-3-*O*-glucoside, quercetin-3-*O*-pentoside, quercetin-3-*O*-rhamnoside and isorhamnetin 3-*O*-rhamnoside	
	Phenolic acids: galloyl-*O*-glucose, gallic acid, di-*O*-galloyl-glucose and derivatives of hydroxycinnamic acid	
R. rhabarbarum and *R. rhaponticum* hybrids leaves and petioles/*n*-butanol	6,8-di-C-β-D-glucosylapigenin, 6-C-β-D-glucosyl-8-C-β-D-arabinosylapigenin, 6-C-β-D-arabinosyl-8-C-β-D-glucosylapigenin, rutin, isovitexin, quercetin-3-*O*-β-D-glucuronide	Krafczyk et al. (2008)
R. rhabarbarum and *R. rhaponticum* hybrids rhizome/ethyl acetate	(+)-catechin, *trans*-resveratrol-4′-*O*-β-D-glucopyranoside, *trans*-piceatannol-3′-*O*-β′-D-glucopyranoside, *trans*-rhaponticin, *trans*-desoxyrhaponticin	Krafczyk et al. (2008)

registered as medicinal product in 1993 (Phytoestrol N, Chemisch-Pharmazeutische Fabrik Göppingen, Carl Müller, Apotheker, GmbH and Co KG, Göppingen, Germany), which was dedicated to alleviate menopausal complaints as an alternative for the hormonal replacement therapy (HRT). At doses ranging from 4 to 1000 mg, different extracts of *R. rhaponticum* are also present in other plant-based herbal products and dietary supplements.

Given the ample evidence that *R. rhaponticum* alleviates some menopause-related complaints, one would expect that also *R. rhabarbarum* possesses similar properties. However, so far, the estrogenic action of the latter has been confirmed only in basic studies. In human hepatoma (HepG2) cells, transiently transfected with ERα, ERβ and ERE-reporter plasmid, some stilbene-derivatives such as piceatannol 3′-*O*-β-D-xylopyranoside, *cis*-rhaponticin and rhapontigenin 3′-*O*-β-D-glucopyranoside isolated from the roots of *R. rhabarbarum* (ethnomedicinal name: *Rhei undulati Rhizoma*) displayed a binding affinity to estrogen receptors (at concentrations of 1 and 10 μM) (Park et al. 2018). The estrogenic activities of three other bioactive constituents of *R. rhabarbarum* roots (i.e. aloe-emodin, rhapontigenin and chrysophanol 1-*O*-β-D-glucopyranoside; 10–50 μg/ml) were also demonstrated in an experimental model of breast cancer cells (MCF-7) (Lee et al. 2018).

Antioxidant properties

Due to a significant contribution of oxidative stress to the pathogenesis of a variety of disorders, antioxidant activity is one of the most investigated properties of natural, plant-derived extracts or individual substances. However, in the case of *R. rhaponticum* and *R. rhabarbarum*, this type of biological activity remains only partly described and evaluated. So far, antioxidant effects of *R. rhaponticum*-derived extracts have been examined in preliminary radical-scavenging assays (based on the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals), mostly at concentrations that are significantly higher than physiologically achievable levels of natural substances and their metabolites (Joo et al. 2014; Kalisz et al. 2020; Park et al. 2008; Raudsepp et al. 2008, 2013; Won Jang et al. 2018; Wu et al. 2017; Zhang et al. 2007) (for details see the Table 5 in supplementary material). In addition to a limited number of studies, different laboratory protocols, diverse ways of data presentation (including a lack of EC_{50} or IC_{50} values) and modifications within the methodology significantly hinder unequivocal interpretation of the existing evidence.

What is the current knowledge of antioxidant effects of *R. rhabarbarum*? Analogously to *R. rhaponticum*, antioxidant assays of the garden rhubarb were preliminary assessed with the use of non-biological tests, but in this case, the literature offers more

Table 3 Studies on molecular mechanisms of biological action, pharmacological relevance and safety of the ERr731® *Rheum rhaponticum* extract

Study type	Study design/experimental system	Main findings	References
In vitro	Study in ERα-expressing yeast cells, ERα-responsive Ishikawa cells and human endometrial HEC-1B cells transiently transfected with the ERα or ERβ	Activation of ERβ; lack of ERα activation in all used cell lines; no anti-estrogenic effects	Wober et al. (2007)
In vitro	Study in bone-derived U2OS cells, stably or transiently expressing the ERβ	Estrogenic effects based on ERβ-dependent activity; activation of the ERα in bone cells	Möller et al. (2007)
Animal	Examination of the safety in male and female beagle dogs, treated with 100, 300 and 1000 mg/kg body weight/day	No toxicity or pathological changes in organs; a slight decrease in glucose levels after a daily dose of 1000 mg/kg b.w.	Kaszkin-Bettag et al. (2008a, b)
Animal	90-day study on the safety in terms of endometrial hyperplasia, in a murine experimental model	A stimulatory activity on proliferation in the uterus was excluded; no effects on the bone mineral density	Keiler et al. (2012)
Animal	Evaluation of dose-dependent effects of ERr731®, alone or in combination with estradiol on growth and proliferation in the uterus of ovariectomized rats	No stimulatory effects on uterine proliferation by ERr731® alone; ERr731® reduced uterine growth induced by estradiol	Papke et al. (2009)
Randomized, double-blind, placebo-controlled prospective study	Clinical trial involving 109 women with menopausal complaints; participants were treated with a daily dose of either one enteric-coated tablet of ERr731® (n = 54) or placebo (n = 55) for 12 weeks	No adverse effects; after 4 weeks; the number and intensity of hot flushes were reduced in the ERr731® group; after 12 weeks both the Menopause Rating Scale II (MRS II) total score and each individual MRS II symptoms were alleviated in the ERr731® group, compared to the placebo group	Heger et al. (2006)
Multicenter, prospective, 48-week observational study (OS) (OS I), followed by a 48-week OS II	Continuation of trial Heger et al. 2006. OS I involved 80 women of the earlier group of 109 participants; 39 were treated with ERr731® and 41 women received placebo. OS II (51 women): 23 received ERr731® and 28 received the placebo. The MRS II score was evaluated after 48 and 96 weeks	OS I: in women receiving the ERr 731 during previous RCT, a further reduction of climacteric symptoms was observed; in women from the placebo group of previous RCT who started the ERr731® therapy, an alleviation of menopausal symptoms was found. OS II: in all women receiving the ERr731®, a further decrease of menopausal symptom scores was found. The long-term use of ERr 731® did not induce adverse effects	Hasper et al. (2009)
Multicenter, placebo-controlled, randomized clinical study	112 perimenopausal women with menopausal symptoms, treated for 12 weeks with ERr 731 (n = 56) or placebo (n = 56). The MRS total score, measured on days 0, 28, 56 and 84	In the study group, the MRS total score was significantly reduced (from 27.0 ± 4.7 points to 12.4 ± 5.3 points), when compared to the placebo group; the alleviation of menopausal symptoms included a reduction in individual MRS scores and hot flushes	Kaszkin-Bettag et al. (2009)

Table 3 continued

Study type	Study design/experimental system	Main findings	References
6-month open observational study	363 menopausal women with climacteric symptoms, receiving one tablet of ERr731® for 6 months. The MRS total score after 6 months	The treatment was well tolerated. In 252 women, an evident decrease (i.e. from 14.5 points to 6.5 points) of the MRS total score was found	Kaszkin-Bettag et al. (2008a, b)
Postmarketing report on data related to safety surveillance and consumer complaints	Research on available reports related to adverse effects in Germany (from 1993 to June 2014) as well as assessment of consumers' complaints in North America and South Africa (from the date of ERr731® preparations launch, to June 2014)	124 reports on adverse effects were recorded in Germany per 140 million daily doses sold between 1993 and 2014. The most common complaints were hypersensitivity (74 reactions) and gastrointestinal symptoms (47 reactions). The sale in North America, from January 2009 to June 2014, attained 13 million tablets, providing 79 consumers' complaints to adverse effects, mostly related to gastrointestinal reactions (23 cases) or the lack of therapeutic/beneficial effects (22 cases). No consumers' complaints were recorded in South Africa, from February 2011 (the date of product implementation) to June 2014	Chang et al. (2016)

information derived from different bioassays (Table 5 in supplementary material). *R. rhabarbarum* contains natural antioxidants, capable of combating oxidative stress at different levels of the cellular antioxidant defense. Its bioactive constituents may act both as scavengers of reactive oxygen species (ROS, e.g. nitric oxide, superoxide anion and hydroxyl radical (Kalpana et al. 2012) and as modulators of cell signalling pathways and gene expression. Molecular mechanisms of these effects include the ability of stilbenes to up-regulate functions of the transcription factor Nrf2 (the nuclear factor (erythroid-derived 2)-like 2) and the Nrf2-mediated pathways. Six stilbenes (i.e. rhaponticin, rhapontigenin, isorhaponticin, desoxyrhaponticin, desoxyrhapontigenin and resveratrol), isolated from the rhizome of *R. rhabarbarum,* significantly reduced the intracellular generation of ROS in RAW 264.7 macrophages (Joo Choi et al. 2014). Furthermore, seven compounds from this plant (i.e. piceatannol, resveratrol, rhapontigenin, desoxyrhapontigenin, pterostilbene, (E)-3,5,4′-trimethoxystilbene and *trans*-stilbene) protected hepatocytes against the arachidonic acid- and iron ions-induced oxidative stress in vitro. Molecular mechanisms of this beneficial action involved the AMP-activated protein kinase (AMPK) pathway, an important regulator of cellular metabolism and energy homeostasis (Dong et al. 2015).

Anti-inflammatory properties

Molecular mechanisms of the anti-inflammatory action of rhubarb-derived substances involve the inhibition of the nuclear factor kappa B (NFκB)-dependent pro-inflammatory pathways. In human umbilical vein endothelial cells (HUVECs), an aqueous extract from *R. rhabarbarum* suppressed the tumor necrosis factor α-induced activation of NF-κB-p65 as well as the expression of adhesion molecules (ICAM-1 and VCAM-1) and the monocyte chemoattractant protein-1 (MCP-1) (Moon et al. 2006). The rhizome-derived stilbenes (rhapontigenin, piceatannol and resveratrol), their derivatives (rhaponticin 2″-*O*-gallate, rhaponticin 6″-*O*-gallate) and a naphthalene glucoside (torachrysone 8-*O*-β-D-glucopyranoside) were found to reduce NO production in macrophages (Matsuda et al. 2000). Other studies on macrophages demonstrated that also aloe-emodin was able to suppress the pro-inflammatory response (Hu et al. 2014). According to available reports, stilbenes isolated from rhizomes of this plant may impair an allergy-induced inflammatory response (Matsuda

Table 4 In vitro evidence of anti-inflammatory activities of *R. rhabarbarum*-derived substances

The examined substances	Experimental model of inflammatory response	Main findings	References
R. rhabarbarum-derived stilbenes	Antigen-stimulated RBL-2H3 cells	Inhibition of RBL-2H3 cell degranulation and secretion of TNF-α and IL-4; 3,5,4′-trimethylpiceatannol (IC_{50} = 2.1 μM) and trimethylresveratrol (IC_{50} = 5.1 μM) most effective. Piceatannol, 3,5,4′-trimethylpiceatannol, resveratrol, and trimethylresveratrol suppressed TNF-α and IL-4 release.	Matsuda et al. (2004)
Rhapontigenin, piceatannol, resveratrol, rhaponticin 2″-*O*-gallate, rhaponticin 6″-*O*-gallate, and torachrysone 8-*O*-β-D-glucopyranoside	Lipopolysaccharide-activated macrophages	Reduction of the NO generation	Matsuda et al. (2000)
Desoxyrhapontigenin	RAW 264.7 macrophages LPS-induced lung inflammation in mice (pre-treated with 2.5 or 10 mg/kg of desoxyrhapontigenin for 7 days, *i.p.*)	Stimulation of the DNA binding of Nrf2; increased expression of antioxidant proteins and enzymes (regulated by Nrf2) Reduction of the LPS-induced inflammation in lung tissue (biochemical and histological changes)	Joo Choi et al. (2014)
Rhapontigenin	HYAL inhibitory assay Experimental model of mast cells	Inhibition of HYAL activity: IC_{50} = 0.14 mM; IC_{50} for sodium cromoglycate was 15.2 mM Inhibition of histamine release and passive cutaneous anaphylaxis reaction	Kim et al. (2000)
Aloe-emodin	RAW 264.7 macrophages	Reduction of NO generation as well as interleukin-6 and -1β (IL-6 and IL-1β) synthesis and secretion	Hu et al. (2014)

et al. 2004). Rhapontigenin was described as an inhibitor of hyaluronidase (HYAL) activity, histamine release from mast cells and passive cutaneous anaphylaxis reaction (Kim et al. 2000). Desoxyrhapontigenin displayed anti-inflammatory properties in vitro and in vivo. It may be also a natural regulator/stimulator of activity of the hemeoxygenase-1 enzyme (HO-1), an important anti-inflammatory, antioxidant and cytoprotective enzyme (Joo Choi et al. 2014). It is believed that the anti-inflammatory and anti-osteoporosis effects of desoxyrhapontigenin are through its inhibitory action on the receptor activator of NF-κB ligand (RANKL) (Tran et al. 2018).

Outcomes of in vitro examinations of anti-inflammatory properties of the isolated compounds (mainly stilbenes and their derivatives) and extracts from the *R. rhabarbarum* plant have been summarized in the Table 4. Besides aforementioned works, anti-inflammatory properties of extracts derived from *R. rhabarbarum* have been also found in animal and clinical studies. The suppression of toll-like receptor 4 pathway by rhein (100 mg/kg b.w.) was demonstrated in an animal model of the lipopolysaccharide (LPS)-induced intestinal injury during sepsis (Zhang et al. 2015b). In a randomized clinical trial involving 120 patients with appendectomy, the therapy (twice a day) with an ointment containing 1 mg/g of *R. rhabarbarum* rhizome extract (methanol/water; 1:1, v/v) significantly reduced inflammation and improved the sutures healing process. Complementary experiments in this study indicated on HUVECs indicated on pro-angiogenic activity of the examined rhubarb extract (at concentrations of 5 and 10 mg/ml) (Li et al. 2016).

Cardioprotective properties

In most cases, cardioprotective effects of plant-derived substances are primarily attributed to their antioxidant and/or anti-inflammatory actions, even though, the biological activity of many natural substances evidently goes beyond these mechanisms. Due to a complex and multifactorial character of etiology and pathophysiology of cardiovascular disorders, research on modern prophylactic and therapeutic strategies encompasses a wide range of aspects related to these diseases. In addition to antioxidant and anti-inflammatory effects, the cardioprotective properties of natural substances include antiplatelet and anticoagulant effects, vasorelaxation and improvement of the blood lipid profile. A hypolipidemic activity of *R. rhaponticum* fibre was evidenced in animals maintained on the high-cholesterol diet (Basu et al. 1993) and in hypercholesterolemic human subjects in studies involving 10 hypercholesterolemic men, consuming a daily dose of 27 g of rhubarb stalk fibre, for 4 weeks. The fibre had also an ability to bind bile acids (Goel et al. 1997) and displayed stimulatory effects on the expression of the cholesterol 7α-hydroxylase gene and excretion of bile acids in mice (Goel et al. 1999). Although the hypolipidemic action of rhubarb was originally attributed to the presence of pectins based on their well-known cholesterol-lowering properties (Fernandez 1995; Fernandez et al. 1992), later works demonstrated that also stilbene compounds, i.e. rhapontin and rhapontigenin might improve the lipid profile. In rats fed a high-cholesterol diet, an intake of these compounds (1–5 mg/kg/day) resulted in a dose-dependent decline of the serum lipid level and a considerable increase of the high-density lipoprotein (HDL) cholesterol level. Additionally, pathological changes in the degenerating fatty liver were significantly reduced by a treatment with either rhaponticin or rhapontigenin (Jo et al. 2014). However, no such promising effects of the rhubarb-fibre diet were found in an experimental model of diabetic rats (treated with 50 g of the rhapontic rhubarb stalk fibre/kg b.w.) (Cheema et al. 2003).

Similarly to lipid-lowering properties of rhapontic rhubarb, also *R. rhabarbarum* showed a cardioprotective potential. The current state of art in this field includes results from in vitro and animal studies, and most of the available data complement each other. It has been found that this plant contains natural inhibitors of the soluble epoxide hydrolase (sEH) enzyme, which is one of the important molecular targets for the therapy of cardiovascular diseases. Preliminary analyses employing a recombinant human sEH demonstrated the following degrees of its inhibition: 49.8%, 107%, 30% and 39%, by the methanol extract, n-hexane, chloroform, and butanol fractions from *R. rhabarbarum* (at 25 μg/ml), respectively. Further experiments revealed that the individual rhubarb-derived compounds modulated the sEH activity by diverse mechanisms. While piceatannol 3′-*O*-β-D-glucopyranoside was a competitive inhibitor of the enzyme, resveratrol, desoxyrhapontigenin, desoxyrhaponticin, rhaponticin, isorhapontin, astringin, chrysophanol-8-*O*-β-D-glucopyranoside and aloe-emodin acted as mixed-type inhibitors. Rhapontigenin and emodin were noncompetitive inhibitors of sEH. The strongest inhibitory effect was found for astringin (IC_{50} = 2.5 ± 0.5 μM), and the weakest one of the inhibitors was desoxyrhaponticin (IC_{50} = 53.2 ± 4.4 μM) (Jo et al. 2016). The anti-obesity and hypolipidemic effects of *R. rhabarbarum*-derived substances were found in vitro and in vivo. A hot water extract from this plant was found to inhibit protein tyrosine phosphatase 1B (PTP1B), an important regulator of the insulin signalling (Lee et al. 2010). Studies in high-fat diet-fed mice revealed the metabolism-regulatory activity of rhubarb extracts and its anthraquinone components, i.e. chrysophanol and physcion. The extract, at one daily dose of 100 mg/kg of b.w., administered for 8 weeks modulated the lipid metabolism in animals (including a stimulation of adiponectin synthesis) and significantly reduced the increase of body weight. In additional experiments, the isolated anthraquinones (30 μM) inhibited PTP1B activity and enhanced the insulin sensitivity in serum-starved 3T3L1 cells (Lee et al. 2012b).

Some basic information on anti-thrombotic activity of *R. rhabarbarum* stilbenes is available as well. Desoxyrhapontigenin and rhapontigenin (100–200 μM) inhibited the arachidonic acid- or collagen-induced aggregation of blood platelets in vitro, but no antiplatelet action was recorded for piceatannol (Ko et al. 1999). Besides the aforementioned effects, anti-diabetic and vasorelaxant activities of *R. rhabarbarum* are postulated. An in vitro screening of the α-glucosidase inhibitory activity of extracts from twenty four plants, suggests that aerial parts of *R. rhabarbarum* contain moderate inhibitors of this

enzyme. The methanol extracts from peel and the peeled stalks inhibited the enzyme with IC_{50} values of 0.013 and 4.94 mg/ml, whereas for the ethyl acetate extracts, the IC_{50} amounted 0.24 and 0.21 mg/ml, respectively. For a comparison, the strongest inhibitor in this study (i.e. the methanol extract from *Cinnamomum zeylanicum*) was characterized by the IC_{50} = 0.009 mg/ml (Kongstad et al. 2015). However, the latest study on α-glucosidase inhibitory activity of phytochemicals isolated from *R. rhabarbarum* has provided diverse results. The IC_{50} values for the most of the isolates exceeded 100 μM; however, the inhibitory actions of δ-viniferin (IC_{50} = 0.5 μM, i.e. 0.23 μg/ml) and rhapontigenin (IC_{50} = 15.4 μM, i.e. 3.97 μg/ml) exceeded the efficiency of acarbose (IC_{50} = 126.8 μM, i.e. 81.8 μg/ml), a well-known inhibitor of this enzyme (Ha et al. 2020).

Desoxyrhapontigenin, emodin and chrysophanol isolated from rhizomes of *R. rhabarbarum* influenced the glucose metabolism in vivo. At oral doses of 0.21 (desoxyrhapontigenin), 0.45 (emodin) and 0.18 (chrysophanol) mg/kg b.w., these compounds reduced the postprandial hyperglycemia in animals by 35.8, 29.5, and 42.3%, respectively (Choi et al. 2005). Among seven stilbene-type compounds from the rhubarb rhizome (i.e. desoxyrhapontigenin, piceid, piceatannol, resveratrol, rhapontigenin, rhaponticin and ε-viniferin), the strongest vasorelaxation of the isolated rat aorta was observed after the treatment with piceatannol (EC_{50} = 2.4 μM) (Yoo et al. 2007). It is suggested that molecular mechanisms of the piceatannol-induced vascular relaxation involve the endothelium-dependent nitric oxide signalling pathway, including the activation of the large conductance, Ca^{2+}-activated K^+ channels (BKCa) (Oh et al. 2007). In vitro vasodilatory properties (also mediated by the endothelium-dependent NO/cGMP pathway) in rat aorta preparations were observed for an aqueous extract of *R. rhabarbarum* rhizomes (Moon et al. 2006). Moreover, both the vasorelaxant and anti-inflammatory effects of this extract were confirmed in vivo, in atherogenic diet-fed rats. The treatment with rhubarb extracts resulted in significant anti-atherogenic effects, including a reduction of low-density lipoprotein-cholesterol and an increase of the HDL-cholesterol in blood plasma. The extracts suppressed the atherogenic diet-induced expression of the vascular NF-κB-p65, adhesion molecules (ICAM-1 and VCAM-1) and E-selectin in the examined animals (Moon et al. 2008). Inhibitory effects of *R. rhabarbarum* stilbenes and their derivatives on functions of the cell adhesion molecules (CAMs) were also described in other papers, which hypothesized that the biological activity of rhubarb-derived substances may be a base for development for new therapeutic strategies (Lee et al. 2012a; Spelman et al. 2011).

Anticancer activity

Both the stilbene (Sirerol et al. 2016) and anthraquinone (Özenver et al. 2018) components of plant extracts display chemopreventive and antitumor properties; however, the anticancer action of the rhubarb-derived extracts has been evaluated hitherto only in vitro. A methanol extract from *R. rhabarbarum* (at concentrations of 70–350 μg/ml) mediated cancer cell death through the activation of the intrinsic (mitochondria-dependent) apoptotic pathway in human adenocarcinoma gastric (AGS) cells (Hong et al. 2015). The exposure of these cells to aloe-emodin or chrysophanol 1-*O*-β-D-glucopyranoside increased the poly(ADP-ribose)polymerase (PARP) cleavage and induced downregulation of the anti-apoptotic protein, Bcl-2. Additionally, chrysophanol 1-*O*-β-D-glucopyranoside slightly stimulated the expression of pro-apoptotic proteins such as Bid and Bax (Trinh et al. 2019). In the MCF-7 human breast cell line, aloe-emodin and rhapontigenin induced the mitochondria-independent apoptosis (mediated by the caspase-8 pathway), while chrysophanol 1-*O*-β-D-glucopyranoside, acted by the mitochondria-dependent apoptotic pathway (Lee et al. 2018). In the same experimental model, the induction of cancer cell apoptosis was also found for desoxyrhapontigenin. Its anticancer effect was through a dilation of endoplasmic reticulum (ER) and up-regulation of the expression of ER stress markers such as a chaperone protein GRP78, inositol-requiring kinase 1α (IRE1α), eukaryotic translation initiation factor 2 (eIF2α), C/EBP homologous protein (CHOP) as well as JNK and p38 kinases (Venkatesan et al. 2016). Furthermore, a hexane extract from *R. rhabarbarum* (20–60 μg/ml) significantly decreased the growth and viability of cancer cells and induced apoptosis in the HN22 and SCC15 oral cancer cell lines (Choi et al. 2011).

In the case of *R. rhaponticum*, the available literature comprises a small number of reports strictly related to anticancer action of the extracts, including a

few mechanistic studies on this issue. The vast majority of data derives from studies on estrogenic properties of this plant; the hormone-like action is believed to be a molecular basis of cancer-preventive or anticancer effects of *R. rhaponticum*. A *R. rhaponticum* extract and its components were found to bind to ERβ in various highly specific cancer cells such as human bone osteosarcoma (U2OS) and human endometrial adenocarcinoma (HEC-1B) cell lines (Kaszkin-Bettag et al. 2008a, b; Möller et al. 2007). In contrast, neither the extract nor its compounds and their metabolites (resveratrol and piceatannol) were able to activate the ERα receptor (Kaszkin-Bettag et al. 2008a, b; Möller et al. 2007). Additional source of data may be works on anticancer action of individual compounds (not isolated from *R. rhabarbarum*), but occurring also in these plants. Among others, such studies were described for piceatannol (Banik et al. 2020).

Antimicrobial and antiviral activities

It is assumed that *R. rhaponticum* and *R. rhabarbarum* contain antimicrobial substances that are active, at least, towards some pathogens (for details, see the Table 6 in supplementary material). The antiviral properties of rhubarbs have been described to a much lesser extent (Nurbaulina et al. 2009). However, despite some encouraging results, the pharmacological significance of antimicrobial action of rhubarbs is difficult to estimate, and main concerns are related to the lack of in vivo examinations. Furthermore, in many cases, beneficial effects were observed only at high, physiologically unachievable concentrations. On the other hand, it is known that various plant preparations are used externally, e.g. onto wounds, burns or ulcers. In these cases, also higher doses of bioactive substances may be clinically useful, if only, the necessary in vivo tests are done.

A comparative assessment of antibacterial actions of the rhizome and roots extracts, prepared from *R. rhaponticum*, *R. palmatum* and *R. rhabarbarum* demonstrated that all of the examined crude extracts (96% ethanol; v/v), were more efficient against the Gram-positive bacteria than those Gram-negative. The most effective one was the preparation from *R. rhabarbarum* (the minimum inhibitory concentration (MIC) = 125-250 μg/ml), but its activity was significantly weaker, compared to gentamicin and cefuroxime. In the case of these antibiotics, the MIC values for reference strains of *Staphylococcus* spp. ranged from 0.12 to 0.49 μg/ml and from 0.49 to 0.98 μg/ml, for gentamicin and cefuroxime, respectively (Kosikowska et al. 2010). Even an infusion from *R. rhaponticum* was found to display antimicrobial effects, though, its activity was weaker than chloramphenicol, a reference bacteriostatic agent (Raudsepp et al. 2013). A screening of antimycobacterial activities of *R. rhaponticum* root extract and its bioactive components such as rhaponticin, desoxyrhaponticin, resveratrol, barbaloin, aloe-emodin and chrysophanol revealed that the anthracene compounds were the most efficient (the MICs ranged between 32 and 64 μg/ml). However, no antimicrobial effects were found for the petiole extract (Smolarz et al. 2013b). Another study evidenced antibacterial properties of the crude, ethyl acetate and aqueous extracts originating from rhapontic rhubarb (Ziad et al. 2011).

Tests employing seventeen bacterial and one fungal strain (Table 6 in supplementary material) revealed antimicrobial activity of the ethanolic extract from the underground organs of *R. rhabarbarum* (Canli et al. 2016). An extract from the petioles was found to possess bacteriostatic, but not bactericidal properties against ATCC strains of *S. aureus*, *S. epidermidis*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* (the MICs ranged from 700 to 900 μg/ml) (Pájaro et al. 2018). Surprisingly, in other comparative studies, based on antimicrobial actions of various extracts and freshly pressed plant juice from different species, the *R. rhabarbarum* preparations had moderate or weak antibacterial properties, except the water extract, which was an efficient inhibitor of growth of two Gram-positive bacteria, i.e. *Bacillus subtilis* and *Bacillus cereus* (Krisch et al. 2008).

Since the therapy of gastrointestinal disorders is one of the ethnomedicinal recommendations for use *R. rhaponticum*, extracts from this plant were tested in terms of anti-*Helicobacter pylori* activity. Nevertheless, literature dealing with antimicrobial properties of this plant has not confirmed its pharmacological efficiency in combating *H. pylori* (Cogo et al. 2010). It seems to that the use of rhubarb-derived substances for disease-preventive purposes, e.g. for maintaining oral hygiene and dental health, can be more promising. Traditionally, the *R. rhabarbarum* root-based preparations have been used for the treatment of dental diseases, and current studies on its inhibitory action on

the dental plaque formation or glycolytic acids production by *Streptococcus mutans* and *Streptococcus sobrinus* confirmed a protective effect of this plant in dental hygiene (Kim et al. 2011). Rhein, one of the anthraquinone components of *R. rhabarbarum* and *R. rhaponticum* roots, displays an ability to combat *Porphyromonas gingivali,* a Gram-negative periodontopathogen, and thus, it is considered potentially useful in maintaining of oral health (Chinsembu 2016). Moreover, it has been demonstrated that rhein may act synergistically with a nitroimidazole antibiotic, metronidazole (Azelmat et al. 2015).

Other biological activities

Hydroxystilbenes isolated from the roots of *R. rhaponticum* displayed hepatoprotective effects in mice exposed to ethanol inhalation. Animals were treated with *trans*-resveratrol (20 mg/kg b.w.) or received a hydroxystilbene-containing *R. rhaponticum* extract (air-dried root in ethanol, 1:10 w/v), administered to an equivalent of the *trans*-resveratrol dose of 20 mg/kg b.w. (Raal et al. 2009).

A methanolic extract from *R. rhaponticum* rhizome inhibited the tyrosinase activity in UV radiation-stimulated human epidermal melanocytes at very low concentrations. According to the authors, inhibitory effects of the examined rhubarb were comparable to the efficiency of kojic acid (IC_{50} = 0.06 µg/ml and 0.02 µg/ml, for the extract and kojic acid, respectively). Additionally, a significant reduction of both the UV-induced secretion of cytokines (IL-1α and TNF-α) and the alpha-melanocyte stimulating hormone (α-MSH) synthesis was found (Silveira et al. 2013).

Advances in studies on *R. rhaponticum* and *R. rhabarbarum*: current state of art

The described rhubarb species contain bioactive phytochemicals with some health-promoting or therapeutic potential. However, only the estrogenic activity of *R. rhaponticum* has been well established in preclinical studies and clinical trials (data summarized in the Table 3), while other activities of this plant have been mainly evidenced by basic studies. Thus, most of the other curative uses of rhapontic rhubarb is still based on ethnomedicinal recommendations. Similar concerns are related to *R. rhabarbarum*—also in this case, results from in vitro works prevail and are supported only by few animal studies. A cross-search in scientific sources and medical databases such as NIH U.S. National Library of Medicine (i.e. ClinicalTrials.gov and PubMed) and SpringerLink indicated that at least several clinical trials including a word "*rhubarb*" have been registered, but no records containing "*rhababarum*" or "*undulatum*" were found. In other scientific resources, only one clinical study on wound-healing and anti-inflammatory properties of *R. rhabarbarum* was found (Li et al. 2016).

One of recent trends in research on rhubarb extracts is their use as pharmacophores. Modern strategies are particularly focused on ecofriendly nanosize materials, produced during the *greener synthesis*/the *green chemistry* processes (Kharissova et al. 2019). Such attempts have been successfully undertaken with other substances of plant origin (Park et al. 2016; Saw et al. 2019). The *R. rhabarbarum*-based silver nanoparticles were found to possess significant antibacterial activity both against *E. coli* (CCM 4517) and *Staphylococcus aureus* (CCM 4516). Additionally, these particles displayed a dose-dependent anti-cancer effect in human epithelial carcinoma (HeLa) cell line (Palem et al. 2016, 2018).

Conclusions

Rheum rhaponticum and *Rheum rhabarbarum* are popular edible plants, commonly cultivated in different regions of the world. A wide range of biological activities of these species has been evidenced by numerous in vitro and several in vivo studies. Both of these species constitute interesting sources of biologically active compounds with health-promoting or therapeutic potential. However, the current knowledge of physiological effects and pharmacological efficacy of these rhubarbs remains incomplete. Therefore, further, more advanced studies (including clinical trials) on pharmacological activity of these rhubarb species are needed.

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Supplementary Table 5. Preliminary evaluations of antioxidant activities of *R. rhaponticum* and *R. rhabarbarum* extracts.

Plant species	Experimental system	Results for the examined substances	Results for the reference compounds	References
R. rhaponticum	DPPH•-scavenging of *R. rhaponticum* root-derived substances	Stronger anti-radical effects of hydroxystilbenes, compared to hydroxyanthraquinones; no information on EC_{50}.		(Raudsepp et al. 2008)
	DPPH•-scavenging effect of *R. rhaponticum* root infusion	AOX (anti-oxidative efficiency) attained up to 87%.	AOX for ascorbic acid (1 mg/ml) and ethanol radical attained 25% and 77%, respectively.	(Raudsepp et al. 2013)
	Analogously prepared infusion and ethanolic extract from the petioles	AOX was 48 and 98%, respectively.		
R. rhabarbarum	DPPH•-scavenging; antioxidant action of isolated rhapontigenin and rhaponticin (5-40 μM) in V79-4 cells	Rhapontigenin was more effective scavenger of intracellular ROS and DPPH•; it enhanced the cellular antioxidant activity and modulated cellular signaling pathways.		(Zhang et al. 2007)
	Superoxide radicals (generated in the xanthine/xanthine oxidase system) and DPPH• scavenging	At 20 μg/ml DPPH•-scavenging efficacy was 72%, and the superoxide scavenging efficacies attained 80%.	EC_{50} for ascorbic acid = 11 μg/ml (DPPH• assay); EC_{50} for butylated hydroxy anisole (BHA) = 32 μg/ml (the superoxide scavenging assay).	(Park et al. 2008).
	ROS-scavenging effects of rhaponticin, rhapontigenin,	Desoxyrhapontigenin IC_{50} = 32.83 μM and 28.22 μM, for ROS and	Resveratrol IC_{50} = 49.07 μM for ROS and 37.82	(Joo Choi et al. 2014)

	isorhaponticin, desoxyrhaponticin, desoxyrhapontigenin and resveratrol from *R. rhabarbarum* rhizome, in RAW 264.7 cells	peroxynitrite generation, respectively; it had also stimulatory effect on antioxidant enzyme expression.	μM for peroxynitrite, respectively.	
	DPPH$^{\bullet}$ scavenging tests for extracts isolated from rhubarb stalks	DPPH$^{\bullet}$ scavenging abilities of the ethyl acetate and methanol extracts (5 μg/ml) attained 94.12% and 96%, respectively.	the DPPH$^{\bullet}$-scavenging ability for butylated hydroxytoluene (BHT) (100 μg/ml) was 96%.	(Wu et al. 2017)
	ABTS$^{\bullet+}$, DPPH$^{\bullet}$ and FRAP analyses of petiole extracts (lyophilized powder, extracted in acidified methanol)	Antioxidant activity parameters of the "*Red Malinowy*" variety attained 18.26, approx. 5.9 and 10 mmol/Trolox equivalents/100 g d.m., for ABTS$^{\bullet+}$, DPPH$^{\bullet}$ and FRAP assays, respectively.		(Kalisz et al. 2020)
	Experimental model of cod liver oil; 7 fractions from the *R. rhabarbarum* petioles (water, 20, 40, 60, 80 and 100% methanol or acetone)	100% methanol fraction showed the highest antioxidant activity, i.e. 72.6 and 92.8% at concentrations of 20 and 100 μg/ml, respectively.	antioxidant efficiency of BHT: 92.2 % at 100 μg/m; α-tocopherol: 84.8 % at 100 μg/ml	(Won Jang et al. 2018)

Supplementary Table 6. Antimicrobial and antiviral activities of *Rheum rhaponticum* and *Rheum rhabarbarum* extracts.

Plant species	Plant part/analysed extracts/compounds	Bacterial /viral strains	Main findings	References
Rheum rhaponticum	Roots/different anthraquinones	*Mycobacterium tuberculosis* H37Ra	MIC: 32 µg/ml (barbaloin); 64 µg/ml (aloe-emodin); 64 µg/ml (chrysophanol)	(Smolarz et al. 2013)
	Roots/different anthraquinones	*Mycobacterium bovis*	MIC: 128 µg/ml (barbaloin); 64 µg/ml (aloe-emodin);64 µg/ml (chrysophanol)	(Smolarz et al. 2013)
	Plant/ crude extract	Twenty strains of *Escherichia coli*: Ec001SGH, Ec002SGH, Ec003SGH, Ec004SGH, Ec007SGH, Ec010SGH, Ec011SGH, Ec012SGH Ec013SGH, Ec016SGH, Ec017SGH, Ec018SGH,Ec019SGH, Ec020SGH, Ec021SGH, Ec023SGH,Ec026SGH, Ec030SGH,Ec031SGH, Ec032SGH	MIC = 5–80 µg/µl MBC = 5-80 µg/µl	(Ziad et al. 2011)
	Plant/ethyl acetate extract	Twenty strains of *Escherichia coli*: Ec001SGH, Ec002SGH, Ec003SGH, Ec004SGH, Ec007SGH, Ec010SGH, Ec011SGH, Ec012SGH Ec013SGH, Ec016SGH, Ec017SGH, Ec018SGH, Ec019SGH, Ec020SGH, Ec021SGH, Ec023SGH, Ec026SGH, Ec030SGH, Ec031SGH, Ec032SGH; and ten strains *Klebsiella pneumoniae*: Kp001SGH, Kp002SGH, Kp005SGH, Kp006SGH, Kp007SGH,Kp008SGH, Kp009SGH,Kp010SGH,	MIC = 5-40µg/µl MIC_{90}= 22µg/µl MBC = 5-80µg/µl	(Ziad et al. 2011)

		Kp013SGH, Kp016SGH		
	Plant/aqueous fractions	Twenty strains of *Escherichia coli*: Ec001SGH, Ec002SGH, Ec003SGH, Ec004SGH, Ec007SGH, Ec010SGH, Ec011SGH, Ec012SGH Ec013SGH, Ec016SGH, Ec017SGH, Ec018SGH, Ec019SGH, Ec020SGH, Ec021SGH, Ec023SGH, Ec026SGH, Ec030SGH, Ec031SGH, Ec032SGH	MIC = 10-80 μg/μl MBC = 40-80 μg/μl	(Ziad et al. 2011)
	Root/tincture	*Campylobacter jejuni*	10 mm of inhibition zone at the dilution of 1:10 (w/v)	(Raudsep et al. 2013)
		Bacillus subtilis	4-10 mm of inhibition zone at the dilution of 1:10 (w/v)	
		Bacillus subtilis, *Listeria monocytogenes*	0,5 mm of inhibition zone at the dilution of 1:80 (w/v)	
	Root/tincture, water infusion	*Kocuria rhizophila*	1-3.5 mm inhibition zone at the dilutions 1:10 and1:20 (w/v)	(Raudsep et al. 2013)
		Escherichia coli	0.5-0.75 mm inhibition zone at the dilutions 1:10 and1:20 (w/v)	
		Lactobacillus acidophilus	2-4 mm inhibition zone at the dilutions 1:10 and 1:20 (w/v)	
Rheum rhabarbarum	Roots/different anthraquinones	*Streptococcus mutans*; *Streptococcus sobrinus*	MIC values 50–200 μg/ml; MBC ≥ 200 μg/ml	(Kim et al. 2011)
	Roots/ethanol extract	*Bacillus subtilis* DSMZ 1971; *Enterobacter aerogenes* ATCC 13048; *Enterococcus durans*; *Enterococcus faecalis* ATCC		(Canli et

		29212; *Enterococcus faecium*; *Escherichia coli* ATCC 25922; *Klebsiella pneumoniae*; *Listeria innocula*; *Listeria monocytogenes*ATCC 7644; *Pseudomonas aeruginosa* DSMZ 50071; *Pseudomonas fluorescence* P1; *Salmonella enteritidis* ATCC 13075; *Salmonella infantis*; *Salmonella kentucky*; *Salmonella typhimurium* SL 1344; *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044	9-29 mm inhibition zones at the dilutions	al. 2016)
	Petioles/70% ethanolic extract	bacteriostatic properties against ATCC strains of *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*	MIC values 700 - 900 µg/ml	(Pájaro et al. 2018)
	Roots/ethanol extract	*Herpes simplex* virus 1 (HSV-1) Herpes simplex virus 2 (HSV-2) *Varicella-zoster* virus (VZV)	ID50%= 0.005 ± 0.0004 µg/ml ID90% = 0.008 ± 0.0007µg/ml	(Nurbaulina et al. 2009)

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration

nutrients

MDPI

Article

Rheum rhaponticum and *Rheum rhabarbarum* Extracts as Modulators of Endothelial Cell Inflammatory Response

Oleksandra Liudvytska [1,*], Michał B. Ponczek [1], Oskar Ciesielski [2,3], Justyna Krzyżanowska-Kowalczyk [4], Mariusz Kowalczyk [4], Aneta Balcerczyk [2] and Joanna Kolodziejczyk-Czepas [1]

1 Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland
2 Department of Sociobiology and Epigenetics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland
3 The Bio-Med-Chem Doctoral School, University of Lodz and Lodz Institutes of the Polish Academy of Sciences, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland
4 Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland
* Correspondence: oleksandra.liudvytska@biol.uni.lodz.pl; Tel.: +48-42-635-44-84

Abstract: Background: Inflammation, endothelial dysfunction, and alterations in blood physiology are key factors contributing to atherosclerosis and other cardiovascular disorders. Hence, modulation of endothelial function and reducing its pro-inflammatory and pro-thrombotic activity is considered one of the most important cardioprotective strategies. This study aimed to evaluate the anti-inflammatory potential of rhubarb extracts isolated from petioles and underground organs of *Rheum rhabarbarum* L. (garden rhubarb) and *R. rhaponticum* L. (rhapontic rhubarb) as well as two stilbenoids, typically found in these plants, i.e., rhapontigenin (RHPG) and its glycoside, rhaponticin (RHPT). Methods: Analysis of the anti-inflammatory effects of the indicated rhubarb-derived substances involved different aspects of the endothelial cells' (HUVECs) response: release of the inflammatory mediators; cyclooxygenase (COX-2) and 5-lipoxygenase (5-LOX) expression as well as the recruitment of leukocytes to the activated HUVECs. The ability of the rhubarb-derived extracts to inhibit COX-2 and 5-LOX activities was examined as well. The study was supplemented with the in silico analysis of major components of the analyzed extracts' interactions with COX-2 and 5-LOX. Results: The obtained results indicated that the examined plant extracts and stilbenes possess anti-inflammatory properties and influence the inflammatory response of endothelial cells. Biochemical and in silico tests revealed significant inhibition of COX-2, with special importance of rhaponticin, as a compound abundant in both plant species. In addition to the reduction in COX-2 gene expression and enzyme activity, a decrease in the cytokine level and leukocyte influx was observed. Biochemical tests and computational analyses indicate that some components of rhubarb extracts may act as COX-2 inhibitors, with marginal inhibitory effect on 5-LOX.

Keywords: *Rheum rhaponticum*; *Rheum rhabarbarum*; rhapontigenin; rhaponticin; anti-inflammatory; cyclooxygenase; lipoxygenase; HR-QTOF/MS; metabolite profiling; molecular docking

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1. Introduction

Recent decades have been characterized by an increasing interest in the role of plant-derived substances in maintaining human health and studies on the physiological effects of phytochemicals occurring in foods and herbal medicines. For example, it has been found that natural, plant-derived compounds, such as resveratrol, curcumin, or epigallocatechin gallate (EGCG), can be helpful in the prophylaxis or treatment of many disorders, including cardiovascular diseases [1–3].

Rheum rhaponticum L. (rhapontic rhubarb, Siberian rhubarb) and *Rheum rhabarbarum* L. (garden rhubarb) belong to the *Rheum* L. genus (Polygonaceae), which includes about

60 herbaceous plants, native to the mountainous and desert regions of the Tibetan Plateau in China, and also growing in Asia and North America [4,5]. Today, rhubarbs are also commonly cultivated in Europe and North America [6] and used as food, medicinal, ornamental, and honey plants. While the edible stalks (petioles) of *R. rhaponticum* and *R. rhabarbarum* are typically known as foods, the roots (rhizome) of these species have been used for centuries in ethnomedicine of different regions of the world [4]. Both ethnomedicinal surveys and research data indicate that rhubarbs are rich in bioactive substances and may display beneficial effects on human health [7]. Numerous rhubarb products are on the market as foods, dietary supplements, and alternative medicines. However, only the phytoestrogenic activity of *R. rhaponticum* has been well evidenced, including basic research, animal studies and clinical trials [8–11]. Other physiologically relevant activities (including the impact on components of the cardiovascular system) of this and other rhubarb species are only partly or even poorly recognized. Moreover, some information on their properties is based only on traditional medicine recommendations. Thus, the biological activity of these plants needs to be examined.

The presented work includes the phytochemical profiling and evaluation of anti-inflammatory effects of extracts originating from petioles and underground parts of two rhubarb species, i.e., *R. rhabarbarum* and *R. rhaponticum* as well as two stilbenes, typically found in these plants—rhapontigenin (RHPG) and its glycoside, rhaponticin (RHPT). Experiments aimed to recognize their effects on the physiology of the endothelium and the possible protective role of these plant-derived substances on the inflammatory response of endothelial cells. The vascular endothelium is actively involved in inflammatory processes and the development of different cardiovascular disorders, e.g., atherosclerosis and thromboembolic complications. Pro-inflammatory factors released by endothelial cells may promote and augment both local and systemic inflammation [12–14]. Therefore, this study evaluated the anti-inflammatory effects of the examined extracts and compounds using an experimental model of human umbilical vein endothelial cells (HUVECs). Anti-inflammatory activities of the examined substances were analyzed at different levels of cell response, including the expression of cyclooxygenase (COX-2) and 5-lipoxygenase (5-LOX) genes, pro-inflammatory cytokine release and changes in the endothelium adhesiveness to leukocytes. Studies on HUVECs were enriched with COX-2 and 5-LOX screening tests and mechanistic in silico analyses of interactions of significant components of the examined extracts with the COX-2 and 5-LOX enzymes.

2. Materials and Methods

2.1. Chemicals

Commercial standards of derivatives of anthraquinones (aloe-emodin, aloe-emodin-8-Glu, emodin, emodin-8-Glu, rhein, rhein-8-Glu, chrysophanol, chrysophanol-8-Glu, physcion, sennoside-A, sennoside-B, sennoside-C, sennoside-D), stilbenes (resveratrol, pterostilbene, pinostilbene, piceatannol, astringin, polydatin, rhapontigenin, rhaponticin, isorhapontigenin, isorhaponticin, deoxyrhapontigenin, deoxyrhaponticin), phenolic acids (glucogallin, gallic-acid), flavones (vicenin-II, vicenin-III, apigenin-7-Glu), flavanones (pinocembrin, pinocembroside), chalcones (phloretin, phloridzin, trilobatin), and catechins (catechin, epicatechin, gallocatechin, procyanidin-B1, procyanidin-B2, procyanidin-B3, and procyanidin-C1), acetonitrile LC-MS grade, formic acid MS-grade, and *tert*-butanol were purchased from Merck (Darmstadt, Germany). Methanol, *n*-hexane, and *n*-butanol, all of analytical grade, were purchased from Fisher Chemical (Loughborough, UK). Ultrapure water was prepared using a Milli-Q water purification system (MerckMillipore). General reagents for bioassays were purchased from Sigma-Aldrich (a part of Merck KGaA, Darmstadt, Germany) and Cayman Chemicals (Ann Arbor, MI, USA). Reagents specific to cell culture, gene expression analyses, and cytokine profiling have been indicated below in the descriptions of the applied methods.

2.2. Plant Material

Petioles and rhizomes of *Rheum rhaponticum* L. were donated from The Botanical Garden of Maria Curie-Skłodowska University in Lublin, whereas analogous organs of *Rheum rhabarbarum* L. were given by The Botanical Garden of Łódź. The voucher samples have been deposited at the Department of Biochemistry and Crop Quality of the Institute.

2.2.1. Extraction Procedure

The plant material was cut into small, approx. 1 cm long pieces and dried in a dryer at 35 °C. The dried material was pulverized using an electric grinder to a homogeneous size and sieved through a 0.5 mm sieve. Powdered plant material was transferred into tightly closed vials and kept in the fridge until the analysis.

The plant material was extracted twice with methanol containing 0.1% formic acid, using an ultrasonic bath at room temperature for 24 h in the dark. The crude methanol extract was defatted using liquid–liquid extraction with *n*-hexane. Afterward, the specific metabolite fraction was prepared using *n*-butanol as an extraction solvent. All collected fractions (hexane, water, and butanol) were monitored for specific metabolites by the UHPLC-HR-MS method. Butanol fractions of petioles and roots were freeze-dried and used in the following research stages, LC-MS analyses and bioactivity assays.

2.2.2. High-Resolution LC-MS Qualitative and Quantitative Analysis

The freeze-dried samples of rhubarbs' butanol extracts (10 mg) were precisely weighed (with an accuracy of 0.1 mg) and dissolved in 80% (*v*/*v*) methanol in 1 mL volumetric flasks. Before analysis, samples were diluted 1:9 with 80% methanol and filtered using Whatman (0.22 μm) filter vials.

High-resolution UHPLC-MS analyses were performed on a Thermo Ultimate 3000 RS chromatographic system coupled/hyphenated with a Bruker Impact II HD (Bruker, Billerica, MA, USA) quadrupole time-of-flight (Q-TOF) mass spectrometer. Chromatographic separations were carried out on a Waters CORTEX T3 column (150 mm × 2.1 mm, 2.7 μm, Milford, MA USA) equipped with a pre-column. The mobile phase A was 0.1% (*v*/*v*) formic acid, and the mobile phase B was acetonitrile containing 0.1% (*v*/*v*) of formic acid. At the beginning of separation, the elution profile was isocratic, 5% of phase B for 0.5 min followed by a linear gradient from 5 to 98% of phase B in 18.5 min and a hold of 98% of phase B for 3 min. After completion of the separation, the column was equilibrated for 5 min with 5% of phase B. The separations were carried out at 35 °C with a constant flow rate of 0.5 mL/min. An injection volume of 5 μL was used.

The flow from the column was split between the charged aerosol detector (Thermo Corona Veo RS) and the ion source of the mass spectrometer type QTOF (Bruker Impact II HD) in a 3-to-1 ratio. This source, designed for electrospray ionization, operated with the following parameters: voltage capillary 2.8 kV; nebulizer pressure 0.7 bar; drying gas flow 6 l/min; drying gas temperature 200 °C; ion energy 4 eV; RF collision cell 700.0 Vpp; transfer time 100.0 μs; and pre-pulse storage 10.0 μs. Negative ions were measured in the *m*/*z* range of 100–1500 with a 5 Hz scanning frequency. MS/MS spectra were obtained in the data-dependent mode, in which two of the most intense ions were fragmented by collision-induced dissociation (CID, Ar collision gas). The collision energy and the ion isolation width for each fragmentation were automatically selected from a predefined list based on the *m*/*z* of the precursor ion. Internal mass calibration for quadrupole and TOF analyzers were based on sodium formate clusters automatically injected in a 10 mM solution in 50% 2-propanol into the ion source immediately before each analysis.

Complete absolute quantitation of all analytes was impossible due to the high diversity of observed metabolites and the lack of appropriate reference standards for most of them. Therefore, wherever possible, we semi-quantified the observed analytes based on the signal from CAD. The dependence of the signal intensity on the mobile phase composition was established using 32 reference standards representing metabolites present in the extracts. Each standard was analyzed at eight concentration levels (from 0.7 to 70 μg/mL) to obtain

3D calibration curves as proposed by [15]. Based on the concentration–response relationship established from this data, a linear universal response model linking retention time and detector response was established and used to provide semi-quantitative data on metabolite contents.

The combined petiole and root samples spiked with 32 reference standards at two concentration levels (4 and 40 µg/mL) were used as quality control samples and analyzed after each block of 10 injections of regular samples. The Bruker Data Analysis, version 4.4 SR1, was used for data analysis and processing. Preliminary identification of rhubarbs' metabolites was performed using high-resolution mass-to-charge (m/z) measurements, with errors not exceeding 5 ppm. Based on these results, summary chemical formulas were calculated. The agreement of these formulas with structural insights gained from MS/MS spectra was validated with computational methods using SIRIUS ver. 4.5.1 software [16].

Identification of detected compounds was mainly based on comparisons with reference spectra and MS/MS spectra interpretation aided by SIRIUS and MetFrag [17].

Each tentatively identified metabolite was assigned an identification confidence level class, as suggested by [18]. The class 1 metabolites were confirmed using authentic reference standards, whereas class 4 compounds were only assigned elemental sum formula and had no identifiable structural features in their MS/MS spectra.

2.3. Cell Culture

HUVECs (human umbilical vein endothelial cells) were cultured in MCDB-131 medium (Life Technologies, Carlsbad, CA, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies, Carlsbad, CA, USA), 10 ng/mL of epidermal growth factor (EGF) (Millipore, Burlington, MA, USA), and 10 mM glutamine (Invitrogen; Carlsbad, CA, USA). The cells were isolated from freshly collected umbilical cords, by collagenase type II digestion, according to Jaffe's protocol [19], and used for the experiments at passages 3-4. The Bioethics Commission at the University of Lodz approved the study protocol (decision No. 15/KBBN-UŁ/III/2019 and 16 (III)/KBBN-UŁ/I/2021-22).

U-937 (ATCC, No. CRL-3253), a human monocyte cell line was cultured in RPMI-1640 medium (Promega, Madison, WI, USA) with 10% heat-inactivated FBS (Life Technologies, Carlsbad, CA, USA) according to a standard suspension cell culture protocol. For stimulation of the inflammatory response of the HUVECs, lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 (Cas No. L4524, Sigma-Aldrich, St. Louis, MI, USA) was used. Indomethacin (CAS No. 53-86-1, Sigma-Aldrich, St. Louis, MI, USA) is a synthetic nonsteroidal indole derivative with anti-inflammatory activity (through cyclooxygenase inhibition). Zileuton (CAS No. 111406-87-2, Cayman Chemical, Ann Arbor, MI, USA) is a leukotriene synthesis inhibitor (5-LOX inhibitor).

2.4. Effects of the Examined Substances on HUVEC Viability (Resazurin-Based Assay)

Cells were seeded into 96-well plates at a density of 1×10^4 cells/well. After 16–24 h, cells were treated with the extracts from petioles and roots of *R. rhaponticum* and *R. rhabarbarum* and stilbenes (RHPG and RHPT), at concentrations of 1–100 µg/mL, for 24 h. After incubation, the cell culture medium was removed, and wells were rinsed twice with 0.02 M phosphate-buffered saline (PBS) containing Ca^{2+}/Mg^{2+} (0.8 mM/0.4 mM), incubated in PBS containing Ca^{2+}/Mg^{2+}, 5.5 mM glucose, and 0.0125 mg/mL resazurin. HUVECs viability was estimated by measurements of the ability of live cells to reduce non-fluorescent resazurin to resorufin, a fluorescent product. After a 3 h incubation, resorufin fluorescence was measured (λ_{ex} = 530 nm, λ_{em} = 590 nm), using the Fluoroscan Ascent microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) [20]. The metabolic activity of control HUVECs (untreated with the examined extracts and stilbenes) was assumed as 100% of cell viability. Samples treated with 1% Triton-X100 were reference samples, with no viable cells (0% of viability). In cell samples treated with the examined extracts or stilbenes, a decrease in cell viability $\geq$ 20% (compared to control/untreated HUVECs) was assumed as a cytotoxic effect.

2.5. Evaluation of the Anti-Inflammatory Properties of the Rhubarb-Derived Substances

2.5.1. Analysis of the Inflammatory-Activated HUVEC Cytokine Profile

Cytokine and chemokine release from LPS-activated endothelial cells was analyzed using the Proteome Profiler Human Cytokine Array (Panel A) (R&D Systems, Minneapolis, USA), simultaneously detecting multiple analytes in cell culture supernatants. The applied panel allowed the identification of 13 substances, i.e., MIF (macrophage migration inhibitory factor), interleukin (IL)-8, Serpin E1, GM-CSF (granulocyte-macrophage colony-stimulating factor), GROα (growth-regulated oncogene α), IL-1α, IL-1β, IL-1ra, IL-6, MCP-1 (monocyte chemoattractant protein-1), MIP-1α (macrophage inflammatory protein 1α), RANTES (C-C motif chemokine ligand 5/regulated on activation, normal T cell expressed and secreted), and TNF-α (tumor necrosis factor alpha). The assay was performed using a cell culture medium, derived from HUVECs (1×10^6 cells on a 6 cm dish), treated for 16 h with the tested substances (extracts or stilbenes), at the selected concentration of 5 μg/mL, followed by 4 h stimulation of the cells with LPS (1 μg/mL). Cell culture supernatants were collected after incubation, centrifuged, and mixed with the biotinylated detection antibody cocktail provided by the manufacturer. The samples were then incubated overnight with the membrane of the cytokine assay kit. After washing off the unbound material, the streptavidin–horseradish peroxidase conjugate and a chemiluminescent cytokine quantification reagent were added. Measurements were performed using a reader Syngen Biotech Azure 300.

2.5.2. Adhesion of Monocytes to the Activated HUVECs

HUVECs were seeded into 6-well plates, at a density of 2×10^6 cells/well. After 16–24 h, when the cells reached 100% confluency and formed paving stones, the medium was changed to a fresh one, containing 1–50 μg/mL extracts from the petioles of *R. rhaponticum* or *R. rhabarbarum*; 1–50 μg/mL extract from the roots of *R. rhaponticum*; 1–30 μg/mL extract from the roots of *R. rhabarbarum*; 1–50 μg/mL RHPT or 1–25 μg/mL RHPG. After 16 h, LPS (at the final concentration of 1 μg/mL) was added, and the incubation was continued for the next 4 h. Analysis of the adhesion of monocytes to the activated endothelial was performed as described previously [21]. U-937 cells were stained with Hoechst 33342 (1 μM), in the dark, for 10 min, and an excess of the fluorescent probe was removed by a series of RPMI-1640 medium washes. Then, the incubation medium from HUVECs was removed, and stained U-937 cells were added to the fresh cell culture medium (1×10^6 monocytes per well). Incubation of monocytes with endothelial cells was performed in the dark, at 37 °C, and lasted 1 h. After that time, the unbound monocytes were removed by aspiration. The monolayers of HUVECs were rinsed twice with 0.02 M PBS containing Ca^{2+}/Mg^{2+} (0.8 mM/0.4 mM). Finally, complexes of cells on the plate were fixed in 2% glutaraldehyde. Then, the stained monocytes were counted using a fluorescence microscope Zeiss (Jena, Germany).

2.6. Evaluation of the Effects of the Examined Substances on COX-2 and 5-LOX Gene Expression

2.6.1. Total RNA Isolation and cDNA Synthesis

Total RNA was isolated using the InviTrap Spin Cell RNA Mini Kit (Stratec Molecular, Berlin, Germany), following the protocol attached to the reagent kit. The purity of the samples (mRNA) was estimated based on optical density (OD) measurements in the BioTek Eon™ microplate spectrophotometer. The OD 260/280 value was >1.8, confirming RNA purity, and the OD 260/230 was >1.5, confirming the absence of residual solvents in the purified RNA.

cDNA synthesis was performed with the PrimeScript RT Master Mix (Perfect Real Time, Takara; Kusatsu, Japan) according to the manufacturer's instructions.

2.6.2. Real-Time Quantitative PCR (RT-qPCR)

Quantitative real-time PCR was performed using the Eco Real-Time PCR System (Illumina; San Diego, CA, USA). The total reaction volume (10 μL) consisted of 0.2 nM of

forward and reverse primers, 1 µL cDNA template, 5 µL Takara BioSYBR Green Master Mix, and 3.6 µL DNAase/RNAase free water. The amplification conditions were as follows: an initial step of 95 °C for 30 s, then 40 cycles of 95 °C for 5 s, and 62 °C for 30 s. The gene-specific primers that were used are presented in Table 1. HPRT1 was used as a reference for gene expression normalization, performed according to the $2^{-\Delta\Delta Ct}$ method [22].

Table 1. Primers sequence used in mRNA qPCR.

Gene	Forward Primer 5′ > 3′	Reverse Primer 5′ > 3′	Amplicon Size (bp)
HPRT1	ATGGACAGGACTGAACGTCTT	TCCAGCAGGTCAGCAAAGAA	113
COX2	GCACTGTTRGGTGGGT	AGAAAACTGCTCAACACCGGAA	94
ALOX5	CGATACTTATGAAAGGCCCAGACC	GGTCTGGGAGACCGTACTGGA	83

2.7. COX and LOX Inhibitor Screening

Analysis of COX and LOX activity was carried out following the protocols of Cayman Chemicals (Ann Arbor, MI, USA). The Cyclooxygenase Colorimetric Inhibitor Screening Assay Kit (Cat. No. 701050) enables the measurement of the peroxidase component of COXs. The peroxidase activity was assayed colorimetrically by monitoring the appearance of the oxidized form of the *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm.

Lipoxygenase Inhibitor Screening Assay Kit (Cat. No. 760700) detects and measures the hydroperoxides produced in the lipoxygenation reaction using a purified LOX enzyme. Absorbance was measured at a wavelength of 500 nm. As a LOX inhibitor, nordihydroguaiaretic acid (NDGA) was used (at the concentration of 100 µM).

2.8. In Silico Studies: Bioactivity, Drug-Likeness, and Molecular Docking

Calculations of bioactivity and drug-likeness of the main compounds detected in *R. rhaponticum* and *R. rhabarbarum* extracts were completed by Molinspiration Cheminformatics website-calculation of Molecular Properties and Bioactivity Score-Predict Bioactivity tool (http://www.molinspiration.com/cgi--bin/properties accessed on 15 October 2021) and SwissTargetPrediction (http://www.swisstargetprediction.ch/ accessed on 15 October 2022). Structures of the compounds were used as ligands to predict binding to COX-2 and 5-LOX crystal structures in Autodock Vina 1.1.2 (http://vina.scripps.edu/ accessed on 12 May 2017) [23]. PDB coordinates of COX-2 (PDB ID: 4COX) [24] accessed on 26 December 2018, with bound indomethacin, and 5-LOX (PDB ID: 3V98, 3V99 and 6N2W) [25] were downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/ accessed on 4 July 2021) [26]. The indomethacin-bound structure was implemented as a reference compound for COX-2 and docked to compute binding energy change for comparison with the experimental crystal structure complex. Structures of ligands were created from planar structures of known compounds drawn in ChemSketch Freeware 2018.2.1 (ACD/Labs: https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/ accessed on 24 April 2019), checked on the pages for the structures available on PubChem (https://pubchem.ncbi.nlm.nih.gov/ accessed on 4 July 2021), and ChemSpider (http://www.chemspider.com/ accessed on 4 July 2021), saved as the MOL format and translated to the MOL2 format using Open Babel 2.4.1 (http://openbabel.org accessed on 26 December 2018). Optimizations of the chemical structure's geometries were calculated in Avogadro 1.2.0 (http://avogadro.cc accessed on 16 December 2018) [27] with the MMFF94 force field [28]. The ligands' and proteins' coordinates were prepared for docking in the ADT software from the MOL2 files as PDBQT files (http://autodock.scripps.edu/resources/adt accessed on 25 December 2018) [29]. The PDB files of all protein structures were purged manually of hetero atoms (HETATM). 10-fold dockings and subsequently parsing of energy results for all compounds were automated by scripts prepared in Python. Autodock Vina docking volume of COX-2 (4COX structure) covered boxes of two similar, opposite indomethacin binding sites with coordinates x, y, z in the centers: 24.864, 24.048,

10.330 and 69.785, 20.297, 7.825, respectively. Coordinates of cube centers for 5-LOX were set as −32.736, 78.963 and 8.039 according to the 3V98 structure and included inside the iron cation complexed with histidine residues as well as binding places of arachidonic acid and NDGA. The dimensions of the boxes were set as 26, 26, and 26 to embrace the active sites of both enzyme molecules. The interactions of ligands and amino acid residues within the binding pocket of the active site were analyzed by LigPlot+ v.2.2 (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/ accessed on 22 December 2022) to generate ligand–protein interaction diagrams [30,31]. The visual assessment and image creation of ligands' docked poses with proteins were set in UCSF Chimera 1.15 (http://www.cgl.ucsf.edu/chimera/ accessed on 22 September 2022) [32] and ChimeraX 1.5 (https://www.cgl.ucsf.edu/chimerax/ accessed on 28 November 2022) [33,34].

2.9. Statistical Analysis

The statistical analysis was performed using the STATISTICA 13.0 PL software (StatSoft Inc., Tulsa, OK, USA). The first step of statistical analysis was the elimination of the uncertain data by the Grubbs' tests (GraphPad Prism 5.01, San Diego, CA, USA). Next, the differences between groups were assessed by the non-parametric Wilcoxon test (for unpaired data), and the Student's *t*-test was used for data with normal distribution. A probability $p < 0.05$ was considered statistically significant. All the values in this work are expressed as mean ± standard deviation (SD).

3. Results

3.1. Phytochemical Profile of the Examined Rhubarb Extracts

Tables 2, S1 and S2 and Figures S1–S4 (Supplementary Materials S1) show the detailed phytochemical profiles of the petioles and roots of *R. rhaponticum* and *R rhabarbarum*, including the semi-quantitative data on the contents of the principal metabolites.

Table 2. Main metabolites detected in the butanol fractions of the petioles and roots of *R. rhabarbarum* and *R. rhaponticum*. A complete dataset is available in Tables S2 and S3.

No	Name	RT (min)	Calc. Formula	Err. (ppm)	*R. rhabarbarum* Petioles (mg/g d.w.)	*R. rhaponticum* Petioles (mg/g d.w.)	ID Level
2	*Unidentified*	2.07	$C_{10}H_{12}O_{10}$	0.7	5.06 ± 1.06	1.71 ± 0.27	4
3	*Unidentified*	2.07	$C_{10}H_{12}O_{11}$	0.7	6.99 ± 1.47	1.97 ± 0.30	4
4	beta-glucogallin	2.22	$C_{13}H_{16}O_{10}$	1.4	14.67 ± 3.79	0.85 ± 0.19	1
5	homocitrate-1	2.86	$C_7H_{10}O_7$	2.8	2.49 ± 1.33	1.07 ± 0.29	3
6	homocitrate-2	3.09	$C_7H_{10}O_7$	2.2	7.16 ± 2.01	5.40 ± 0.90	3
7	gentisoyl-Hex	3.70	$C_{13}H_{16}O_9$	−0.2	ND	1.59 ± 0.12	3
11	Trp	6.46	$C_{11}H_{12}N_2O_2$	−0.9	2.63 ± 0.66	ND	2
13	syringoyl-Hex-2	7.57	$C_{15}H_{20}O_{10}$	0.6	1.36 ± 0.39	4.21 ± 0.99	3
14	procyanidin-B1	7.76	$C_{30}H_{26}O_{12}$	0.6	1.60 ± 0.44	0.05 ± 0.02	1
16	*Unidentified*	8.51	$C_{12}H_{22}O_8$	−0.8	ND	1.33 ± 0.34	4
17	catechin	8.81	$C_{15}H_{14}O_6$	0.7	24.71 ± 3.36	0.26 ± 0.05	1
23	eucomic-acid-2	9.56	$C_{11}H_{12}O_6$	0.0	1.79 ± 0.35	0.40 ± 0.07	2
25	*Unidentified*	9.93	$C_8H_{14}O_5$	−0.8	0.43 ± 0.03	1.43 ± 0.21	4
31	sinapoyl-Hex-2	11.31	$C_{17}H_{22}O_{10}$	0.0	3.53 ± 0.77	ND	3
35	methyl-butyl-Hex-Pent	12.09	$C_{17}H_{32}O_{12}$	−0.1	1.30 ± 0.23	ND	3
42	resveratrol-Hex-1 (resveratroloside)	13.24	$C_{20}H_{22}O_8$	1.0	1.24 ± 0.17	ND	2
43	tetrahomocitrate	13.43	$C_{10}H_{16}O_7$	1.6	0.33 ± 0.18	0.88 ± 0.10	3
44	*Unidentified*	13.69	$C_{21}H_{24}O_{12}$	0.7	0.77 ± 0.12	ND	4
46	myrcetin-Hex-dHex	14.08	$C_{27}H_{30}O_{17}$	1.7	3.15 ± 0.73	1.21 ± 0.08	3
47	myrcetin-HexA	14.10	$C_{21}H_{18}O_{14}$	0.2	ND	0.74 ± 0.05	3
48	vicenin-III	14.14	$C_{26}H_{28}O_{14}$	1.2	1.76 ± 0.41	1.74 ± 0.11	2
54	*Unidentified*	14.69	$C_{21}H_{22}O_{12}$	1.0	0.82 ± 0.16	ND	4
55	galloyl-catechin-1	15.02	$C_{22}H_{18}O_{10}$	0.9	0.63 ± 0.18	0.21 ± 0.01	2

Table 2. *Cont.*

No	Name	RT (min)	Calc. Formula	Err. (ppm)	*R. rhabarbarum* Petioles (mg/g d.w.)	*R. rhaponticum* Petioles (mg/g d.w.)	ID Level
62	rutin	15.83	$C_{27}H_{30}O_{16}$	1.8	17.69 ± 1.85	11.60 ± 0.95	1
65	quercetin-HexA	16.02	$C_{21}H_{18}O_{13}$	0.3	ND	4.66 ± 0.21	3
66	quercetin-Hex-2 (isoquercetrin)	16.20	$C_{21}H_{20}O_{12}$	1.4	4.41 ± 0.65	2.74 ± 0.01	2
70	rhapontin	16.82	$C_{21}H_{24}O_{9}$	2.0	3.46 ± 0.57	0.35 ± 0.11	1
75	quercetin-Pent-2 (avicularin)	17.51	$C_{20}H_{18}O_{11}$	1.5	1.12 ± 0.20	ND	2
102	deoxyrhapontigenin-Hex-1	21.85	$C_{21}H_{24}O_{8}$	0.4	1.29 ± 0.25	ND	3
103	(aloe-)emodin-anthrone-malonyl-Hex-1	22.09	$C_{24}H_{24}O_{12}$	0.6	0.69 ± 0.10	0.37 ± 0.02	3
107	torachrysone-Hex-1	22.45	$C_{20}H_{24}O_{9}$	0.2	1.42 ± 0.35	0.30 ± 0.04	3
109	(aloe-)emodin-dianthrone-di(malonyl-Hex)-1	22.85	$C_{48}H_{46}O_{24}$	0.7	1.16 ± 0.26	0.36 ± 0.09	3
112	apigenin-7-Glu	23.02	$C_{21}H_{20}O_{10}$	−0.3	0.85 ± 0.10	0.55 ± 0.11	1
113	pinocembrine-Hex-5	23.07	$C_{21}H_{22}O_{9}$	−0.3	1.78 ± 0.22	0.41 ± 0.08	2
116	(aloe-)emodin-anthrone-malonyl-Hex-2	24.50	$C_{24}H_{24}O_{12}$	−0.9	28.90 ± 2.80	4.32 ± 0.36	3
118	emodin-malonyl-Hex-2	24.55	$C_{24}H_{22}O_{13}$	−0.9	ND	2.34 ± 0.20	3
121	torachrysone-Ac-Hex-2	24.79	$C_{22}H_{26}O_{10}$	−0.3	6.51 ± 0.84	1.47 ± 0.15	3
123	nataloe-emodin-8-Me-Ac-Hex	25.50	$C_{24}H_{26}O_{11}$	0.4	1.08 ± 0.11	0.43 ± 0.07	4
129	(aloe-)emodin-dianthrone-di(malonyl-Hex)-2	27.81	$C_{48}H_{46}O_{24}$	1.2	2.72 ± 0.25	0.97 ± 0.11	3
130	physcion-anthrone-malonyl-Hex	27.92	$C_{25}H_{26}O_{12}$	1.4	1.78 ± 0.22	ND	3
131	physcion-Ac-Hex-3	28.01	$C_{24}H_{24}O_{11}$	0.7	1.67 ± 0.33	0.79 ± 0.11	3
133	(aloe-)emodin-dianthrone-di(malonyl-Hex)-3	28.70	$C_{48}H_{46}O_{24}$	1.5	2.23 ± 0.28	0.84 ± 0.09	3
136	*Unidentified*	29.11	$C_{21}H_{18}O_{10}$	0.4	1.36 ± 0.13	0.68 ± 0.08	4
137	emodin-dianthrone-malonyl-Hex-1	29.68	$C_{39}H_{34}O_{16}$	1.1	1.91 ± 0.08	0.85 ± 0.14	3
140	emodin-dianthrone-malonyl-Hex-2	29.93	$C_{39}H_{34}O_{16}$	0.3	1.60 ± 0.12	0.36 ± 0.05	3
143	emodin-dianthrone-malonyl-Hex-3	30.44	$C_{39}H_{34}O_{16}$	−0.2	1.30 ± 0.09	0.86 ± 0.06	3
147	emodin-dianthrone-malonyl-Hex-4	30.64	$C_{39}H_{34}O_{16}$	0.0	1.64 ± 0.19	0.74 ± 0.09	3
149	(aloe-)emodin-anthrone-2	30.85	$C_{15}H_{12}O_{4}$	0.9	10.48 ± 1.80	1.98 ± 0.18	3
151	emodin	31.11	$C_{15}H_{10}O_{5}$	0.9	4.77 ± 2.22	2.60 ± 0.14	1
155	emodin-dianthrone-1?	31.78	$C_{30}H_{22}O_{8}$	0.5	1.20 ± 0.13	0.58 ± 0.10	3
159	emodin-dianthrone-2	32.03	$C_{30}H_{22}O_{8}$	0.0	1.22 ± 0.06	0.43 ± 0.07	3
164	18:2-LPC-2	32.29	$C_{27}H_{52}NO_{9}P$	0.5	1.69 ± 0.22	0.80 ± 0.06	2
166	18:2-LPE-2	32.33	$C_{23}H_{44}NO_{7}P$	0.3	1.19 ± 0.15	0.67 ± 0.05	2
171	*Unidentified*	32.64	$C_{49}H_{78}O_{14}$	−0.2	ND	0.84 ± 0.27	4
173	16:0-LPC-2	32.71	$C_{25}H_{52}NO_{9}P$	0.4	0.92 ± 0.43	0.94 ± 0.30	2
174	16:0-LPE-2	32.71	$C_{21}H_{44}NO_{7}P$	0.3	0.64 ± 0.30	0.68 ± 0.22	2

No	Name	RT (min)	Calc. Formula	Err. (ppm)	*R. rhabarbarum* Roots (mg/g d.w.)	*R. rhaponticum* Roots (mg/g d.w.)	ID Level
1	Hex-Hex	1.12	$C_{12}H_{22}O_{11}$	−0.4	23.7 ± 1.58	110.9 ± 6.11	3
2	malate	1.28	$C_{4}H_{6}O_{5}$	1.5	4.6 ± 0.44	ND	2
3	citrate	1.43	$C_{6}H_{8}O_{7}$	0.2	0.8 ± 0.15	1.5 ± 0.11	2
4	tyrosine	1.68	$C_{9}H_{11}NO_{3}$	1.7	0.6 ± 0.03	0.8 ± 0.12	1
5	beta-glucogallin	1.92	$C_{13}H_{16}O_{10}$	0.7	31.2 ± 2.39	6.4 ± 0.23	1
6	beta-glucogallin-2	2.21	$C_{13}H_{16}O_{10}$	1.2	1.0 ± 0.14	3.0 ± 0.28	3
7	gallate	2.41	$C_{7}H_{6}O_{5}$	4.4	1.2 ± 0.24	ND	1
8	beta-glucogallin-3	2.44	$C_{13}H_{16}O_{10}$	0.9	1.4 ± 0.11	4.5 ± 0.35	3
17	Trp	5.74	$C_{11}H_{12}N_{2}O_{2}$	0.1	0.9 ± 0.11	0.8 ± 0.05	1
28	digalloyl-Hex-5	7.60	$C_{20}H_{20}O_{14}$	−0.9	6.1 ± 0.43	8.5 ± 0.46	3
30	catechin	7.83	$C_{15}H_{14}O_{6}$	−0.9	10.1 ± 0.84	1.8 ± 0.11	1
31	*Unidentified*	7.98	$C_{21}H_{22}O_{11}$	−0.8	ND	1.2 ± 0.08	4
32	*Unidentified*	8.05	$C_{17}H_{30}O_{13}$	1.8	1.2 ± 0.29	ND	4
33	digalloyl-Hex-7	8.08	$C_{20}H_{20}O_{14}$	1.9	3.1 ± 0.30	1.5 ± 0.07	3
34	coumaroyl-Hex-3	8.15	$C_{15}H_{18}O_{8}$	−2.1	2.1 ± 0.28	ND	3
36	resveratrol-diHex-1	8.61	$C_{26}H_{32}O_{13}$	1.2	0.6 ± 0.17	0.7 ± 0.03	3
39	digalloyl-Hex-8	8.86	$C_{20}H_{20}O_{14}$	−0.8	ND	0.7 ± 0.06	3

Table 2. *Cont.*

No	Name	RT (min)	Calc. Formula	Err. (ppm)	*R. rhabarbarum* Roots (mg/g d.w.)	*R. rhaponticum* Roots (mg/g d.w.)	ID Level
40	benzoyl-Hex-Pent	8.93	$C_{18}H_{24}O_{11}$	2,0	1.5 ± 0.05	ND	3
42	procyanidin-B2	9.25	$C_{30}H_{26}O_{12}$	−0.8	ND	1.3 ± 0.18	1
43	hydroxybenozyl-galloyl-Hex-1	9.27	$C_{20}H_{20}O_{12}$	−1.8	ND	0.7 ± 0.08	3
46	*Unidentified*	9.44	$C_{17}H_{24}O_{9}$	2.3	1.1 ± 0.24	ND	4
51	piceatannol/oxyresveratrol-diHex-2	10.02	$C_{26}H_{32}O_{14}$	2.0	0.9 ± 0.09	1.3 ± 0.02	3
61	(epi-)catechin-(epi-)catechin-gallate-1	10.71	$C_{37}H_{30}O_{16}$	−1.4	1.1 ± 0.17	ND	3
62	*Unidentified*	10.83	$C_{35}H_{34}O_{15}$	−0.5	ND	1.2 ± 0.10	4
64	piceatannol-Hex-1	11.12	$C_{20}H_{22}O_{9}$	1.0	6.7 ± 0.55	4.2 ± 0.27	3
66	piceatannol-Hex-2	11.30	$C_{20}H_{22}O_{9}$	−0.4	1.8 ± 0.26	2.7 ± 0.24	3
69	resveratrol-Hex-1 (resveratroloside)	11.62	$C_{20}H_{22}O_{8}$	0.1	24.5 ± 1.98	21.1 ± 1.14	2
74	hydroxybenozyl-galloyl-Hex-4	11.87	$C_{20}H_{20}O_{12}$	−1.4	ND	0.7 ± 0.06	3
75	resveratrol-diHex-2	11.91	$C_{26}H_{32}O_{13}$	−0.1	0.5 ± 0.06	1.6 ± 0.27	3
78	*Unidentified*	12.04	$C_{29}H_{34}O_{17}$	0.1	ND	1.3 ± 0.14	4
79	trihydroxyresveratrol	12.07	$C_{14}H_{12}O_{6}$	−0.6	ND	1.1 ± 0.18	3
84	astringin	12.68	$C_{20}H_{22}O_{9}$	0.4	65.3 ± 4.38	99.3 ± 4.20	1
88	*Unidentified*	13.06	$C_{21}H_{28}O_{13}$	−1.2	5.1 ± 0.79	4.9 ± 0.44	4
89	polyflavanostilbene-A	13.10	$C_{42}H_{38}O_{19}$	−0.4	4.7 ± 0.45	ND	3
90	digalloyl-procyanidin-B2-1	13.12	$C_{44}H_{34}O_{20}$	0.4	ND	3.8 ± 0.69	3
91	polydatin	13.20	$C_{20}H_{22}O_{8}$	−0.1	14.0 ± 0.72	11.3 ± 0.56	1
96	piceatannol-galloyl-Hex-1	13.35	$C_{27}H_{26}O_{13}$	−0.1	1.7 ± 0.25	ND	3
99	galloyl-catechin-2	13.47	$C_{22}H_{18}O_{10}$	−0.2	6.7 ± 0.52	8.0 ± 0.36	3
108	*Unidentified*	13.91	$C_{43}H_{40}O_{19}$	−0.5	1.7 ± 0.08	ND	4
110	piceatannol-Pent	14.01	$C_{19}H_{20}O_{8}$	−0.4	3.4 ± 0.44	4.7 ± 0.50	3
112	piceatannol-galloyl-Hex-2	14.10	$C_{27}H_{26}O_{13}$	−0.4	4.4 ± 1.09	11.3 ± 0.78	3
113	(iso-)rhapontigenin-Hex-1	14.20	$C_{21}H_{24}O_{9}$	−0.4	18.5 ± 0.55	17.7 ± 0.73	3
115	pinocembrine-Hex-2	14.34	$C_{21}H_{22}O_{9}$	−1.2	ND	1.6 ± 0.11	3
116	trans-piceatannol	14.43	$C_{14}H_{12}O_{4}$	−0.5	2.5 ± 0.50	4.0 ± 1.17	1
121	rhapontin	14.84	$C_{21}H_{24}O_{9}$	−0.3	184.0 ± 10.93	151.3 ± 7.77	1
129	(iso-)rhapontigenin-galloyl-Hex-1	15.32	$C_{28}H_{28}O_{13}$	0.1	3.8 ± 0.70	5.0 ± 1.77	3
134	eriodictyol-Hex	15.79	$C_{21}H_{22}O_{11}$	0.1	1.0 ± 0.18	2.6 ± 0.28	3
140	(iso-)rhapontigenin-galloyl-Hex-2	16.21	$C_{28}H_{28}O_{13}$	1.1	13.3 ± 0.58	9.4 ± 0.74	3
142	(iso-)rhapontigenin-malonyl-Hex-1	16.40	$C_{24}H_{26}O_{12}$	−0.1	11.2 ± 1.09	5.7 ± 0.41	3
145	piceatannol-hydroxybenzoyl-Hex	16.66	$C_{27}H_{26}O_{11}$	0.7	0.6 ± 0.11	ND	3
147	emodin-Hex-3	16.71	$C_{21}H_{20}O_{10}$	1.1	1.2 ± 0.20	12.8 ± 0.68	3
158	*Unidentified*	17.31	$C_{42}H_{34}O_{9}$	1.2	6.7 ± 0.59	5.6 ± 0.49	4
161	*Unidentified*	17.49	$C_{42}H_{34}O_{9}$	1.1	3.5 ± 0.13	2.3 ± 0.13	4
163	piceatannol-dimer (cararosinol-D)	17.55	$C_{28}H_{22}O_{8}$	1.5	ND	1.5 ± 0.40	2
167	piceatannol-coumaroyl-Hex-2	17.68	$C_{29}H_{28}O_{11}$	1.0	3.9 ± 0.36	5.1 ± 0.24	3
168	*Unidentified*	17.77	$C_{24}H_{28}O_{11}$	1.5	1.2 ± 0.32	ND	4
169	piceatannol-feruloyl-Hex	17.83	$C_{30}H_{30}O_{12}$	1.0	0.6 ± 0.08	3.8 ± 0.24	3
172	(aloe-)emodin-galloyl-Hex	18.07	$C_{28}H_{24}O_{14}$	0.9	ND	2.0 ± 0.30	3
180	(iso-)rhapontigenin	18.56	$C_{15}H_{14}O_{4}$	2.3	12.6 ± 0.79	10.7 ± 0.85	3
193	deoxyrhapontigenin-Hex-1	19.18	$C_{21}H_{24}O_{8}$	1.7	138.6 ± 8.55	31.7 ± 2.08	3
199	chrysophanol-Hex-1	19.39	$C_{21}H_{20}O_{9}$	1.2	4.2 ± 0.82	17.4 ± 0.91	3
201	apigenin-7-Glu	19.51	$C_{21}H_{20}O_{10}$	1.0	1.1 ± 0.17	3.5 ± 0.19	1
203	(iso-)rhapontigenin-coumaroyl-Hex-2	19.67	$C_{30}H_{30}O_{11}$	0.9	3.9 ± 0.60	3.0 ± 0.29	3
205	chrysophanol-Hex-2	19.81	$C_{21}H_{20}O_{9}$	1.3	2.5 ± 0.26	12.5 ± 0.73	3
208	(iso-)rhapontigenin-feruloyl-Hex-2	19.93	$C_{31}H_{32}O_{12}$	1.0	ND	2.0 ± 0.40	3
209	deoxyrhapontigenin-galloyl-Hex-1	20,00	$C_{28}H_{28}O_{12}$	1.4	6.8 ± 0.74	1.8 ± 0.22	3
210	flavanol-piceatannol-dimer	20.01	$C_{29}H_{24}O_{8}$	1.6	ND	3.4 ± 0.43	3
212	rhapontigenin-coumaroyl-Hex-2	20.42	$C_{30}H_{30}O_{11}$	1.7	ND	2.1 ± 0.13	3
214	deoxyrhapontigenin-malonyl-Hex	20.44	$C_{24}H_{26}O_{11}$	1.4	3.0 ± 0.31	1.2 ± 0.13	3
218	rhapontigenin-feruloyl-Hex-3	20.72	$C_{31}H_{32}O_{12}$	1.6	ND	0.7 ± 0.13	3
221	(aloe-)emodin-anthrone-malonyl-Hex(-CO2)-2	20.94	$C_{23}H_{24}O_{10}$	1.3	2.2 ± 0.16	ND	3
222	resveratrol-dimer-1	20.95	$C_{28}H_{22}O_{6}$	1.4	3.3 ± 0.29	3.4 ± 0.16	3

Table 2. *Cont.*

No	Name	RT (min)	Calc. Formula	Err. (ppm)	*R. rhabarbarum* Roots (mg/g d.w.)	*R. rhaponticum* Roots (mg/g d.w.)	ID Level
223	torachrysone-malonyl-Hex(-CO2)-3	21.08	$C_{22}H_{26}O_{10}$	1.4	5.1 ± 0.46	5.0 ± 0.29	3
227	physcion-Hex-1 (rheochrysin)	21.24	$C_{21}H_{20}O_8$	0.1	ND	2.2 ± 0.38	3
229	resveratrol-piceatannol-mixed-tetramer	21.33	$C_{38}H_{52}O_{25}$	−2.0	0.8 ± 0.07	2.9 ± 0.59	3
231	chrysophanol-Ac-Hex-3	21.45	$C_{23}H_{22}O_{10}$	2.7	6.0 ± 0.52	ND	3
232	chrysophanol-physcion-dianthr.-di(malonyl-Hex)-1	21.45	$C_{48}H_{44}O_{24}$	1.0	ND	16.0 ± 0.72	3
240	chrysophanol-malonyl-Hex(-CO2)-4	21.82	$C_{23}H_{22}O_{10}$	0.2	5.8 ± 0.36	ND	3
241	*Unidentified*	21.82	$C_{46}H_{44}O_{22}$	0.8	ND	4.7 ± 1.40	4
242	chrysophanol-physcion-dianthr.-di(malonyl-Hex)-2	21.82	$C_{48}H_{44}O_{24}$	1.7	ND	11.5 ± 1.15	3
252	chrysophanol-dianthrone-di(malonyl-Hex)-2	22.49	$C_{48}H_{46}O_{22}$	1.5	1.0 ± 0.13	ND	3
258	resveratrol-dimer-2	23.10	$C_{28}H_{22}O_6$	2.7	8.4 ± 0.61	9.4 ± 0.57	3
267	chrysophanol-anthrone-malonyl-Hex-4	23.45	$C_{24}H_{24}O_{11}$	−0.6	ND	4.4 ± 0.33	3
272	chrysophanol-dianthrone-malonyl-diHex-8	23.56	$C_{45}H_{44}O_{19}$	1.9	0.7 ± 0.08	ND	3
278	deoxyrhapontigenin	23.89	$C_{15}H_{14}O_3$	3.6	13.4 ± 1.16	2.2 ± 0.21	1
286	chrysophanol-dianthrone-di(malonyl-Hex)-6	24.25	$C_{48}H_{46}O_{22}$	1.2	0.8 ± 0.11	ND	3
291	chrysophanol-dianthrone-di(malonyl-Hex)-7	24.47	$C_{48}H_{46}O_{22}$	1.8	1.5 ± 0.25	ND	3
309	chrysophanol-dianthrone-di(malonyl-Hex)-9	26.55	$C_{48}H_{46}O_{22}$	1.7	0.9 ± 0.03	ND	3
322	emodin	29.75	$C_{15}H_{10}O_5$	0.9	0.8 ± 0.12	2.8 ± 0.11	1
324	chrysophanol-dianthr.-malonyl-Hex-2	29.91	$C_{39}H_{34}O_{14}$	−0.9	3.2 ± 0.26	ND	3
327	chrysophanol-physcion-dianthr.-malonyl-Hex-2	30.11	$C_{40}H_{36}O_{15}$	−0.7	1.2 ± 0.14	ND	3

Qualitatively, the chemical composition of the petiole butanol extracts (Tables 2, S1 and S3, Figures S1 and S3) of *R. rhabarbarum* and *R. rhaponticum* were very similar. Over 50% of observed metabolites were identical between the two extracts. Generally, they represented typical classes of compounds observed previously in rhubarbs [35–38]. The main anthraquinones were emodin (Tables 2 and S1, peak 151) and its anthrone derivative (peak 149), as well as numerous hexosides of both these compounds, mainly occurring as malonic or succinic acid esters (peaks 103, 106, 111, 116, 118, 120). Compared to derivatives of emodin, metabolites derived from physcion (rheochrysidin/methoxy-emodin) were relatively infrequent and restricted to acetyl-hexoside (peak 131), malonyl-hexoside, and hexoside of anthrone (peaks 130 and 139) as well as several mixed dianthrones with emodin. A relatively rare anthraquinone, a methoxylated derivative of nataloe-emodin, was observed in both species as malonyl-hexoside (peak 123). Of particular interest may be the tentatively identified dihexosides of emodin dianthrones, which represent structures analogous to sennosides from medicinal rhubarbs, but often exist as malonic acid esters (for example, peaks 109, 125, 127, 128, 129, 132, 133). They were observed as multiple isobaric peaks, presumably due to variable stereochemistry and various attached hexoses. Similar dianthrones were also formed between molecules of physcion and emodin.

Naphthalene derivatives were represented by torachrysone compounds (peaks 115, 121, 148). Compounds from the stilbene group were also detected: hexosides of resveratrol (resveratroloside and piceid, peaks 42 and 71), piceatannol (astringin, peak 39), and rhapontigenin (rhaponticin, peak 70). Flavonoid glycosides were represented by several compounds mainly derived from quercetin, apigenin, kaempferol, isorhamnetin, and pinocembrin. Apigenin derivatives were observed only as *C*-glycosides (peaks 45, 48, 56), and the remaining flavonoids were present as *O*-glycosides. Moreover, a whole group of typical polyphenolic compounds found in plants was also observed—phenolic acid hexosides, catechins, lignans, amino acids, and simple organic acids. The petiole extracts also contained small amounts of polar lipids and free triterpenes. A sulfonated catechin (peak 24) was observed in *R. rhaponticum* but was completely missing from *R. rhabarbarum*. Similarly absent were also flavonol derivatives of hexuronic acid (peaks 47 and 65).

The phytochemical composition of root extracts was less similar between the species, yet nearly 40% of the identified compounds were identical (Tables 2, S2 and S3, Figures S2 and S4 and 1). Compared with the petioles, in the root extracts (Tables 2, S2 and S3, Figures S2 and S4) the diversity of anthraquinone derivatives was smaller. The main compounds of this group present in the roots were chrysophanol malonyl hexosides (peaks 224 and 240). Chrysophanol hexoside esterified with gallic acid was also present (peak 225). Additionally, smaller signals from emodin hexosides, free emodin and its anthrone and dianthrone, and physcion hexoside and mixed emodin dianthrones with chrysophanol and physcion were observed. As in the petioles, the same torachrysone derivatives were present in the roots of both *Rheum* species.

In contrast, the observed variety of stilbene derivatives in the roots was greater. As shown in Figures S2 and S4, Tables 2, S2 and S3 this group appeared to be a dominant type of metabolite observed in the root extracts, with astringin, rhaponticin, and deoxyrhaponticin forming prominent peaks on the chromatogram. The leading derivatives of this group were hexosides of resveratrol (peaks 69 and 91), piceatannol (peak 84), rhapontigenin (peak 121), and also deoxyrhapontigenin (peak 191). These hexosides were accompanied by numerous other derivatives, including malonic, gallic, coumaric, and ferulic acid esters. Pentosides and di-hexosides of stilbenes were also detected. As expected, the pool of flavonoid glycosides in the root was limited to a few derivatives of quercetin (peaks 126 and 128), apigenin (peak 130), and naringenin (peak 106). Phenolic acid derivatives were mainly detected as gallic and hydroxybenzoic acid esters of their hexosides.

Principal metabolites mentioned above are summarized in Table 2, while extensive tables containing complete data can be found in the Supplementary files (Tables S1 and S2).

A summary of qualitative similarities and dissimilarities between the extracts from both species and plant parts is shown in Venn diagrams in Figure 1 and Table S3 in the Supplementary Materials.

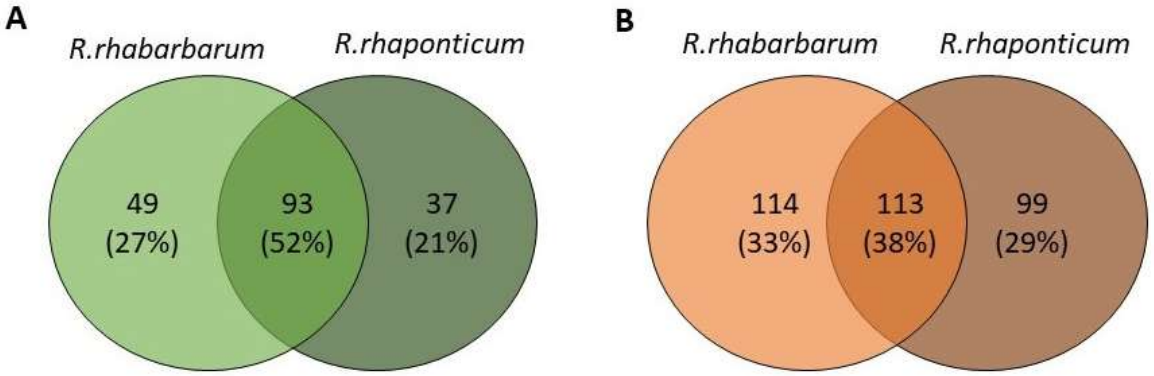


Figure 1. Qualitative similarities between the extracts from the petioles (**A**) and roots (**B**) of *R. rhabarbarum* and *R. rhaponticum*. Numbers indicate common and unique metabolites for each species.

The quantitative analysis of the prepared extract was complicated due to the lack of appropriate calibration reference substances. In the petiole extracts, for 179 identified compounds, 10 reference substances were commercially available, whereas, in the root/rhizome extracts with 346 identified metabolites, just 14 reference substances were available. For this reason, we decided to use a semi-quantitative approach employing a universal charged aerosol detector (CAD). However, the response of all aerosol evaporative detectors varies as a function of mobile phase composition [15]. During isocratic separation, all the analytes at the same concentration should produce identical responses. In contrast, the higher percentage of the organic solvent, the higher the signal for the analytes will be observed during gradient elution. In the case of rhubarb extracts, utilization of isocratic elution is not feasible because samples are very complex. There are two ways of correcting signal increases during the gradient elution. One possibility is to provide the detector with a constant mobile phase concentration throughout the analysis, using the secondary pump running a reverse gradient through a separate, identical column.

Both columns' outflows are mixed before the detector, providing a constant concentration of the mobile phase [39,40]. While relatively simple in application and providing nearly perfect results [39], this approach requires additional, carefully set equipment and uses significant volumes of solvents. As an alternative, the so-called 3D calibration can compensate for signal changes during the elution [15]. We used a modified version of the second approach, applying a linear correlation between the increase in the organic component of the mobile phase and the increase in calibration slope coefficient for 32 reference standards eluting at different times throughout the separation. This approach allowed for a relatively reliable semi-quantitation of the metabolites detected in the extracts. The test set of 10 metabolites analyzed at two concentration levels (4 and 40 µg/mL) indicated deviations not exceeding 30% in the worst cases (for chrysophanol-8-glucoside) but usually reaching 10–15%, which was acceptable for our purposes. Because the CAD signal is one-dimensional, semi-quantitative contents data were calculated only for well-separated chromatographic peaks containing one primary component. This condition was difficult to achieve due to the richness of metabolites in the extracts, mainly in the roots. In a few cases of multi-component peaks considered particularly important for the study, the CAD peak area was divided based on ion intensities in corresponding HR-MS peaks. However, the results obtained in this way had decreased accuracy.

3.2. Effects of the Examined Substances on HUVEC Viability

The viability of HUVECs treated with extracts from the petioles and roots of *R. rhaponticum* and *R. rhabarbarum*, two stilbenes (RHPG and RHPT), and reference compounds (indomethacin and zileuton) at concentrations of 1–100 µg/mL was analyzed by the resazurin reduction assay (Figure 2). A 24 h treatment with extracts from the petioles from *R. rhabarbarum* and *R. rhaponticum* did not affect the viability of HUVECs in the concentration range of 1–100 µg/mL (Figure 2A). In contrast, in samples treated with extracts from the roots at concentrations higher than 50 µg/mL and 30 µg/mL for *R. rhaponticum* and *R. rhabarbarum*, respectively, a decrease in cell viability was observed (Figure 2B). A 24 h treatment with stilbenes up to 100 µg/mL for RHPT did not affect cell viability; however, RHPG treatment decreased cell viability at concentrations higher than 25 µg/mL (IC_{50} = 46.9 µg/mL) (Figure 2C). An incubation of HUVECs with the non-steroidal anti-inflammatory drug, indomethacin, at concentrations above 80 µg/mL (IC_{50} = 93.13 µg/mL), resulted in a sharp decrease in cell viability, while zileuton showed no effect in the range of the tested concentrations (Figure 2D). A decrease in cell viability ≥ 20% (compared to the control/untreated HUVECs) was assumed as a cytotoxic effect.

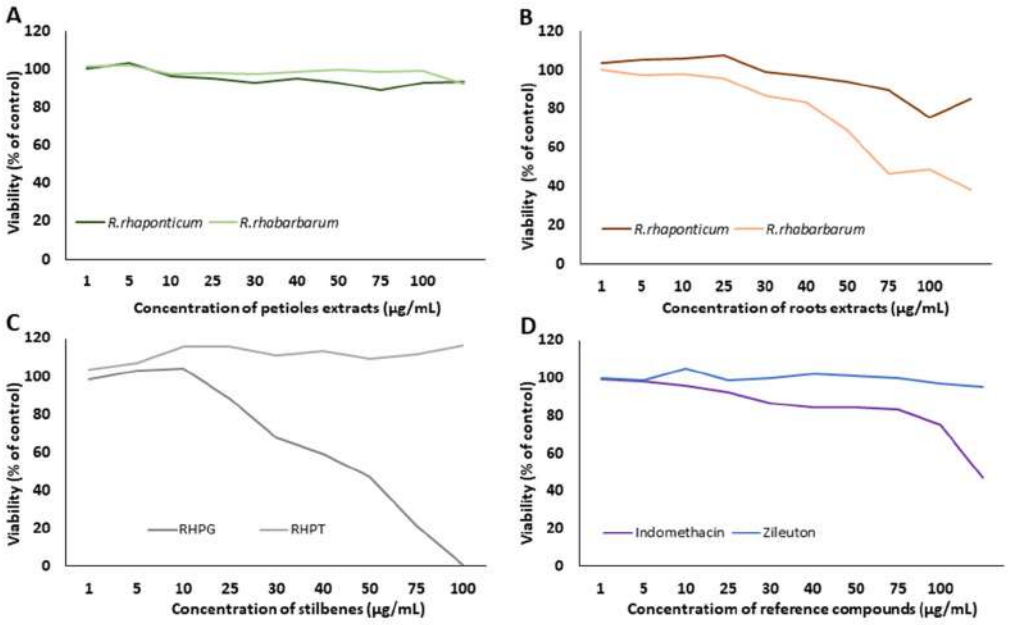


Figure 2. *Cont.*

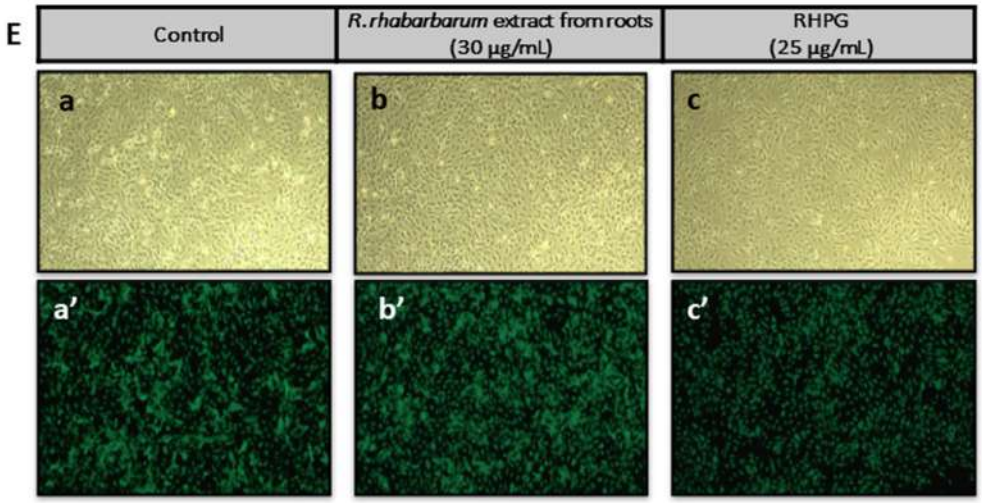


Figure 2. Effects of the examined substances on HUVEC viability. The figure presents the viability curves of HUVECs after 24 h of incubation with the petiole extracts from *R. rhaponticum* and *R. rhabarbarum* (**A**), the root extracts from *R. rhaponticum* and *R. rhabarbarum* (**B**), RHPG and RHPT (**C**) and reference anti-inflammatory drugs (indomethacin and zileuton) (**D**), at concentrations of 1–100 µg/mL, established in the resazurin-based assay. Results derive from four independent experiments. Panel (**E**) presents the effects of *R. rhabarbarum* root extract (**b**,**b′**) and RHPG (**c**,**c′**) at concentrations of 30 and 25 µg/mL, respectively. The (**a**,**b**,**c**) panels are the images of HUVECs in transmitted light, whereas the (**a′**,**b′**,**c′**) panels represents the same HUVECs samples obtained from the fluorescence microscope. HUVECs were stained with 3 µM Calcein-AM; images were acquired with 4× magnification.

Additionally, microscopic analyses of the HUVECs treated with *R. rhabarbarum* root extracts (30 µg/mL) and RHPG (25 µg/mL) were performed. Images were taken under transmitted light (Figure 2E, panels: a, b, and c) as well as a fluorescence microscope (Figure 2E, panels a′–c′), using the Calcein-AM fluorescent probe (3 µM). The obtained results confirmed the cellular safety of the examined substances at the selected concentrations.

The viability test results verified the cellular safety of the examined substances. Based on above data, concentrations not affecting cell viability were chosen for further experiments (Table 3).

Table 3. The in vitro assessment of cytotoxicity of the examined rhubarb extracts and stilbenes towards HUVECs. Data are presented as mean ± SD, $n = 4$. The viability of control (untreated) HUVECs was assumed as 100%.

Extract/Compounds	Concentration (µg/mL)	% of Cell Viability ± SD
R. rhaponticum petiole extract	1	102.94 ± 4.62
	5	96.24 ± 4.55
	50	88.81 ± 2.83
R. rhabarbarum petiole extract	1	101.94 ± 5.61
	5	97.38 ± 6.54
	50	98.52 ± 7.58
R. rhaponticum root extract	1	104.99 ± 4.97
	5	105.43 ± 4.49
	50	89.54 ± 5.47
R. rhabarbarum root extract	1	97.34 ± 4.43
	5	97.86 ± 6.95
	30	83.45 ± 7.06

Table 3. *Cont.*

Extract/Compounds	Concentration (μg/mL)	% of Cell Viability ± SD
Rhapontigenin	1	98.59 ± 14.9
	5	103.12 ± 12.10
	25	88.32 ± 7.75
Rhaponticin	1	103.38 ± 18.84
	5	106.72 ± 12.70
	50	109.32 ± 17.01
Indomethacin	5	95.83 ± 5.63
Zileuton	5	105.28 ± 3.56

3.3. Evaluation of the Anti-Inflammatory Properties of the Examined Substances

Anti-inflammatory effects of the examined extracts and stilbenes were monitored at a cytophysiological level (cytokine release from HUVECs and interactions with monocytes) as well as at a molecular level of intracellular processes (the expression of pro-inflammatory enzyme genes).

To evaluate the effects of the examined substances on cytokine secretion and interactions with monocytes, HUVECs were pre-incubated with the extracts or stilbenes, and then stimulated with LPS. In these assays, the anti-inflammatory efficiency of the examined extracts and stilbenes was estimated by comparing the cytokine level or monocyte influx in the HUVEC samples pre-incubated with the plant substances and activated by LPS, to cells stimulated with LPS in the absence of the examined extracts and stilbenes.

Analyses of COX-2 and 5-LOX gene expression required the use of two experimental models. The plant-derived substances are exogenous factors that may themselves influence gene expression, as a part of the cell adaptive response. Therefore, in the first of the used experimental models, the expression of COX-2 and 5-LOX was studied in HUVECs pre-incubated with the examined plant substances, without subsequent stimulation with LPS. This assay enabled verification if the examined extracts influenced the HUVECs at their physiological state (under physiological conditions, with no exposure to pro-inflammatory stimuli). Effects of the rhubarb extracts and stilbenes on COX-2 and 5-LOX gene expression were evaluated by comparison with control samples, i.e., native HUVECs (untreated with the plant substances or LPS).

The second experimental model was designed to study the anti-inflammatory properties of rhubarb extracts and stilbenes under inflammatory conditions (i.e., in the LPS-stimulated cells). HUVECS were pre-incubated with the extracts or stilbenes, and then stimulated with LPS. The anti-inflammatory action of the examined substances was evaluated by comparison of the COX-2 and 5-LOX genes expression in these samples to the gene expression in HUVECs treated with LPS in the absence of the rhubarb extracts or stilbenes.

3.3.1. Effects of the Rhubarb-Derived Compounds and Stilbenes on the Cytokine Secretory Profile of Endothelial Cells

The cytokine secretory profile of HUVECs was analyzed using the Proteome Profiler Human Cytokine Array, allowing the detection of growth factors, chemokines, interleukins as well as other factors that are essential both for the development and modulation of the inflammatory response of different cells. HUVECs were treated for 16 h with rhubarb-derived extracts and stilbenes at the selected concentration of 5 μg/mL and then stimulated with LPS (1 μg/mL) to induce an inflammatory response. The obtained results indicated that the examined substances might modulate the pro-inflammatory response of the HUVECs. Visualization of the proteome profiler membrane (Figure 3A) revealed changes in the secretion of 9 (out of 13 disclosed) cytokines. RHPT, the petiole extracts of both rhubarb species and the root extract from *R. rhaponticum* completely inhibited the release of the following cytokines: CCL5/RANTES, CXCL10/IP-10, CXCL12/SDF-1, and IL-18/IL-IF4. Furthermore, about 80% inhibition of the CCL5/RANTES release was observed in HUVECs

treated with RHPG (Figure 3C). However, the petiole extracts of both species increased IL-8 secretion by about 50% (Figure 3D). An enhanced release of IL-8 was also found in cells treated with the *R. rhaponticum* root extract.

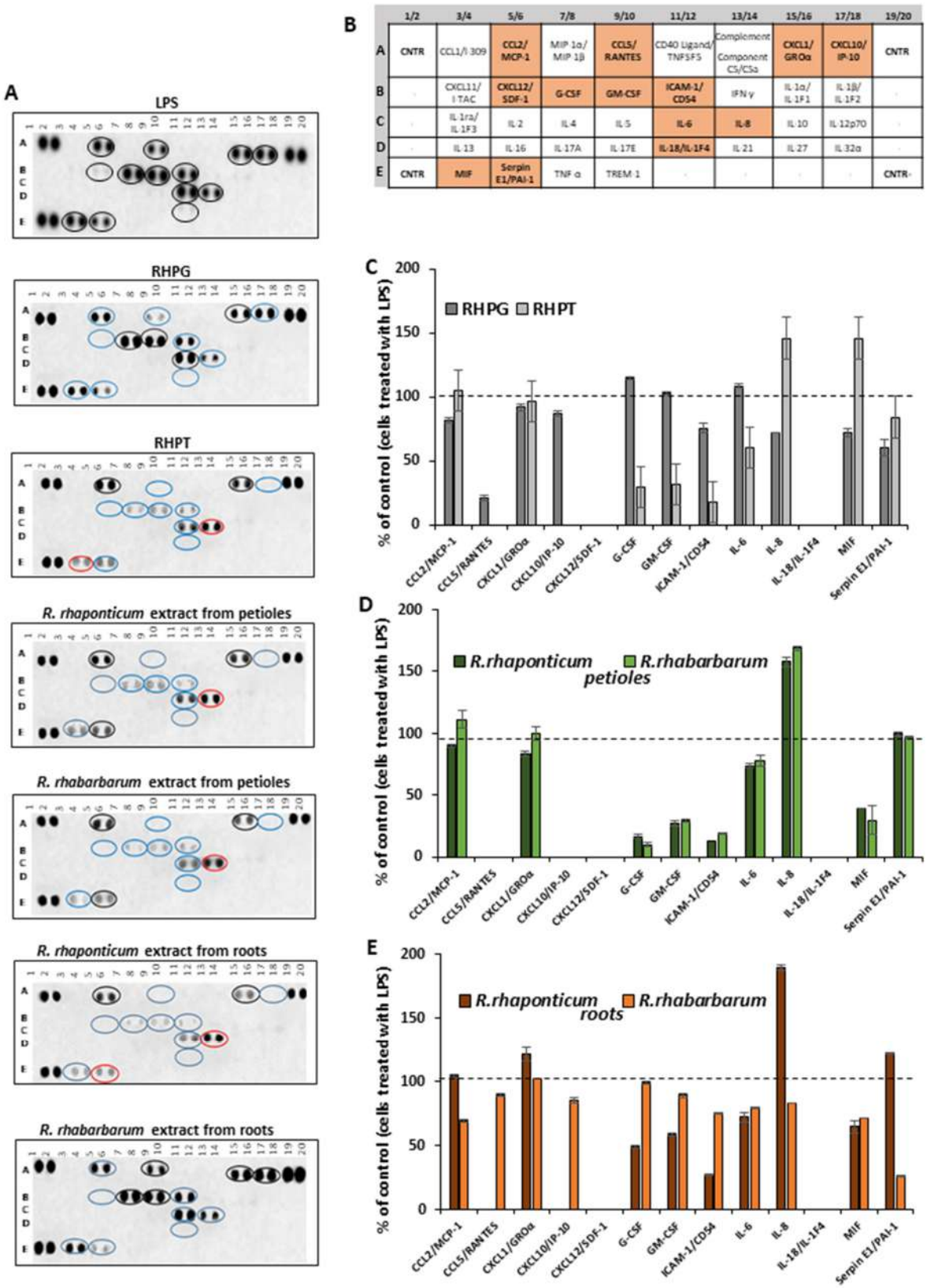


Figure 3. Effects of the examined rhubarb extracts and stilbenes on the HUVEC cytokine secretory

profile, detected by the Proteome Profiler Human Cytokine Array. Panel (**A**) contains a representative dot-blot pattern of cytokines, released into the cell culture medium by HUVECs pre-incubated with the rhubarb extracts or the stilbenes (5 μg/mL), and stimulated by LPS (1 μg/mL). Panel (**B**) refers to the table for the Human Cytokine Array coordinates. The graphs show results calculated into percentage, based on the positive control (i.e., cells treated with LPS in the absence of the examined substances), which was assumed as 100% of cytokine secretion. Panel (**C**)—data for stilbenes; panel (**D**)—results for the petiole extracts; panel (**E**)—results for the root extracts.

In cells treated with RHPT, the release of G-CSF and CM-CSF was reduced by about 60% when compared to cells treated with LPS in the absence of the examined plant-derived substances. However, in those samples, an increase in the level of IL-8 and MIF by over 30% was observed as well (Figure 3C). A similar effect was also found in cells treated with *R. rhaponticum* and *R. rhabarbarum* extracts from the petioles, including a significant decrease in G-CSF and CM-CSF, increased IL-8 release and the detection of MIF in the cell supernatant. In addition to typical cytokines, the used assay enabled the detection of the Serpin E1/PAI-1 (plasminogen activator inhibitor-1), an important regulator of hemostasis, the complement pathway and extracellular matrix remodeling. In this case, the most active one was the *R. rhabarbarum* root extract, reducing this protein release by about 80% (Figure 3E).

3.3.2. Effects of the Rhubarb-Derived Extracts and Stilbenes on Endothelial Cell–Monocyte Interactions

Interactions of endothelial cells and leukocytes play a crucial role in the development of inflammation and in the amplification of inflammatory processes. Therefore, the assessment of the anti-inflammatory activity of the examined rhubarb extracts and stilbenes also included studies on their effects on the recruitment of monocytes to the activated HUVECs. A 16 h pre-treatment of HUVECs with the rhubarb-derived extracts and stilbenes, followed by a 3 h incubation with (1 μg/mL) LPS, significantly decreased the recruitment of U-937 monocytes to the activated endothelial cells (Figure 4), as confirmed by the microscope observations of Hoechst 33342-stained monocytes (Figure 4A). The root extracts of both *R. rhaponticum* and *R. rhabarbarum* and the stilbenes (RHPG and RHPT) displayed similar effects (Figure 4B,C,F,G, respectively) on monocyte recruitment. However, no significant changes in endothelial cell–monocyte interactions were found in samples treated with the *R. rhaponticum* extract from the petioles at a concentration of 1 μg/mL (Figure 4D). On the other hand, in HUVECs treated with the *R. rhabarbarum* extract from the petioles, applied at the same concentration (i.e., 1 μg/mL), the inhibition of monocyte influx was observed (Figure 4E).

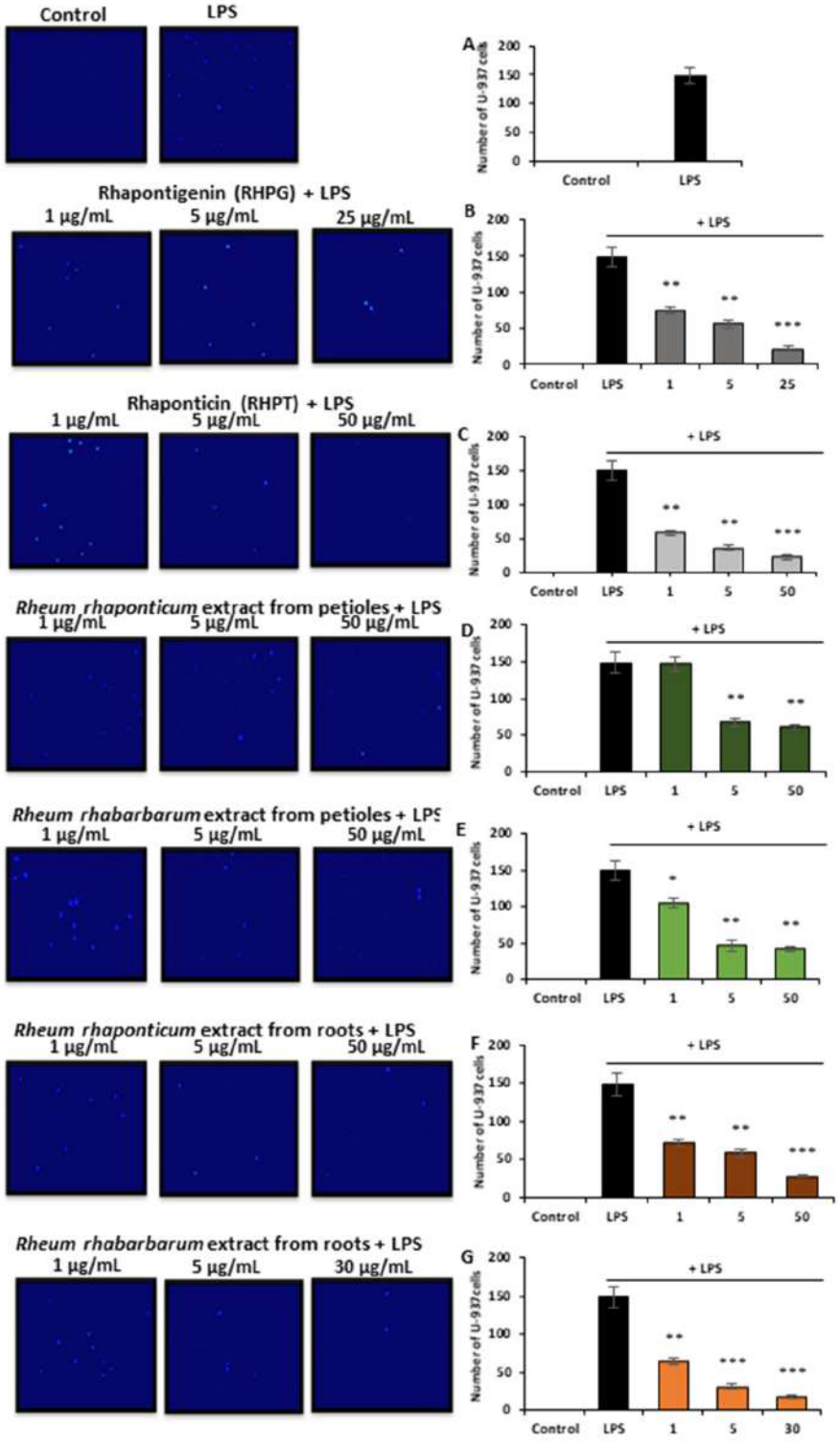


Figure 4. The effect of the examined rhubarb extracts and stilbenes on the recruitment of U-937 monocytes to the LPS-activated HUVECs. The image panel represents the pictures obtained from the fluorescence (UV) microscope; magnification 20×. Calculations of the adhered monocytes have been presented as follows: control/untreated and LPS-treated cells (**A**), RHPG (**B**), RHPT (**C**), *R. rhaponticum* extract from the petioles (**D**), *R. rhabarbarum* extract from the petioles (**E**), *R. rhaponticum* extract from the roots (**F**), *R. rhabarbarum* extract from the roots (**G**). Data are presented as means ± SD; $n = 3$. Statistical significance (HUVECs pre-incubated with plant substances and activated by LPS versus HUVECs activated by LPS in the absence of the examined substances): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.3.3. Effects of the Rhubarb-Derived Extracts and Stilbenes on Cyclooxygenase (COX-2) Expression in HUVECs

Comparing the results in both experimental models, it was noted that the extracts from the petioles of *R. rhaponticum* and *R. rhabarbarum* affected COX-2 gene expression (Figure 5A,B). In the unstimulated HUVECs, the maximal observed decrease in mRNA level expression was about 30% ($p < 0.05$, Figure 5A). Some fluctuations in COX-2 gene expression were observed in cells pre-incubated with the *R. rhaponticum* and *R. rhabarbarum* root extracts. RHPT had no effect on COX-2 mRNA levels in the unstimulated cells ($p > 0.05$), and the RHPG suppressed COX-2 gene expression maximally by approximately 20-25% ($p < 0.05$, Figure 5A).

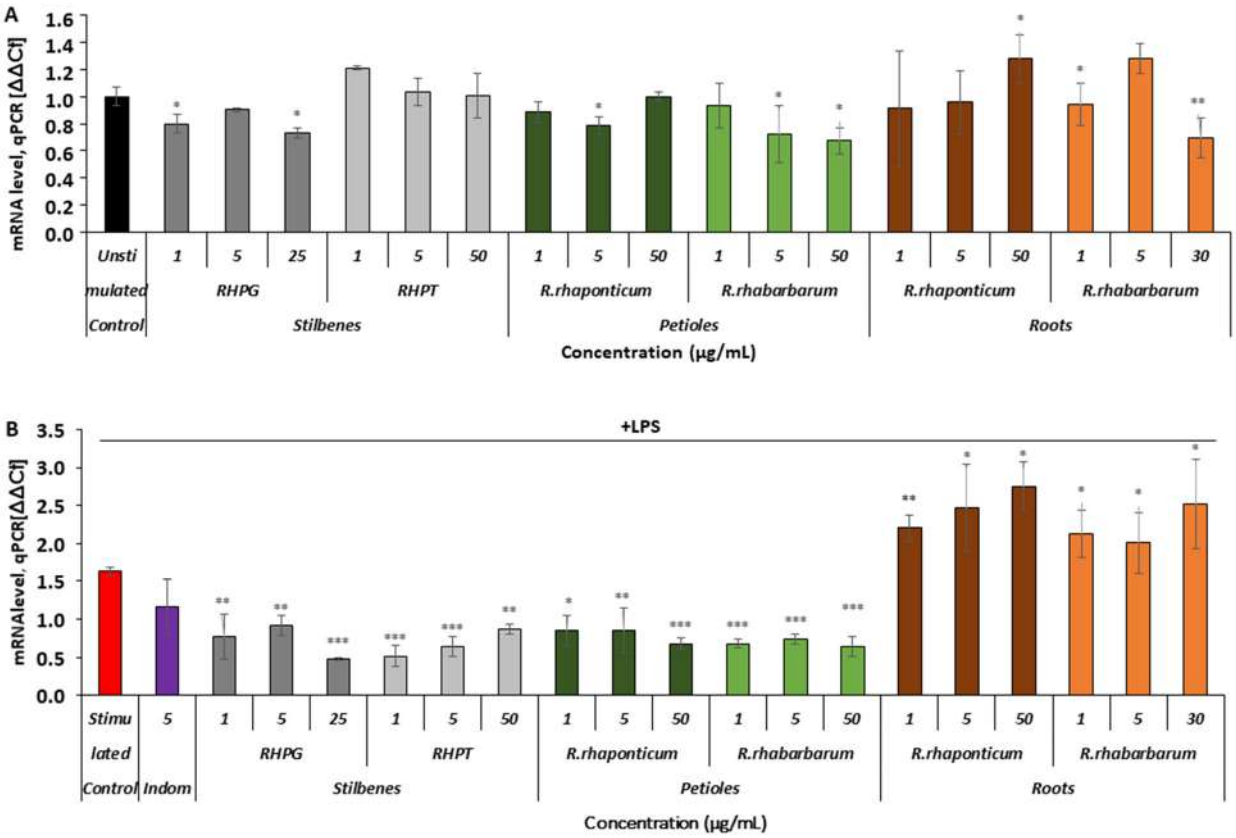


Figure 5. Effects of the rhubarb extracts and stilbenes on COX-2 gene expression in the unstimulated and inflammatory-activated HUVECs. Panel (**A**)—data derived from experiments involving HUVECs treated with stilbenes (RHPG and RHPT) or rhubarb extracts, without subsequent pro-inflammatory stimulation with LPS. Panel (**B**)—COX-2 gene expression in HUVECs treated with the stilbenes (RHPG and RHPT) or rhubarb extracts and stimulated with LPS; $n = 5$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Indom—indomethacin, a reference compound (COX inhibitor).

In the LPS-activated cells (Figure 5B), a decrease in COX-2 mRNA levels in the presence of the petiole extracts and stilbenes was more evident. The gene suppression level in most samples of HUVECs treated with these preparations attained or even exceeded 50%. In contrast, the *R. rhaponticum* and *R. rhabarbarum* root extracts increased the level of COX-2 gene expression (Figure 5B).

3.3.4. Effects of the Rhubarb-Derived Extracts and Stilbenes on 5-Lipoxygenase (*ALOX5*) Gene Expression in HUVECs

In the unstimulated cells (Figure 6A), only the *R. rhaponticum* petiole extract influenced the *ALOX5* mRNA level in the full range of tested concentrations (1–50 μg/mL; * $p < 0.01$

and *** $p < 0.001$). The remaining substances displayed no effects or only slightly reduced the mRNA levels.

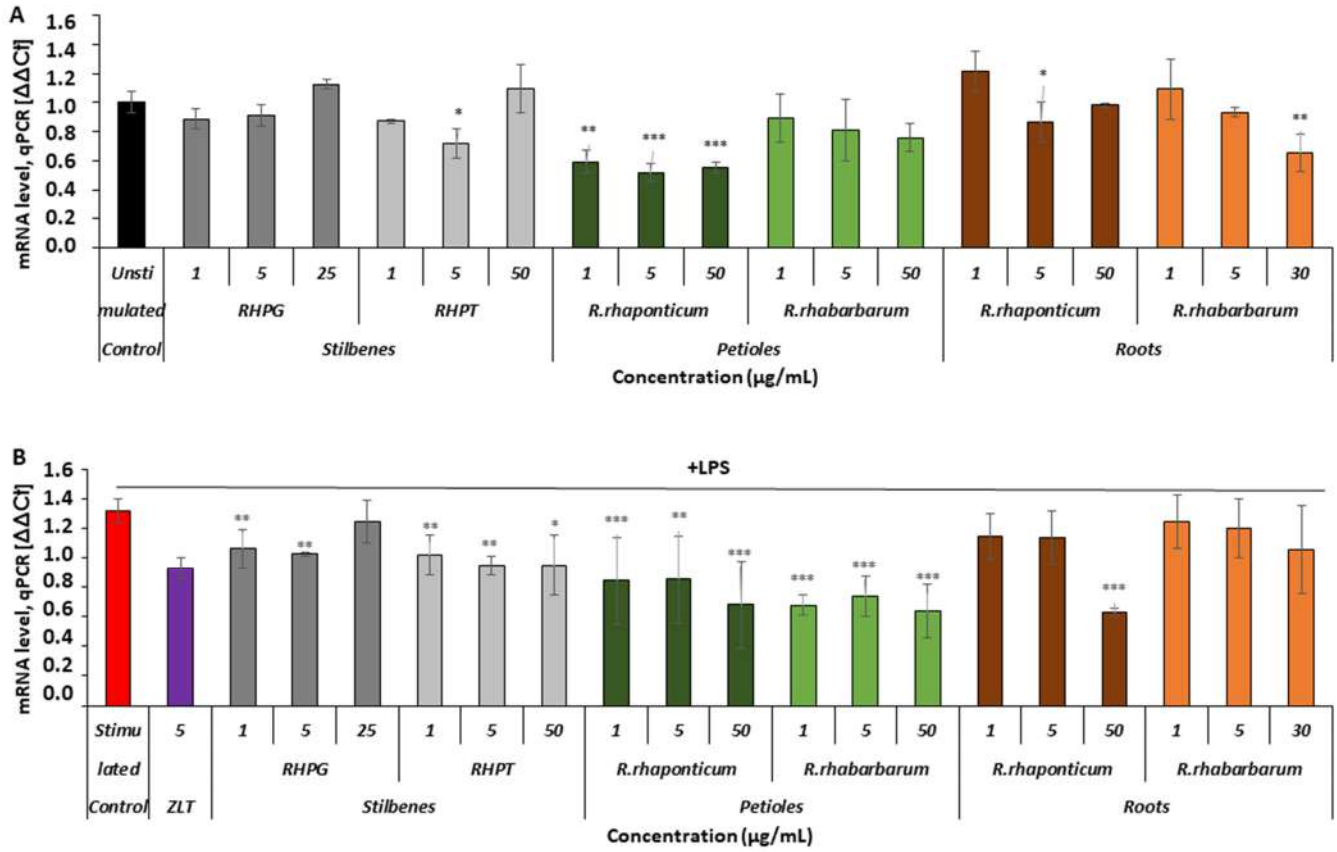


Figure 6. Effects of the rhubarb extracts and stilbenes on *ALOX5* gene expression in unstimulated and inflammatory-activated HUVECs. Panel (**A**)—*ALOX5* gene expression in HUVECs treated with stilbenes (RHPG and RHPT) or rhubarb extracts, without subsequent pro-inflammatory stimulation with LPS. Panel (**B**)—*ALOX5* gene expression in HUVECs pre-incubated with stilbenes (RHPG and RHPT) or rhubarb extracts, and stimulated with LPS; $n = 5$; (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). ZLT -zileuton, a reference compound (5-LOX inhibitor).

In the activated HUVECs (Figure 6B), extracts from the petioles of both rhubarb species and stilbenes markedly inhibited *ALOX5* gene expression, compared to this gene's expression level in cells activated by LPS in the absence of the examined rhubarb-derived substances. The *R. rhaponticum* root extract decreased *ALOX5* gene expression only at its highest concentration, i.e., 50 μg/mL, whereas the *R. rhabarbarum* root extract did not affect this gene's expression (Figure 6B).

3.4. COX-2 and 5-LOX Inhibitor Screening

To assess whether the examined extracts and selected components can acts as anti-inflammatory agents at a level of COX-2 and 5-LOX enzyme activity, a colorimetric inhibitor screening test was performed. The reference compound was indomethacin.

The experiments revealed that the activity of the pro-inflammatory enzyme COX-2 was most effectively reduced by the root extracts of both species of rhubarb, used at a concentration of 50 μg/mL (Figure 7A, *** $p < 0.001$). In these samples, the enzyme activity was reduced by about 80%, when compared to the native (untreated) COX-2. The IC50 values for the extracts from the roots of *R. rhaponticum* and *R. rhabarbarum* were 19.16 μg/mL and 19.44 μg/mL, respectively. The COX-2-inhibitory effect (about 30% of enzyme activity reduction) was also found in the samples treated with RHPG at the same concentration

(i.e., 50 μg/mL; *** $p < 0.001$). RHPT and extracts from the petioles of *R. rhaponticum* and *R. rhabarbarum* were considerably weaker inhibitors of this enzyme (Figure 7A).

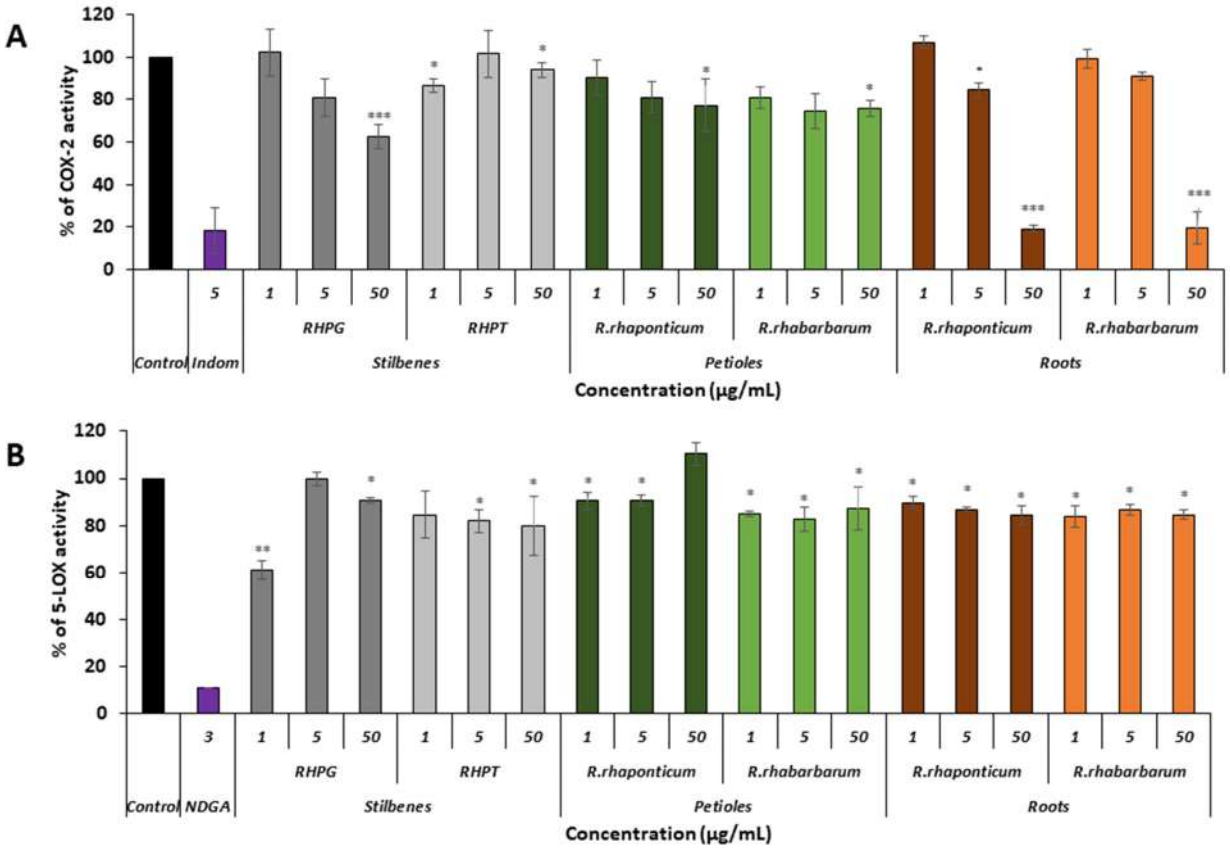


Figure 7. Effect of stilbenes (RHPG and RHPT) as well as the petiole and root extracts of *Rheum rhaponticum* and *Rheum rhabarbarum* on the activity of COX-2 (panel **A**) and 5-LOX (panel **B**); $n = 7$. The activity of native enzymes (untreated with any of the examined substances) was assumed as 100%; * $p < 0.5$; ** $p < 0.01$; *** $p < 0.001$.

In the case of 5-LOX, most of the examined substances displayed slight inhibitory activities (Figure 7B). The most effective was RHPG, at a concentration of 1 μg/mL (*** $p < 0.001$). The reference inhibitor was nordihydroguaiaretic acid (NDGA), contained in the kit at a concentration of 3 μg/mL.

3.5. In Silico Studies: Bioactivity, Drug-Likeness, and Molecular Docking

The most common organic chemical compounds identified in the plant extracts were subjected to computational drug-likeness analysis and their ability to bind to COX-2 and 5-LOX protein molecules in the vicinity of their active sites as potential competitive inhibitors (Table 4). All compounds generally met the criteria for drug candidates by the Molinspiration Molecular Properties and Bioactivity Score. According to SwissTargetPrediction some of them were probable targets for COX-2 or 5-LOX (Figure S5 in the Supplementary Materials S2). All of them showed a significant ability to bind to COX-2 but only a few had an affinity for 5-LOX in Autodock Vina molecular docking. Many of them, including piceatannol-galloylglucoside, viniferin (resveratrol-dehydrodimer), emodin, emodin anthrone, deoxyrhaponticin, rhaponticin, resveratrol 3-*O*-beta-glucopyranoside, digalloyl glucoside, piceid (polydatin), chrysophanol-8-glucoside, and astringin, showed particularly high negative values for the change in binding energy in the active site (range from −9.3 to −8.5 kcal·mol^{-1}), indicating an exoergic process of high-affinity, compared to indomethacin (−9.4 kcal·mol^{-1}), an inhibitor and non-steroidal anti-inflammatory drug. In contrast, most compounds bound weakly at the 5-LOX active site, often with positive changes in binding energy (Table 4).

Table 4. Computational analysis of the most abundant compounds found in *R. rhaponticum* and *R. rhabarbarum*, native substrate native arachidonic acid and inhibitors: indomethacin and NDGA: MW—predicted molecular weight, PSA—molecular polar surface area, HA—the number of heavy atoms, MBS PI—protease inhibitor Molinspiration bioactivity score v2014.03, MBS EI—enzyme inhibitor Molinspiration bioactivity score v2014.03, ΔG°—molecular docking predicted standard free energy change in ligand binding—values for COX-2 up, 5-LOX bottom, respectively, LE—ligand efficiency (LE = −RTlnKd/HA or −ΔG°/HA), LELP = milog P/LE.

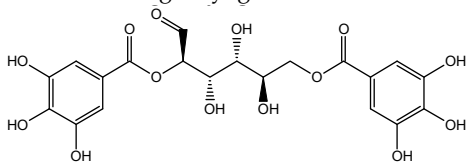
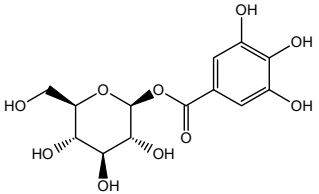
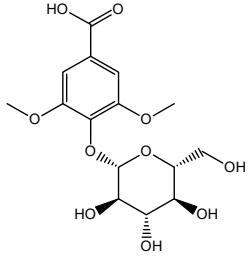
(No.)	Compound Names, Chemical Structure and SMILES	MW (Da) PSA	HA	milogP	MBS PI	MBS EI	ΔG°_{bind} (kcal·mol^{-1}) COX-2 5-LOX	LELELP
(1)	digalloyl glucoside  c1c(C(=O)OC[C@H]([C@H]([C@H]([C@H](OC(=O)c2cc(c(c(O)c2)O)O)C=O)O)O)O)cc(c(c1O)O)O	484.37 251.73	34	−0.31	0.01	0.13	−8.5 ± 0.2 −1.5 ± 1.6	0.25 −1.24 0.04 −7.75
(2)	glucogallin  c1(cc(cc(c1O)O)C(=O)O[C@H]1[C@@H]([C@H]([C@@H]([C@H](O1)CO)O)O)O)O	332.26 177.13	23	−1.48	0.07	0.42	−7.7 ± 0.1 −5.9 ± 1.0	0.33 −4.48 0.26 −5.69
(3)	syringoyl 1-*O*-glucopyranoside  [C@@H]1([C@@H]([C@H]([C@@H](O[C@@H]1CO)Oc1c(cc(cc1OC)C(=O)O)OC)O)O)O	360.31 155.15	25	−0.84	−0.02	0.30	−7.0 ± 0.2 −2.8 ± 0.6	0.28 −3.00 0.11 −7.64

Table 4. *Cont.*

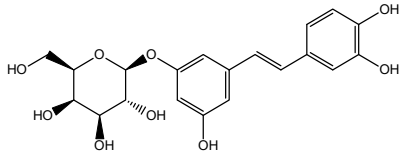
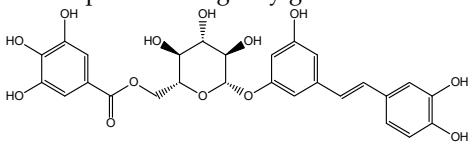
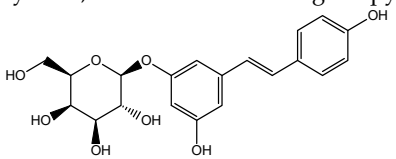
(No.)	Compound Names, Chemical Structure and SMILES	MW (Da) PSA	HA	milogP	MBS PI	MBS EI	ΔG°_{bind} (kcal·mol^{-1}) COX-2 5-LOX	LELELP
(4)	astringin  c1c(O)cc(cc1/C=C/c1cc(c(cc1)O)O)O[C@H]1[C@@H]([C@H]([C@H]([C@H](O1)CO)O)O)O	406.39 160.06	29	0.71	0.04	0.34	−8.1 ± 0.1 0.4 ± 1.4	0.28 2.54 −0.01 −71.00
(5)	piceatannol-galloylglucoside  c1c(c(cc(c1)/C=C/c1cc(cc(c1)O)O[C@H]1[C@@H] ([C@H]([C@@H]([C@H](O1)COC(=O)c1cc(c(c(c1)O)O)O)O)O)O)O)O	558.49 226.82	40	1.88	−0.06	0.12	−9.3 ± 0.2 3.5 ± 2.7	0.23 8.17 −0.09 −20.89
(6)	piceid (polydatin; resveratrol 3-*O*-beta-glucopyranoside)  C1=CC(=CC=C1/C=C/C2=CC(=CC(=C2)O[C@H]3 [C@@H]([C@H]([C@@H]([C@H](O3)CO)O)O)O)O)O	390.39 139.84	28	1.20	0.05	0.34	−8.5 ± 0.2 0.5 ± 1.6	0.30 4.00 −0.018 −66.67

Table 4. *Cont.*

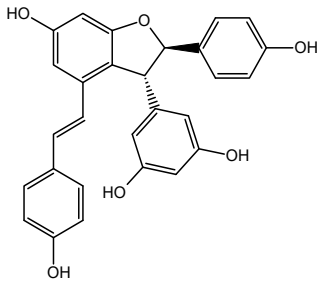
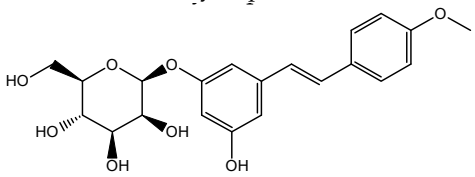
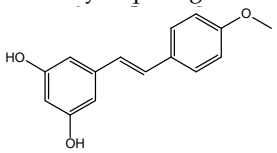
(No.)	Compound Names, Chemical Structure and SMILES	MW (Da) PSA	HA	milogP	MBS PI	MBS EI	ΔG°_{bind} (kcal·mol^{-1}) COX-2 5-LOX	LELELP
(7)	viniferin (resveratrol-dehydrodimer)  c1cc(ccc1/C=C/c1cc(cc2c1[C@H]([C@@H](O2)c1ccc(cc1)O)c1cc(cc(c1)O)O)O)O	454.48 110.37	34	4.77	−0.4	0.20	−8.8 ± 0.1 7.0 ± 0.1	0.26 18.35 −0.21 −22.71
(8)	deoxyrhaponticin  c1c(ccc(c1)/C=C/c1cc(cc(c1)O[C@H]1[C@H]([C@H]([C@@H]([C@H](O1)CO)O)O)O)O)OC	404.42 128.84	26	1.74	0.01	0.30	−8.8 ± 0.1 1.2 ± 1.5	0.34 5.12 −0.05 −34.8
(9)	deoxyrhapontigenin 	243.27 49.69	18	3.52	−0.40	−0.01	−7.6 ± 0.1 −5.2 ± 0.1	0.42 8.38 0.29 12.14

Table 4. *Cont.*

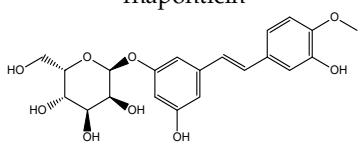
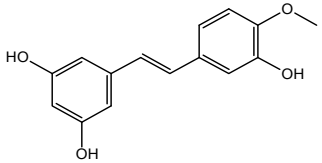
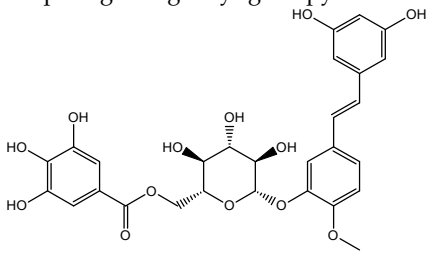
(No.)	Compound Names, Chemical Structure and SMILES	MW (Da) PSA	HA	milogP	MBS PI	MBS EI	ΔG°_{bind} (kcal·mol^{-1}) COX-2 5-LOX	LELELP
(10)	rhaponticin  c1c(cc(cc1O[C@H]1[C@H]([C@H]([C@@H]([C@@H](O1)CO)O)O)O)/C=C/c1ccc(c(c1)O)OC)O	420.41 149.07	30	1.02	−0.02	0.30	−8.5 ± 0.2 0.4 ± 3.5	0.29 42.57 −0.01 −102.0
(11)	rhapontigenin  c1c(c(cc(c1)/C=C/c1cc(cc(c1)O)O)O)O	244.25 80.91	18	2.50	−0.34	0.07	−7.7 ± 0.1 −5.6 ± 0.2	0.43 5.81 0.31 8.06
(12)	rhapontigenin-galloyl-glucopyranoside  c1c(c(cc(c1)/C=C/c1cc(cc(c1)O)O)O[C@H]1[C@@H]([C@H]([C@@H]([C@H](O1)COC(=O)c1cc(c(c(c1)O)O)O)O)O)O)OC	572.52 215.83	41	1.96	−0.10	0.05	−7.8 ± 0.2 5.60 ± 3.1	0.19 10.32 −0.14 −14

Table 4. *Cont.*

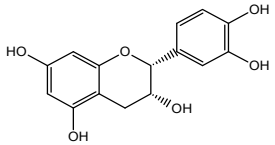
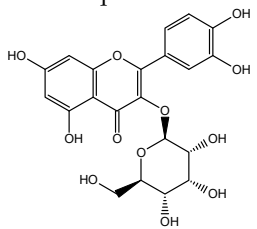
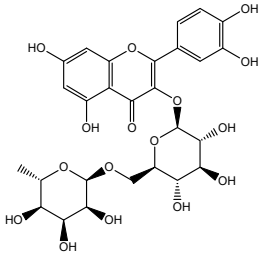
(No.)	Compound Names, Chemical Structure and SMILES	MW (Da) PSA	HA	milogP	MBS PI	MBS EI	ΔG°_{bind} (kcal·mol^{-1}) COX-2 5-LOX	LELELP
(13)	(epi)catechin  c1(cc(c2c(c1)O[C@@H]([C@@H](C2)O)c1cc(c(cc1)O)O)O)O	290.27 110.37	21	1.37	0.26	0.47	−8.1 ± 0.1 −4.3 ± 0.1	0.39 3.51 0.21 6.2
(14)	isoquercitrin  c1(cc(c2c(c1)[OH+]C(=C(C2=O)O[C@@H]1[C@@H]([C@@H]([C@@H]([C@H](O1)CO)O)O)O)c1ccc(c(c1)O)O)O)O	465.39 211.69	33	−3.57	−0.04	0.29	−7.0 ± 0.2 4.5 ± 2.0	0.21 −17.0 −0.14 25.5
(15)	rutin  C[C@H]1[C@@H]([C@H]([C@H]([C@@H](O1)OC[C@@H]2[C@H]([C@@H]([C@H]([C@@H](O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O)O	610.52 269.43	43	−1.06	−0.07	0.12	−7.9 ± 0.1 11.3 ± 1.5	0.18 −5.09 −0.26 4.08

Table 4. *Cont.*

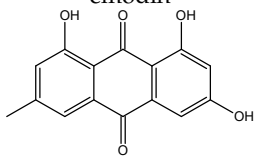
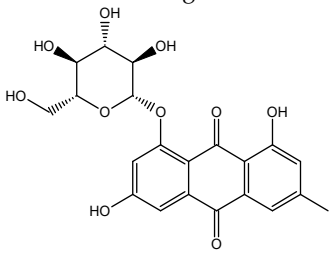
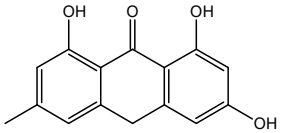
(No.)	Compound Names, Chemical Structure and SMILES	MW (Da) PSA	HA	milogP	MBS PI	MBS EI	ΔG°_{bind} (kcal·mol^{-1}) COX-2 5-LOX	LELELP
(16)	emodin  c1c(cc2c(c1O)C(=O)c1c(C2=O)cc(cc1O)C)O	270.24 94.83	20	3.01	−0.21	0.21	−8.9 ± 0.1 −6.4 ± 0.1	0.45 6.67 0.32 9.41
(17)	emodin 8-*O*-glucoside  [C@@H]1([C@H](Oc2c3c(C(=O)c4c(C3=O)c (cc(c4)C)O)cc(c2)O)O[C@@H]([C@H]([C@@H]1O)O)CO)O	432.38 173.98	31	0.96	0.05	0.41	−6.9 ± 0.3 0.7 ± 0.8	0.22 4.36 −0.02 −48.00
(18)	emodin anthrone  c1c(cc2c(c1O)C(=O)c1c(C2)cc(cc1O)C)O	256.26 77.75	19	3.25	−0.29	0.30	−8.8 ± 0.0 −7.2 ± 0.2	0.46 7.06 0.38 8.55

Table 4. *Cont.*

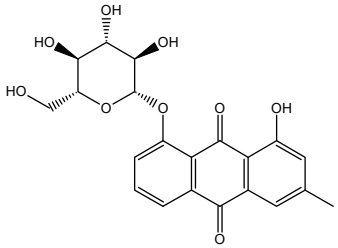
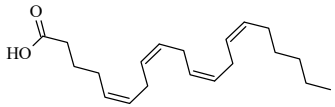
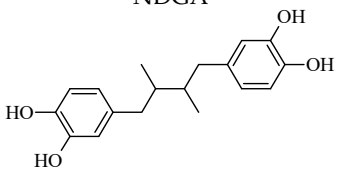
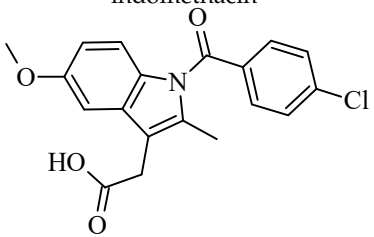
(No.)	Compound Names, Chemical Structure and SMILES	MW (Da) PSA	HA	milogP	MBS PI	MBS EI	ΔG°_{bind} (kcal·mol^{-1}) COX-2 5-LOX	LELELP
(19)	chrysophanol-8-glucoside  c1ccc2c(c1O[C@H]1[C@@H]([C@H]([C@@H]([C@H](O1)CO)O)O)O)C(=O)c1c(C2=O)cc(cc1O)C	416.38 153.75	30	1.49	0.06	0.39	-8.4 ± 0.0 0.7 ± 3.2	0.28 5.32 -0.02 -74.50
(20)	arachidonic acid  OC(=O)CCC/C=C\C/C=C\C/C=C\C/C=C\CCCCC	304.47 37.30	22	6.42	0.19	0.35	-7.4 ± 0.2 -5.1 ± 0.3	0.34 18.88 0.23 27.91
(21)	NDGA  CC(CC1=CC(=C(C=C1)O)O)C(C)CC2=CC(=C(C=C2)O)O	302.37 80.91	22	3.48	0.01	0.13	-8.0 ± 0.2 -5.2 ± 0.5	0.34 10.24 0.035 99.43

Table 4. *Cont.*

(No.)	Compound Names, Chemical Structure and SMILES	MW (Da) PSA	HA	milogP	MBS PI	MBS EI	ΔG°_{bind} (kcal·mol^{-1}) COX-2 5-LOX	LELELP
(22)	indomethacin  C(=O)(n1c(C)c(c2cc(ccc12)OC)CC(=O)O)c1ccc(Cl)cc1	357.79 68.64	25	3.99	−0.11	0.30	−9.4 ± 1.3 0.8 ± 1.3	0.38 10.50 −0.03 −133.00

Due to the high content of rhaponticin in the examined extracts (Table 2), worthy docking results, and drug-likeness parameters, we focused further on the analysis of computational results obtained for this compound compared to known inhibitors, such as indomethacin and NDGA, and the natural substrate arachidonate. Rhaponticin fits in the COX-2 hydrophobic binding pocket of the substrate arachidonate, in the area where also non-steroidal anti-inflammatory drugs and inhibitors, such as indomethacin and NDGA, attach. According to LigPlot+ v.2.2 and visual inspection on UCSF Chimera and ChimeraX rhaponticin conformers bound near amino acid residues Tyr385, Ser530, Glu524, and Arg120 stabilized by polar interactions, such as hydrogen bonds, van der Waals interactions, and hydrophobic effects, with Val116, Val359, Leu531, and other residues (Figure 8).

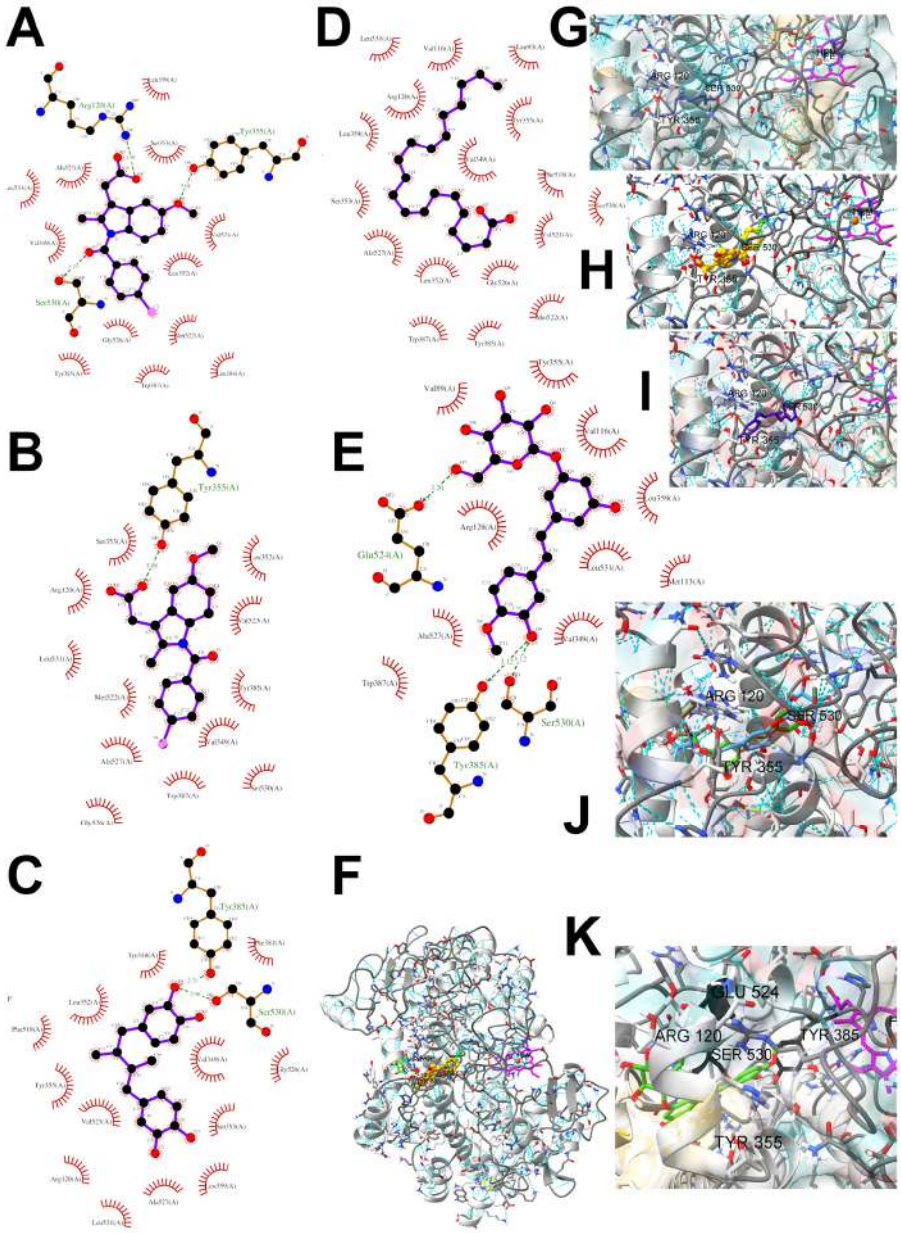


Figure 8. Ligand binding analysis in the area of hydrophobic COX-2 active site pocket 1 according to the crystal structure 4COX where the location of indomethacin was determined experimentally. The panels from A to F are LigPlot+ v.2.2 analysis: (**A**) experimental indomethacin binding with amino acid residues in the 4COX crystal structure, (**B**) docking studies of indomethacin, (**C**) NDGA docking, (**D**) arachidonate docking (**E**) rhaponticin docking. The panels from F to K show the spatial structure of the COX-2 monomer. (**F**) A view of the whole monomer-bound indomethacin molecules (yellow,

orange, crystal structure, and docked, respectively), rhaponticin molecules (green and plum) and arachidonate (khaki). Heme moiety of the peroxidase site (pink) with an iron cation (orange) visible on the right. The panels from G to K show the zoomed-in substrate–inhibitor binding site with ligands (**G**) arachidonate (light navy) (**H**) indomethacin molecules (yellow, orange, crystal structure, and docked, respectively), (**I**) NDGA (dark navy) (**J**) rhaponticin 10 molecules (**K**) rhaponticin bound—one molecule (light green). Hydrogen bonds are marked by light blue dashed lines. Glu536, Ser530 and Tyr385 are marked in dark gray.

According to the 4COX crystallographic structure, bound indomethacin in the chain A binding pocket site 1 could interact by hydrogen bonding with the Arg120 side chain -NH1 (hydrogen donor), Tyr355 (hydroxyl group donor) - ligand carbonyl oxygen of carboxyl group as an acceptor. The Ser353 hydroxyl group could be the acceptor and donor with the carboxyl of the ligand. The Ser 530 hydroxyl of the side chain forms a hydrogen bond with the carbonyl oxygen of indomethacin, and methoxy oxygen can be an acceptor of the hydroxyl hydrogen of Tyr355. Many van der Waals and hydrophobic effects could be important for this ligand stabilization including: the indol group with Val523, Ser353, Ala527, Phe518, Val349, Leu352, a phenyl ring with Phe381, Trp387, Tyr385, Gly526, Leu384, Met522, Ser530, and the methyl group Leu531. The Gly526 residue could interact with an indomethacin chlorine atom (Figure 8A). Indomethacin and NDGA docked in the same location showing similar interactions (Figure 8B,C) with rhaponticin.

4. Discussion

From ancient times to modern history, plants (including different rhubarb species) have been used to treat or support the treatment of different diseases. *R. rhabarbarum*-based preparations were administered as purgative agents as well as to treat liver, spleen, and stomach dysfunctions. Traditional medicine recommendations for the therapeutic use of *R. rhaponticum* include purgative effects, gastrointestinal and reproductive system disorders, injuries, heartache, and pain in the pericardium [7]. Currently, *R. rhaponticum* is primarily known as a component of the preparations dedicated to alleviating menopausal complaints, especially the ERr731® extract, which has been registered as a medicinal product. The phytoestrogenic activity of the preparation based on the rhapontic rhubarb rhizome has become the leading research trend on this species. In contrast, other biological activities of both the aforementioned species have been elucidated and described to a significantly lesser extent. A rich phytochemical profile of rhubarbs containing different groups of flavonoids, stilbenes, phenolic acids, anthraquinones, and naphthalene derivatives suggests the possibility of beneficial, pleiotropic activity through different molecular pathways in the human body [4].

The selection of raw materials with diversified and the highest possible content of bioactive ingredients is very important in both functional food production and for medicinal purposes as well. Most studies on the chemical composition of *Rheum rhabarbarum* or *Rheum rhaponticum* mainly focus on the petioles/stalks, consumed as a vegetable and used in culinary preparations, such as soups, pies, tarts, jams, jellies, compotes, juices, candies, and others [41]. Therefore, one of the goals of this study was to screen, fully characterize the possible components in complex samples and quantify the principal compounds in *Rheum rhabarbarum* and *Rheum rhaponticum* organs, such as the petioles and roots, which will enable chemical fingerprinting and metabolomic identification of these species. According to previous studies [35–38], we also identified several metabolites from the most important biologically active classes, including anthraquinones, anthrones, and phenolic compounds: stilbenes, flavonoids, phenolic acids, cinnamic acid derivatives, and tannins. Despite superficial similarities in qualitative comparisons, the two investigated species appear to differ significantly in their contents of active ingredients. The contents of rhaponticin in the petioles and deoxyrhaponticin in the roots best illustrate this observation. In the petioles, garden rhubarb contains 10 times more rhaponticin than rhaponic rhubarb (Table 2). Furthermore, the petioles of garden rhubarb also contain an isomer of

rhaponticin, only present in rhapontic rhubarb in minuscule quantities. Similarly, the levels of deoxyrhaponticin in the roots of *R. rhabarbarum* are much higher than in the roots of *R. rhaponticum*. The levels of rhaponticin are similar in these organs and do not seem to correlate with the levels of deoxyrhaponticin. Therefore, deoxyrhaponticin is presumably not a derivative of rhaponticin but an independently synthesized metabolite.

The present work evaluated the anti-inflammatory effects of plant extracts from two popular, edible rhubarbs (*R. rhaponticum* and *R. rhabarbarum*) in the context of their cardioprotective potential. The anti-inflammatory properties of rhubarb extracts isolated from the petioles and underground organs of *R. rhabarbarum* and *R. rhaponticum*, as well as of the two stilbenoids usually found in these plants, i.e., rhapontigenin and its glycoside, rhaponticin, were examined using an experimental model of vein endothelial cells (HUVECs). The endothelial cells are not only an integral part of blood vessel physiology and vascular homeostasis, but also participate in many other physiological and pathological processes, including cardiovascular diseases. Numerous studies have suggested that dietary plant-derived substances, especially those with antioxidant activity, may be a helpful and promising strategy for preventing cardiovascular disorders [42,43]. On the other hand, a growing number of critical opinions have emphasized that the antioxidant action is one of many mechanisms of beneficial effects of plant-derived compounds on the human body. Inflammation, endothelial dysfunction, and changes in blood physiology form a closely related network of pathological interactions resulting in pro-inflammatory and adhesive properties of the vessel wall. Inflammation is a key event in the pathophysiology of various cardiovascular disorders - it affects the functionality of both the blood vessel wall and blood components. For this reason, besides the antioxidant action, other properties of natural substances, including their anti-inflammatory actions may be pivotal for their health-promoting or even therapeutic effects [44–46].

The first step of the study involved cytotoxicity tests and confirmed cellular safety of the *R. rhaponticum* extracts from the roots and petioles, *R. rhabarbarum* petiole extract, and rhaponticin, at concentrations of 1–50 μg/mL. In the case of *R. rhabarbarum* root extracts and rhapontigenin, a safe concentration range was lower and amounted to 1–30 and 1–25 μg/mL, respectively. Application of the examined extracts at the mentioned concentrations corresponds to nano- or micromolar concentrations of their bioactive ingredients in the tested samples, which is consistent with the literature data on physiologically relevant levels of the plant-derived substances. It has been established that the blood plasma levels of natural plant-derived compounds or their metabolites achieve concentrations from nanomoles to a few micromoles per liter [47].

The literature provides very little information on the anti-inflammatory actions of extracts derived from garden and/or rhapontic rhubarb. An aqueous extract from *R. rhabarbarum* suppressed the tumor necrosis factor α-induced activation of NF-κB-p65 as well as the expression of adhesion molecules (ICAM-1 and VCAM-1) and the monocyte chemoattractant protein-1 (MCP-1) [48]. More data has been reported from studies focused on rhubarb-derived stilbenes or other single compounds. For instance, six stilbenes (rhaponticin, rhapontigenin, isorhaponticin, desoxyrhaponticin, desoxyrhapontigenin and resveratrol) isolated from the *R. rhabarbarum* rhizome reduced the reactive oxygen species production in RAW 264.7 macrophages [49]. The ability of rhaponticin (1 μM) to inhibit the MAPK/NF-κB signaling pathways in LPS-stimulated endothelial cells has been recently reported [50]. It has been suggested that the anti-inflammatory action of natural stilbenoids (pinosylvin, monomethylpinosylvin, resveratrol, pterostilbene, piceatannol, and rhapontigenin) is possibly mediated via inhibition of the PI3K/Akt pathway [51]. The PI3K/Akt pathway is activated in low-grade inflammation [52], commonly associated with chronic inflammatory processes occurring in cardiovascular disorders, e.g., atherosclerosis. Therefore, an effective modulation of this pathway by natural substances provides a promising basis for further research. In other work, aloe-emodin from *R. rhabarbarum* suppressed LPS-induced iNOS expression, degradation of IκBα and phosphorylation of ERK, p38, JNK, and Akt in macrophages [53].

Our experiments indicated that the examined extracts and substances possessed anti-inflammatory properties and modulated the pro-inflammatory response of endothelial cells at the level of gene expression, cytokine release and interactions with leukocytes. However, the activities of the preparations from the petioles and root extracts differed significantly. For example, extracts from the petioles of *R. rhaponticum* and *R. rhabarbarum*, as well as RHPG and RHPG significantly reduced the expression of the *COX-2* gene in LPS-activated HUVECs. On the other hand, the roots extracts increased the level of gene expression. Similar diversity in anti-inflammatory efficiency was observed in 5-LOX (*ALOX5*) gene expression. The *R. rhaponticum* and *R. rhabarbarum* petiole extracts, together with RHPG and RHPG, evidently reduced the expression of the *ALOX5* gene, while the root extract from *R. rhaponticum* suppressed the *ALOX5 gene* only at the highest used concentration (i.e., 50 μg/mL), and the *R. rhabarbarum* root extract was inefficient at this level of modulation of the 5-LOX enzyme action.

The inhibitor screening tests, carried out in the next stages of the present study, demonstrated that the examined substances might also act at the level of the active enzyme COX-2 and partly reduced its activity. The examined substances inhibited COX-2 enzyme activity with varied efficiency. Their inhibitory activity towards 5-LOX was significantly weaker. This observation was consistent with results obtained in silico, where most of the tested compounds showed much better COX-2 inhibitory abilities than 5-LOX, referring to such chemical compounds as piceatannol-galloylglucoside, viniferin, emodin, emodin anthrone, deoxyrhaponticin, rhaponticin, resveratrol 3-*O*-beta-glucopyranoside, digalloyl glucoside, piceid and, astringin, and chrysophanol-8-glucoside. Rhaponticin was most abundant in the roots of both rhubarb species (184.0 ± 10.93 and 151.3 ± 7.77 mg per g of extract, respectively), one to two orders of magnitude more than the other compounds (Table 2). Taking into account that the root extracts of both species of rhubarb clearly inhibited COX-2, especially visible at the highest concentration used, and the docking indicated strong binding to the active site (-8.5 ± 0.2 kcal·mol^{-1}), with no such effect of 5-LOX, it might be assumed that it was the most important compound responsible for the selective anti-inflammatory effect (Figure 8) binding in COX-2 hydrophobic pockets where arachidonate attaches as a substrate.

The COX-2 hydrophobic pocket is important for arachidonate hydrocarbon chain binding with amino acid residues: Val116, Arg120, Leu352, Gly326, Leu 359, Leu 531, Leu93, Val523, and Tyr385 (Figure 8D). Tyr385 is a key player in catalytic activity. A hydroperoxide oxidizes the heme to a ferryl-oxo derivative and Tyr385 forms a tyrosyl radical which attacks arachidonate, oxidizing and detaching its 13-pro(S) hydrogen to initiate the COX cycle. Blocking of the Tyr385 residue by non-steroidal anti-inflammatory drugs inhibits the whole arachidonate transformation process. Rhaponticin and other stilbene-carbohydrate derivatives, located in the hydrophobic pocket and stabilized by hydrogen bonding, might act as inhibitors analogous to non-steroidal anti-inflammatory drugs, such as indomethacin and NDGA, occupying this place and blocking it from binding and oxidizing arachidonate (Figure 8).

Conversely to the results obtained at the level of pro-inflammatory enzymes gene expression, at the level of enzyme activity, the root extracts were more effective than extracts isolated from the petioles. The IC_{50} values for extracts from the roots of *R. rhaponticum* and *R. rhabarbarum* were 19.16 μg/mL and 19.44 μg/mL, respectively. The IC_{50} values for RHPG and RHPT attained concentrations > 50 μg/mL. Our work provides the first evidence of the ability of rhubarb extracts to inhibit the COX-2 enzyme. However, some information on the cyclooxygenase inhibitory effects is available for RHPG. Based on the in vitro evaluation of the effect of dietary stilbenes on 5-lipoxygenase and cyclooxygenase activities [54], Kutil and co-authors (2015) reported that this stilbene can inhibit COX-2 activity (IC_{50} = 36.1 μM). On the other hand, our in vitro studies on the stilbene effects on 5-LOX activity suggested weak inhibitory efficiency of this enzyme of both RHPG and RHPT (IC_{50} > 50 μg/mL). According to the literature, the LOX-inhibitory efficiency of

rhapontigenin was characterized by an IC_{50} of 10.7 μM, and for rhaponticin, the IC_{50} was 34.3 μM [55,56].

Furthermore, our work demonstrated that the examined extracts and stilbenes might reduce or modulate the release of pro-inflammatory cytokines from HUVECs. Results of the HUVEC cytokine profiling demonstrated that most of the examined substances (including RHPT, the petiole extracts of *R. rhaponticum* and *R. rhabarbarum*, and the root extract from *R. rhaponticum*) inhibited the release of crucial pro-inflammatory factors such as CCL5/RANTES, CXCL10/IP-10, CXCL12/SDF-1, and IL-18/IL-IF4. On the other hand, an increase in the IL-8 level was found for both stilbenes and most of the examined extracts (except for the *R. rhabarbarum* root extract). This latter observation may be a result of both a divergence in mechanisms of action of the examined plant-derived substances and the complexity of the immune response, including multiple pathways of control for IL-8 synthesis and secretion [57]. Regulation of the synthesis and release of IL-8 by natural substances has only partly been elucidated, and the available evidence is inconsistent. For example, a reduction in IL-8 release from THP-1 monocytes was demonstrated for *Castanea sativa* Mill., *Alchemilla vulgaris* L., and *Salix alba* L. [58]. However, in another study, green tea extracts induced IL-8 mRNA and protein expression in Caco-2 cells [59]

Chronic and severe inflammatory processes occurring in the cardiovascular system influence the physiology of endothelial cells and impair their modulatory functions. The endothelium is not only a regulator of blood hemostasis, but also a modulator of vascular tone, redox balance, blood flow and fluidity, as well as immune response. For this reason, endothelial dysfunction is considered a systemic pathological state of the vasculature. The inflammatory-activated endothelial cells lose their anti-coagulant and modulatory properties in favor of pro-coagulant activity and pro-atherogenic effects. They also release numerous cytokines, chemokines, and growth factors, leading to an augmentation of the inflammatory response and an increase in endothelium permeability [11,60,61].

Our work provides new evidence of the biological activity of rhubarb-derived species and indicates that the examined plant extracts and stilbenes display anti-inflammatory effects. Using a combination of in vitro studies and in silico analyses, we have demonstrated, for the first time, the anti-inflammatory potential of rhubarb extracts and stilbenes in the context of endothelium physiology and the prevention of its dysfunction. The examined plant-derived substances displayed a protective action towards the undesired, inflammation-triggered changes in the endothelium physiology. They reduced the endothelial cell pro-inflammatory response at different molecular levels. Rhubarb-derived extracts-displayed the ability to modulate the pro-inflammatory gene expression, inhibited the COX-2 enzyme activity, suppressed the release of pro-inflammatory cytokines, and reduced the macrophage influx to endothelial cells. The obtained results provide a promising background for further studies, including in vivo work.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu15040949/s1, Figure S1: UHPLC profile of the butanol extract obtained from the petioles of *Rheum rhabarbarum* Supplementary Materials S1; Figure S2: UHPLC profile of the butanol extract obtained from *Rheum rhabarbarum* roots/rhizomes Supplementary Materials S1; Figure S3: UHPLC profile of the butanol extract obtained from the petioles of *Rheum rhaponticum* Supplementary Materials S1; Figure S4: UHPLC profile of the butanol extract obtained from *Rheum rhaponticum* roots/rhizomes Supplementary Materials S1; Supplementary Materials S2; Table S1: Supplementary Table S1. Comparative analysis of metabolites identified in butanol extracts obtained from the petioles of *Rheum rhaponticum* and *Rheum rhabarbarum*. Table S2: Supplementary Table S2. Comparative analysis of metabolites identified in butanol extracts obtained from the roots/rhizomes of *Rheum rhaponticum* and *Rheum rhabarbarum*; Table S3: Supplementary Table S3. Qualitative similarities and unique metabolites observed between the extracts from petioles (A) and roots (B) of *R. rhabarbarum* and *R. rhaponticum*. The supplementary materials S2 file contains detailed information on the estimation of the most probable macromolecular targets of rhubarb compounds (the Figure S5).

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Supplementary materials S1

SUPPLEMENTARY MATERIALS S1

Rheum rhaponticum and *Rheum rhabarbarum extracts* As Modulators of Endothelial Cell Inflammatory Response

Oleksandra Liudvytska [1,*], Michał B. Ponczek [1], Oskar Ciesielski [2,3], Justyna Krzyżanowska-Kowalczyk [4], Mariusz Kowalczyk [4], Aneta Balcerczyk [2] and Joanna Kolodziejczyk-Czepas [1]

[1] Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland
[2] Department of Sociobiology and Epigenetics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland
[3] The Bio-Med-Chem Doctoral School, University of Lodz and Lodz Institutes of the Polish Academy of Sciences, University of Lodz, Banacha 12/16, 90-237, Lodz, Poland
[4] Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland
* Correspondence: oleksandra.liudvytska@biol.uni.lodz.pl; Tel.: +48-42-635-44-84

Contents:

Figure S4. UHPLC profile of the butanol extract obtained from *Rheum rhaponticum* roots/rhizomes (upper panel - CAD detector signal, lower panel - MS chromatogram using the negative ESI mode, the numbers correspond to the numbers of compounds tentatively identified in Table S2).

Table S1. Comparative analysis of metabolites identified in butanol extracts obtained from the petioles of *Rheum rhaponticum* and *Rheum rhabarbarum*.

Table S2. Comparative analysis of metabolites identified in butanol extracts obtained from the roots/rhizomes of *Rheum rhaponticum* and *Rheum rhabarbarum*.

Table S3. Qualitative similarities and unique metabolites observed between the extracts from petioles (A) and roots (B) of *R. rhabarbarum* and *R. rhaponticum*.

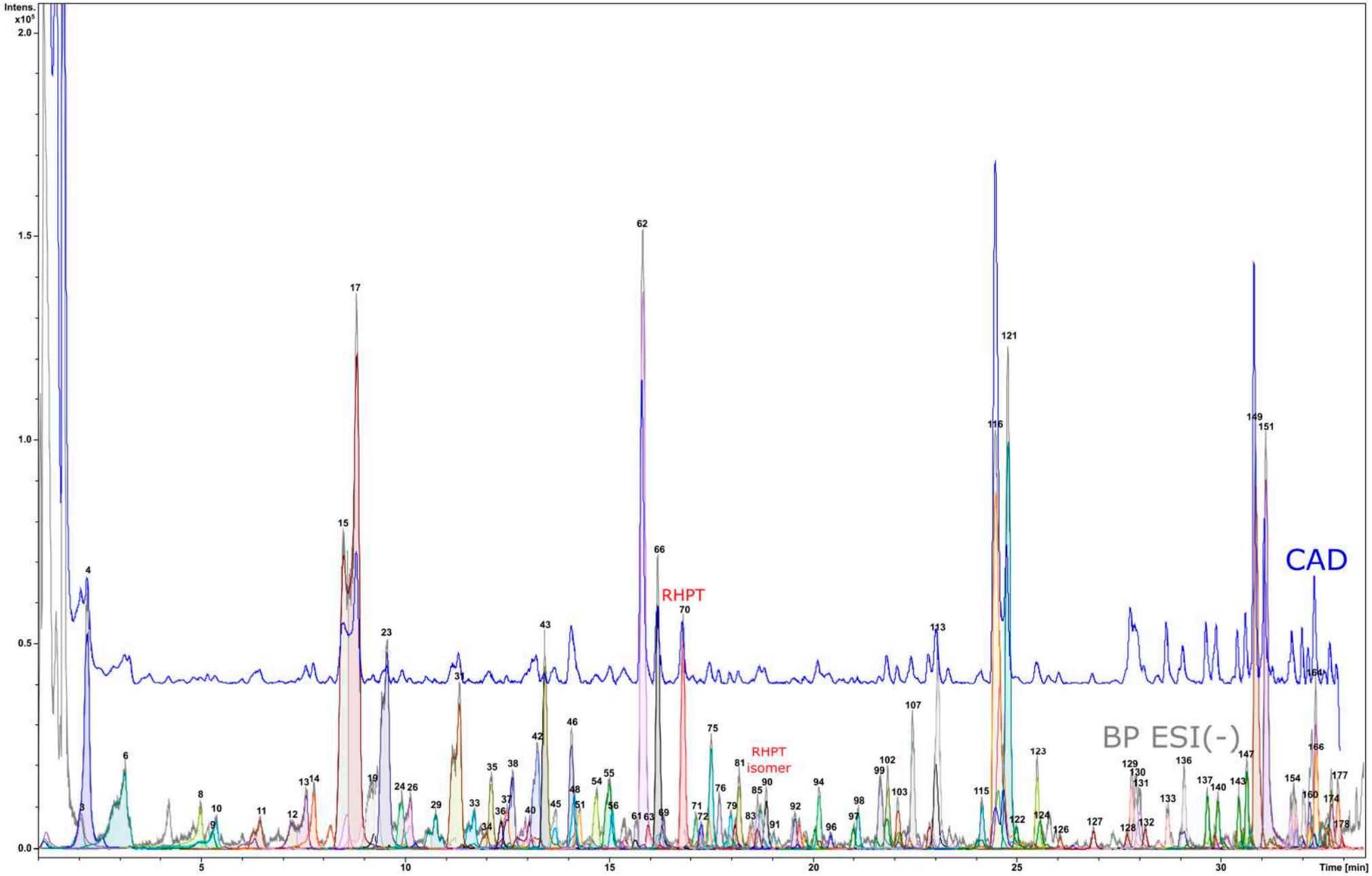


Figure S1. UHPLC profile of the butanol extracts obtained from the petioles of *Rheum rhabarbarum* (upper panel - CAD detector signal, lower panel - MS chromatogram using negative ESI mode, the numbers correspond to the numbers of compounds tentatively identified in Table S1).

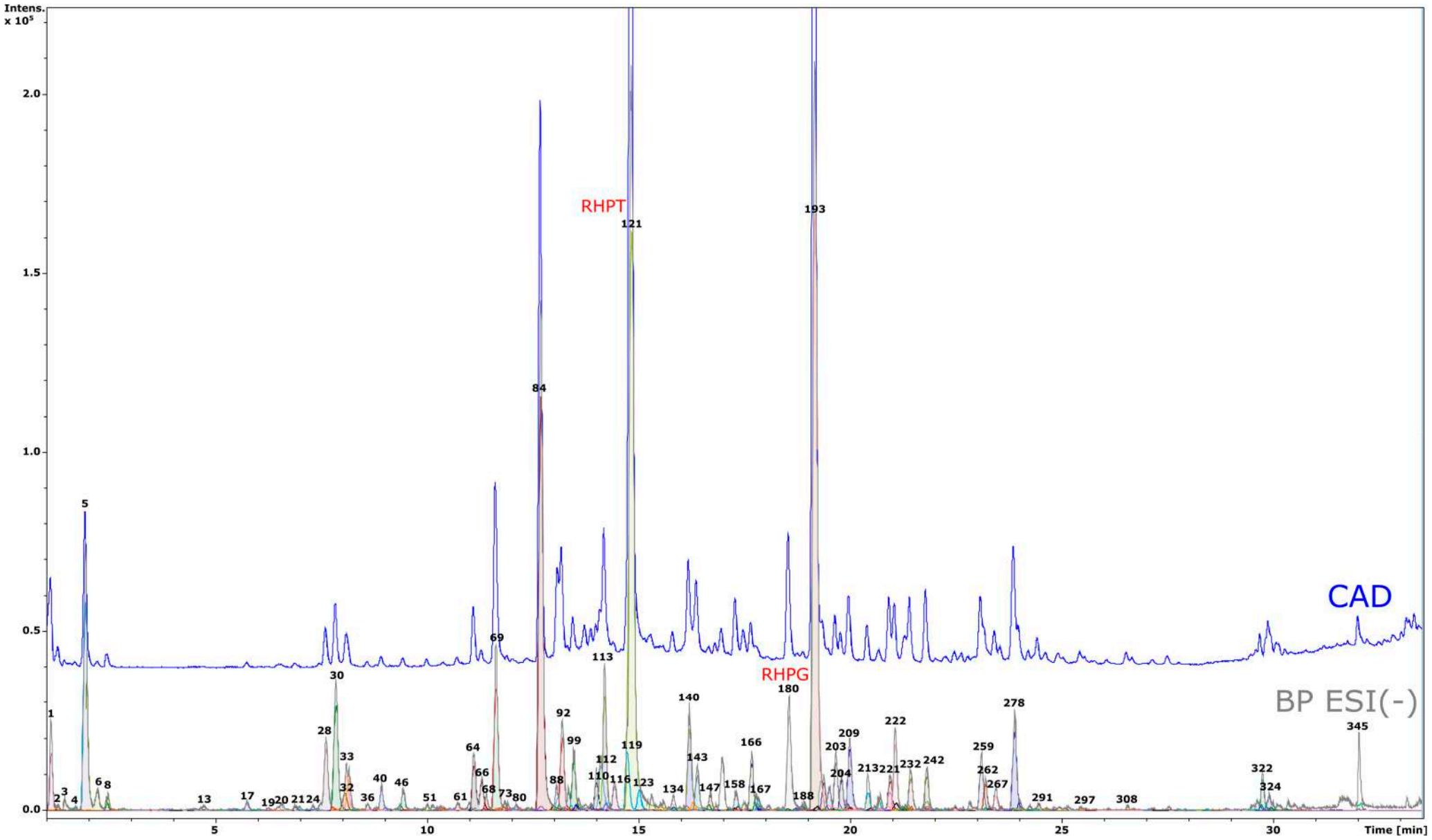


Figure S2. UHPLC profile of the butanol extracts obtained from *Rheum rhabarbarum* roots/rhizomes (upper panel - CAD detector signal, lower panel - MS chromatogram using negative ESI mode, the numbers correspond to the numbers of compounds tentatively identified in Table S2).

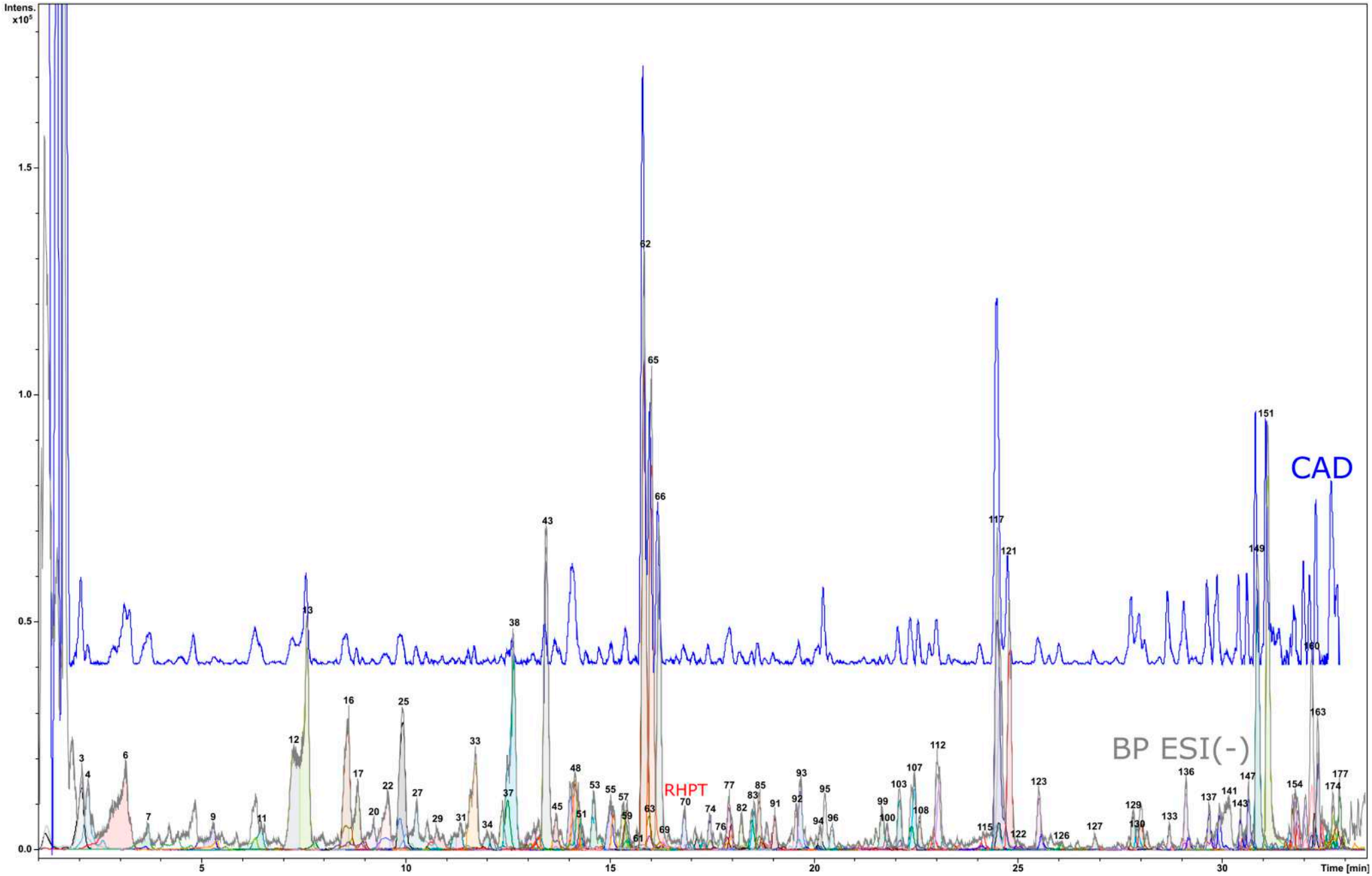


Figure S3. UHPLC profile of the butanol extracts obtained from the petioles of *Rheum rhaponticum* (upper panel - CAD detector signal, lower panel - MS chromatogram using negative ESI mode, the numbers correspond to the numbers of compounds tentatively identified in Table S1).

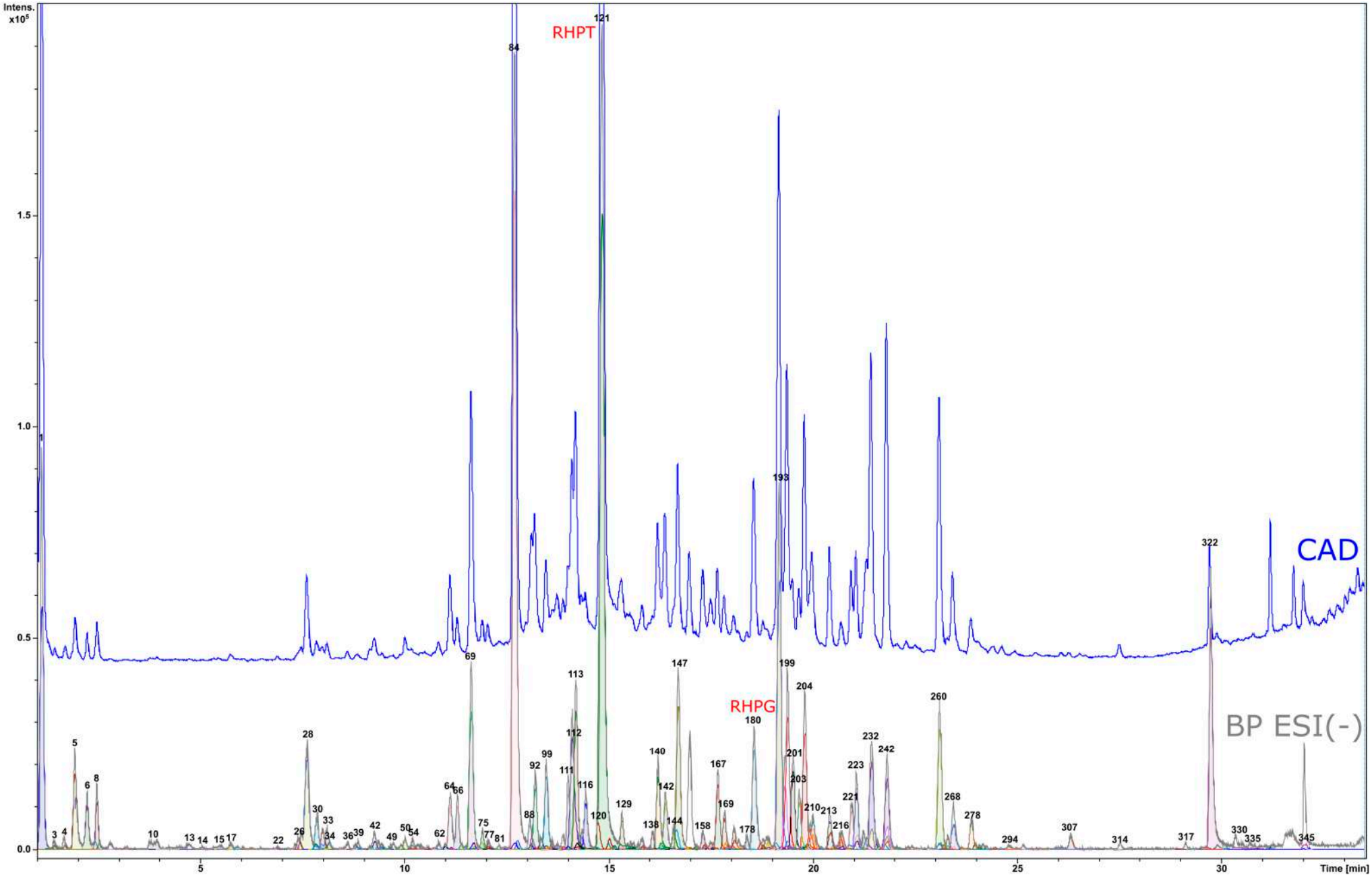


Figure S4. UHPLC profile of the butanol extracts obtained from *Rheum rhaponticum* roots/rhizomes (upper panel - CAD detector signal, lower panel - MS chromatogram using the negative ESI mode, the numbers correspond to the numbers of compounds tentatively identified in Table S2).

Supplementary table S1

No	Name	RT [min]	Formula	Adduct	Precursor	Err. [ppm]	mσ	rdb	R.rhabarbarum	[mg/g d.w.]	R.rhaponticum	[mg/g d.w.]	ID level	NEG MS2 Spectrum
1	*Unidentified*	2,00	$C_{10}H_{12}O_{10}$	M-H	291,0358	1,3	13,2	5	+				4	115.0011 (8.3; -$C_6H_8O_6$), 133.0119 (7.0; -$C_6H_6O_5$), 175.0244 (100.0; -$C_4H_4O_4$)
2	*Unidentified*	2,07	$C_{10}H_{12}O_{10}$	M-H	291,0358	0,7	14,9	5	+	5.06 ± 1.06	+	1.71 ± 0.27	4	115.0011 (8.3; -$C_6H_8O_6$), 133.0119 (7.0; -$C_6H_6O_5$), 175.0244 (100.0; -$C_4H_4O_4$)
3	*Unidentified*	2,07	$C_{10}H_{12}O_{11}$	M-H	307,0307	0,7	12,7	5	+	6.99 ± 1.47	+	1.97 ± 0.30	4	111.0062 (16.1; -$C_5H_8O_8$), 115.0032 (21.0; -$C_6H_8O_7$), 133.0120 (100.0; -$C_6H_6O_6$), 191.0212 (6.6; -$C_4H_4O_4$)
4	**beta-glucogallin**	2,22	$C_{13}H_{16}O_{10}$	M-H	331,0671	1,4	6,5	6	+	14.67 ± 3.79	+	0.85 ± 0.19	1	123.0079 (2.0; -$C_7H_{12}O_7$), 151.0025 (5.0; -$C_6H_{12}O_6$), 169.0141 (19.1; -$C_6H_{10}O_5$), 193.0140 (1.1; -$C_4H_{10}O_5$), 211.0251 (15.4; -$C_4H_8O_4$), 241.0357 (1.5; -$C_3H_6O_3$), 271.0462 (29.8; -$C_2H_4O_2$), 313.0566 (0.9; -H_2O), 331.0673 (100.0)
5	homocitrate-1	2,86	$C_7H_{10}O_7$	M-H	205,0354	2,8	23,5	3	+	2.49 ± 1.33	+	1.07 ± 0.29	3	111.0065 (100.0; -$C_2H_6O_4$), 143.0355 (27.3; -CH_2O_3)
6	homocitrate-2	3,09	$C_7H_{10}O_7$	M-H	205,0354	2,2	16,0	3	+	7.16 ± 2.01	+	5.40 ± 0.90	3	111.0064 (100.0; -$C_2H_6O_4$), 143.0330 (75.2; -CH_2O_3), 173.0100 (12.4; -CH_4O), 205.0349 (68.5)
7	gentisoyl-Hex	3,70	$C_{13}H_{16}O_9$	M-H	315,0722	-0,2	13,9	6			+	1.59 ± 0.12	3	195.0323 (37.8; -$C_4H_8O_4$), 255.0512 (94.2; -$C_2H_4O_2$), 315.0719 (100.0)
8	**gallocatechin**	5,01	$C_{15}H_{14}O_7$	M-H	305,0667	1,6	19,7	9	+	0.32 ± 0.05			1	125.0238 (28.1; -$C_9H_8O_4$), 179.0335 (21.9; -$C_6H_6O_3$), 204.0419 (16.1; -$C_4H_5O_3$), 219.0671 (32.8; -$C_3H_2O_3$), 237.0765 (17.1; -C_3O_2), 243.0666 (14.6; -CH_2O_3), 261.0759 (47.3; -CO_2), 305.0655 (100.0)
9	saliciloyl-Hex-1	5,30	$C_{13}H_{16}O_8$	M-H	299,0772	-0,1	13,3	6			+	0.16 ± 0.03	3	137.0234 (19.1; -$C_6H_{10}O_5$), 151.0395 (4.0; -$C_5H_8O_5$), 161.0446 (1.4; -$C_7H_6O_3$), 179.0349 (34.4; -$C_4H_8O_4$), 209.0454 (9.3; -$C_3H_6O_3$), 239.0564 (41.6; -$C_2H_4O_2$), 299.0770 (100.0)
10	*Unidentified*	5,37	$C_{11}H_{10}O_9$	M-H	285,0252	0,9	21,1	7	+	0.50 ± 0.04			4	115.0031 (30.2; -$C_7H_6O_5$), 133.0124 (100.0; -$C_7H_4O_4$)
11	Trp	6,46	$C_{11}H_{12}N_2O_2$	M-H	203,0826	-0,9	11,5	7	+	2.63 ± 0.66			2	116.0495 (11.5; -$C_3H_5NO_2$), 129.0573 (0.4; -$C_2H_4NO_2$), 142.0654 (5.3; -CH_3NO_2), 159.0922 (7.5; -CO_2), 186.0559 (4.3; -H_3N), 203.0827 (100.0)
12	syringoyl-Hex-1	7,25	$C_{15}H_{20}O_{10}$	M-H	359,0984	-0,1	18,9	6			+		3	182.0220 (7.7; -$C_7H_{13}O_5$), 197.0454 (74.3; -$C_6H_{10}O_5$), 211.0615 (17.5; -$C_5H_8O_5$), 224.0332 (5.4; -$C_5H_{11}O_4$), 239.0561 (79.2; -$C_4H_8O_4$), 269.0660 (5.2; -$C_3H_6O_3$), 299.0779 (19.5; -$C_2H_4O_2$), 344.0750 (21.4; -CH_3), 359.0985 (100.0)
13	syringoyl-Hex-2	7,57	$C_{15}H_{20}O_{10}$	M-H	359,0984	0,6	4,3	6	+	1.36 ± 0.39	+	4.21 ± 0.99	3	197.0452 (67.3; -$C_6H_{10}O_5$), 211.0612 (19.4; -$C_5H_8O_5$), 239.0560 (74.9; -$C_4H_8O_4$), 269.0664 (8.1; -$C_3H_6O_3$), 299.0768 (20.4; -$C_2H_4O_2$), 344.0744 (17.3; -CH_3), 359.0982 (100.0)
14	**procyanidin-B1**	7,76	$C_{30}H_{26}O_{12}$	M-H	577,1352	0,6	23,0	18	+	1.60 ± 0.44	+	0.05 ± 0.02	1	125.0241 (5.5; -$C_{24}H_{20}O_9$), 161.0237 (4.5; -$C_{21}H_{20}O_9$), 203.0722 (4.3; -$C_{18}H_{14}O_9$), 205.0508 (4.2; -$C_{19}H_{16}O_8$), 245.0821 (24.9; -$C_{16}H_{12}O_8$), 273.0406 (6.7; -$C_{16}H_{16}O_6$), 289.0725 (100.0; -$C_{15}H_{12}O_6$), 299.0567 (3.8; -$C_{14}H_{14}O_6$), 329.0664 (3.8; -$C_{13}H_{12}O_5$), 339.0883 (12.7; -$C_{11}H$
15	catechin	8,51	$C_{15}H_{14}O_6$	M-H	289,0718	0,7	20,8	9	+		+		2	179.0348 (7.3; -$C_6H_6O_2$), 203.0711 (21.9; -$C_3H_2O_3$), 221.0818 (13.2; -C_3O_2), 245.0817 (64.9; -CO_2), 271.0613 (4.4; -H_2O), 289.0717 (100.0)
16	*Unidentified*	8,51	$C_{12}H_{22}O_8$	M-H	293,1242	-0,8	75,5	2			+	1.33 ± 0.34	4	113.0233 (0.7; -$C_7H_{16}O_5$), 131.0697 (6.3; -$C_6H_{10}O_5$), 173.0812 (1.7; -$C_4H_8O_4$), 293.1245 (100.0)
17	**catechin**	8,81	$C_{15}H_{14}O_6$	M-H	289,0718	0,7	20,0	9	+	24.71 ± 3.36	+	0.26 ± 0.05	1	125.0243 (3.0; -$C_9H_8O_3$), 151.0397 (2.5; -$C_7H_6O_3$), 165.0196 (3.2; -$C_7H_8O_2$), 179.0360 (8.1; -$C_6H_6O_2$), 203.0721 (23.0; -$C_3H_2O_3$), 205.0513 (18.6; -$C_4H_4O_2$), 221.0829 (12.6; -C_3O_2), 227.0724 (5.7; -CH_2O_3), 245.0829 (71.0; -CO_2), 271.0618 (4.2; -H_2O), 289.0730 (100.0)
18	benzyl-Hex-Pent	8,95	$C_{18}H_{26}O_{10}$	M-H	401,1453	-0,3	38,8	6	+				3	179.0720 (31.4; -$C_8H_{14}O_7$), 239.0925 (100.0; -$C_6H_{10}O_5$), 401.1457 (18.2)
19	coumaroyl-Hex-1	9,13	$C_{15}H_{18}O_8$	M-H	325,0929	-1,0	4,9	7	+	0.25 ± 0.12			3	117.0336 (7.4; -$C_7H_{12}O_7$), 119.0494 (7.7; -$C_7H_{10}O_7$), 145.0282 (100.0; -$C_6H_{12}O_6$), 161.0606 (3.7; -$C_5H_8O_6$), 163.0396 (26.2; -$C_6H_{10}O_5$), 177.0548 (1.2; -$C_5H_8O_5$), 187.0400 (11.1; -$C_4H_{10}O_5$), 205.0506 (10.6; -$C_4H_8O_4$), 229.0514 (1.2; -$C_2H_8O_4$), 235.0615 (2.2; -$C_3H_6O_3$), 265.0
20	catechin-trimer (procyanidin-C1)	9,23	$C_{45}H_{38}O_{18}$	M-H	865,1985	-2,5	30,4	27	+	0.45 ± 0.23			2	125.0244 (10.9; -$C_{39}H_{32}O_{15}$), 161.0246 (9.2; -$C_{36}H_{32}O_{15}$), 243.0311 (31.5; -$C_{32}H_{30}O_{13}$), 261.0412 (20.0; -$C_{32}H_{28}O_{12}$), 289.0728 (100.0; -$C_{30}H_{24}O_{12}$), 407.0785 (91.6; -$C_{23}H_{22}O_{10}$), 425.0900 (14.3; -$C_{23}H_{20}O_9$), 525.0852 (11.7; -$C_{16}H_{20}O_8$)
21	coumaroyl-Hex-2	9,24	$C_{15}H_{18}O_8$	M-H	325,0929	-0,2	15,7	7	+		+	0.04 ± 0.02	3	119.0491 (2.9; -$C_7H_{10}O_7$), 145.0278 (100.0; -$C_6H_{12}O_6$), 163.0401 (13.4; -$C_6H_{10}O_5$), 187.0402 (11.4; -$C_4H_{10}O_5$), 205.0511 (9.0; -$C_4H_8O_4$), 235.0615 (2.7; -$C_3H_6O_3$), 265.0707 (15.9; -$C_2H_4O_2$), 325.0942 (14.0)
22	eucomic_acid-1	9,44	$C_{11}H_{12}O_6$	M-H	239,0561	0,0	8,1	6	+		+		2	149.0594 (7.5; -$C_2H_2O_4$), 177.0555 (33.0; -CH_2O_3), 179.0349 (59.3; -$C_2H_4O_2$), 195.0663 (9.5; -CO_2), 221.0455 (5.5; -H_2O), 239.0562 (100.0)
23	eucomic_acid-2	9,56	$C_{11}H_{12}O_6$	M-H	239,0561	0,0	8,1	6	+	1.79 ± 0.35	+	0.40 ± 0.07	2	149.0595 (7.8; -$C_2H_2O_4$), 177.0555 (33.1; -CH_2O_3), 179.0348 (62.2; -$C_2H_4O_2$), 195.0663 (9.5; -CO_2), 221.0455 (5.7; -H_2O), 239.0562 (100.0)
24	(epi-)catechin-sulfate	9,88	$C_{15}H_{14}O_9S$	M-H	369,0286	-0,7	35,7	9			+		2	230.9973 (60.4; -$C_7H_6O_3$), 245.0815 (23.0; -CO_5S), 289.0724 (100.0; -O_3S), 369.0287 (98.0)
25	*Unidentified*	9,93	$C_8H_{14}O_5$	M-H	189,0768	-0,8	8,5	2	+	0.43 ± 0.03	+	1.43 ± 0.21	4	115.0024 (38.4; -$C_4H_{10}O$), 145.0857 (5.3; -CO_2), 189.0771 (100.0)
26	*Unidentified*	10,14	$C_{12}H_{10}O_6$	M-H	249,0405	-0,4	10,7	8	+	0.22 ± 0.04			4	205.0507 (100.0; -CO_2), 249.0413 (1.5)
27	*Unidentified*	10,27	$C_{19}H_{24}O_{14}$	M-H	475,1093	-0,6	21,9	8			+	0.40 ± 0.04	4	153.0546 (5.2; -$C_{11}H_{14}O_{11}$), 197.0452 (100.0; -$C_{10}H_{14}O_9$), 211.0620 (7.3; -$C_9H_{12}O_9$), 239.0555 (36.1; -$C_8H_{12}O_8$), 269.0670 (6.8; -$C_7H_{10}O_7$), 299.0767 (8.2; -$C_6H_8O_6$), 344.0746 (2.6; -$C_5H_7O_4$), 359.0983 (70.9; -$C_4H_4O_4$)
28	**procyanidin-B2**	10,52	$C_{30}H_{26}O_{12}$	M-H	577,1352	0,6	23,0	18			+		1	125.0241 (5.5; -$C_{24}H_{20}O_9$), 161.0237 (4.5; -$C_{21}H_{20}O_9$), 203.0722 (4.3; -$C_{18}H_{14}O_9$), 205.0508 (4.2; -$C_{19}H_{16}O_8$), 245.0821 (24.9; -$C_{16}H_{12}O_8$), 273.0406 (6.7; -$C_{16}H_{16}O_6$), 289.0725 (100.0; -$C_{15}H_{12}O_6$), 299.0567 (3.8; -$C_{14}H_{14}O_6$), 329.0664 (3.8; -$C_{13}H_{12}O_5$), 339.0883 (12.7; -$C_{11}H$
29	feruloyl-Hex-2	10,76	$C_{16}H_{20}O_9$	M-H	355,1035	0,0	28,6	7	+	0.12 ± 0.03			3	160.0163 (13.1; -$C_7H_{15}O_6$), 175.0401 (100.0; -$C_6H_{12}O_6$), 193.0502 (25.2; -$C_6H_{10}O_5$), 217.0504 (5.2; -$C_4H_{10}O_5$), 235.0610 (7.8; -$C_4H_8O_4$), 295.0812 (4.8; -$C_2H_4O_2$), 355.1012 (3.8)
30	sinapoyl-Hex-1	11,16	$C_{17}H_{22}O_{10}$	M-H	385,1140	-0,2	13,0	7	+				3	164.0476 (1.7; -$C_8H_{13}O_7$), 190.0277 (24.6; -$C_7H_{15}O_6$), 205.0510 (100.0; -$C_6H_{12}O_6$), 221.0825 (2.4; -$C_5H_8O_6$), 223.0617 (10.2; -$C_6H_{10}O_5$), 232.0389 (1.9; -$C_5H_{13}O_5$), 247.0617 (3.8; -$C_4H_{10}O_5$), 265.0715 (4.1; -$C_4H_8O_4$), 325.0934 (2.3; -$C_2H_4O_2$), 385.1149 (1.2)
31	sinapoyl-Hex-2	11,31	$C_{17}H_{22}O_{10}$	M-H	385,1140	0,0	4,3	7	+	3.53 ± 0.77			3	164.0472 (1.5; -$C_8H_{13}O_7$), 175.0046 (1.7; -$C_8H_{18}O_6$), 179.0712 (1.2; -$C_7H_{10}O_7$), 190.0278 (25.6; -$C_7H_{15}O_6$), 205.0510 (100.0; -$C_6H_{12}O_6$), 223.0616 (10.2; -$C_6H_{10}O_5$), 247.0617 (3.1; -$C_4H_{10}O_5$), 265.0721 (3.8; -$C_4H_8O_4$), 295.0843 (0.5; -$C_3H_6O_3$), 325.0925 (1.9; -$C_2H_4O_2$), 341.
32	(epi-)catechin-1	11,54	$C_{15}H_{14}O_6$	M-H	289,0718	0,8	13,1	9			+	0.18 ± 0.02	2	221.0812 (12.7; -C_3O_2), 245.0812 (61.9; -CO_2), 271.0613 (9.6; -H_2O), 289.0712 (100.0)
33	**epi-catechin**	11,66	$C_{15}H_{14}O_6$	M-H	289,0718	1,4	1,4	9			+	0.21 ± 0.09	1	151.0395 (4.3; -$C_7H_6O_3$), 179.0343 (7.5; -$C_6H_6O_2$), 203.0705 (24.9; -$C_3H_2O_3$), 221.0822 (12.6; -C_3O_2), 245.0816 (67.8; -CO_2), 271.0609 (3.5; -H_2O), 289.0716 (100.0)
34	sinapoyl-Hex-3	12,01	$C_{17}H_{22}O_{10}$	M-H	385,1140	0,6	19,2	7			+	0.09 ± 0.05	3	164.0479 (8.9; -$C_8H_{13}O_7$), 179.0710 (9.8; -$C_7H_{10}O_7$), 190.0265 (5.6; -$C_7H_{15}O_6$), 193.0150 (2.9; -$C_8H_{16}O_5$), 205.0505 (22.1; -$C_6H_{12}O_6$), 208.0377 (41.1; -$C_7H_{13}O_5$), 223.0610 (100.0; -$C_6H_{10}O_5$), 265.0722 (2.8; -$C_4H_8O_4$)
35	methyl-butyl-Hex-Pent	12,09	$C_{17}H_{32}O_{12}$	M-H	427,1821	-0,1	92,8	2	+	1.30 ± 0.23			3	131.0330 (1.7; -$C_{12}H_{24}O_8$), 161.0458 (1.8; -$C_{11}H_{22}O_7$), 215.0562 (1.3; -$C_8H_{20}O_6$), 233.0671 (6.3; -$C_8H_{18}O_5$), 249.1343 (78.3; -$C_6H_{10}O_6$), 293.0874 (1.1; -$C_6H_{14}O_3$), 381.1765 (100.0; -CH_2O_2)
36	*Unidentified*	12,40	$C_{10}H_{16}O_7$	M-H	247,0823	0,7	11,8	3			+	0.15 ± 0.08	4	111.0069 (100.0; -$C_5H_{12}O_4$), 173.0103 (7.6; -$C_4H_{10}O$), 185.0810 (9.3; -CH_2O_3)
37	benzoyloxy-hydroxypropyl-HexA	12,50	$C_{16}H_{20}O_{10}$	M-H	371,0984	0,4	14,7	7	+	0.21 ± 0.07	+		3	121.0280 (14.7; -$C_9H_{14}O_8$), 231.0501 (8.6; -$C_7H_8O_3$), 249.0613 (100.0; -$C_7H_6O_2$), 289.0726 (3.0; -CH_6O_4), 311.0785 (3.4; -$C_2H_4O_2$), 371.0977 (36.6)
38	hydroxyoctanoic-acid	12,62	$C_8H_{14}O_5$	M-H	189,0768	0,9	8,0	2	+		+	0.58 ± 0.14	3	127.0755 (7.0; -CH_2O_3), 171.0657 (100.0; -H_2O), 189.0767 (5.8)
39	piceatannol-Hex-3	12,90	$C_{20}H_{22}O_9$	M-H	405,1191	0,3	41,0	10	+	0.28 ± 0.06			3	201.0551 (2.3; -$C_8H_{12}O_6$), 243.0666 (100.0; -$C_6H_{10}O_5$)
40	procyanidin-B1	13,02	$C_{30}H_{26}O_{12}$	M-H	577,1352	1,2	31,8	18	+				2	125.0236 (7.5; -$C_{24}H_{20}O_9$), 245.0820 (27.8; -$C_{16}H_{12}O_8$), 289.0719 (100.0; -$C_{15}H_{12}O_6$), 339.0861 (10.8; -$C_{11}H_{10}O_6$), 369.0615 (17.6; -$C_{11}H_{12}O_4$), 399.0714 (24.0; -$C_{10}H_{10}O_3$), 407.0773 (53.5; -$C_8H_{10}O_4$), 459.0940 (12.4; -C_8H_6O), 489.1027 (12.4; -C_7H_4)
41	*Unidentified*	13,16	$C_{29}H_{40}O_{16}$	M-H	643,2244	-0,2	47,6	10	+	0.49 ± 0.21	+	0.10 ± 0.03	4	166.0255 (18.2; -$C_{21}H_{33}O_{12}$), 181.0512 (45.8; -$C_{20}H_{30}O_{12}$), 205.0865 (20.6; -$C_{17}H_{26}O_{13}$), 338.1144 (48.1; -$C_9H_{21}O_{11}$), 369.1329 (31.1; -$C_8H_{18}O_{10}$), 387.1441 (100.0; -$C_8H_{16}O_9$)
42	resveratrol-Hex-1 (resveratroloside)	13,24	$C_{20}H_{22}O_8$	M+HCOOH-H	435,1297	-1,0	20,6	10	+	1.24 ± 0.17			2	185.0590 (0.2; -$C_9H_{14}O_8$), 227.0716 (100.0; -$C_7H_{12}O_7$), 269.0798 (0.3; -$C_5H_{10}O_6$), 389.1251 (4.1; -CH_2O_2)
43	tetrahomocitrate	13,43	$C_{10}H_{16}O_7$	M-H	247,0823	1,6	1,1	3	+	0.33 ± 0.18	+	0.88 ± 0.10	3	111.0070 (26.9; -$C_5H_{12}O_4$), 173.0084 (6.5; -$C_4H_{10}O$), 185.0816 (47.1; -CH_2O_3), 247.0820 (100.0)
44	*Unidentified*	13,69	$C_{21}H_{24}O_{12}$	M-H	467,1195	0,7	26,1	10	+	0.77 ± 0.12			4	125.0226 (59.4; -$C_{15}H_{18}O_9$), 183.0300 (15.3; -$C_{13}H_{16}O_7$), 197.0453 (30.5; -$C_{12}H_{14}O_7$), 327.0715 (65.0; -$C_7H_8O_3$), 341.0891 (100.0; -$C_6H_6O_3$)
45	vicenin-I	13,70	$C_{26}H_{28}O_{14}$	M-H	563,1406	1,1	25,8	13	+	0.43 ± 0.07	+	0.48 ± 0.19	2	325.0712 (5.6; -$C_8H_{14}O_8$), 353.0661 (100.0; -$C_7H_{14}O_7$), 383.0763 (84.5; -$C_6H_{12}O_6$), 413.0873 (10.6; -$C_5H_{10}O_5$), 425.0869 (17.8; -$C_4H_{10}O_5$), 443.0979 (40.4; -$C_4H_8O_4$), 473.1084 (40.9; -$C_3H_6O_3$), 503.1197 (23.5; -$C_2H_4O_2$), 545.1308 (9.3; -H_2O), 563.1396 (62.1)
46	myrcetin-Hex-dHex	14,08	$C_{27}H_{30}O_{17}$	M-H	625,1410	1,7	24,2	13	+	3.15 ± 0.73	+	1.21 ± 0.08	3	178.9982 (0.8; -$C_{19}H_{26}O_{12}$), 271.0241 (2.2; -$C_{13}H_{22}O_{11}$), 287.0195 (1.4; -$C_{13}H_{22}O_{10}$), 316.0220 (100.0; -$C_{12}H_{21}O_9$), 419.1706 (2.7; -$C_5H_2O_9$), 581.2231 (2.8; -$C_{27}H_{29}O_{17}$), 625.1403 (25.3)
47	myrcetin-HexA	14,10	$C_{21}H_{18}O_{14}$	M-H	493,0624	0,2	3,4	13			+	0.74 ± 0.05	3	151.0017 (1.1; -$C_{14}H_{14}O_{10}$), 178.9984 (3.6; -$C_{13}H_{14}O_9$), 227.0342 (0.6; -$C_8H_{10}O_{10}$), 271.0246 (1.6; -$C_7H_{10}O_8$), 299.0195 (3.3; -$C_6H_{10}O_7$), 317.0302 (100.0; -$C_6H_8O_6$), 493.0625 (0.7)
48	vicenin-III	14,14	$C_{26}H_{28}O_{14}$	M-H	563,1406	1,2	6,7	13	+	1.76 ± 0.41	+	1.74 ± 0.11	2	325.0714 (4.0; -$C_8H_{14}O_8$), 353.0665 (93.1; -$C_7H_{14}O_7$), 365.0664 (6.4; -$C_6H_{14}O_7$), 383.0769 (79.6; -$C_6H_{12}O_6$), 413.0876 (12.8; -$C_5H_{10}O_5$), 425.0871 (14.1; -$C_4H_{10}O_5$), 443.0981 (60.4; -$C_4H_8O_4$), 473.1085 (50.7; -$C_3H_6O_3$), 503.1192 (19.9; -$C_2H_4O_2$), 545.1292 (8.9; -H_2O), 563.
49	lyoniresinol-Hex-1	14,18	$C_{28}H_{38}O_{13}$	M+HCOOH-H	627,2294	-0,1	12,1	10	+				3	(3.1; -$C_8H_{16}O_5$), 459.1281 (6.7; -$C_6H_{16}O_5$), 475.1614 (1.1; -$C_5H_{12}O_5$), 489.1754 (1.0; -$C_4H_{10}O_5$), 535.1816 (5.4; -$C_3H_8O_3$), 551.1767 (3.7; -$C_3H_8O_2$), 566.1997 (17.3; -$C_2H_5O_2$), 581.2231 (87.1; -CH_2O_2)
50	*Unidentified*	14,19	$C_8H_{14}O_5$	M-H	189,0768	1,7	8,5	2			+		4	111.0084 (28.2; -$C_3H_{10}O_2$), 171.0650 (100.0; -H_2O), 189.0733 (56.4)
51	myrcetin-Hex	14,28	$C_{21}H_{20}O_{13}$	M-H	479,0831	1,1	18,8	12	+		+		3	151.0039 (0.3; -$C_{14}H_{16}O_9$), 178.9979 (0.7; -$C_{13}H_{16}O_8$), 271.0244 (2.9; -$C_7H_{12}O_7$), 287.0199 (2.1; -$C_7H_{12}O_6$), 316.0223 (100.0; -$C_6H_{11}O_5$), 479.0827 (12.3)
52	syringoyl-galloyl-Hex	14,32	$C_{22}H_{24}O_{14}$	M-H	511,1093	0,2	24,2	11			+		3	151.0017 (13.3; -$C_{15}H_{20}O_{10}$), 169.0135 (10.4; -$C_{15}H_{18}O_9$), 327.0708 (23.9; -$C_8H_8O_5$), 341.0887 (9.9; -$C_7H_6O_5$), 452.0957 (5.0; -$C_2H_3O_2$), 467.1194 (18.8; -CO_2), 511.1088 (100.0)
53	*Unidentified*	14,60	$C_8H_{14}O_5$	M-H	189,0768	0,8	10,2	2			+		4	127.0751 (17.1; -CH_2O_3), 171.0656 (100.0; -H_2O), 189.0774 (1.6)
54	*Unidentified*	14,69	$C_{21}H_{22}O_{12}$	M-H	465,1039	1,0	19,6	11	+	0.82 ± 0.16			4	151.0020 (17.0; -$C_{14}H_{18}O_8$), 285.0397 (5.4; -$C_6H_{12}O_6$), 303.0505 (77.0; -$C_6H_{10}O_5$), 313.0925 (100.0; -$C_7H_4O_4$), 339.0722 (3.2; -$C_6H_6O_3$), 465.1033 (31.3)
55	galloyl-catechin-1	15,02	$C_{22}H_{18}O_{10}$	M-H	441,0827	0,9	23,8	14	+	0.63 ± 0.18	+	0.21 ± 0.01	2	289.0712 (100.0; -$C_7H_4O_4$), 303.0503 (5.2; -$C_7H_6O_3$), 331.0458 (3.0; -$C_{22}H_{17}O_{10}$), 441.0818 (1.6)
56	**vicenin-III**	15,08	$C_{26}H_{28}O_{14}$	M-H	563,1406	2,0	28,8	13	+	0.38 ± 0.11	+	0.16 ± 0.01	1	(100.0)
57	*Unidentified*	15,32	$C_{21}H_{24}O_{11}$	M-H	451,1246	-0,1	9,1	10			+		4	138.0306 (2.8; -$C_{14}H_{17}O_8$), 182.0220 (14.7; -$C_{13}H_{17}O_6$), 197.0451 (36.2; -$C_{12}H_{14}O_6$), 281.0653 (4.3; -$C_8H_{10}O_4$), 311.0779 (9.3; -$C_7H_8O_3$), 327.0716 (35.2; -$C_7H_8O_2$), 341.0877 (100.0; -$C_6H_6O_2$), 421.0759 (3.3; -C_2H_6), 436.1008 (32.5; -CH_3), 451.1244 (92.7)
58	*Unidentified*	15,39	$C_{10}H_{16}O_7$	M-H	247,0823	0,8	18,6	3			+	0.31 ± 0.09	4	111.0065 (65.2; -$C_5H_{12}O_4$), 185.0818 (100.0; -CH_2O_3), 247.0831 (96.3)
59	*Unidentified*	15,39	$C_{21}H_{24}O_{11}$	M-H	451,1246	-0,1	19,7	10			+		4	138.0306 (2.8; -$C_{14}H_{17}O_8$), 182.0220 (14.7; -$C_{13}H_{17}O_6$), 197.0451 (36.2; -$C_{12}H_{14}O_6$), 281.0653 (4.3; -$C_8H_{10}O_4$), 311.0779 (9.3; -$C_7H_8O_3$), 327.0716 (35.2; -$C_7H_8O_2$), 341.0877 (100.0; -$C_6H_6O_2$), 421.0759 (3.3; -C_2H_6), 436.1008 (32.5; -CH_3), 451.1244 (92.7)
60	quercetin-Hex-dHex-1	15,43	$C_{27}H_{30}O_{16}$	M-H	609,1461	0,2	6,5	13			+	0.49 ± 0.14	3	211.0407 (0.8; -$C_{14}H_{22}O_{13}$), 227.0349 (1.7; -$C_{14}H_{22}O_{12}$), 239.0346 (0.6; -$C_{13}H_{22}O_{12}$), 257.0453 (100.0; -$C_{13}H_{20}O_{11}$), 283.0248 (27.6; -$C_{12}H_{22}O_{10}$), 301.0352 (66.7; -$C_{12}H_{20}O_9$), 317.0311 (0.5; -$C_{12}H_{20}O_8$), 343.0447 (0.6; -$C_{10}H_{18}O_8$)
61	*Unidentified*	15,68	$C_{17}H_{32}O_{10}$	M+HCOOH-H	441,1978	2,1	23,8	2	+				4	233.0655 (7.3; -$C_9H_{20}O_5$), 245.1389 (3.6; -$C_6H_{12}O_7$), 263.1493 (96.4; -$C_6H_{10}O_6$), 395.1915 (100.0; -CH_2O_2)
62	**rutin**	15,83	$C_{27}H_{30}O_{16}$	M-H	609,1461	1,8	0,9	13	+	17.69 ± 1.85	+	11.60 ± 0.95	1	125.0240 (0.0; -$C_{21}H_{24}O_{13}$), 151.0021 (0.3; -$C_{20}H_{26}O_{12}$), 178.9982 (0.7; -$C_{19}H_{26}O_{11}$), 243.0296 (0.3; -$C_{14}H_{22}O_{11}$), 255.0297 (1.4; -$C_{13}H_{22}O_{11}$), 271.0244 (3.1; -$C_{13}H_{22}O_{10}$), 283.0241 (0.4; -$C_{12}H_{22}O_{10}$), 300.0271 (100.0; -$C_{12}H_{21}O_9$), 301.0337 (50.2; -$C_{12}H_{20}O_9$), 313.0352 (0.3; -
63	quercetin-hydroxy-succinyl-Hex	15,96	$C_{25}H_{24}O_{16}$	M-H	579,0992	1,1	22,9	14	+		+		3	178.9969 (0.9; -$C_{17}H_{20}O_{11}$), 255.0306 (1.1; -$C_{11}H_{16}O_{11}$), 271.0245 (1.5; -$C_{11}H_{16}O_{10}$), 283.0242 (0.6; -$C_{10}H_{16}O_{10}$), 300.0276 (100.0; -$C_{10}H_{15}O_9$), 313.0355 (0.5; -$C_9H_{14}O_9$), 343.0465 (1.0; -$C_8H_{12}O_8$), 445.0776 (0.5; -$C_4H_6O_5$), 463.0882 (53.1; -$C_4H_4O_4$)
64	benzyl-Hex-Hex	15,97	$C_{19}H_{28}O_{11}$	M-H	431,1559	2,3	28,4	6	+				3	271.0677 (28.5; -$C_{11}H_{12}O$), 311.0561 (100.0; -$C_2H_{16}O_5$), 341.0656 (43.7; -$CH_{14}O_4$)
65	quercetin-HexA	16,02	$C_{21}H_{18}O_{13}$	M-H	477,0675	0,3	10,9	13			+	4.66 ± 0.21	3	151.0025 (0.8; -$C_{14}H_{14}O_9$), 163.0028 (0.1; -$C_{13}H_{14}O_9$), 178.9987 (1.9; -$C_{13}H_{14}O_8$), 211.0403 (0.3; -$C_8H_{10}O_{10}$), 229.0507 (0.4; -$C_8H_8O_9$), 239.0351 (0.3; -$C_7H_{10}O_9$), 245.0458 (0.5; -$C_8H_8O_8$), 255.0305 (0.9; -$C_7H_{10}O_8$), 266.0227 (0.1; -$C_6H_{11}O_8$), 273.0407 (1.5; -$C_7H_8O_7$), 283.0
66	quercetin-Hex-2 (isoquercetrin)	16,20	$C_{21}H_{20}O_{12}$	M-H	463,0882	1,4	2,3	12	+	4.41 ± 0.65	+	2.74 ± 0.01	2	151.0022 (0.2; -$C_{14}H_{16}O_8$), 178.9983 (0.5; -$C_{13}H_{16}O_7$), 227.0348 (0.1; -$C_8H_{12}O_8$), 243.0292 (0.4; -$C_8H_{12}O_7$), 255.0294 (1.7; -$C_7H_{12}O_7$), 271.0244 (3.4; -$C_7H_{12}O_6$), 283.0247 (0.4; -$C_6H_{12}O_6$), 300.0271 (100.0; -$C_6H_{11}O_5$), 301.0337 (44.8; -$C_6H_{10}O_5$), 343.0453 (0.4; -$C_4H_8O_4$), 44
67	coumaroyl-galloyl-Hex-6	16,31	$C_{22}H_{22}O_{12}$	M-H	477,1039	2,1	41,9	12	+				3	169.0149 (24.9; -$C_{15}H_{16}O_7$), 205.0494 (39.6; -$C_{11}H_{12}O_8$), 235.0613 (15.5; -$C_{10}H_{10}O_7$), 265.0710 (100.0; -$C_9H_8O_6$), 313.0571 (34.7; -$C_9H_8O_3$), 331.0676 (15.6; -$C_9H_6O_2$), 417.0835 (12.1; -$C_2H_4O_2$), 477.1027 (71.5)
68	*Unidentified*	16,31	$C_{30}H_{30}O_{18}$	M-H	677,1359	0,9	28,6	16	+				4	255.0657 (59.2; -$C_{15}H_{18}O_{14}$), 297.0754 (3.7; -$C_{13}H_{16}O_{13}$), 459.1289 (100.0; -$C_7H_6O_8$)
69	*Unidentified*	16,33	$C_{18}H_{24}O_{10}$	M+HCOOH-H	445,1352	2,3	18,7	7	+				4	193.0878 (3.3; -$C_8H_{12}O_9$), 222.0521 (3.1; -$C_8H_{15}O_7$), 237.0764 (100.0; -$C_7H_{12}O_7$), 279.0298 (2.7; -$C_3H_{18}O_7$), 283.0251 (17.0; -$C_4H_{18}O_6$), 307.0223 (4.2; -$C_2H_{18}O_6$)
70	**rhapontin**	16,82	$C_{21}H_{24}O_9$	M+HCOOH-H	465,1402	2,0	1,8	10	+	3.46 ± 0.57	+	0.35 ± 0.11	1	172.0527 (0.1; -$C_{11}H_{17}O_9$), 185.0611 (0.1; -$C_{10}H_{16}O_9$), 199.0413 (0.1; -$C_{10}H_{18}O_8$), 214.0628 (0.1; -$C_9H_{15}O_8$), 242.0573 (6.5; -$C_8H_{15}O_7$), 257.0811 (100.0; -$C_7H_{12}O_7$), 281.0800 (0.0; -$C_5H_{12}O_7$)
71	resveratrol-Hex-2	17,13	$C_{20}H_{22}O_8$	M-H	389,1242	-2,1	24,5	10	+	0.34 ± 0.12			2	209.1179 (3.6; -$C_8H_4O_5$), 227.0720 (100.0; -$C_6H_{10}O_5$)
72	feruloyl-galloyl-Hex	17,20	$C_{23}H_{24}O_{13}$	M-H	507,1144	1,5	30,0	12	+				3	151.0020 (8.8; -$C_{16}H_{20}O_9$), 211.0247 (4.2; -$C_{14}H_{16}O_7$), 273.0766 (15.5; -$C_8H_{10}O_8$), 337.0924 (4.8; -$C_7H_6O_5$), 463.1251 (4.1; -CO_2), 507.1135 (100.0)
73	*Unidentified*	17,26	$C_{25}H_{28}O_{13}$	M-H	535,1457	1,4	11,7	12	+	0.16 ± 0.07			4	241.0497 (7.3; -$C_{11}H_{18}O_9$), 257.0813 (100.0; -$C_{10}H_{14}O_9$)
74	kaempferol-Hex-dHex (nicotiflorin)	17,44	$C_{27}H_{30}O_{15}$	M-H	593,1512	1,2	24,4	13	+		+	0.26 ± 0.05	2	151.0020 (0.2; -$C_{20}H_{26}O_{11}$), 227.0342 (1.0; -$C_{14}H_{22}O_{11}$), 229.0507 (1.4; -$C_{14}H_{20}O_{11}$), 255.0293 (3.6; -$C_{13}H_{22}O_{10}$), 285.0396 (100.0; -$C_{12}H_{20}O_9$), 300.0269 (0.7; -$C_{12}H_{21}O_8$), 327.0514 (2.7; -$C_{10}H_{18}O_8$), 357.0608 (0.5; -$C_9H_{16}O_7$), 593.1506 (29.0)
75	quercetin-Pent-2 (avicularin)	17,51	$C_{20}H_{18}O_{11}$	M-H	433,0776	1,5	13,9	12	+	1.12 ± 0.20			2	151.0019 (0.3; -$C_{13}H_{14}O_7$), 178.9986 (0.7; -$C_{12}H_{14}O_6$), 243.0292 (0.4; -$C_7H_{10}O_6$), 255.0293 (2.3; -$C_6H_{10}O_6$), 271.0245 (4.0; -$C_6H_{10}O_5$), 283.0243 (0.9; -$C_5H_{10}O_5$), 300.0271 (100.0; -$C_5H_9O_4$), 301.0340 (65.0; -$C_5H_8O_4$), 343.0450 (0.2; -$C_3H_6O_3$), 415.0671 (0.1; -H_2O), 433.077
76	syringaresinol-Hex (acanthoside-B)	17,71	$C_{28}H_{36}O_{13}$	M-H	579,2083	1,7	17,3	11	+	0.54 ± 0.13			2	166.0255 (4.2; -$C_{20}H_{29}O_9$), 181.0500 (32.7; -$C_{19}H_{26}O_9$), 205.0865 (1.7; -$C_{16}H_{22}O_{10}$), 301.0374 (0.9; -$C_{13}H_{26}O_6$), 356.1280 (1.4; -$C_8H_{15}O_7$), 371.1499 (3.8; -$C_7H_{12}O_7$), 387.1079 (11.1; -$C_8H_{16}O_5$), 402.1314 (33.9; -$C_7H_{13}O_5$), 417.1548 (100.0; -$C_6H_{10}O_5$), 549.1624 (0.7; -C_2H_6)
77	quercetin-dHex-1 (quercitrin)	17,93	$C_{21}H_{20}O_{11}$	M-H	447,0933	-0,9	20,4	12			+	0.35 ± 0.08	2	151.0029 (0.3; -$C_{14}H_{16}O_7$), 178.9986 (0.7; -$C_{13}H_{16}O_6$), 227.0356 (0.3; -$C_8H_{12}O_7$), 243.0302 (0.4; -$C_8H_{12}O_6$), 255.0307 (2.3; -$C_7H_{12}O_6$), 271.0256 (3.6; -$C_7H_{12}O_5$), 284.0330 (3.8; -$C_6H_{11}O_5$), 300.0282 (100.0; -$C_6H_{11}O_4$), 301.0353 (72.3; -$C_6H_{10}O_4$), 343.0471 (0.2; -$C_4H_8O_3$), 42
78	*Unidentified*	17,97	$C_{24}H_{26}O_{12}$	M-H	505,1352	-0,9	82,9	12	+	0.12 ± 0.02	+	0.21 ± 0.05	4	119.0498 (10.5; -$C_{16}H_{18}O_{11}$), 163.0394 (67.7; -$C_{15}H_{18}O_9$), 197.0455 (48.7; -$C_{15}H_{16}O_7$), 239.0575 (5.8; -$C_{13}H_{14}O_6$), 281.0653 (6.1; -$C_{11}H_{12}O_5$), 299.0563 (33.4; -$C_8H_{14}O_6$), 327.0726 (49.4; -$C_{10}H_{10}O_3$), 341.0884 (100.0; -$C_9H_8O_3$), 431.0999 (5.0; -$C_3H_6O_2$), 446.1235 (9.6; -C_2H
79	isorhamnetin-Hex-dHex (narcissin)	17,99	$C_{28}H_{32}O_{16}$	M-H	623,1618	0,9	8,5	13	+	0.24 ± 0.04	+	0.15 ± 0.03	2	255.0281 (0.8; -$C_{14}H_{24}O_{11}$), 271.0245 (3.1; -$C_{14}H_{24}O_{10}$), 315.0506 (100.0; -$C_{12}H_{20}O_9$), 357.0621 (1.4; -$C_{10}H_{18}O_8$), 417.1559 (1.3; -$C_6H_6O_8$), 595.1668 (1.0; -CO), 623.1619 (8.8)
80	tetrahydroxy-dimethylanthraquinone-1	18,10	$C_{16}H_{12}O_6$	M-H	299,0561	1,1	12,9	11	+				3	189.0559 (100.0; -$C_5H_2O_3$), 215.0334 (26.0; -$C_4H_4O_2$), 255.0675 (28.9; -CO_2), 299.0543 (21.4)
81	eriodictyol-Hex	18,22	$C_{21}H_{22}O_{11}$	M-H	449,1089	0,5	3,5	11	+	0.51 ± 0.16			3	135.0433 (3.0; -$C_{13}H_{14}O_9$), 151.0020 (13.1; -$C_{14}H_{18}O_7$), 243.0660 (0.6; -$C_7H_{10}O_7$), 269.0817 (1.1; -$C_5H_8O_7$), 287.0558 (100.0; -$C_6H_{10}O_5$), 297.0978 (3.6; -$C_7H_4O_4$), 323.0781 (1.0; -$C_6H_6O_3$), 431.1333 (0.5; -$C_{21}H_{21}O_{11}$), 449.1085 (13.3)
82	*Unidentified*	18,22	$C_{22}H_{24}O_{12}$	M-H	479,1195	-0,6	20,7	11			+	0.15 ± 0.11	4	137.0231 (6.9; -$C_{15}H_{18}O_9$), 179.0345 (8.3; -$C_{13}H_{16}O_8$), 197.0458 (62.9; -$C_{13}H_{14}O_7$), 211.0609 (16.5; -$C_{12}H_{12}O_7$), 239.0561 (100.0; -$C_{11}H_{12}O_6$), 251.0560 (6.7; -$C_{10}H_{12}O_6$), 269.0663 (20.7; -$C_{10}H_{10}O_5$), 281.0665 (23.7; -$C_9H_{10}O_5$), 299.0776 (42.6; -$C_9H_8O_4$), 327.0719 (16.8; -C
83	aloesone-Hex-2	18,47	$C_{19}H_{22}O_9$	M-H	393,1191	1,6	19,4	9	+	0.06 ± 0.01	+	0.06 ± 0.01	2	187.0770 (0.3; -$C_7H_{10}O_7$), 231.0660 (100.0; -$C_6H_{10}O_5$), 273.0772 (0.1; -$C_4H_8O_4$), 393.1186 (0.1)
84	kaempferide-Hex	18,53	$C_{22}H_{22}O_{11}$	M-H	461,1089	1,9	20,9	12	+	0.07 ± 0.02	+	0.07 ± 0.01	3	189.0564 (14.8; -$C_{11}H_{12}O_8$), 213.0561 (40.1; -$C_9H_{12}O_8$), 231.0670 (39.0; -$C_9H_{10}O_7$), 255.0669 (100.0; -$C_7H_{10}O_7$), 281.0468 (2.6; -$C_6H_{12}O_6$), 299.0567 (37.7; -$C_6H_{10}O_5$), 357.0974 (1.5; -$C_3H_4O_4$), 377.0884 (1.3; -$C_4H_4O_2$), 401.0878 (4.2; -$C_2H_4O_2$), 419.0995 (4.3; -C_2H_2O), 46
85	isorhamnetin-Hex	18,65	$C_{22}H_{22}O_{12}$	M-H	477,1039	1,0	11,0	12	+	0.23 ± 0.07	+	0.32 ± 0.06	3	271.0241 (7.8; -$C_8H_{14}O_6$), 299.0194 (94.3; -$C_7H_{14}O_5$), 314.0428 (100.0; -$C_6H_{11}O_5$), 342.0362 (2.3; -$C_5H_{11}O_4$), 357.0609 (3.4; -$C_4H_8O_4$), 449.1081 (3.1; -CO), 462.0814 (1.3; -CH_3), 477.1033 (78.1)
86	tetrahydroxy-dimethylanthraquinone-2	18,65	$C_{16}H_{12}O_6$	M-H	299,0561	0,9	13,0	11	+	0.16 ± 0.05	+		3	189.0548 (41.0; -$C_5H_2O_3$), 213.0553 (100.0; -$C_3H_2O_3$), 231.0653 (34.5; -C_3O_2), 255.0661 (38.0; -CO_2), 299.0559 (87.8)
87	(iso-)rhapontigenin-malonyl-Hex-1	18,73	$C_{24}H_{26}O_{12}$	M-H	505,1352	1,2	6,1	12	+	0.26 ± 0.08			3	224.0475 (1.2; -$C_{10}H_{17}O_9$), 241.0505 (12.9; -$C_{10}H_{16}O_8$), 257.0814 (100.0; -$C_9H_{12}O_8$), 299.0915 (0.3; -$C_7H_{10}O_7$), 401.1252 (0.4; -$C_3H_4O_4$)
88	**phloridzin**	18,80	$C_{21}H_{24}O_{10}$	M+HCOOH-H	481,1352	2,3	19,9	10	+		+		1	167.0339 (11.7; -$C_{13}H_{16}O_6$), 217.0131 (8.8; -$C_{10}H_{18}O_5$), 273.0765 (100.0; -$C_6H_{10}O_5$), 341.0666 (66.3; -$C_3H_{10}O_3$)
89	methyl-hexyl-Hex-Pent	18,83	$C_{18}H_{34}O_{10}$	M+HCOOH-H	455,2134	1,4	4,4	2	+	0.35 ± 0.13			3	161.0450 (2.2; -$C_{13}H_{26}O_7$), 191.0548 (1.3; -$C_{12}H_{24}O_6$), 233.0659 (5.2; -$C_{10}H_{22}O_5$), 277.1651 (100.0; -$C_6H_{10}O_6$), 293.0880 (1.3; -$C_8H_{18}O_3$), 409.2074 (91.5; -CH_2O_2)
90	rhapontin	18,83	$C_{21}H_{24}O_9$	M-H	419,1348	1,5	26,8	10	+	0.29 ± 0.10	+	0.05 ± 0.00	2	225.0549 (1.3; -$C_7H_{14}O_6$), 242.0574 (13.4; -$C_7H_{13}O_5$), 257.0812 (100.0; -$C_6H_{10}O_5$)
91	*Unidentified*	19,05	$C_{24}H_{26}O_{12}$	M-H	505,1352	-0,9	22,5	12			+	0.11 ± 0.01	4	119.0491 (18.5; -$C_{16}H_{18}O_{11}$), 163.0392 (30.6; -$C_{15}H_{18}O_9$), 177.0547 (14.5; -$C_{14}H_{16}O_9$), 183.0301 (28.8; -$C_{16}H_{18}O_7$), 197.0454 (16.4; -$C_{15}H_{16}O_7$), 299.0569 (6.3; -$C_8H_{14}O_6$), 312.0499 (9.7; -$C_{11}H_{13}O_3$), 327.0721 (100.0; -$C_{10}H_{10}O_3$), 341.0877 (89.0; -$C_9H_8O_3$), 490.1131 (6.0; -C
92	pinocembrine-Hex-4	19,55	$C_{21}H_{22}O_9$	M-H	417,1191	0,8	18,2	11	+	0.26 ± 0.16	+	0.12 ± 0.02	2	211.0766 (0.7; -$C_7H_{10}O_7$), 226.0633 (0.6; -$C_7H_{11}O_6$), 239.0702 (0.4; -$C_6H_{10}O_6$), 255.0660 (100.0; -$C_6H_{10}O_5$), 297.0766 (1.6; -$C_4H_8O_4$), 417.1186 (69.4)
93	emodin-Hex-3	19,67	$C_{21}H_{20}O_{10}$	M-H	431,0984	1,4	13,1	12	+		+	0.30 ± 0.04	2	225.0560 (0.5; -$C_7H_{10}O_7$), 240.0428 (6.7; -$C_7H_{11}O_6$), 253.0514 (0.3; -$C_6H_{10}O_6$), 269.0454 (78.5; -$C_6H_{10}O_5$), 280.0377 (0.8; -$C_5H_{11}O_5$), 293.0452 (0.4; -$C_4H_{10}O_5$), 311.0559 (1.0; -$C_4H_8O_4$), 323.0547 (0.1; -$C_3H_8O_4$), 431.0979 (100.0)
94	*Unidentified*	20,15	$C_{30}H_{30}O_{18}$	M-H	677,1359	1,1	5,9	16	+	0.81 ± 0.30	+	0.12 ± 0.03	4	211.0760 (0.3; -$C_{16}H_{18}O_{16}$), 255.0661 (58.7; -$C_{15}H_{18}O_{14}$), 279.0651 (0.4; -$C_{13}H_{18}O_{14}$), 297.0772 (2.5; -$C_{13}H_{16}O_{13}$), 399.1078 (0.4; -$C_9H_{10}O_{10}$), 417.1186 (2.4; -$C_9H_8O_9$), 441.1191 (0.4; -$C_7H_8O_9$), 459.1290 (100.0; -$C_7H_6O_8$), 593.1539 (0.2; -$C_{30}H_{29}O_{18}$)

95	diferuloyl-Suc (helonioside-A)	20,26	$C_{32}H_{38}O_{17}$	M-H	693,2036	-0,4	16,3	14			+	0.68 ± 0.12	2	132.0206 (2.6; -$C_{24}H_{33}O_{15}$), 134.0356 (2.3; -$C_{24}H_{31}O_{15}$), 160.0161 (20.1; -$C_{23}H_{33}O_{14}$), 175.0401 (100.0; -$C_{22}H_{30}O_{14}$), 193.0511 (36.2; -$C_{22}H_{28}O_{13}$), 207.0658 (1.0; -$C_{21}H_{26}O_{13}$), 235.0631 (2.3; -$C_{20}H_{26}O_{12}$), 247.0623 (2.4; -$C_{19}H_{26}O_{12}$), 265.0720 (12.2; -$C_{19}H_{24}O_{11}$), 307.0832 (2
96	aleosone-malonyl-Hex	20,44	$C_{22}H_{24}O_{12}$	M-H	479,1195	-0,7	20,9	11			+	0.05 ± 0.02	3	187.0764 (0.6; -$C_{10}H_{12}O_{10}$), 231.0663 (100.0; -$C_{9}H_{12}O_{8}$)
97	dicoumaroyl-Hex-3	20,99	$C_{24}H_{24}O_{10}$	M-H	471,1297	0,3	22,4	13	+	0.14 ± 0.05			3	145.0286 (15.7; -$C_{15}H_{18}O_{8}$), 163.0394 (43.4; -$C_{15}H_{16}O_{7}$), 187.0409 (5.1; -$C_{13}H_{16}O_{7}$), 307.0824 (100.0; -$C_{9}H_{8}O_{3}$), 471.1298 (7.4)
98	*Unidentified*	21,10	$C_{22}H_{32}O_{9}$	M-H	439,1974	-0,6	5,7	7	+	0.23 ± 0.05			4	218.1311 (0.7; -$C_{8}H_{13}O_{7}$), 233.1544 (43.9; -$C_{7}H_{10}O_{7}$), 277.1444 (100.0; -$C_{6}H_{10}O_{5}$), 395.2055 (0.6; -CO_{2}), 439.1973 (22.9)
99	quercetin	21,65	$C_{15}H_{10}O_{7}$	M-H	301,0354	-0,1	2,7	11	+	0.29 ± 0.06	+	0.09 ± 0.04	2	121.0285 (1.0; -$C_{8}H_{4}O_{5}$), 151.0028 (9.3; -$C_{8}H_{6}O_{3}$), 178.9993 (15.0; -$C_{7}H_{6}O_{2}$), 193.0146 (1.0; -$C_{6}H_{4}O_{2}$), 229.0507 (2.2; -$C_{2}O_{3}$), 245.0459 (1.6; -$C_{2}O_{2}$), 257.0469 (2.2; -CO_{2}), 273.0412 (8.6; -CO), 301.0365 (100.0)
100	*Unidentified*	21,80	$C_{22}H_{34}O_{11}$	M-H	473,2028	-1,1	5,3	6			+	0.05 ± 0.03	4	163.0419 (29.6; -$C_{13}H_{26}O_{8}$), 247.1366 (100.0; -$C_{7}H_{14}O_{8}$), 429.2079 (91.4; -$C_{22}H_{33}O_{11}$)
101	dicoumaroyl-Hex-4	21,81	$C_{24}H_{24}O_{10}$	M-H	471,1297	0,4	25,8	13	+				3	145.0286 (91.6; -$C_{15}H_{18}O_{8}$), 163.0395 (41.7; -$C_{15}H_{16}O_{7}$), 187.0403 (17.1; -$C_{13}H_{16}O_{7}$), 205.0503 (38.8; -$C_{13}H_{14}O_{6}$), 235.0606 (24.6; -$C_{12}H_{12}O_{5}$), 265.0713 (78.3; -$C_{11}H_{10}O_{4}$), 307.0822 (100.0; -$C_{9}H_{8}O_{3}$), 471.1291 (14.3)
102	deoxyrhapontigenin-Hex-1	21,85	$C_{21}H_{24}O_{8}$	M+HCOOH-H	449,1453	0,4	9,1	10	+	1.29 ± 0.25			3	226.0627 (1.8; -$C_{8}H_{15}O_{7}$), 241.0869 (100.0; -$C_{7}H_{12}O_{7}$), 255.0647 (0.1; -$C_{7}H_{14}O_{6}$), 265.0860 (0.1; -$C_{5}H_{12}O_{7}$), 283.0971 (0.1; -$C_{5}H_{10}O_{6}$)
103	(aloe-)emodin-anthrone-malonyl-Hex-1	22,09	$C_{24}H_{24}O_{12}$	M-H	503,1195	0,6	11,9	13	+	0.69 ± 0.10	+	0.37 ± 0.02	3	211.0751 (0.4; -$C_{10}H_{12}O_{10}$), 255.0661 (100.0; -$C_{9}H_{12}O_{8}$), 279.0676 (0.5; -$C_{7}H_{12}O_{8}$), 297.0762 (2.3; -$C_{7}H_{10}O_{7}$), 417.1193 (2.7; -$C_{3}H_{2}O_{3}$), 437.1234 (0.3; -$H_{2}O_{4}$), 459.1293 (8.9; -CO_{2})
104	deoxyrhapontigenin-Hex-2	22,09	$C_{21}H_{24}O_{8}$	M+HCOOH-H	449,1453	0,5	22,7	10	+				3	241.0869 (100.0; -$C_{7}H_{12}O_{7}$)
105	*Unidentified*	22,33	$C_{26}H_{26}O_{10}$	M-H	497,1453	0,3	23,2	14	+				4	117.0332 (2.7; -$C_{18}H_{20}O_{9}$), 145.0279 (10.6; -$C_{17}H_{20}O_{8}$), 163.0413 (5.5; -$C_{17}H_{18}O_{7}$), 189.0561 (100.0; -$C_{15}H_{16}O_{7}$), 307.0821 (35.7; -$C_{11}H_{10}O_{3}$)
106	emodin-malonyl-Hex-1	22,38	$C_{24}H_{22}O_{13}$	M-H	517,0988	-0,7	20,9	14	+		+	0.21 ± 0.03	3	225.0561 (1.8; -$C_{10}H_{12}O_{10}$), 237.0570 (1.4; -$C_{9}H_{12}O_{10}$), 240.0439 (1.8; -$C_{10}H_{13}O_{9}$), 249.0566 (0.7; -$C_{8}H_{12}O_{10}$), 255.0673 (1.8; -$C_{9}H_{10}O_{9}$), 265.0517 (5.1; -$C_{8}H_{12}O_{9}$), 269.0465 (100.0; -$C_{9}H_{12}O_{8}$), 282.0537 (2.8; -$C_{8}H_{11}O_{8}$), 293.0471 (8.2; -$C_{7}H_{12}O_{8}$), 311.0568 (1.5; -$C_{7}H_{10}O_{7}$), 4
107	torachrysone-Hex-1	22,45	$C_{20}H_{24}O_{9}$	M-H	407,1348	0,2	1,0	9	+	1.42 ± 0.35	+	0.30 ± 0.04	3	203.0717 (0.1; -$C_{8}H_{12}O_{6}$), 215.0351 (0.5; -$C_{8}H_{16}O_{5}$), 230.0592 (8.8; -$C_{7}H_{13}O_{5}$), 245.0826 (100.0; -$C_{6}H_{10}O_{5}$), 269.0820 (0.1; -$C_{4}H_{10}O_{5}$), 407.1364 (0.1)
108	diferuloyl-Ac-Suc (helonioside-B)	22,59	$C_{34}H_{40}O_{18}$	M-H	735,2142	-1,1	13,9	15			+	0.45 ± 0.04	2	132.0211 (6.2; -$C_{26}H_{35}O_{16}$), 160.0165 (21.6; -$C_{25}H_{35}O_{15}$), 175.0404 (100.0; -$C_{24}H_{32}O_{15}$), 193.0514 (34.8; -$C_{24}H_{30}O_{14}$), 265.0725 (8.5; -$C_{21}H_{26}O_{12}$), 307.0822 (5.4; -$C_{19}H_{24}O_{11}$), 319.0835 (6.3; -$C_{18}H_{24}O_{11}$), 337.0941 (19.6; -$C_{18}H_{22}O_{10}$), 499.1455 (7.8; -$C_{12}H_{12}O_{5}$), 517.1615 (5
109	(aloe-)emodin-dianthrone-di(malonyl-Hex)-1	22,85	$C_{48}H_{46}O_{24}$	M-H	1005,2306	0,7	10,3	26	+	1.16 ± 0.26	+	0.36 ± 0.09	3	917.2516 (0.2; -$C_{2}O_{4}$)
110	*Unidentified*	22,92	$C_{25}H_{26}O_{13}$	M-H	533,1301	-0,1	24,9	13	+				4	211.0756 (0.5; -$C_{11}H_{14}O_{11}$), 255.0664 (100.0; -$C_{10}H_{14}O_{9}$), 279.0653 (1.2; -$C_{8}H_{14}O_{9}$), 297.0766 (5.8; -$C_{8}H_{12}O_{8}$), 417.1193 (85.6; -$C_{4}H_{4}O_{4}$), 471.1311 (1.3; -$CH_{2}O_{3}$), 515.1185 (1.1; -$H_{2}O$)
111	(aloe-)emodin-succinyl-Hex	22,94	$C_{25}H_{24}O_{14}$	M-H	547,1093	-0,2	25,4	14	+		+		3	269.0463 (83.6; -$C_{10}H_{14}O_{9}$), 311.0578 (5.4; -$C_{8}H_{12}O_{8}$), 413.0875 (2.3; -$C_{4}H_{6}O_{5}$), 431.0988 (100.0; -$C_{4}H_{4}O_{4}$), 529.0993 (1.6; -$C_{25}H_{23}O_{14}$), 547.1080 (1.9; -$C_{25}H_{23}O_{14}$)
112	**apigenin-7-Glu**	23,02	$C_{21}H_{20}O_{10}$	M-H	431,0984	-0,3	3,0	12	+	0.85 ± 0.10	+	0.55 ± 0.11	1	225.0559 (0.7; -$C_{7}H_{10}O_{7}$), 240.0441 (1.0; -$C_{7}H_{11}O_{6}$), 265.0505 (1.2; -$C_{5}H_{10}O_{6}$), 269.0457 (100.0; -$C_{6}H_{10}O_{5}$), 282.0540 (0.8; -$C_{5}H_{9}O_{5}$), 293.0461 (1.7; -$C_{4}H_{10}O_{5}$), 311.0565 (4.6; -$C_{4}H_{8}O_{4}$), 371.0753 (0.3; -$C_{2}H_{4}O_{2}$), 413.0880 (0.5; -$H_{2}O$), 431.0988 (51.2)
113	pinocembrine-Hex-5	23,07	$C_{21}H_{22}O_{9}$	M-H	417,1191	-0,3	16,2	11	+	1.78 ± 0.22	+	0.41 ± 0.08	2	211.0768 (0.8; -$C_{7}H_{10}O_{7}$), 239.0719 (0.3; -$C_{6}H_{10}O_{6}$), 251.0722 (0.6; -$C_{5}H_{10}O_{6}$), 255.0670 (100.0; -$C_{6}H_{10}O_{5}$), 269.0828 (0.3; -$C_{5}H_{8}O_{5}$), 279.0668 (1.2; -$C_{4}H_{10}O_{5}$), 297.0780 (5.1; -$C_{4}H_{8}O_{4}$), 399.1093 (0.5; -$H_{2}O$), 417.1201 (56.4)
114	*Unidentified*	23,15	$C_{28}H_{28}O_{16}$	M-H	619,1305	0,0	26,0	15	+				4	255.0667 (100.0; -$C_{13}H_{16}O_{12}$), 297.0764 (2.8; -$C_{11}H_{14}O_{11}$), 459.1298 (63.7; -$C_{5}H_{4}O_{6}$)
115	torachrysone-Ac-Hex-1	24,16	$C_{22}H_{26}O_{10}$	M-H	449,1453	-0,3	3,6	10	+	0.53 ± 0.12	+	0.08 ± 0.02	3	215.0354 (0.6; -$C_{10}H_{18}O_{6}$), 230.0584 (8.6; -$C_{9}H_{15}O_{6}$), 245.0820 (100.0; -$C_{8}H_{12}O_{6}$), 269.0813 (0.6; -$C_{6}H_{12}O_{6}$), 287.0924 (0.4; -$C_{6}H_{10}O_{5}$), 389.1249 (2.2; -$C_{2}H_{4}O_{2}$), 449.1422 (0.4)
116	(aloe-)emodin-anthrone-malonyl-Hex-2	24,50	$C_{24}H_{24}O_{12}$	M-H	503,1195	-0,9	21,9	13	+	28.90 ± 2.80	+	4.32 ± 0.36	3	211.0767 (0.6; -$C_{10}H_{12}O_{10}$), 227.0726 (0.5; -$C_{10}H_{12}O_{9}$), 239.0709 (0.3; -$C_{9}H_{12}O_{9}$), 251.0722 (1.0; -$C_{8}H_{12}O_{9}$), 255.0665 (100.0; -$C_{9}H_{12}O_{8}$), 267.0666 (0.4; -$C_{8}H_{12}O_{8}$), 269.0811 (0.4; -$C_{8}H_{10}O_{8}$), 279.0673 (1.2; -$C_{7}H_{12}O_{8}$), 297.0773 (4.4; -$C_{7}H_{10}O_{7}$), 399.1077 (0.8; -$C_{3}H_{4}O_{4}$), 41
117	rhein-dianthrone-di(malonyl-Hex)	24,51	$C_{48}H_{46}O_{25}$	M-H	1021,2255	-0,6	17,6	26	+				3	255.0667 (4.3; -$C_{33}H_{34}O_{21}$), 269.0462 (48.3; -$C_{33}H_{36}O_{20}$), 293.0459 (1.2; -$C_{31}H_{36}O_{20}$), 311.0570 (2.8; -$C_{31}H_{34}O_{19}$), 413.0886 (0.3; -$C_{27}H_{28}O_{16}$), 431.0989 (2.4; -$C_{27}H_{26}O_{15}$), 459.1304 (7.8; -$C_{25}H_{22}O_{15}$), 473.1097 (100.0; -$C_{25}H_{24}O_{14}$)
118	emodin-malonyl-Hex-2	24,55	$C_{24}H_{22}O_{13}$	M-H	517,0988	-0,9	2,4	14	+		+	2.34 ± 0.20	3	225.0567 (0.5; -$C_{10}H_{12}O_{10}$), 240.0435 (0.9; -$C_{10}H_{13}O_{9}$), 253.0517 (0.4; -$C_{9}H_{12}O_{9}$), 265.0510 (1.4; -$C_{8}H_{12}O_{9}$), 269.0460 (100.0; -$C_{9}H_{12}O_{8}$), 282.0538 (1.0; -$C_{8}H_{11}O_{8}$), 293.0460 (2.1; -$C_{7}H_{12}O_{8}$), 309.1141 (1.3; -$C_{5}H_{4}O_{9}$), 311.0569 (4.7; -$C_{7}H_{10}O_{7}$), 323.0579 (0.2; -$C_{6}H_{10}O_{7}$), 33
119	(aloe-)emodin-anthrone-Ac-Hex-3	24,67	$C_{23}H_{24}O_{10}$	M-H	459,1297	-0,1	27,4	12			+		3	255.0663 (100.0; -$C_{8}H_{12}O_{6}$), 267.0651 (1.7; -$C_{7}H_{12}O_{6}$), 279.0652 (2.0; -$C_{6}H_{12}O_{6}$), 297.0770 (7.4; -$C_{6}H_{10}O_{5}$), 381.0968 (1.4; -$C_{2}H_{6}O_{3}$), 399.1085 (30.7; -$C_{2}H_{4}O_{2}$), 413.1633 (1.6; -$C_{23}H_{23}O_{10}$), 417.1192 (4.0; -$C_{2}H_{2}O$), 459.1297 (56.2)
120	(aloe-)emodin-anthrone-malonyl-Hex-3	24,68	$C_{24}H_{24}O_{12}$	M-H	503,1195	-0,4	12,8	13	+				3	211.0767 (0.6; -$C_{10}H_{12}O_{10}$), 227.0726 (0.5; -$C_{10}H_{12}O_{9}$), 239.0709 (0.3; -$C_{9}H_{12}O_{9}$), 251.0722 (1.0; -$C_{8}H_{12}O_{9}$), 255.0665 (100.0; -$C_{9}H_{12}O_{8}$), 267.0666 (0.4; -$C_{8}H_{12}O_{8}$), 269.0811 (0.4; -$C_{8}H_{10}O_{8}$), 279.0673 (1.2; -$C_{7}H_{12}O_{8}$), 297.0773 (4.4; -$C_{7}H_{10}O_{7}$), 399.1077 (0.8; -$C_{3}H_{4}O_{4}$), 41
121	torachrysone-Ac-Hex-2	24,79	$C_{22}H_{26}O_{10}$	M-H	449,1453	-0,3	4,3	10	+	6.51 ± 0.84	+	1.47 ± 0.15	3	202.0639 (0.1; -$C_{10}H_{15}O_{7}$), 215.0352 (0.7; -$C_{10}H_{18}O_{6}$), 230.0582 (12.5; -$C_{9}H_{15}O_{6}$), 245.0818 (100.0; -$C_{8}H_{12}O_{6}$), 269.0811 (0.1; -$C_{6}H_{12}O_{6}$), 287.0933 (0.1; -$C_{6}H_{10}O_{5}$), 449.1461 (0.0)
122	*Unidentified*	24,97	$C_{28}H_{28}O_{16}$	M-H	619,1305	-0,2	53,4	15	+				3	255.0663 (100.0; -$C_{13}H_{16}O_{12}$), 279.0685 (1.0; -$C_{11}H_{16}O_{12}$), 297.0771 (4.0; -$C_{11}H_{14}O_{11}$), 417.1164 (4.7; -$C_{7}H_{6}O_{7}$), 459.1297 (78.1; -$C_{5}H_{4}O_{6}$)
123	nataloe-emodin-8-Me-Ac-Hex	25,50	$C_{24}H_{26}O_{11}$	M-H	489,1402	0,4	3,3	12	+	1.08 ± 0.11	+	0.43 ± 0.07	4	227.0345 (0.6; -$C_{11}H_{18}O_{7}$), 242.0585 (4.7; -$C_{10}H_{15}O_{7}$), 257.0817 (9.1; -$C_{9}H_{12}O_{7}$), 270.0530 (5.8; -$C_{9}H_{15}O_{6}$), 285.0766 (100.0; -$C_{8}H_{12}O_{6}$), 327.0882 (0.3; -$C_{6}H_{10}O_{5}$), 356.0901 (0.1; -$C_{5}H_{9}O_{4}$), 489.1408 (0.2)
124	*Unidentified*	25,56	$C_{28}H_{28}O_{16}$	M-H	619,1305	0,6	5,4	15	+		+	0.12 ± 0.02	3	255.0661 (100.0; -$C_{13}H_{16}O_{12}$), 297.0766 (3.1; -$C_{11}H_{14}O_{11}$), 399.1084 (0.5; -$C_{7}H_{8}O_{8}$), 417.1178 (2.7; -$C_{7}H_{6}O_{7}$), 441.1174 (0.5; -$C_{5}H_{6}O_{7}$), 459.1295 (64.1; -$C_{5}H_{4}O_{6}$)
125	(aloe-)emodin-dianthrone-malonyl-diHex-4	26,02	$C_{45}H_{44}O_{21}$	M-H	919,2302	1,2	17,6	24			+	0.16 ± 0.01	3	(0.7; -$C_{3}H_{2}O_{3}$), 875.2404 (2.6; -CO_{2})
126	emodin-physcion-dianthrone-di(malonyl-Hex)-1	26,05	$C_{49}H_{48}O_{24}$	M-H	1019,2463	1,4	16,4	26	+	0.38 ± 0.03	+	0.13 ± 0.01	3	225.0556 (0.8; -$C_{35}H_{38}O_{21}$), 239.0709 (0.8; -$C_{34}H_{36}O_{21}$), 254.0580 (32.8; -$C_{34}H_{37}O_{20}$), 255.0645 (11.3; -$C_{34}H_{36}O_{20}$), 268.0735 (1.6; -$C_{33}H_{35}O_{20}$), 280.0738 (0.5; -$C_{32}H_{35}O_{20}$), 296.0691 (1.1; -$C_{32}H_{35}O_{19}$), 398.0998 (0.5; -$C_{28}H_{29}O_{16}$), 416.1107 (2.8; -$C_{28}H_{27}O_{15}$), 458.1211 (100.
127	(aloe-)emodin-dianthrone-malonyl-diHex-5	26,86	$C_{45}H_{44}O_{21}$	M-H	919,2302	0,5	23,3	24	+	0.29 ± 0.07	+	0.17 ± 0.04	3	875.2412 (0.4; -CO_{2})
128	(aloe-)emodin-dianthrone-malonyl-diHex-6	27,70	$C_{45}H_{44}O_{21}$	M-H	919,2302	0,5	6,7	24	+	0.14 ± 0.03			3	671.1771 (0.5; -$C_{9}H_{12}O_{8}$), 713.1885 (0.4; -$C_{7}H_{10}O_{7}$), 833.2288 (0.1; -$C_{3}H_{2}O_{3}$), 875.2403 (0.4; -CO_{2})
129	(aloe-)emodin-dianthrone-di(malonyl-Hex)-2	27,81	$C_{48}H_{46}O_{24}$	M-H	1005,2306	1,2	11,8	26	+	2.72 ± 0.25	+	0.97 ± 0.11	3	917.2517 (0.2; -$C_{2}O_{4}$)
130	physcion-anthrone-malonyl-Hex	27,92	$C_{25}H_{26}O_{12}$	M-H	517,1352	1,4	6,0	13	+	1.78 ± 0.22	+		3	226.0643 (0.9; -$C_{11}H_{15}O_{9}$), 254.0588 (13.2; -$C_{10}H_{15}O_{8}$), 269.0820 (100.0; -$C_{9}H_{12}O_{8}$), 293.0829 (0.4; -$C_{7}H_{12}O_{8}$), 311.0925 (1.4; -$C_{7}H_{10}O_{7}$), 413.1245 (0.3; -$C_{3}H_{4}O_{4}$), 431.1343 (0.9; -$C_{3}H_{2}O_{3}$), 458.1213 (1.1; -$C_{2}H_{3}O_{2}$), 473.1447 (2.4; -CO_{2})
131	physcion-Ac-Hex-3	28,01	$C_{24}H_{24}O_{11}$	M-H	487,1246	0,7	4,2	13	+	1.67 ± 0.33	+	0.79 ± 0.11	3	240.0426 (8.1; -$C_{10}H_{15}O_{7}$), 268.0370 (2.0; -$C_{9}H_{15}O_{6}$), 279.0662 (3.8; -$C_{7}H_{12}O_{7}$), 283.0608 (100.0; -$C_{8}H_{12}O_{6}$), 292.0377 (4.5; -$C_{7}H_{15}O_{6}$), 297.0749 (1.8; -$C_{7}H_{10}O_{6}$), 307.0608 (4.0; -$C_{6}H_{12}O_{6}$), 325.0716 (0.7; -$C_{6}H_{10}O_{5}$)
132	(aloe-)emodin-dianthrone-malonyl-diHex-7	28,14	$C_{45}H_{44}O_{21}$	M-H	919,2302	0,7	12,4	24	+	0.52 ± 0.12	+	0.21 ± 0.06	3	671.1775 (0.5; -$C_{9}H_{12}O_{8}$), 713.1866 (0.4; -$C_{7}H_{10}O_{7}$), 875.2432 (0.3; -CO_{2})
133	(aloe-)emodin-dianthrone-di(malonyl-Hex)-3	28,70	$C_{48}H_{46}O_{24}$	M-H	1005,2306	1,5	14,2	26	+	2.23 ± 0.28	+	0.84 ± 0.09	3	713.1863 (0.5; -$C_{10}H_{12}O_{10}$), 875.2402 (0.1; -$C_{4}H_{2}O_{5}$), 917.2461 (0.2; -$C_{48}H_{45}O_{24}$)
134	emodin-physcion-dianthrone-di(malonyl-Hex)-2	29,01	$C_{49}H_{48}O_{24}$	M-H	1019,2463	0,9	25,1	26	+	0.36 ± 0.08	+	0.18 ± 0.02	3	225.0562 (0.9; -$C_{35}H_{38}O_{21}$), 238.0641 (0.8; -$C_{34}H_{37}O_{21}$), 254.0586 (34.4; -$C_{34}H_{37}O_{20}$), 267.0665 (1.6; -$C_{33}H_{36}O_{20}$), 280.0743 (0.6; -$C_{32}H_{35}O_{20}$), 296.0695 (1.2; -$C_{32}H_{35}O_{19}$), 398.1008 (0.5; -$C_{28}H_{29}O_{16}$), 416.1114 (3.3; -$C_{28}H_{27}O_{15}$), 441.1188 (0.4; -$C_{26}H_{26}O_{15}$), 458.1218 (100.0
135	emodin-physcion-dianthrone-di(malonyl-Hex)-3	29,11	$C_{49}H_{48}O_{24}$	M-H	1019,2463	0,3	27,7	26	+	0.36 ± 0.08		0.18 ± 0.02	3	225.0562 (0.9; -$C_{35}H_{38}O_{21}$), 238.0641 (0.8; -$C_{34}H_{37}O_{21}$), 254.0586 (34.4; -$C_{34}H_{37}O_{20}$), 267.0665 (1.6; -$C_{33}H_{36}O_{20}$), 280.0743 (0.6; -$C_{32}H_{35}O_{20}$), 296.0695 (1.2; -$C_{32}H_{35}O_{19}$), 398.1008 (0.5; -$C_{28}H_{29}O_{16}$), 416.1114 (3.3; -$C_{28}H_{27}O_{15}$), 441.1188 (0.4; -$C_{26}H_{26}O_{15}$), 458.1218 (100.0
136	*Unidentified*	29,11	$C_{21}H_{18}O_{10}$	M-H	429,0827	0,4	14,4	13	+	1.36 ± 0.13	+	0.68 ± 0.08	4	135.0294 (3.0; -$C_{17}H_{10}O_{5}$), 255.0660 (9.8; -$C_{6}H_{6}O_{6}$), 267.0666 (16.2; -$C_{5}H_{6}O_{6}$), 293.0453 (100.0; -$C_{4}H_{8}O_{5}$), 321.0404 (32.1; -$C_{3}H_{8}O_{4}$), 351.0511 (21.8; -$C_{2}H_{6}O_{3}$), 367.0814 (6.9; -$CH_{2}O_{3}$), 383.0766 (22.2; -$CH_{2}O_{2}$), 411.0740 (11.0; -$H_{2}O$)
137	emodin-dianthrone-malonyl-Hex-1	29,68	$C_{39}H_{34}O_{16}$	M-H	757,1774	1,1	27,2	23	+	1.91 ± 0.08	+	0.85 ± 0.14	3	225.0552 (0.7; -$C_{25}H_{24}O_{13}$), 239.0711 (1.0; -$C_{24}H_{22}O_{13}$), 254.0586 (32.9; -$C_{24}H_{23}O_{12}$), 267.0667 (1.6; -$C_{23}H_{22}O_{12}$), 280.0732 (0.5; -$C_{22}H_{21}O_{12}$), 296.0693 (1.2; -$C_{22}H_{21}O_{11}$), 309.0784 (0.2; -$C_{21}H_{20}O_{11}$), 340.0949 (0.3; -$C_{20}H_{17}O_{10}$), 398.1015 (0.6; -$C_{18}H_{15}O_{8}$), 416.1120 (2.4; -C
138	emodin-physcion-dianthrone-di(malonyl-Hex)-4	29,85	$C_{49}H_{48}O_{24}$	M-H	1019,2463	0,1	27,3	26	+	0.20 ± 0.03	+	0.13 ± 0.02	3	225.0565 (0.9; -$C_{35}H_{38}O_{21}$), 239.0714 (0.8; -$C_{34}H_{36}O_{21}$), 254.0588 (33.4; -$C_{34}H_{37}O_{20}$), 267.0670 (1.6; -$C_{33}H_{36}O_{20}$), 296.0694 (1.3; -$C_{32}H_{35}O_{19}$), 398.1012 (0.7; -$C_{28}H_{29}O_{16}$), 416.1116 (2.8; -$C_{28}H_{27}O_{15}$), 440.1113 (0.4; -$C_{26}H_{27}O_{15}$), 458.1220 (100.0; -$C_{26}H_{25}O_{14}$), 523.1395 (0.7
139	physcion-anthrone-Hex-4	29,87	$C_{23}H_{28}O_{8}$	M-H	431,1711	0,6	23,2	10	+	0.30 ± 0.04	+	0.23 ± 0.03	3	225.0545 (0.2; -$C_{8}H_{14}O_{6}$), 254.0586 (13.9; -$C_{7}H_{13}O_{5}$), 269.0819 (100.0; -$C_{6}H_{10}O_{5}$), 296.0706 (0.3; -$C_{5}H_{11}O_{4}$), 311.0924 (1.7; -$C_{4}H_{8}O_{4}$), 416.1105 (1.5; -CH_{3}), 431.1348 (9.2)
140	emodin-dianthrone-malonyl-Hex-2	29,93	$C_{39}H_{34}O_{16}$	M-H	757,1774	0,3	8,5	23	+	1.60 ± 0.12	+	0.36 ± 0.05	3	225.0551 (0.8; -$C_{25}H_{24}O_{13}$), 238.0642 (0.9; -$C_{24}H_{23}O_{13}$), 254.0586 (34.1; -$C_{24}H_{23}O_{12}$), 268.0736 (1.7; -$C_{23}H_{21}O_{12}$), 280.0748 (0.6; -$C_{22}H_{21}O_{12}$), 296.0698 (1.3; -$C_{22}H_{21}O_{11}$), 398.1012 (0.8; -$C_{18}H_{15}O_{8}$), 416.1112 (2.3; -$C_{18}H_{13}O_{7}$), 440.1117 (0.5; -$C_{16}H_{13}O_{7}$), 458.1219 (100.0; -C
141	*Unidentified*	30,14	$C_{19}H_{18}O_{4}$	M-H	309,1132	-1,2	62,7	11			+	0.22 ± 0.05	4	267.1021 (3.6; -$C_{2}H_{2}O$), 309.1137 (100.0)
142	emodin-physcion-dianthrone-malonyl-Hex-1	30,38	$C_{40}H_{36}O_{16}$	M-H	771,1931	0,4	12,9	23	+				3	226.0616 (0.3; -$C_{26}H_{25}O_{13}$), 254.0580 (100.0; -$C_{25}H_{25}O_{12}$), 268.0740 (1.0; -$C_{24}H_{23}O_{12}$), 416.1122 (0.7; -$C_{19}H_{15}O_{7}$), 440.1127 (0.6; -$C_{17}H_{15}O_{7}$), 523.1395 (3.3; -$C_{9}H_{12}O_{8}$), 685.1910 (0.6; -$C_{3}H_{2}O_{3}$), 727.1995 (0.7; -$C_{40}H_{35}O_{16}$)
143	emodin-dianthrone-malonyl-Hex-3	30,44	$C_{39}H_{34}O_{16}$	M-H	757,1774	-0,2	8,1	23	+	1.30 ± 0.09	+	0.86 ± 0.06	3	225.0558 (0.8; -$C_{25}H_{24}O_{13}$), 238.0642 (0.8; -$C_{24}H_{23}O_{13}$), 254.0586 (33.8; -$C_{24}H_{23}O_{12}$), 267.0663 (1.5; -$C_{23}H_{22}O_{12}$), 280.0734 (0.6; -$C_{22}H_{21}O_{12}$), 297.0775 (1.0; -$C_{22}H_{20}O_{11}$), 398.1020 (0.5; -$C_{18}H_{15}O_{8}$), 416.1110 (2.3; -$C_{18}H_{13}O_{7}$), 440.1112 (0.4; -$C_{16}H_{13}O_{7}$), 458.1215 (100.0; -C
144	*Unidentified*	30,49	$C_{11}H_{15}NO_{4}$	M-H	224,0928	-0,3	71,1	5			+		4	194.0815 (5.6; -$CH_{2}O$), 224.0922 (100.0)
145	*Unidentified*	30,54	$C_{10}H_{13}NO_{3}$	M-H	194,0823	-0,3	26,8	5			+		4	194.0823 (100.0)
146	emodin-dianthrone-Hex-6	30,56	$C_{36}H_{32}O_{13}$	M-H	671,1770	0,2	7,1	21	+		+		3	225.0552 (0.5; -$C_{22}H_{22}O_{10}$), 238.0625 (0.7; -$C_{21}H_{21}O_{10}$), 254.0585 (57.2; -$C_{21}H_{21}O_{9}$), 267.0651 (2.1; -$C_{20}H_{20}O_{9}$), 281.2456 (0.6; -$C_{36}H_{31}O_{13}$), 298.0835 (2.2; -$C_{19}H_{17}O_{8}$), 325.0716 (0.5; -$C_{18}H_{18}O_{7}$), 370.1079 (0.3; -$C_{16}H_{13}O_{6}$), 386.1013 (0.5; -$C_{16}H_{13}O_{5}$), 399.1064 (0.6; -C15H1
147	emodin-dianthrone-malonyl-Hex-4	30,64	$C_{39}H_{34}O_{16}$	M-H	757,1774	0,0	14,2	23	+	1.64 ± 0.19	+	0.74 ± 0.09	3	225.0551 (0.8; -$C_{25}H_{24}O_{13}$), 238.0642 (0.9; -$C_{24}H_{23}O_{13}$), 254.0586 (34.1; -$C_{24}H_{23}O_{12}$), 268.0736 (1.7; -$C_{23}H_{21}O_{12}$), 280.0748 (0.6; -$C_{22}H_{21}O_{12}$), 296.0698 (1.3; -$C_{22}H_{21}O_{11}$), 398.1012 (0.8; -$C_{18}H_{15}O_{8}$), 416.1112 (2.3; -$C_{18}H_{13}O_{7}$), 440.1117 (0.5; -$C_{16}H_{13}O_{7}$), 458.1219 (100.0; -C
148	torachrysone	30,76	$C_{14}H_{14}O_{4}$	M-H	245,0819	0,1	10,8	8	+		+		3	215.0357 (1.7; -$C_{2}H_{6}$), 230.0587 (49.4; -CH_{3}), 245.0821 (100.0)
149	(aloe-)emodin-anthrone-2	30,85	$C_{15}H_{12}O_{4}$	M-H	255,0663	0,9	1,4	10	+	10.48 ± 1.80	+	1.98 ± 0.18	3	187.0766 (0.1; -$C_{3}O_{2}$), 211.0761 (0.9; -CO_{2}), 227.0715 (0.1; -CO), 255.0661 (100.0)
150	emodin-physcion-dianthrone-malonyl-Hex-3	31,03	$C_{40}H_{36}O_{16}$	M-H	771,1931	0,2	20,6	23	+		+		3	225.0556 (0.8; -$C_{26}H_{26}O_{13}$), 238.0642 (0.5; -$C_{25}H_{25}O_{13}$), 254.0587 (100.0; -$C_{25}H_{25}O_{12}$), 268.0733 (1.9; -$C_{24}H_{23}O_{12}$), 280.0744 (0.5; -$C_{23}H_{23}O_{12}$), 297.0770 (0.6; -$C_{23}H_{22}O_{11}$), 416.1118 (1.4; -$C_{19}H_{15}O_{7}$), 458.1219 (37.9; -$C_{17}H_{13}O_{6}$), 491.1144 (0.3; -$C_{10}H_{16}O_{9}$), 523.1390 (4.5; -C
151	**emodin**	31,11	$C_{15}H_{10}O_{5}$	M-H	269,0455	0,9	3,1	11	+	4.77 ± 2.22	+	2.60 ± 0.14	1	197.0614 (0.1; -$C_{2}O_{3}$), 225.0558 (1.9; -CO_{2}), 241.0511 (0.7; -CO), 269.0458 (100.0)
152	18:3-LPC-1	31,65	$C_{27}H_{50}NO_{9}P$	M-H	562,3150	0,1	32,1	4			+		2	224.0692 (9.1; -$C_{20}H_{34}O_{4}$), 242.0796 (2.1; -$C_{20}H_{32}O_{3}$), 277.2168 (100.0; -$C_{9}H_{20}NO_{7}P$), 502.2938 (13.3; -$C_{2}H_{4}O_{2}$)
153	*Unidentified*	31,65	$C_{37}H_{60}O_{18}$	M-H	791,3707	-0,5	15,5	8			+		4	179.0560 (1.9; -$C_{31}H_{48}O_{12}$), 221.0677 (2.2; -$C_{29}H_{46}O_{11}$), 235.0826 (5.2; -$C_{28}H_{44}O_{11}$), 253.0936 (1.4; -$C_{28}H_{42}O_{10}$), 277.2175 (100.0; -$C_{19}H_{30}O_{16}$), 287.0781 (1.3; -$C_{25}H_{44}O_{10}$), 305.0883 (1.5; -$C_{25}H_{42}O_{9}$), 323.0995 (1.4; -$C_{25}H_{40}O_{8}$), 379.1248 (1.0; -$C_{22}H_{36}O_{7}$), 397.1358 (34.3; -C
154	18:3-DGMG-2	31,72	$C_{33}H_{56}O_{14}$	M+HCOOH-H	721,3652	0,3	11,1	6	+	0.26 ± 0.08	+	0.21 ± 0.03	2	179.0561 (1.5; -$C_{28}H_{46}O_{10}$), 221.0662 (1.4; -$C_{26}H_{44}O_{9}$), 235.0820 (5.8; -$C_{25}H_{42}O_{9}$), 245.0673 (0.9; -$C_{24}H_{44}O_{9}$), 253.0926 (1.7; -$C_{25}H_{40}O_{8}$), 263.0782 (1.6; -$C_{24}H_{42}O_{8}$), 277.2169 (100.0; -$C_{16}H_{28}O_{14}$), 287.0777 (1.0; -$C_{22}H_{42}O_{8}$), 305.0884 (1.3; -$C_{22}H_{40}O_{7}$), 323.0992 (0.6; -C22H3
155	emodin-dianthrone-1?	31,78	$C_{30}H_{22}O_{8}$	M-H	509,1242	0,5	6,8	20	+	1.20 ± 0.13	+	0.58 ± 0.10	3	227.2010 (0.7; -$C_{30}H_{21}O_{8}$), 254.0588 (100.0; -$C_{15}H_{11}O_{4}$), 279.2353 (0.6; -$C_{30}H_{21}O_{8}$), 421.4051 (0.6; -$C_{30}H_{21}O_{8}$), 491.1142 (0.6; -$H_{2}O$)
156	18:3-LPC-2	31,84	$C_{27}H_{50}NO_{9}P$	M-H	562,3150	0,7	18,7	4	+		+		2	224.0692 (9.1; -$C_{20}H_{34}O_{4}$), 242.0796 (2.1; -$C_{20}H_{32}O_{3}$), 277.2168 (100.0; -$C_{9}H_{20}NO_{7}P$), 502.2938 (13.3; -$C_{2}H_{4}O_{2}$)
157	18:3-LPE	31,84	$C_{23}H_{42}NO_{7}P$	M-H	474,2626	0,5	24,3	4	+		+		2	196.0379 (5.9; -$C_{18}H_{30}O_{2}$), 214.0470 (1.5; -$C_{18}H_{28}O$), 277.2169 (100.0; -$C_{5}H_{12}NO_{5}P$), 474.2628 (2.7)
158	*Unidentified*	31,99	$C_{31}H_{50}O_{13}$	M-H	629,3179	-0,1	23,9	7	+		+	0.28 ± 0.05	4	253.0923 (8.3; -$C_{22}H_{32}O_{5}$), 277.2171 (100.0; -$C_{13}H_{20}O_{11}$)
159	emodin-dianthrone-2	32,03	$C_{30}H_{22}O_{8}$	M-H	509,1242	0,0	7,5	20	+	1.22 ± 0.06	+	0.43 ± 0.07	3	225.0555 (0.2; -$C_{16}H_{12}O_{5}$), 254.0585 (100.0; -$C_{15}H_{11}O_{4}$), 281.2493 (0.2; -$C_{30}H_{21}O_{8}$), 465.1284 (0.2; -$C_{30}H_{21}O_{8}$), 491.1117 (0.4; -$H_{2}O$)
160	18:2-LPC-1	32,18	$C_{27}H_{52}NO_{9}P$	M-H	564,3307	0,2	9,9	3	+	0.24 ± 0.03	+		2	224.0693 (3.7; -$C_{20}H_{36}O_{4}$), 242.0790 (2.5; -$C_{20}H_{34}O_{3}$), 279.2326 (100.0; -$C_{9}H_{20}NO_{7}P$), 504.3090 (6.3; -$C_{2}H_{4}O_{2}$)
161	18:3-MGMG-1	32,18	$C_{27}H_{46}O_{9}$	M+HCOOH-H	559,3124	0,5	10,5	5	+	0.32 ± 0.04	+	0.51 ± 0.11	2	235.0823 (0.5; -$C_{19}H_{32}O_{4}$), 253.0932 (7.0; -$C_{19}H_{30}O_{3}$), 277.2170 (100.0; -$C_{10}H_{18}O_{9}$)
162	18:2-LPE-1	32,19	$C_{23}H_{44}NO_{7}P$	M-H	476,2783	0,1	4,1	3	+	0.15 ± 0.02			2	196.0374 (0.9; -$C_{18}H_{32}O_{2}$), 214.0492 (1.5; -$C_{18}H_{30}O$), 279.2330 (100.0; -$C_{5}H_{12}NO_{5}P$), 476.2774 (0.6)
163	*Unidentified*	32,25	$C_{18}H_{12}O_{4}$	M-H	291,0663	-0,7	12,8	13	+		+		4	221.1553 (0.2; -$C_{18}H_{11}O_{4}$), 236.1039 (0.3; -$C_{18}H_{11}O_{4}$), 247.0777 (0.5; -CO_{2}), 291.0665 (100.0)
164	18:2-LPC-2	32,29	$C_{27}H_{52}NO_{9}P$	M-H	564,3307	0,5	5,9	3	+	1.69 ± 0.22	+	0.80 ± 0.06	2	224.0694 (8.0; -$C_{20}H_{36}O_{4}$), 242.0796 (2.2; -$C_{20}H_{34}O_{3}$), 279.2328 (100.0; -$C_{9}H_{20}NO_{7}P$), 504.3095 (14.0; -$C_{2}H_{4}O_{2}$)
165	ketostearic acid-1	32,29	$C_{18}H_{34}O_{3}$	M-H	297,2435	0,3	20,6	2	+		+		3	183.1386 (17.1; -$C_{7}H_{14}O$), 279.2322 (8.7; -$H_{2}O$), 297.2434 (100.0)
166	18:2-LPE-2	32,33	$C_{23}H_{44}NO_{7}P$	M-H	476,2783	0,3	15,5	3	+	1.19 ± 0.15	+	0.67 ± 0.05	2	196.0382 (5.7; -$C_{18}H_{32}O_{2}$), 214.0488 (1.4; -$C_{18}H_{30}O$), 279.2327 (100.0; -$C_{5}H_{12}NO_{5}P$), 476.2777 (3.6)
167	hydroxy-hexadecanedioic-acid-1	32,43	$C_{16}H_{30}O_{5}$	M-H	301,2020	0,6	14,6	2	+		+		3	239.2012 (68.8; -$CH_{2}O_{3}$), 283.1886 (100.0; -$H_{2}O$)
168	16:0-DGMG	32,54	$C_{31}H_{58}O_{14}$	M+HCOOH-H	699,3809	0,1	11,6	3	+		+		2	235.0819 (8.0; -$C_{23}H_{44}O_{9}$), 255.2328 (100.0; -$C_{16}H_{28}O_{14}$), 281.2484 (89.8; -$C_{14}H_{26}O_{14}$), 397.1356 (32.4; -$C_{17}H_{34}O_{4}$), 415.1455 (15.9; -$C_{17}H_{32}O_{3}$)
169	(aloe-)emodin-physcion-dianthrone-1	32,57	$C_{31}H_{24}O_{8}$	M-H	523,1398	-0,2	5,5	20	+		+		3	254.0583 (100.0; -$C_{16}H_{13}O_{4}$)
170	18:2-MGMG	32,62	$C_{27}H_{48}O_{9}$	M+HCOOH-H	561,3280	0,4	35,2	4	+		+		2	253.0937 (5.8; -$C_{8}H_{38}O_{11}$), 279.2327 (100.0; -$C_{10}H_{18}O_{9}$)
171	*Unidentified*	32,64	$C_{49}H_{78}O_{14}$	M-H	889,5319	-0,2	146,2	11			+	0.84 ± 0.27	4	235.0826 (0.7; -$C_{40}H_{62}O_{7}$), 253.0929 (4.1; -$C_{40}H_{60}O_{6}$), 277.2175 (100.0; -$C_{31}H_{48}O_{12}$)
172	(aloe-)emodin-physcion-dianthrone-2	32,67	$C_{31}H_{24}O_{8}$	M-H	523,1398	-2,9	28,9	20	+	0.53 ± 0.25	+		3	254.0583 (100.0; -$C_{16}H_{13}O_{4}$)
173	16:0-LPC-2	32,71	$C_{25}H_{52}NO_{9}P$	M-H	540,3307	0,4	8,7	1	+	0.92 ± 0.43	+	0.94 ± 0.30	2	224.0691 (8.7; -$C_{18}H_{36}O_{4}$), 242.0804 (2.3; -$C_{18}H_{34}O_{3}$), 255.2328 (100.0; -$C_{9}H_{20}NO_{7}P$), 480.3096 (22.4; -$C_{2}H_{4}O_{2}$)
174	16:0-LPE-2	32,71	$C_{21}H_{44}NO_{7}P$	M-H	452,2783	0,3	3,8	1	+	0.64 ± 0.30	+	0.68 ± 0.22	2	196.0379 (6.2; -$C_{16}H_{32}O_{2}$), 214.0479 (1.4; -$C_{16}H_{30}O$), 255.2328 (100.0; -$C_{5}H_{12}NO_{5}P$), 452.2779 (5.4)
175	21:0-LPS	32,74	$C_{27}H_{54}NO_{9}P$	M-H	566,3463	0,7	11,5	2	+		+	0.14 ± 0.09	2	224.0695 (8.3; -$C_{20}H_{38}O_{4}$), 242.0807 (2.1; -$C_{20}H_{36}O_{3}$), 281.2488 (100.0; -$C_{9}H_{20}NO_{7}P$), 506.3255 (15.5; -$C_{2}H_{4}O_{2}$)
176	*Unidentified*	32,81	$C_{31}H_{48}O_{7}$	M-H	531,3327	0,9	39,0	8	+				4	415.3218 (100.0; -$C_{4}H_{4}O_{4}$), 531.3328 (7.0)
177	36:5-MGDG	32,83	$C_{45}H_{76}O_{10}$	M+HCOOH-H	821,5421	-0,9	76,4	8			+		2	253.0944 (7.5; -$C_{37}H_{60}O_{4}$), 277.2177 (97.0; -$C_{28}H_{48}O_{10}$), 279.2330 (100.0; -$C_{28}H_{46}O_{10}$)
178	*Unidentified*	32,88	$C_{22}H_{36}O_{6}$	M-H	395,2439	-1,9	27,3	5			+		4	279.2334 (100.0; -$C_{4}H_{4}O_{4}$)
179	*Unidentified*	32,92	$C_{25}H_{42}O_{8}$	M-H	469,2807	0,0	28,6	5	+				4	279.2323 (100.0; -$C_{7}H_{10}O_{6}$), 451.2708 (5.6; -$H_{2}O$)

Supplementary table S2

No	Name	RT_min	Formula	Adduct	Precursor	Err. ppm	mσ	rdb	R.rhabarbarum	[mg/g d.w.]	R.rhaponticum	[mg/g d.w.]	ID level	NEG MS2 Spectrum
1	Hex-Hex	1,12	$C_{12}H_{22}O_{11}$	M+HCOOH-H	387,1144	-0,4	1,8	2	+	23.7 ± 1.58	+	110.9 ± 6.11	3	179.0563 (18.5; $-C_7H_{12}O_7$), 281.0883 (0.6; $-C_3H_6O_4$), 341.1090 (100.0; $-CH_2O_2$), 161.0452 (2.2; $-C_7H_{14}O_8$)
2	malate	1,28	$C_4H_6O_5$	M-H	133,0142	1,5	6,0	2	+	4.6 ± 0.44	+		2	133.0122 (100.0), 115.0023 (83.0; $-H_2O$)
3	citrate	1,43	$C_6H_8O_7$	M-H	191,0197	0,2	8,2	3	+	0.8 ± 0.15	+	1.5 ± 0.11	2	111.0091 (22.3; $-CH_4O_4$), 129.0168 (12.4; $-CH_2O_3$), 154.9965 (7.4; $-H_4O_2$), 173.0107 (15.1; $-H_2O$), 191.0200 (100.0)
4	**tyrosine**	1,68	$C_9H_{11}NO_3$	M-H	180,0666	1,7	3,7	5	+	0.6 ± 0.03	+	0.8 ± 0.12	1	119.0479 (18.0; $-CH_3NO_2$), 163.0401 (41.4; $-H_3N$), 180.0671 (100.0)
5	**beta-glucogallin**	1,92	$C_{13}H_{16}O_{10}$	M-H	331,0670	0,7	2,9	6	+	31.2 ± 2.39	+	6.4 ± 0.23	1	169.0136 (24.5; $-C_6H_{10}O_5$), 211.0245 (20.2; $-C_4H_8O_4$), 271.0458 (37.2; $-C_2H_4O_2$), 331.0667 (100.0)
6	beta-glucogallin-2	2,21	$C_{13}H_{16}O_{10}$	M-H	331,0670	1,2	13,6	6	+	1.0 ± 0.14	+	3.0 ± 0.28	3	169.0143 (55.5; $-C_6H_{10}O_5$), 271.0463 (17.9; $-C_2H_4O_2$), 331.0669 (100.0)
7	**gallate**	2,41	$C_7H_6O_5$	M-H	169,0142	4,4	11,8	5	+	1.2 ± 0.24	+		1	125.0229 (73.7; $-CO_2$), 169.0135 (100.0)
8	beta-glucogallin-3	2,44	$C_{13}H_{16}O_{10}$	M-H	331,0670	0,9	13,8	6	+	1.4 ± 0.11	+	4.5 ± 0.35	3	169.0129 (12.0; $-C_6H_{10}O_5$), 211.0239 (26.8; $-C_4H_8O_4$), 241.0355 (12.6; $-C_3H_6O_3$), 271.0455 (100.0; $-C_2H_4O_2$), 331.0649 (6.9)
9	*Unidentified*	2,49	$C_{13}H_{16}O_8$	M-H	299,0772	0,6	21,6	6			+		4	137.0215 (100.0; $-C_6H_{10}O_5$)
10	beta-glucogallin-4	3,93	$C_{13}H_{16}O_{10}$	M-H	331,0670	0,4	17,1	6			+		3	125.0233 (12.5; $-C_7H_{10}O_7$), 169.0139 (100.0; $-C_6H_{10}O_5$), 331.0639 (4.1)
11	tyrosol-Hex-1	4,31	$C_{14}H_{20}O_7$	M+HCOOH-H	345,1191	0,7	9,8	5			+		3	137.0650 (65.4; $-C_7H_{12}O_7$), 299.0776 (100.0; $-C_2H_6O$)
12	digalloyl-Hex-2	4,49	$C_{20}H_{20}O_{14}$	M-H	483,0780	0,0	4,5	11	+				3	125.0230 (13.7; $-C_{14}H_{14}O_{11}$), 169.0137 (100.0; $-C_{13}H_{14}O_9$), 313.0566 (14.1; $-C_7H_6O_5$), 331.0675 (4.3; $-C_7H_4O_4$), 483.0770 (0.9)
13	saliciloyl-Hex-1	4,73	$C_{13}H_{16}O_8$	M-H	299,0772	0,0	13,0	6	+				3	137.0226 (24.8; $-C_6H_{10}O_5$), 179.0338 (42.2; $-C_4H_8O_4$), 209.0439 (7.7; $-C_3H_6O_3$), 239.0566 (59.2; $-C_2H_4O_2$), 299.0771 (100.0)
14	*Unidentified*	5,04	$C_{13}H_{16}O_8$	M-H	299,0772	0,7	20,1	6			+		4	137.0220 (100.0; $-C_6H_{10}O_5$), 299.0781 (45.4)
15	digalloyl-Hex-4	5,44	$C_{20}H_{20}O_{14}$	M-H	483,0780	1,7	20,9	11	+		+		3	169.0135 (16.6; $-C_{13}H_{14}O_9$), 193.0142 (7.5; $-C_{11}H_{14}O_9$), 211.0245 (34.2; $-C_{11}H_{12}O_8$), 241.0347 (8.3; $-C_{10}H_{10}O_7$), 253.0350 (1.6; $-C_9H_{10}O_7$), 271.0454 (100.0; $-C_9H_8O_6$), 295.0443 (3.3; $-C_{20}H_{19}O_{14}$), 313.0562 (47.7; $-C_{20}H_{19}O_{14}$), 331.0668 (13.8; $-C_{20}H_{19}O_{14}$), 423
16	*Unidentified*	5,48	$C_{13}H_{16}O_8$	M-H	299,0772	0,9	4,0	6			+		4	137.0220 (100.0; $-C_6H_{10}O_5$), 299.0781 (44.2)
17	**Trp**	5,74	$C_{11}H_{12}N_2O_2$	M-H	203,0826	0,1	7,0	7	+	0.9 ± 0.11	+	0.8 ± 0.05	1	116.0498 (12.6; $-C_3H_5NO_2$), 142.0641 (6.6; $-CH_3NO_2$), 159.0903 (4.3; $-CO_2$), 203.0828 (100.0)
18	*Unidentified*	5,90	$C_{13}H_{16}O_8$	M-H	299,0772	1,1	1,6	6			+		4	137.0220 (100.0; $-C_6H_{10}O_5$), 299.0781 (44.2)
19	tyrosol-Hex-2	6,21	$C_{14}H_{20}O_7$	M+HCOOH-H	345,1191	-0,3	18,1	5			+		3	137.0650 (65.4; $-C_7H_{12}O_7$), 299.0776 (100.0; $-C_2H_6O$)
20	catechin-Hex-2	6,48	$C_{21}H_{24}O_{11}$	M-H	451,1245	-0,4	12,0	10	+				3	245.0820 (27.3; $-C_7H_{10}O_7$), 289.0723 (100.0; $-C_6H_{10}O_5$)
21	*Unidentified*	6,88	$C_{17}H_{28}O_{13}$	M-H	439,1457	2,1	7,4	4	+	0.2 ± 0.12			4	131.0321 (9.7; $-C_{12}H_{20}O_9$), 149.0429 (16.6; $-C_{12}H_{18}O_8$), 191.0556 (32.2; $-C_{10}H_{16}O_7$), 233.0671 (27.8; $-C_8H_{14}O_6$), 293.0876 (100.0; $-C_6H_{10}O_4$), 313.0563 (13.2; $-C_4H_{14}O_4$)
22	**procyanidin-B1**	6,88	$C_{30}H_{26}O_{12}$	M-H	577,1351	0,2	0,7	18	+	0.2 ± 0.11	+		1	125.0236 (5.7; $-C_{24}H_{20}O_9$), 161.0235 (4.0; $-C_{21}H_{20}O_9$), 245.0819 (23.1; $-C_{16}H_{12}O_8$), 289.0719 (100.0; $-C_{15}H_{12}O_6$), 339.0879 (12.3; $-C_{11}H_{10}O_6$), 381.0983 (5.6; $-C_9H_8O_5$), 407.0774 (73.2; $-C_8H_{10}O_4$), 425.0882 (5.0; $-C_8H_8O_3$), 451.1038 (3.0; $-C_6H_6O_3$), 577.1366 (
23	*Unidentified*	6,98	$C_{12}H_8O_7$	M-H	263,0197	2,7	10,6	9	+				4	121.0276 (100.0; $-C_7H_{12}O_7$)
24	*Unidentified*	7,30	$C_{11}H_{14}O_7S$	M-H	289,0387	2,3	3,4	5	+				4	121.0277 (100.0; $-C_{12}H_{20}O_{11}$), 191.0561 (14.7; $-C_{12}H_{14}O_7$), 233.0667 (6.0; $-C_{10}H_{12}O_6$), 271.0441 (6.5; $-C_8H_{14}O_5$), 293.0875 (17.5; $-C_8H_8O_4$), 415.1248 (5.5; $-CH_2O_2$)
25	*Unidentified*	7,33	$C_{12}H_{22}O_8$	M-H	293,1241	0,2	1,8	2			+		4	131.0701 (9.6; $-C_6H_{10}O_5$), 173.0795 (5.5; $-C_4H_8O_4$), 293.1248 (100.0)
26	*Unidentified*	7,40	$C_{21}H_{22}O_{11}$	M-H	449,1089	0,0	5,2	11			+	0.5 ± 0.05	4	163.0026 (1.4; $-C_{13}H_{18}O_7$), 225.0556 (100.0; $-C_7H_{12}O_8$), 243.0641 (0.9; $-C_7H_{10}O_7$), 269.0461 (5.4; $-C_6H_{12}O_6$), 357.1559 (0.6; $-C_{21}H_{21}O_{11}$)
27	**procyanidin-B3**	7,44	$C_{30}H_{26}O_{12}$	M-H	577,1351	-0,3	4,5	18	+				1	125.0238 (5.9; $-C_{24}H_{20}O_9$), 161.0243 (4.3; $-C_{21}H_{20}O_9$), 245.0821 (22.7; $-C_{16}H_{12}O_8$), 289.0721 (100.0; $-C_{15}H_{12}O_6$), 339.0874 (15.9; $-C_{11}H_{10}O_6$), 381.0988 (6.3; $-C_9H_8O_5$), 407.0777 (88.6; $-C_8H_{10}O_4$), 425.0885 (6.6; $-C_8H_8O_3$), 451.1030 (2.8; $-C_6H_6O_3$)
28	digalloyl-Hex-5	7,60	$C_{20}H_{20}O_{14}$	M-H	483,0780	-0,9	4,2	11	+	6.1 ± 0.43	+	8.5 ± 0.46	3	169.0139 (13.5; $-C_{13}H_{14}O_9$), 211.0250 (11.8; $-C_{11}H_{12}O_8$), 241.0357 (7.5; $-C_{10}H_{10}O_7$), 271.0462 (100.0; $-C_9H_8O_6$), 313.0570 (49.3; $-C_7H_6O_5$), 331.0676 (18.4; $-C_7H_4O_4$), 483.0784 (38.3)
29	galloyl-arbutin	7,81	$C_{19}H_{20}O_{11}$	M-H	423,0932	-0,3	8,5	10			+		3	169.0131 (5.2; $-C_{12}H_{14}O_6$), 211.0257 (1.5; $-C_{10}H_{12}O_5$), 241.0343 (1.0; $-C_9H_{10}O_4$), 253.0343 (1.1; $-C_8H_{10}O_4$), 271.0444 (1.2; $-C_8H_8O_3$), 313.0561 (25.0; $-C_6H_6O_2$), 423.0931 (100.0)
30	**catechin**	7,83	$C_{15}H_{14}O_6$	M-H	289,0717	-0,9	3,5	9	+	10.1 ± 0.84	+	1.8 ± 0.11	1	179.0351 (7.3; $-C_6H_6O_2$), 203.0716 (24.6; $-C_3H_2O_3$), 221.0822 (15.7; $-C_3O_2$), 245.0822 (70.9; $-CO_2$), 271.0617 (4.4; $-H_2O$), 289.0722 (100.0)
31	*Unidentified*	7,98	$C_{21}H_{22}O_{11}$	M-H	449,1089	-0,8	18,0	11			+	1.2 ± 0.08	4	225.0557 (100.0; $-C_7H_{12}O_8$), 269.0460 (4.6; $-C_6H_{12}O_6$), 289.0729 (1.7; $-C_6H_8O_5$)
32	*Unidentified*	8,05	$C_{17}H_{30}O_{13}$	M-H	441,1613	1,8	1,6	3	+	1.2 ± 0.29			4	149.0449 (16.9; $-C_{12}H_{20}O_8$), 191.0560 (32.1; $-C_{10}H_{18}O_7$), 233.0667 (31.4; $-C_8H_{16}O_6$), 293.0875 (100.0; $-C_6H_{12}O_4$), 351.0005 (10.8; $-C_3H_{22}O_2$)
33	digalloyl-Hex-7	8,08	$C_{20}H_{20}O_{14}$	M-H	483,0780	1,9	4,3	11	+	3.1 ± 0.30	+	1.5 ± 0.07	3	169.0140 (13.4; $-C_{13}H_{14}O_9$), 211.0250 (10.5; $-C_{11}H_{12}O_8$), 241.0357 (7.4; $-C_{10}H_{10}O_7$), 271.0462 (100.0; $-C_9H_8O_6$), 313.0570 (49.7; $-C_7H_6O_5$), 331.0677 (18.5; $-C_7H_4O_4$), 483.0785 (39.0)
34	coumaroyl-Hex-3	8,15	$C_{15}H_{18}O_8$	M-H	325,0928	-2,1	17,0	7	+	2.1 ± 0.28	+		3	145.0280 (100.0; $-C_6H_{12}O_6$), 187.0411 (29.6; $-C_4H_{10}O_5$), 205.0510 (15.5; $-C_4H_8O_4$), 325.0906 (34.5)
35	*Unidentified*	8,30	$C_{20}H_{18}O_{16}$	M-H	513,0522	1,9	38,5	12	+				4	169.0136 (74.6; $-C_{13}H_{12}O_{11}$), 191.0352 (45.1; $-C_{10}H_{10}O_{12}$), 207.0301 (42.9; $-C_{10}H_{10}O_{11}$), 223.0246 (67.7; $-C_{10}H_{10}O_{10}$), 235.0249 (100.0; $-C_9H_{10}O_{10}$), 253.0354 (36.1; $-C_9H_8O_9$), 279.0143 (19.8; $-C_8H_{10}O_8$)
36	resveratrol-diHex-1	8,61	$C_{26}H_{32}O_{13}$	M+HCOOH-H	597,1824	1,2	5,7	11	+	0.6 ± 0.17	+	0.7 ± 0.03	3	227.0709 (100.0; $-C_{13}H_{22}O_{12}$), 389.1233 (10.1; $-C_7H_{12}O_7$)
37	coumaroyl-Hex-4	8,80	$C_{15}H_{18}O_8$	M-H	325,0928	2,3	13,0	7	+		+		3	119.0484 (4.1; $-C_7H_{10}O_7$), 145.0282 (10.0; $-C_6H_{12}O_6$), 163.0391 (19.8; $-C_6H_{10}O_5$), 177.0555 (3.2; $-C_5H_8O_5$), 205.0504 (47.6; $-C_4H_8O_4$), 235.0612 (16.5; $-C_3H_6O_3$), 265.0718 (100.0; $-C_2H_4O_2$), 325.0944 (1.8)
38	*Unidentified*	8,80	$C_{13}H_{16}O_7$	M+HCOOH-H	329,0878	2,2	3,3	6	+				4	207.0654 (100.0; $-C_3H_6O_5$)
39	digalloyl-Hex-8	8,86	$C_{20}H_{20}O_{14}$	M-H	483,0780	-0,8	1,2	11			+	0.7 ± 0.06	3	169.0140 (53.4; $-C_{13}H_{14}O_9$), 313.0569 (100.0; $-C_7H_6O_5$), 331.0674 (14.3; $-C_7H_4O_4$), 483.0783 (7.7)
40	benzoyl-Hex-Pent	8,93	$C_{18}H_{24}O_{11}$	M+HCOOH-H	461,1300	2,0	2,9	7	+	1.5 ± 0.05			3	121.0277 (100.0; $-C_{12}H_{20}O_{11}$), 191.0561 (14.7; $-C_{12}H_{14}O_7$), 233.0667 (6.0; $-C_{10}H_{12}O_6$), 271.0441 (6.5; $-C_8H_{14}O_5$), 293.0875 (17.5; $-C_8H_8O_4$), 415.1248 (5.5; $-CH_2O_2$)
41	piceatannol/oxyresveratrol-diHex-1	9,12	$C_{26}H_{32}O_{14}$	M+HCOOH-H	613,1774	-1,1	28,3	11			+		3	243.0656 (100.0; $-C_{13}H_{22}O_{12}$), 315.0868 (0.8; $-C_{10}H_{18}O_{10}$), 405.1188 (17.1; $-C_7H_{12}O_7$)
42	**procyanidin-B2**	9,25	$C_{30}H_{26}O_{12}$	M-H	577,1351	-0,8	6,6	18	+		+	1.3 ± 0.18	1	125.0232 (5.1; $-C_{24}H_{20}O_9$), 161.0227 (4.3; $-C_{21}H_{20}O_9$), 245.0812 (25.6; $-C_{16}H_{12}O_8$), 289.0711 (100.0; $-C_{15}H_{12}O_6$), 339.0871 (16.4; $-C_{11}H_{10}O_6$), 381.0966 (6.2; $-C_9H_8O_5$), 407.0765 (79.9; $-C_8H_{10}O_4$)
43	hydroxybenozyl-galloyl-Hex-1	9,27	$C_{20}H_{20}O_{12}$	M-H	451,0882	-1,8	4,2	11			+	0.7 ± 0.08	3	137.0224 (2.0; $-C_{13}H_{14}O_9$), 169.0141 (11.1; $-C_{13}H_{14}O_7$), 241.0358 (3.4; $-C_{10}H_{10}O_5$), 253.0357 (1.8; $-C_9H_{10}O_5$), 313.0571 (100.0; $-C_7H_6O_3$), 407.0987 (4.9; $-CO_2$), 451.0891 (30.1)
44	feruloyl-Hex-1	9,36	$C_{16}H_{20}O_9$	M-H	355,1034	-2,1	9,0	7	+		+		3	160.0160 (12.9; $-C_7H_{15}O_6$), 175.0402 (100.0; $-C_6H_{12}O_6$), 193.0509 (16.1; $-C_6H_{10}O_5$), 235.0613 (8.4; $-C_4H_8O_4$), 295.0828 (4.8; $-C_2H_4O_2$)
45	aloesone/cassiachromone-Hex-1	9,43	$C_{19}H_{22}O_9$	M-H	393,1191	-1,6	4,3	9			+		3	189.0563 (4.4; $-C_8H_{12}O_6$), 231.0667 (100.0; $-C_6H_{10}O_5$), 393.1209 (3.1)
46	*Unidentified*	9,44	$C_{17}H_{24}O_9$	M-H	371,1347	2,3	5,5	6	+	1.1 ± 0.24			4	163.0754 (100.0; $-C_7H_{12}O_7$), 211.0958 (19.7; $-C_6H_8O_5$)
47	*Unidentified*	9,48	$C_{18}H_{30}O_9$	M-H	389,1817	-0,8	15,3	4			+		4	181.0888 (1.1; $-C_8H_{16}O_6$), 209.1198 (2.5; $-C_6H_{12}O_6$), 227.1287 (7.2; $-C_6H_{10}O_5$), 371.1694 (1.7; $-H_2O$), 389.1817 (100.0)
48	aloesol-Hex-1	9,65	$C_{19}H_{24}O_9$	M+HCOOH-H	441,1402	-1,9	6,5	8			+		3	189.0565 (7.4; $-C_9H_{16}O_8$), 215.0736 (2.1; $-C_7H_{14}O_8$), 233.0824 (100.0; $-C_7H_{12}O_7$)
49	coumaroyl-Hex-5	9,72	$C_{15}H_{18}O_8$	M-H	325,0928	1,9	10,8	7	+		+		3	145.0282 (13.5; $-C_6H_{12}O_6$), 163.0397 (24.3; $-C_6H_{10}O_5$), 205.0510 (63.1; $-C_4H_8O_4$), 235.0616 (18.7; $-C_3H_6O_3$), 265.0719 (100.0; $-C_2H_4O_2$), 325.0919 (2.1)
50	*Unidentified*	9,91	$C_{21}H_{22}O_{13}$	M-H	481,0987	-2,0	4,9	11	+		+		4	169.0140 (12.2; $-C_{14}H_{16}O_8$), 241.0360 (5.3; $-C_{11}H_{12}O_6$), 313.0574 (100.0; $-C_8H_8O_4$), 437.1084 (8.6; $-CO_2$), 481.0998 (23.1)
51	piceatannol/oxyresveratrol-diHex-2	10,02	$C_{26}H_{32}O_{14}$	M+HCOOH-H	613,1774	2,0	9,7	11	+	0.9 ± 0.09	+	1.3 ± 0.02	3	201.0549 (0.2; $-C_{15}H_{24}O_{13}$), 243.0665 (100.0; $-C_{13}H_{22}O_{12}$), 285.0745 (0.2; $-C_{11}H_{20}O_{11}$), 405.1197 (73.3; $-C_7H_{12}O_7$), 427.0774 (0.4; $-C_{27}H_{33}O_{16}$), 567.1724 (2.2; $-CH_2O_2$)
52	*Unidentified*	10,14	$C_{10}H_{14}O_5S$	M-H	245,0489	2,1	7,9	4	+				4	165.0921 (0.8; $-O_3S$), 245.0490 (100.0)
53	feruloyl-Hex-5	10,19	$C_{16}H_{20}O_9$	M-H	355,1034	-1,3	10,2	7			+		3	175.0398 (45.1; $-C_6H_{12}O_6$), 193.0510 (63.2; $-C_6H_{10}O_5$), 235.0612 (100.0; $-C_4H_8O_4$), 265.0712 (18.6; $-C_3H_6O_3$), 295.0822 (71.4; $-C_2H_4O_2$)
54	**epi-catechin**	10,20	$C_{15}H_{14}O_6$	M-H	289,0717	-1,4	6,7	9			+		1	179.0354 (14.6; $-C_6H_6O_2$), 203.0732 (30.3; $-C_3H_2O_3$), 221.0835 (20.6; $-C_3O_2$), 227.0738 (11.0; $-CH_2O_3$), 245.0824 (78.4; $-CO_2$), 289.0726 (100.0)
55	methyl-butyl-Hex-Pent	10,27	$C_{16}H_{30}O_{10}$	M+HCOOH-H	427,1821	1,2	8,7	2	+		+		3	233.0657 (7.1; $-C_8H_{18}O_5$), 249.1343 (80.1; $-C_6H_{10}O_6$), 381.1766 (100.0; $-CH_2O_2$)
56	*Unidentified*	10,31	$C_{21}H_{22}O_{11}$	M-H	449,1089	-1,3	14,4	11		0.2 ± 0.04	+		4	125.0238 (4.8; $-C_{15}H_{16}O_8$), 178.9979 (7.0; $-C_{13}H_{18}O_6$), 243.0661 (6.9; $-C_7H_{10}O_7$), 259.0609 (100.0; $-C_7H_{10}O_6$), 269.0453 (99.6; $-C_6H_{12}O_6$), 287.0563 (74.3; $-C_6H_{10}O_5$), 313.0567 (12.9; $-C_8H_8O_2$), 342.0591 (4.8; $-C_7H_7O$), 449.1087 (32.9)
57	trigalloyl-Hex	10,38	$C_{27}H_{24}O_{18}$	M-H	635,0889	-0,8	5,2	16	+	0.2 ± 0.01			3	169.0142 (14.1; $-C_{20}H_{18}O_{13}$), 211.0250 (5.1; $-C_{18}H_{16}O_{12}$), 295.0465 (8.4; $-C_{14}H_{12}O_{10}$), 313.0571 (82.8; $-C_{14}H_{10}O_9$), 465.0682 (100.0; $-C_7H_6O_5$)
58	*Unidentified*	10,40	$C_{21}H_{22}O_{10}$	M+HCOOH-H	479,1195	0,3	12,1	11	+	0.2 ± 0.02			4	149.0231 (2.9; $-C_{14}H_{18}O_9$), 255.0666 (100.0; $-C_7H_{12}O_8$), 273.0775 (7.4; $-C_7H_{10}O_7$)
59	*Unidentified*	10,53	$C_{17}H_{28}O_{11}$	M+HCOOH-H	453,1613	0,7	11,8	4	+				4	131.0333 (54.1; $-C_{13}H_{22}O_9$), 149.0435 (39.7; $-C_{13}H_{20}O_8$), 293.0893 (100.0; $-C_7H_{12}O_4$)
60	*Unidentified*	10,58	$C_{21}H_{22}O_{12}$	M-H	465,1038	-1,5	8,9	11	+				4	169.0143 (13.7; $-C_{14}H_{16}O_7$), 313.0571 (50.1; $-C_8H_8O_3$), 421.1146 (100.0; $-CO_2$), 447.0944 (11.7; $-H_2O$), 465.1048 (19.8)
61	(epi-)catechin-(epi-)catechin-gallate-1	10,71	$C_{37}H_{30}O_{16}$	M-H	729,1461	-1,4	3,0	23	+	1.1 ± 0.17			3	125.0239 (8.8; $-C_{31}H_{24}O_{13}$), 245.0826 (12.6; $-C_{23}H_{16}O_{12}$), 269.0460 (35.7; $-C_{22}H_{20}O_{11}$), 289.0724 (100.0; $-C_{22}H_{16}O_{10}$), 339.0881 (10.8; $-C_{18}H_{14}O_{10}$), 407.0781 (76.9; $-C_{15}H_{14}O_8$), 433.0937 (23.5; $-C_{13}H_{12}O_8$), 451.1043 (10.3; $-C_{13}H_{10}O_7$), 541.1157 (3.9; $-C_7H_8O_6$)
62	*Unidentified*	10,83	$C_{35}H_{34}O_{15}$	M-H	693,1824	-0,5	5,6	19			+	1.2 ± 0.10	4	125.0234 (22.2; $-C_{29}H_{28}O_{12}$), 161.0232 (18.6; $-C_{26}H_{28}O_{12}$), 243.0663 (100.0; $-C_{21}H_{22}O_{11}$), 283.0613 (19.7; $-C_{19}H_{22}O_{10}$), 335.0929 (25.1; $-C_{15}H_{18}O_{10}$), 361.0719 (42.6; $-C_{14}H_{20}O_9$), 487.1409 (11.6; $-C_7H_{10}O_7$), 541.1341 (10.8; $-C_8H_8O_3$)
63	benzoyloxy-hydroxypropyl-HexA	11,00	$C_{16}H_{20}O_{10}$	M-H	371,0983	-0,9	0,9	7	+		+		3	121.0293 (11.0; $-C_9H_{14}O_8$), 231.0517 (8.4; $-C_7H_8O_3$), 249.0613 (100.0; $-C_7H_6O_2$), 371.0975 (32.0)
64	piceatannol-Hex-1	11,12	$C_{20}H_{22}O_9$	M-H	405,1191	1,0	1,5	10	+	6.7 ± 0.55	+	4.2 ± 0.27	3	175.0762 (0.8; $-C_9H_{10}O_7$), 243.0667 (100.0; $-C_6H_{10}O_5$)
65	*Unidentified*	11,18	$C_{24}H_{28}O_{13}$	M-H	523,1457	0,5	28,5	11	+				4	169.0127 (7.2; $-C_{17}H_{22}O_8$), 209.0814 (8.5; $-C_{13}H_{14}O_9$), 313.0562 (43.6; $-C_{11}H_{14}O_4$), 371.1349 (11.9; $-C_7H_4O_4$), 463.1241 (100.0; $-C_2H_4O_2$), 487.1244 (15.6; $-H_4O_2$), 505.1341 (46.8; $-H_2O$), 523.1453 (76.9)
66	piceatannol-Hex-2	11,30	$C_{20}H_{22}O_9$	M-H	405,1191	-0,4	22,3	10	+	1.8 ± 0.26	+	2.7 ± 0.24	3	175.0762 (0.8; $-C_9H_{10}O_7$), 243.0667 (100.0; $-C_6H_{10}O_5$)
67	*Unidentified*	11,35	$C_{16}H_{24}O_7$	M+HCOOH-H	373,1504	0,6	3,0	5	+				4	165.0906 (7.1; $-C_7H_{12}O_7$), 327.1444 (100.0; $-CH_2O_2$)
68	dihydrokaempferol-1	11,40	$C_{15}H_{12}O_6$	M-H	287,0561	-0,4	1,6	10	+		+		3	125.0230 (12.3; $-C_9H_6O_3$), 177.0551 (5.2; $-C_5H_2O_3$), 201.0551 (9.3; $-C_3H_2O_3$), 243.0665 (24.0; $-CO_2$), 259.0613 (100.0; $-CO$), 287.0559 (33.6)
69	resveratrol-Hex-1 (resveratroloside)	11,62	$C_{20}H_{22}O_8$	M+HCOOH-H	435,1296	0,1	2,4	10	+	24.5 ± 1.98	+	21.1 ± 1.14	2	227.0713 (100.0; $-C_7H_{12}O_7$), 389.1244 (3.8; $-CH_2O_2$)
70	(epi-)catechin-(epi-)catechin-gallate-2	11,67	$C_{37}H_{30}O_{16}$	M-H	729,1461	-0,5	10,0	23	+		+		3	125.0228 (6.5; $-C_{31}H_{24}O_{13}$), 245.0820 (8.8; $-C_{23}H_{16}O_{12}$), 271.0614 (16.9; $-C_{22}H_{18}O_{11}$), 289.0720 (100.0; $-C_{22}H_{16}O_{10}$), 339.0886 (5.5; $-C_{18}H_{14}O_{10}$), 381.0986 (4.9; $-C_{16}H_{12}O_9$), 389.0668 (6.5; $-C_{15}H_{16}O_9$), 407.0776 (86.3; $-C_{15}H_{14}O_8$), 451.1033 (7.2; $-C_{13}H_{10}O_7$)
71	(iso-)rhapontigenin-diHex-1	11,73	$C_{28}H_{36}O_{16}$	M-H	627,1930	0,1	11,4	11	+		+		3	224.0479 (0.8; $-C_{14}H_{27}O_{13}$), 242.0582 (5.8; $-C_{14}H_{25}O_{12}$), 257.0819 (100.0; $-C_{13}H_{22}O_{12}$), 299.0935 (0.6; $-C_{11}H_{20}O_{11}$), 419.1333 (0.3; $-C_7H_{12}O_7$)
72	*Unidentified*	11,78	$C_{28}H_{24}O_7$	M+HCOOH-H	517,1504	-0,3	9,3	17	+				4	255.0661 (100.0; $-C_{14}H_{14}O_5$), 349.1078 (40.7; $-C_8H_8O_4$), 377.1030 (8.2; $-C_7H_8O_3$), 471.1468 (3.7; $-CH_2O_2$)
73	*Unidentified*	11,84	$C_{17}H_{30}O_{11}$	M+HCOOH-H	455,1770	0,4	7,6	3	+	0.3 ± 0.05			4	115.0746 (20.5; $-C_{12}H_{20}O_{11}$), 149.0436 (21.1; $-C_{13}H_{22}O_8$), 191.0566 (44.6; $-C_{11}H_{20}O_7$), 233.0680 (35.3; $-C_9H_{18}O_6$), 293.0875 (100.0; $-C_7H_{14}O_4$), 409.1703 (4.1; $-CH_2O_2$)
74	hydroxybenozyl-galloyl-Hex-4	11,87	$C_{20}H_{20}O_{12}$	M-H	451,0882	-1,4	8,3	11			+	0.7 ± 0.06	3	137.0225 (6.5; $-C_{13}H_{14}O_9$), 169.0147 (15.7; $-C_{13}H_{14}O_7$), 179.0355 (14.4; $-C_{11}H_{12}O_8$), 211.0245 (18.8; $-C_{11}H_{12}O_6$), 239.0563 (62.6; $-C_9H_8O_6$), 313.0556 (32.7; $-C_7H_6O_3$), 451.0896 (100.0)
75	resveratrol-diHex-2	11,91	$C_{26}H_{32}O_{13}$	M+HCOOH-H	597,1824	-0,1	5,3	11	+	0.5 ± 0.06	+	1.6 ± 0.27	3	227.0713 (100.0; $-C_{13}H_{22}O_{12}$), 323.0987 (16.0; $-C_{15}H_{14}O_5$)
76	hexyl-diHex-1	12,00	$C_{18}H_{34}O_{11}$	M+HCOOH-H	471,2083	1,4	16,4	2	+		+		3	113.0227 (1.3; $-C_{14}H_{30}O_{10}$), 143.0335 (1.5; $-C_{13}H_{28}O_9$), 161.0446 (8.6; $-C_{13}H_{26}O_8$), 179.0566 (1.9; $-C_{13}H_{24}O_7$), 203.0564 (1.6; $-C_{11}H_{24}O_7$), 221.0687 (1.6; $-C_{11}H_{22}O_6$), 245.0678 (4.1; $-C_9H_{22}O_6$), 263.1501 (100.0; $-C_7H_{12}O_7$), 310.1097 (4.3; $-C_4H_{17}O_6$), 425.2026 (
77	coumaroyl-galloyl-Hex-2	12,03	$C_{22}H_{22}O_{12}$	M-H	477,1038	-1,1	27,7	12			+		3	163.0385 (6.0; $-C_{13}H_{14}O_9$), 169.0134 (8.6; $-C_{15}H_{16}O_7$), 241.0366 (2.8; $-C_{12}H_{12}O_5$), 313.0559 (100.0; $-C_9H_8O_3$), 433.1141 (3.5; $-CO_2$), 477.1031 (48.4)
78	*Unidentified*	12,04	$C_{29}H_{34}O_{17}$	M-H	653,1723	0,1	27,3	13	+		+	1.3 ± 0.14	4	243.0664 (100.0; $-C_{15}H_{22}O_{13}$), 387.1087 (3.7; $-C_9H_{14}O_9$), 405.1190 (4.8; $-C_9H_{12}O_8$), 447.1303 (29.3; $-C_7H_{10}O_7$), 609.1809 (0.4; $-CO_2$)
79	trihydroxyresveratrol	12,07	$C_{14}H_{12}O_6$	M-H	275,0561	-0,6	3,3	9			+	1.1 ± 0.18	3	172.0510 (12.6; $-C_3H_3O_4$), 187.0773 (38.0; $-C_2O_4$), 231.0667 (100.0; $-CO_2$)
80	naringenin-C-Hex-1	12,12	$C_{21}H_{22}O_{10}$	M-H	433,1140	0,3	23,9	11	+				3	227.0711 (11.2; $-C_7H_{10}O_7$), 241.0863 (7.5; $-C_6H_8O_7$), 267.0659 (10.1; $-C_5H_{10}O_6$), 295.0612 (100.0; $-C_4H_{10}O_5$), 313.0717 (59.1; $-C_4H_8O_4$), 325.0715 (56.6; $-C_3H_8O_4$), 343.0822 (20.1; $-C_3H_6O_3$), 387.1660 (6.8; $-C_{21}H_{21}O_{10}$), 397.0926 (7.8; $-H_4O_2$), 415.1025 (7.1;
81	aloesone/cassiachromone-Hex-2	12,32	$C_{19}H_{22}O_9$	M-H	393,1191	-0,8	2,5	9			+		3	189.0571 (28.9; $-C_8H_{12}O_6$), 231.0668 (100.0; $-C_6H_{10}O_5$)
82	*Unidentified*	12,47	$C_{28}H_{38}O_{13}$	M+HCOOH-H	627,2294	0,0	19,7	10	+		+		4	233.0810 (10.4; $-C_{16}H_{26}O_{11}$), 299.0913 (8.4; $-C_{12}H_{24}O_{10}$), 371.1131 (9.8; $-C_9H_{20}O_8$), 373.1289 (11.0; $-C_9H_{18}O_8$), 386.1361 (13.8; $-C_8H_{17}O_8$), 401.1594 (12.5; $-C_7H_{14}O_8$), 404.1466 (19.0; $-C_8H_{15}O_7$), 419.1703 (100.0; $-C_7H_{12}O_7$), 535.1819 (6.1; $-C_3H_8O_3$), 551.173

83	hexyl-diHex-2	12,49	$C_{18}H_{34}O_{11}$	M+HCOOH-H	471,2083	-1,6	14,4	2			+		3	113.0227 (1.3; $-C_{14}H_{30}O_{10}$), 143.0335 (1.5; $-C_{13}H_{28}O_9$), 161.0446 (8.6; $-C_{13}H_{26}O_8$), 179.0566 (1.9; $-C_{13}H_{24}O_7$), 203.0564 (1.6; $-C_{11}H_{24}O_7$), 221.0687 (1.6; $-C_{11}H_{22}O_6$), 245.0678 (4.1; $-C_9H_{22}O_6$), 263.1501 (100.0; $-C_7H_{12}O_7$), 310.1097 (4.3; $-C_4H_{17}O_6$), 425.2026 (
84	**astringin**	12,68	$C_{20}H_{22}O_9$	M-H	405,1191	0,4	3,9	10	+	65.3 ± 4.38	+	99.3 ± 4.20	1	201.0559 (0.7; $-C_8H_{12}O_6$), 225.0560 (0.4; $-C_6H_{12}O_6$), 243.0662 (100.0; $-C_6H_{10}O_5$)
85	*Unidentified*	12,77	$C_{22}H_{24}O_{11}$	M-H	463,1245	-0,5	6,5	11			+		4	177.0189 (45.1; $-C_{13}H_{18}O_7$), 225.0558 (68.4; $-C_8H_{14}O_8$), 239.0715 (100.0; $-C_7H_{12}O_8$), 257.0817 (17.9; $-C_7H_{10}O_7$), 283.0607 (14.4; $-C_6H_{12}O_6$), 421.1127 (2.0; $-C_2H_2O$), 463.1202 (0.8; $-C_{22}H_{23}O_{11}$)
86	galloyl-(epi-)catechin-galloyl-(epi-)catechin-(epi-)catechin-1	12,95	$C_{59}H_{46}O_{26}$	M-H	#######	-1,0	46,6	37			+		3	125.0231 (61.6; $-C_{53}H_{40}O_{23}$), 169.0147 (73.0; $-C_{52}H_{40}O_{21}$), 243.0290 (57.8; $-C_{46}H_{38}O_{21}$), 269.0453 (60.8; $-C_{44}H_{36}O_{21}$), 289.0711 (100.0; $-C_{44}H_{32}O_{20}$), 303.0508 (70.0; $-C_{44}H_{34}O_{19}$), 407.0778 (89.3; $-C_{37}H_{30}O_{18}$), 677.1297 (11.7; $-C_{22}H_{20}O_{13}$)
87	*Unidentified*	12,97	$C_{21}H_{22}O_{12}$	M-H	465,1038	-0,6	37,5	11	+				4	151.0018 (18.6; $-C_{14}H_{18}O_8$), 239.0716 (3.0; $-C_6H_{10}O_9$), 257.0813 (15.4; $-C_6H_8O_8$), 283.0977 (6.8; $-C_4H_6O_8$), 303.0511 (75.4; $-C_6H_{10}O_5$), 313.0924 (100.0; $-C_7H_4O_4$), 331.0975 (4.1; $-H_6O_8$), 339.0717 (2.6; $-C_6H_6O_3$), 421.1121 (1.3; $-CO_2$), 465.1033 (30.8)
88	*Unidentified*	13,06	$C_{21}H_{28}O_{13}$	M-H	487,1457	-1,2	3,3	8	+	5.1 ± 0.79	+	4.9 ± 0.44	4	147.0435 (100.0; $-C_{12}H_{20}O_{11}$), 191.0561 (16.0; $-C_{14}H_{16}O_7$), 233.0676 (7.0; $-C_{12}H_{14}O_6$), 293.0878 (16.2; $-C_{10}H_{10}O_4$)
89	polyflavanostilbene-A	13,10	$C_{42}H_{38}O_{19}$	M-H	845,1934	-0,4	5,3	24	+	4.7 ± 0.45	+		3	125.0230 (20.2; $-C_{36}H_{32}O_{16}$), 169.0131 (24.6; $-C_{35}H_{32}O_{14}$), 243.0650 (66.9; $-C_{28}H_{26}O_{15}$), 269.0445 (35.7; $-C_{27}H_{28}O_{14}$), 287.0554 (37.0; $-C_{27}H_{26}O_{13}$), 335.0916 (30.8; $-C_{22}H_{22}O_{14}$), 361.0707 (42.0; $-C_{21}H_{24}O_{13}$), 391.0812 (100.0; $-C_{20}H_{22}O_{12}$), 405.0970 (74.8; -C
90	digalloyl-procyanidin-B2-1	13,12	$C_{44}H_{34}O_{20}$	M-H	881,1570	0,4	8,7	28			+	3.8 ± 0.69	3	125.0235 (7.8; $-C_{38}H_{28}O_{17}$), 169.0136 (7.1; $-C_{37}H_{28}O_{15}$), 245.0455 (8.3; $-C_{31}H_{24}O_{15}$), 269.0456 (45.5; $-C_{29}H_{24}O_{15}$), 289.0719 (71.7; $-C_{29}H_{20}O_{14}$), 363.0874 (7.2; $-C_{23}H_{18}O_{14}$), 389.0667 (10.2; $-C_{22}H_{20}O_{13}$), 407.0773 (100.0; $-C_{22}H_{18}O_{12}$), 433.0930 (25.4; $-C_{20}H_{16}O$
91	**polydatin**	13,20	$C_{20}H_{22}O_8$	M+HCOOH-H	435,1296	-0,1	4,9	10	+	14.0 ± 0.72	+	11.3 ± 0.56	1	185.0613 (0.8; $-C_9H_{14}O_8$), 227.0714 (100.0; $-C_7H_{12}O_7$), 273.0771 (36.1; $-C_6H_{10}O_5$), 389.1240 (5.4; $-CH_2O_2$), 435.1310 (5.2)
92	digalloyl-procyanidin-B2-2	13,21	$C_{44}H_{34}O_{20}$	M-H	881,1570	-0,5	33,2	28	+				3	125.0235 (7.8; $-C_{38}H_{28}O_{17}$), 169.0136 (7.1; $-C_{37}H_{28}O_{15}$), 245.0455 (8.3; $-C_{31}H_{24}O_{15}$), 269.0456 (45.5; $-C_{29}H_{24}O_{15}$), 289.0719 (71.7; $-C_{29}H_{20}O_{14}$), 363.0874 (7.2; $-C_{23}H_{18}O_{14}$), 389.0667 (10.2; $-C_{22}H_{20}O_{13}$), 407.0773 (100.0; $-C_{22}H_{18}O_{12}$), 433.0930 (25.4; $-C_{20}H_{16}O$
93	dicaffeoyl-Hex-1	13,25	$C_{24}H_{24}O_{12}$	M-H	503,1195	0,1	3,5	13	+				3	169.0139 (12.0; $-C_{17}H_{18}O_7$), 189.0557 (16.1; $-C_{13}H_{14}O_9$), 241.0353 (3.6; $-C_{14}H_{14}O_5$), 253.0358 (1.8; $-C_{13}H_{14}O_5$), 273.0758 (0.4; $-C_9H_{10}O_7$), 313.0566 (100.0; $-C_{11}H_{10}O_3$), 503.1195 (20.0)
94	piceatannol/oxyresveratrol-malonyl-Hex	13,28	$C_{23}H_{24}O_{12}$	M-H	491,1195	-1,9	7,3	12			+		3	243.0657 (100.0; $-C_9H_{12}O_8$), 405.1149 (1.0; $-C_3H_3O_3$), 447.1280 (4.6; $-CO_2$)
95	(iso-)rhapontigenin-diHex-2	13,30	$C_{27}H_{34}O_{14}$	M-H	581,1875	-0,4	23,7	11	+		+		3	221.0673 (2.4; $-C_{19}H_{20}O_7$), 241.0505 (18.2; $-C_{13}H_{24}O_{10}$), 257.0816 (100.0; $-C_{12}H_{20}O_{10}$), 299.0903 (2.7; $-C_{10}H_{18}O_9$), 537.1480 (1.2; $-C_{27}H_{33}O_{14}$)
96	piceatannol-galloyl-Hex-1	13,35	$C_{27}H_{26}O_{13}$	M-H	557,1300	-0,1	2,1	15	+	1.7 ± 0.25	+		3	243.0665 (100.0; $-C_{13}H_{14}O_9$), 313.0572 (52.6; $-C_{14}H_{12}O_4$), 405.1195 (18.6; $-C_7H_4O_4$), 557.1311 (73.6)
97	*Unidentified*	13,39	$C_{17}H_{32}O_{10}$	M+HCOOH-H	441,1977	0,2	4,2	14	+				4	149.0432 (3.7; $-C_{13}H_{24}O_7$), 161.0461 (2.6; $-C_{12}H_{24}O_7$), 215.0532 (2.2; $-C_9H_{22}O_6$), 233.0662 (4.2; $-C_9H_{20}O_5$), 263.1497 (96.2; $-C_6H_{10}O_6$), 289.0707 (3.4; $-C_3H_{20}O_6$), 395.1917 (100.0; $-CH_2O_2$)
98	**rutin**	13,45	$C_{27}H_{30}O_{16}$	M-H	609,1461	0,5	24,3	13	+		+		1	178.9993 (0.6; $-C_{19}H_{26}O_{11}$), 243.0292 (0.7; $-C_{14}H_{22}O_{11}$), 255.0295 (1.5; $-C_{13}H_{22}O_{11}$), 271.0242 (3.5; $-C_{13}H_{22}O_{10}$), 283.0242 (0.6; $-C_{12}H_{22}O_{10}$), 300.0272 (100.0; $-C_{12}H_{21}O_9$), 301.0341 (48.2; $-C_{12}H_{20}O_9$), 343.0452 (1.7; $-C_{10}H_{18}O_8$), 457.1360 (0.2; $-C_7H_4O_4$), 609.1
99	galloyl-catechin-2	13,47	$C_{22}H_{18}O_{10}$	M-H	441,0827	-0,2	3,7	14	+	6.7 ± 0.52	+	8.0 ± 0.36	3	125.0237 (7.6; $-C_{16}H_{12}O_7$), 169.0139 (40.3; $-C_{15}H_{12}O_5$), 245.0822 (25.2; $-C_8H_4O_6$), 271.0617 (7.4; $-C_7H_6O_5$), 289.0720 (100.0; $-C_7H_4O_4$), 303.0513 (5.0; $-C_7H_6O_3$), 331.0463 (2.5; $-C_6H_6O_2$)
100	coumaroyl-galloyl-Hex-3	13,52	$C_{22}H_{22}O_{12}$	M-H	477,1038	-2,3	10,6	12			+		3	151.0029 (6.9; $-C_{15}H_{18}O_8$), 169.0120 (7.3; $-C_{15}H_{16}O_7$), 193.0127 (3.5; $-C_{13}H_{16}O_7$), 211.0242 (3.0; $-C_{13}H_{14}O_6$), 241.0488 (7.7; $-C_8H_{12}O_8$), 253.0867 (25.4; $-C_6H_8O_9$), 265.0693 (5.1; $-C_9H_8O_6$), 285.0388 (6.4; $-C_7H_{12}O_6$), 331.0667 (2.6; $-C_9H_6O_2$), 459.0930 (2.7; -H
101	galloyl-(epi-)catechin-galloyl-(epi-)catechin-(epi-)catechin-2	13,53	$C_{59}H_{46}O_{26}$	M-H	#######	-1,1	###	37			+		3	125.0231 (61.6; $-C_{53}H_{40}O_{23}$), 169.0147 (73.0; $-C_{52}H_{40}O_{21}$), 243.0290 (57.8; $-C_{46}H_{38}O_{21}$), 269.0453 (60.8; $-C_{44}H_{36}O_{21}$), 289.0711 (100.0; $-C_{44}H_{32}O_{20}$), 303.0508 (70.0; $-C_{44}H_{34}O_{19}$), 407.0778 (89.3; $-C_{37}H_{30}O_{18}$), 677.1297 (11.7; $-C_{22}H_{20}O_{13}$)
102	hydroxyphenyl-butanone-galloyl-Hex-1?_lindleyin-1	13,59	$C_{23}H_{26}O_{11}$	M-H	477,1402	0,9	16,7	11	+				2	125.0227 (0.9; $-C_{17}H_{20}O_8$), 163.0756 (0.9; $-C_{13}H_{14}O_9$), 169.0134 (9.0; $-C_{16}H_{20}O_6$), 211.0242 (0.6; $-C_{14}H_{18}O_5$), 241.0346 (1.6; $-C_{13}H_{16}O_4$), 269.0453 (1.8; $-C_8H_{16}O_6$), 313.0561 (42.0; $-C_{10}H_{12}O_2$), 477.1396 (100.0)
103	pinocembrine-Hex-1	13,61	$C_{21}H_{22}O_9$	M-H	417,1191	-0,7	2,7	11	+				3	176.0856 (0.7; $-C_{10}H_9O_7$), 213.0561 (5.4; $-C_8H_{12}O_6$), 227.0685 (0.8; $-C_7H_{10}O_6$), 255.0662 (100.0; $-C_6H_{10}O_5$), 297.0764 (1.0; $-C_4H_8O_4$)
104	*Unidentified*	13,62	$C_{22}H_{22}O_{11}$	M-H	461,1089	-0,9	17,8	12			+		4	213.0558 (3.2; $-C_9H_{12}O_8$), 255.0662 (100.0; $-C_7H_{10}O_7$), 299.0204 (0.8; $-C_7H_{14}O_4$), 417.1178 (0.2; $-CO_2$)
105	(iso-)rhapontigenin-diHex-4	13,76	$C_{27}H_{34}O_{14}$	M+HCOOH-H	627,1930	-1,3	15,5	11			+		3	161.0442 (0.7; $-C_{22}H_{26}O_{11}$), 221.0658 (4.9; $-C_{20}H_{22}O_9$), 241.0496 (5.1; $-C_{14}H_{26}O_{12}$), 249.0609 (3.6; $-C_{19}H_{22}O_8$), 257.0811 (100.0; $-C_{13}H_{22}O_{12}$), 299.0897 (0.6; $-C_{11}H_{20}O_{11}$), 323.0980 (2.2; $-C_{16}H_{16}O_6$), 401.1230 (1.6; $-C_7H_{14}O_8$), 419.1353 (1.4; $-C_7H_{12}O_7$), 461.14
106	naringenin-C-Hex-2	13,79	$C_{21}H_{22}O_{10}$	M-H	433,1140	-0,7	86,4	11	+				2	125.0257 (9.5; $-C_{15}H_{16}O_7$), 227.0711 (12.8; $-C_7H_{10}O_7$), 258.0541 (16.4; $-C_7H_{11}O_5$), 273.0759 (100.0; $-C_6H_8O_5$), 307.0804 (4.8; $-C_6H_6O_3$)
107	cinnamoyl-Hex	13,83	$C_{15}H_{18}O_7$	M+HCOOH-H	355,1034	1,9	5,5	7			+		3	147.0437 (100.0; $-C_7H_{12}O_7$)
108	*Unidentified*	13,91	$C_{43}H_{40}O_{19}$	M-H	859,2091	-0,5	31,0	24	+	1.7 ± 0.08	+		4	125.0231 (62.5; $-C_{37}H_{34}O_{16}$), 169.0140 (100.0; $-C_{36}H_{34}O_{14}$), 269.0465 (57.7; $-C_{28}H_{30}O_{14}$), 349.1090 (74.9; $-C_{22}H_{22}O_{14}$), 375.0874 (50.5; $-C_{21}H_{24}O_{13}$), 405.0982 (79.6; $-C_{20}H_{22}O_{12}$), 417.0984 (60.9; $-C_{19}H_{22}O_{12}$), 441.1338 (42.9; $-C_{16}H_{18}O_{13}$), 483.1447 (65.2; -C $C_7H_4O_4$)
109	catechin-catechin-gallate-3	13,92	$C_{37}H_{30}O_{16}$	M-H	729,1461	-0,1	16,4	23	+				3	125.0227 (10.7; $-C_{31}H_{24}O_{13}$), 169.0134 (7.8; $-C_{30}H_{24}O_{11}$), 245.0816 (12.2; $-C_{23}H_{16}O_{12}$), 269.0464 (33.3; $-C_{22}H_{20}O_{11}$), 289.0718 (100.0; $-C_{22}H_{16}O_{10}$), 339.0875 (11.0; $-C_{18}H_{14}O_{10}$), 407.0774 (76.6; $-C_{15}H_{14}O_8$), 433.0921 (19.2; $-C_{13}H_{12}O_8$), 577.1340 (6.9; $-C_7H_4O_4$
110	piceatannol-Pent	14,01	$C_{19}H_{20}O_8$	M-H	375,1085	-0,4	2,1	10	+	3.4 ± 0.44	+	4.7 ± 0.50	3	175.0758 (0.2; $-C_8H_8O_6$), 201.0558 (0.7; $-C_7H_{10}O_5$), 225.0556 (0.4; $-C_5H_{10}O_5$), 243.0663 (100.0; $-C_5H_8O_4$), 375.1099 (0.2)
111	dihydrokaempferol-Pent	14,02	$C_{20}H_{20}O_{10}$	M-H	419,0983	-0,4	3,4	11	+				3	151.0018 (3.7; $-C_{13}H_{16}O_6$), 180.0068 (14.8; $-C_{12}H_{15}O_5$), 243.0666 (60.0; $-C_6H_8O_6$), 259.0611 (8.0; $-C_6H_8O_5$), 269.0459 (100.0; $-C_5H_{10}O_5$), 286.0492 (6.5; $-C_5H_9O_4$), 375.1093 (2.4; $-CO_2$), 419.0986 (19.4)
112	piceatannol-galloyl-Hex-2	14,10	$C_{27}H_{26}O_{13}$	M-H	557,1300	-0,4	6,1	15	+	4.4 ± 1.09	+	11.3 ± 0.78	3	169.0138 (6.5; $-C_{20}H_{20}O_8$), 243.0664 (100.0; $-C_{13}H_{14}O_9$), 313.0568 (61.0; $-C_{14}H_{12}O_4$), 405.1192 (11.9; $-C_7H_4O_4$), 557.1303 (26.8)
113	(iso-)rhapontigenin-Hex-1	14,20	$C_{21}H_{24}O_9$	M+HCOOH-H	465,1402	-0,4	2,6	10	+	18.5 ± 0.55	+	17.7 ± 0.73	3	224.0484 (0.9; $-C_8H_{17}O_8$), 242.0584 (15.4; $-C_8H_{15}O_7$), 257.0820 (100.0; $-C_7H_{12}O_7$)
114	coumaroyl-galloyl-Hex-4	14,24	$C_{22}H_{22}O_{12}$	M-H	477,1038	-0,6	4,5	12	+				3	145.0278 (4.8; $-C_{13}H_{16}O_{10}$), 151.0018 (5.5; $-C_{15}H_{18}O_8$), 169.0138 (33.1; $-C_{15}H_{16}O_7$), 205.0505 (28.6; $-C_{11}H_{12}O_8$), 211.0250 (21.4; $-C_{13}H_{14}O_6$), 235.0613 (25.7; $-C_{10}H_{10}O_7$), 253.0346 (14.4; $-C_{11}H_{12}O_5$), 265.0715 (100.0; $-C_9H_8O_6$), 271.0617 (17.6; $-C_7H_{10}O_7$), 30
115	pinocembrine-Hex-2	14,34	$C_{21}H_{22}O_9$	M-H	417,1191	-1,2	58,5	11			+	1.6 ± 0.11	3	185.0605 (0.5; $-C_9H_{12}O_7$), 213.0559 (4.7; $-C_8H_{12}O_6$), 239.0707 (1.0; $-C_6H_{10}O_6$), 255.0665 (100.0; $-C_6H_{10}O_5$), 281.0675 (1.6; $-C_8H_8O_2$)
116	**trans-piceatannol**	14,43	$C_{14}H_{12}O_4$	M-H	243,0662	-0,5	3,9	9	+	2.5 ± 0.50	+	4.0 ± 1.17	1	159.0439 (1.0; $-C_4H_4O_2$), 175.0758 (1.1; $-C_3O_2$), 201.0557 (4.9; $-C_2H_2O$), 215.0712 (0.7; -CO), 225.0557 (2.4; $-H_2O$), 243.0663 (100.0)
117	coumaroyl-galloyl-Hex-5	14,53	$C_{22}H_{22}O_{12}$	M-H	477,1038	-1,7	41,3	12	+		+		3	151.0029 (6.9; $-C_{15}H_{18}O_8$), 169.0120 (7.3; $-C_{15}H_{16}O_7$), 193.0127 (3.5; $-C_{13}H_{16}O_7$), 211.0242 (3.0; $-C_{13}H_{14}O_6$), 241.0488 (7.7; $-C_8H_{12}O_8$), 253.0867 (25.4; $-C_6H_8O_9$), 265.0693 (5.1; $-C_9H_8O_6$), 285.0388 (6.4; $-C_7H_{12}O_6$), 331.0667 (2.6; $-C_9H_6O_2$), 459.0930 (2.7; -H
118	*Unidentified*	14,71	$C_{13}H_{14}O_4$	M-H	233,0819	-0,1	6,7	7			+		4	161.0592 (1.0; $-C_3H_4O_2$), 189.0543 (28.9; $-C_2H_4O$), 233.0814 (100.0)
119	resveratrol-galloyl-Hex-2	14,73	$C_{27}H_{26}O_{12}$	M-H	541,1351	0,9	1,9	15	+		+		3	125.0232 (0.9; $-C_{21}H_{20}O_9$), 151.0024 (0.8; $-C_{20}H_{22}O_8$), 169.0136 (11.5; $-C_{20}H_{20}O_7$), 211.0248 (1.0; $-C_{18}H_{18}O_6$), 227.0712 (17.5; $-C_{13}H_{14}O_9$), 241.0355 (3.0; $-C_{17}H_{16}O_5$), 253.0351 (1.8; $-C_{16}H_{16}O_5$), 313.0564 (93.6; $-C_{14}H_{12}O_3$), 389.1239 (0.9; $-C_7H_4O_4$), 541.1348
120	*Unidentified*	14,74	$C_{18}H_{32}O_{11}$	M+HCOOH-H	469,1926	-0,2	34,0	3	+				4	125.0214 (12.3; $-C_{13}H_{28}O_{10}$), 129.0900 (39.9; $-C_{12}H_{20}O_{11}$), 149.0428 (17.7; $-C_{14}H_{24}O_8$), 191.0562 (28.4; $-C_{12}H_{22}O_7$), 233.0652 (27.3; $-C_{10}H_{20}O_6$), 257.0808 (100.0; $-C_4H_{20}O_9$), 293.0867 (60.2; $-C_8H_{16}O_4$), 331.0979 (2.2; $-C_{19}H_{33}O_{13}$), 407.0998 (5.3; $-H_{14}O_3$), 423
121	**rhapontin**	14,84	$C_{21}H_{24}O_9$	M+HCOOH-H	465,1402	-0,3	1,8	10	+	184.0 ± 10.93	+	151.3 ± 7.77	1	224.0482 (0.6; $-C_8H_{17}O_8$), 241.0508 (6.3; $-C_8H_{16}O_7$), 257.0818 (100.0; $-C_7H_{12}O_7$)
122	*Unidentified*	14,99	$C_{28}H_{32}O_{16}$	M-H	623,1617	0,0	10,4	13			+		4	249.0553 (3.1; $-C_{12}H_{22}O_{13}$), 253.0504 (100.0; $-C_{13}H_{22}O_{12}$), 266.0579 (3.3; $-C_{12}H_{21}O_{12}$), 277.0504 (12.1; $-C_{11}H_{22}O_{12}$), 289.0513 (0.6; $-C_{10}H_{22}O_{12}$), 295.0612 (3.8; $-C_{11}H_{20}O_{11}$), 307.0618 (0.7; $-C_{10}H_{20}O_{11}$), 319.0630 (0.6; $-C_9H_{20}O_{11}$), 519.1144 (0.5; $-C_4H_8O_3$)
123	resveratrol-galloyl-Hex-3	15,02	$C_{27}H_{26}O_{12}$	M-H	541,1351	-0,3	1,8	15	+		+		3	169.0139 (11.5; $-C_{20}H_{20}O_7$), 227.0715 (25.7; $-C_{13}H_{14}O_9$), 241.0357 (3.1; $-C_{17}H_{16}O_5$), 253.0357 (1.9; $-C_{16}H_{16}O_5$), 313.0569 (81.4; $-C_{14}H_{12}O_3$), 541.1355 (100.0)
124	*Unidentified*	15,06	$C_{36}H_{36}O_{15}$	M-H	707,1981	-0,2	10,4	19			+		4	125.0232 (100.0; $-C_{30}H_{30}O_{12}$), 161.0228 (56.3; $-C_{27}H_{30}O_{12}$), 243.0669 (33.2; $-C_{22}H_{24}O_{11}$), 269.0449 (49.2; $-C_{21}H_{26}O_{10}$), 287.0560 (49.9; $-C_{21}H_{24}O_9$), 375.0861 (44.6; $-C_{14}H_{20}O_9$), 405.0990 (45.6; $-C_{13}H_{18}O_8$), 419.1123 (46.0; $-C_{12}H_{16}O_8$), 527.1365 (29.8; $-C_6H_{12}O$
125	resveratrol-Ac-Hex	15,14	$C_{22}H_{24}O_9$	M-H	431,1347	0,2	17,6	11	+		+		3	185.0619 (1.7; $-C_{10}H_{14}O_7$), 227.0708 (100.0; $-C_8H_{12}O_6$), 243.0673 (2.3; $-C_8H_{12}O_5$)
126	quercetin-dHex-1 (quercitrin)	15,18	$C_{21}H_{20}O_{11}$	M-H	447,0932	0,9	3,3	12			+		2	178.9974 (1.3; $-C_{13}H_{16}O_6$), 227.0355 (1.2; $-C_8H_{12}O_7$), 255.0297 (5.5; $-C_7H_{12}O_6$), 271.0240 (4.1; $-C_7H_{12}O_5$), 284.0319 (26.8; $-C_6H_{11}O_5$), 300.0271 (100.0; $-C_6H_{11}O_4$), 301.0340 (78.0; $-C_6H_{10}O_4$), 447.0925 (24.8)
127	*Unidentified*	15,29	$C_{42}H_{38}O_{19}$	M-H	845,1934	1,2	40,0	24			+		4	125.0235 (24.1; $-C_{36}H_{32}O_{16}$), 243.0654 (100.0; $-C_{28}H_{26}O_{15}$), 265.0609 (33.4; $-C_{13}H_{15}O_9$), 293.0819 (25.3; $-C_{24}H_{24}O_{15}$), 303.0506 (30.4; $-C_{27}H_{26}O_{12}$), 335.0906 (37.3; $-C_{22}H_{22}O_{14}$), 361.0730 (38.2; $-C_{21}H_{24}O_{13}$), 379.0829 (28.5; $-C_{21}H_{22}O_{12}$), 405.0975 (61.5; $-C_{19}$
128	quercetin-dHex-2 (quercitrin)	15,32	$C_{21}H_{20}O_{11}$	M-H	447,0932	-0,1	13,3	12			+		2	227.0347 (3.7; $-C_8H_{12}O_7$), 255.0298 (9.4; $-C_7H_{12}O_6$), 284.0323 (100.0; $-C_6H_{11}O_5$), 285.0387 (35.9; $-C_6H_{10}O_5$), 327.0507 (2.5; $-C_4H_8O_4$), 447.0930 (38.2)
129	(iso-)rhapontigenin-galloyl-Hex-1	15,32	$C_{28}H_{28}O_{13}$	M-H	571,1457	0,1	4,5	15	+	3.8 ± 0.70	+	5.0 ± 1.77	3	169.0137 (12.7; $-C_{21}H_{22}O_8$), 243.0660 (8.0; $-C_{14}H_{16}O_9$), 257.0820 (15.0; $-C_{13}H_{14}O_9$), 313.0568 (80.8; $-C_{15}H_{14}O_4$), 327.0721 (4.4; $-C_{14}H_{12}O_4$), 556.1231 (2.2; $-CH_3$), 571.1459 (100.0)
130	malonyl-vitexin	15,35	$C_{24}H_{22}O_{13}$	M-H	517,0987	-0,1	33,6	14			+		4	165.0547 (2.6; $-C_{15}H_{12}O_{10}$), 243.0668 (2.0; $-C_{10}H_{10}O_9$), 255.0670 (1.5; $-C_9H_{10}O_9$), 282.0523 (2.0; $-C_8H_{11}O_8$), 311.0559 (100.0; $-C_7H_{10}O_7$), 353.0659 (3.8; $-C_5H_8O_6$), 430.0891 (7.8; $-C_3H_3O_3$), 473.1081 (46.3; $-CO_2$), 517.0983 (39.7)
131	(iso-)rhapontigenin-Pent	15,54	$C_{20}H_{22}O_8$	M+HCOOH-H	435,1296	0,0	12,0	10	+		+		3	242.0579 (16.6; $-C_7H_{13}O_6$), 257.0817 (100.0; $-C_6H_{10}O_6$), 389.1256 (1.0; $-CH_2O_2$)
132	coumaroyl-galloyl-Hex-6	15,61	$C_{22}H_{22}O_{12}$	M-H	477,1038	-1,0	29,3	12			+		3	240.0423 (0.8; $-C_8H_{13}O_8$), 255.0660 (2.5; $-C_7H_{10}O_8$), 265.0507 (6.0; $-C_6H_{12}O_8$), 269.0454 (100.0; $-C_7H_{12}O_7$), 282.0534 (6.1; $-C_6H_{11}O_7$), 293.0455 (15.2; $-C_5H_{12}O_7$), 311.0561 (1.8; $-C_5H_{10}O_6$), 323.0566 (0.7; $-C_4H_{10}O_6$), 335.0562 (0.8; $-C_3H_{10}O_6$), 386.1012 (0.6; -
133	*Unidentified*	15,74	$C_{22}H_{22}O_{10}$	M-H	445,1140	0,4	54,8	12			+		4	137.0220 (28.5; $-C_{15}H_{16}O_7$), 145.0270 (31.0; $-C_{13}H_{16}O_8$), 307.0820 (100.0; $-C_7H_6O_3$)
134	eriodictyol-Hex	15,79	$C_{21}H_{22}O_{11}$	M-H	449,1089	0,1	4,9	11	+	1.0 ± 0.18	+	2.6 ± 0.28	3	151.0027 (13.9; $-C_{14}H_{18}O_7$), 243.0653 (9.3; $-C_7H_{10}O_7$), 287.0562 (100.0; $-C_6H_{10}O_5$), 297.0979 (4.3; $-C_7H_4O_4$), 449.1110 (11.8)
135	*Unidentified*	15,82	$C_{20}H_{32}O_{13}$	M-H	479,1770	-0,6	29,5	5	+				4	139.0746 (100.0; $-C_{12}H_{20}O_{11}$), 293.0868 (37.7; $-C_9H_{14}O_4$)
136	*Unidentified*	15,84	$C_{43}H_{40}O_{19}$	M-H	859,2091	0,8	26,2	24	+	0.5 ± 0.07	+		4	125.0242 (47.2; $-C_{37}H_{34}O_{16}$), 169.0134 (84.8; $-C_{36}H_{34}O_{14}$), 269.0463 (61.7; $-C_{28}H_{30}O_{14}$), 287.0547 (100.0; $-C_{28}H_{28}O_{13}$), 393.0983 (55.0; $-C_{21}H_{22}O_{12}$), 405.0968 (41.3; $-C_{20}H_{22}O_{12}$), 419.1151 (31.4; $-C_{19}H_{20}O_{12}$), 527.1350 (48.9; $-C_{13}H_{16}O_{10}$)
137	*Unidentified*	15,98	$C_{23}H_{24}O_{11}$	M-H	475,1245	-0,6	25,5	12			+		4	137.0225 (30.4; $-C_{16}H_{18}O_8$), 175.0389 (53.5; $-C_{13}H_{16}O_8$), 193.0502 (19.5; $-C_{13}H_{14}O_7$), 311.0561 (100.0; $-C_6H_{12}O_5$), 337.0922 (58.9; $-C_7H_6O_3$)
138	aloesone/cassiachromone-Hex-3	16,07	$C_{19}H_{22}O_9$	M-H	393,1191	1,2	1,6	9	+		+		2	187.0768 (0.3; $-C_7H_{10}O_7$), 231.0658 (100.0; $-C_6H_{10}O_5$), 255.0671 (0.1; $-C_4H_{10}O_5$), 273.0770 (0.1; $-C_4H_8O_4$), 393.1184 (0.1)
139	tetrahydroxy-dimethylanthraquinone	16,15	$C_{16}H_{12}O_6$	M-H	299,0561	0,6	3,8	11	+				3	189.0566 (92.0; $-C_5H_2O_3$), 213.0559 (79.8; $-C_3H_2O_3$), 231.0650 (44.1; $-C_3O_2$), 255.0644 (100.0; $-CO_2$), 299.0568 (37.8)
140	(iso-)rhapontigenin-galloyl-Hex-2	16,21	$C_{28}H_{28}O_{13}$	M-H	571,1457	1,1	8,1	15	+	13.3 ± 0.58	+	9.4 ± 0.74	3	169.0135 (12.6; $-C_{21}H_{22}O_8$), 257.0817 (16.0; $-C_{13}H_{14}O_9$), 313.0563 (75.9; $-C_{15}H_{14}O_4$), 556.1219 (12.0; $-CH_3$), 571.1450 (100.0)
141	resveratrol-Hex-2	16,29	$C_{20}H_{22}O_8$	M+HCOOH-H	435,1296	1,0	6,8	10	+				3	167.0351 (9.5; $-C_{13}H_{16}O_6$), 179.0369 (7.3; $-C_{12}H_{16}O_6$), 227.0697 (3.6; $-C_7H_{12}O_7$), 273.0774 (100.0; $-C_6H_{10}O_5$)
142	(iso-)rhapontigenin-malonyl-Hex-1	16,40	$C_{24}H_{26}O_{12}$	M-H	505,1351	-0,1	12,4	12	+	11.2 ± 1.09	+	5.7 ± 0.41	3	187.0759 (0.8; $-C_{12}H_{14}O_{10}$), 215.0699 (1.7; $-C_{11}H_{14}O_9$), 239.0704 (1.2; $-C_9H_{14}O_9$), 257.0815 (100.0; $-C_9H_{12}O_8$), 299.0917 (0.7; $-C_7H_{10}O_7$), 443.1339 (0.3; $-CH_2O_3$), 461.1451 (1.0; $-CO_2$)
143	digalloyl-procyanidin-B2-3	16,41	$C_{44}H_{34}O_{20}$	M-H	881,1570	1,7	21,8	28	+		+		3	125.0228 (18.6; $-C_{38}H_{28}O_{17}$), 169.0133 (26.4; $-C_{37}H_{28}O_{15}$), 289.0712 (100.0; $-C_{29}H_{20}O_{14}$), 303.0503 (40.3; $-C_{29}H_{22}O_{13}$), 407.0766 (45.8; $-C_{22}H_{18}O_{12}$)
144	*Unidentified*	16,58	$C_{27}H_{26}O_{11}$	M+HCOOH-H	571,1457	0,8	3,8	15			+		4	169.0133 (6.7; $-C_{21}H_{22}O_8$), 242.0570 (1.4; $-C_{14}H_{17}O_9$), 257.0810 (9.0; $-C_{13}H_{14}O_9$), 313.0560 (100.0; $-C_{15}H_{14}O_4$), 571.1453 (4.8)
145	piceatannol-hydroxybenzoyl-Hex	16,66	$C_{27}H_{26}O_{11}$	M-H	525,1402	0,7	9,0	15	+	0.6 ± 0.11	+		3	201.0542 (1.2; $-C_{15}H_{16}O_8$), 243.0655 (100.0; $-C_{13}H_{14}O_7$), 281.0660 (25.4; $-C_{14}H_{12}O_4$), 387.1073 (0.5; $-C_7H_6O_3$), 405.1178 (1.4; $-C_7H_4O_2$)
146	pinocembrine-Hex-4	16,68	$C_{21}H_{22}O_9$	M-H	417,1191	1,2	22,8	11	+				3	211.0757 (0.9; $-C_7H_{10}O_7$), 226.0638 (0.8; $-C_7H_{11}O_6$), 255.0660 (100.0; $-C_6H_{10}O_5$), 266.0585 (0.6; $-C_5H_{11}O_5$), 297.0764 (1.4; $-C_4H_8O_4$), 417.1190 (74.4
147	emodin-Hex-3	16,71	$C_{21}H_{20}O_{10}$	M-H	431,0983	1,1	1,6	12	+	1.2 ± 0.20	+	12.8 ± 0.68	3	225.0557 (0.5; $-C_7H_{10}O_7$), 240.0426 (7.3; $-C_7H_{11}O_6$), 253.0500 (0.3; $-C_6H_{10}O_6$), 269.0452 (78.3; $-C_6H_{10}O_5$), 280.0366 (0.7; $-C_5H_{11}O_5$), 293.0449 (0.4; $-C_4H_{10}O_5$), 311.0561 (1.0; $-C_4H_8O_4$), 413.0860 (0.1; $-H_2O$), 431.0979 (100.0)
148	*Unidentified*	16,84	$C_{43}H_{40}O_{18}$	M-H	843,2141	1,2	11,3	24	+	1.5 ± 0.20	+		4	125.0230 (77.6; $-C_{37}H_{34}O_{15}$), 161.0233 (24.2; $-C_{34}H_{34}O_{15}$), 169.0137 (100.0; $-C_{36}H_{34}O_{13}$), 241.0866 (25.2; $-C_{28}H_{26}O_{15}$), 269.0455 (54.3; $-C_{28}H_{30}O_{13}$), 287.0564 (30.2; $-C_{28}H_{28}O_{12}$), 333.1137 (69.5; $-C_{22}H_{22}O_{14}$), 359.0924 (45.8; $-C_{21}H_{24}O_{13}$), 389.1029 (38.4; -C (68.6; $-C_{15}H_{12}O_7$), 551.1560 (20.3; $-C_{14}H_{12}O_7$), 565.1695 (23.1; $-C_{13}H_{10}O_7$), 629.1991 (11.2; $-CH_{10}O_{12}$), 673.1928 (50.2; $-C_7H_6O_5$), 691.2008 (38.9; $-C_7H_4O_4$)
149	*Unidentified*	16,88	$C_{20}H_{32}O_9$	M-H	415,1973	2,0	46,0	5			+		4	207.1385 (55.7; $-C_7H_{12}O_7$), 371.1700 (100.0; $-C_2H_4O$)
150	*Unidentified*	17,08	$C_{22}H_{20}O_{11}$	M-H	459,0932	0,9	3,9	13			+		4	169.0129 (0.5; $-C_{15}H_{14}O_6$), 211.0244 (1.4; $-C_{13}H_{12}O_5$), 225.0545 (0.2; $-C_8H_{10}O_8$), 237.0548 (0.8; $-C_7H_{10}O_8$), 249.0568 (0.4; $-C_6H_{10}O_8$), 253.0503 (100.0; $-C_7H_{10}O_7$), 266.0580 (29.7; $-C_6H_9O_7$), 291.0662 (1.4; $-C_4H_8O_7$), 295.0607 (3.3; $-C_5H_8O_6$), 308.0679 (0.4; -
151	*Unidentified*	17,08	$C_{31}H_{32}O_{13}$	M-H	611,1770	0,4	14,8	16			+		4	295.0611 (10.8; $-C_{14}H_{20}O_8$), 407.1126 (24.8; $-C_8H_{12}O_6$), 449.1233 (100.0; $-C_6H_{10}O_5$), 491.1374 (7.5; $-C_4H_8O_4$)
152	*Unidentified*	17,10	$C_{24}H_{28}O_{11}$	M-H	491,1558	1,0	28,4	11	+				4	203.0711 (0.7; $-C_{12}H_{16}O_8$), 245.0818 (1.5; $-C_{10}H_{14}O_7$), 269.0816 (3.5; $-C_8H_{14}O_7$), 272.0686 (5.0; $-C_9H_{15}O_6$), 287.0922 (100.0; $-C_8H_{12}O_6$), 473.1462 (0.9; $-H_2O$)
153	dihydroxy-dimethoxy-stilbene-Hex-1	17,12	$C_{22}H_{26}O_9$	M+HCOOH-H	479,1558	0,7	23,8	10	+		+		3	197.0613 (0.5; $-C_{10}H_{18}O_9$), 241.0494 (1.7; $-C_9H_{18}O_7$), 256.0736 (4.0; $-C_8H_{15}O_7$), 271.0973 (100.0; $-C_7H_{12}O_7$)
154	*Unidentified*	17,14	$C_{30}H_{30}O_{12}$	M-H	581,1664	1,7	11,0	16			+		4	241.0498 (1.0; $-C_{16}H_{20}O_8$), 243.0661 (7.2; $-C_{16}H_{18}O_8$), 269.0813 (2.9; $-C_{14}H_{16}O_8$), 283.0970 (1.5; $-C_{13}H_{14}O_8$), 295.0612 (19.7; $-C_{13}H_{18}O_7$), 309.0750 (1.5; $-C_{12}H_{16}O_7$), 337.0726 (2.3; $-C_{11}H_{16}O_6$), 377.1031 (18.3; $-C_8H_{12}O_6$), 404.0893 (1.9; $-C_7H_{13}O_5$), 419.1133 (
155	deoxyrhapontigenin-diHex-2	17,16	$C_{27}H_{34}O_{13}$	M+HCOOH-H	611,1981	3,8	39,9	11	+				3	161.0444 (8.4; $-C_{22}H_{26}O_{10}$), 179.0554 (3.7; $-C_{22}H_{24}O_9$), 225.0557 (5.5; $-C_{14}H_{26}O_{12}$), 241.0873 (100.0; $-C_{13}H_{22}O_{12}$), 323.0990 (33.9; $-C_{16}H_{16}O_5$), 361.1078 (4.2; $-C_6H_{18}O_{10}$), 403.1190 (5.8; $-C_4H_{16}O_9$)
156	*Unidentified*	17,27	$C_{23}H_{26}O_{12}$	M-H	493,1351	1,4	21,7	11	+				4	224.0471 (1.1; $-C_9H_{17}O_9$), 241.0509 (7.3; $-C_9H_{16}O_8$), 257.0815 (100.0; $-C_8H_{12}O_8$), 287.0925 (3.1; $-C_7H_{10}O_7$), 431.0993 (1.0; $-C_2H_6O_2$)
157	resveratrol-dimer-diHex	17,27	$C_{40}H_{42}O_{16}$	M-H	777,2400	1,7	29,6	20			+		3	333.0773 (1.7; $-C_{20}H_{28}O_{11}$), 347.0916 (6.0; $-C_{19}H_{26}O_{11}$), 359.0924 (4.7; $-C_{18}H_{26}O_{11}$), 369.1114 (1.7; $-C_{16}H_{24}O_{12}$), 411.1236 (3.1; $-C_{14}H_{22}O_{11}$), 435.1250 (2.8; $-C_{12}H_{22}O_{11}$), 453.1337 (100.0; $-C_{12}H_{20}O_{10}$), 597.1742 (0.6; $-C_6H_{12}O_6$)
158	*Unidentified*	17,31	$C_{42}H_{34}O_9$	M+HCOOH-H	727,2184	1,2	26,5	26	+	6.7 ± 0.59	+	5.6 ± 0.49	4	227.0709 (15.3; $-C_{29}H_{24}O_8$), 255.0658 (22.9; $-C_{28}H_{24}O_7$), 331.0970 (100.0; $-C_{22}H_{20}O_7$), 347.0920 (23.7; $-C_{22}H_{20}O_6$), 359.0920 (23.5; $-C_{21}H_{20}O_6$), 453.1336 (9.9; $-C_{15}H_{14}O_5$)
159	piceatannol-coumaroyl-Hex-1	17,35	$C_{29}H_{28}O_{11}$	M-H	551,1558	1,2	9,3	16	+		+		3	145.0281 (10.0; $-C_{20}H_{22}O_9$), 243.0658 (100.0; $-C_{15}H_{16}O_7$), 307.0826 (29.5; $-C_{14}H_{12}O_4$), 405.1165 (6.8; $-C_9H_6O_2$), 551.1554 (11.5)
160	piceatannol-sinapoyl-Hex	17,38	$C_{31}H_{32}O_{13}$	M-H	611,1770	0,2	23,6	16	+		+		3	205.0509 (8.3; $-C_{20}H_{22}O_9$), 243.0655 (100.0; $-C_{17}H_{20}O_9$), 367.1027 (8.7; $-C_{14}H_{12}O_4$)
161	*Unidentified*	17,49	$C_{42}H_{34}O_9$	M+HCOOH-H	727,2184	1,1	32,5	26	+	3.5 ± 0.13	+	2.3 ± 0.13	4	227.0706 (18.9; $-C_{29}H_{24}O_8$), 255.0659 (24.2; $-C_{28}H_{24}O_7$), 331.0974 (100.0; $-C_{22}H_{20}O_7$), 347.0924 (24.0; $-C_{22}H_{20}O_6$), 359.0919 (33.5; $-C_{21}H_{20}O_6$), 453.1337 (11.2; $-C_{15}H_{14}O_5$), 493.1303 (2.3; $-C_{13}H_{14}O_4$), 587.1719 (1.3; $-C_7H_8O_3$), 681.2145 (0.8; $-CH_2O_2$)

162	*Unidentified*	17,52	$C_{35}H_{34}O_{13}$	M-H	661,1926	1,8	11,8	19			+		4	241.0500 (8.0; -$C_{21}H_{24}O_9$), 323.0569 (2.1; -$C_{17}H_{22}O_7$), 341.0667 (4.6; -$C_{17}H_{20}O_6$), 362.0799 (3.7; -$C_{14}H_{19}O_7$), 389.1026 (2.1; -$C_{12}H_{16}O_7$), 415.1179 (3.4; -$C_{10}H_{14}O_7$), 453.1354 (3.1; -$C_7H_{12}O_7$), 465.1185 (5.7; -$C_{10}H_{12}O_4$), 499.1395 (100.0; -$C_6H_{10}O_5$)
163	piceatannol-dimer (cararosinol-D)	17,55	$C_{28}H_{22}O_8$	M-H	485,1241	1,5	7,3	18			+	1.5 ± 0.40	2	375.0870 (100.0; -$C_6H_6O_2$), 485.1220 (46.9)
164	resveratrol-dimer-Hex-1	17,56	$C_{34}H_{32}O_{11}$	M-H	615,1871	0,7	9,2	19	+				3	313.0550 (1.1; -$C_{10}H_{24}O_{10}$), 333.0762 (2.2; -$C_{14}H_{18}O_6$), 347.0918 (9.8; -$C_{13}H_{16}O_6$), 359.0915 (10.2; -$C_{12}H_{16}O_6$), 369.1138 (2.2; -$C_{10}H_{14}O_7$), 409.1434 (1.7; -$C_7H_{10}O_7$), 436.1289 (1.9; -$C_6H_{11}O_6$), 453.1340 (100.0; -$C_6H_{10}O_5$)
165	*Unidentified*	17,61	$C_{30}H_{30}O_{12}$	M-H	581,1664	2,8	18,1	16			+		4	241.0498 (1.0; -$C_{16}H_{20}O_8$), 243.0661 (7.2; -$C_{16}H_{18}O_8$), 269.0813 (2.9; -$C_{14}H_{16}O_8$), 283.0970 (1.5; -$C_{13}H_{14}O_8$), 295.0612 (19.7; -$C_{13}H_{18}O_7$), 309.0750 (1.5; -$C_{12}H_{16}O_7$), 337.0726 (2.3; -$C_{11}H_{16}O_6$), 377.1031 (18.3; -$C_8H_{12}O_6$), 404.0893 (1.9; -$C_7H_{13}O_5$), 419.1133 (
166	(iso-)rhapontigenin-malonyl-Hex-2	17,66	$C_{24}H_{26}O_{12}$	M-H	505,1351	1,5	38,8	12	+		+		3	224.0477 (1.3; -$C_{10}H_{17}O_9$), 241.0503 (14.7; -$C_{10}H_{16}O_8$), 257.0814 (100.0; -$C_9H_{12}O_8$), 281.0816 (0.5; -$C_7H_{12}O_8$), 299.0925 (0.4; -$C_7H_{10}O_7$), 401.1232 (0.6; -$C_3H_4O_4$)
167	piceatannol-coumaroyl-Hex-2	17,68	$C_{29}H_{28}O_{11}$	M-H	551,1558	1,0	3,4	16	+	3.9 ± 0.36	+	5.1 ± 0.24	3	145.0276 (2.4; -$C_{20}H_{22}O_9$), 163.0388 (1.0; -$C_{20}H_{20}O_8$), 201.0558 (1.0; -$C_{17}H_{18}O_8$), 225.0553 (0.4; -$C_{15}H_{18}O_8$), 243.0659 (100.0; -$C_{15}H_{16}O_7$), 307.0821 (12.7; -$C_{14}H_{12}O_4$), 387.1080 (0.4; -$C_9H_8O_3$), 405.1187 (2.5; -$C_9H_6O_2$)
168	*Unidentified*	17,77	$C_{24}H_{28}O_{11}$	M-H	491,1558	1,5	6,5	11	+	1.2 ± 0.32			4	245.0813 (1.8; -$C_{10}H_{14}O_7$), 269.0819 (2.0; -$C_8H_{14}O_7$), 272.0676 (3.8; -$C_9H_{15}O_6$), 287.0917 (100.0; -$C_8H_{12}O_6$), 329.1023 (0.5; -$C_6H_{10}O_5$), 445.1128 (0.6; -C_2H_6O), 473.1405 (0.4; -$C_{24}H_{27}O_{11}$)
169	piceatannol-feruloyl-Hex	17,83	$C_{30}H_{30}O_{12}$	M-H	581,1664	1,0	2,0	16	+	0.6 ± 0.08	+	3.8 ± 0.24	3	160.0150 (0.8; -$C_{21}H_{25}O_9$), 175.0395 (5.0; -$C_{20}H_{22}O_9$), 193.0505 (2.9; -$C_{20}H_{20}O_8$), 243.0658 (100.0; -$C_{16}H_{18}O_8$), 287.0916 (1.2; -$C_{14}H_{14}O_7$), 337.0921 (9.3; -$C_{14}H_{12}O_4$), 405.1184 (1.8; -$C_{10}H_8O_3$)
170	ebracteatoside-D	17,89	$C_{19}H_{34}O_{11}$	M+HCOOH-H	483,2083	1,1	2,4	3	+				2	143.1071 (100.0; -$C_{12}H_{20}O_{11}$), 191.0552 (79.0; -$C_{13}H_{24}O_7$), 233.0672 (45.6; -$C_{11}H_{22}O_6$), 293.0870 (83.6; -$C_9H_{18}O_4$)
171	(iso-)rhapontigenin-Ac-Hex	17,93	$C_{23}H_{26}O_{10}$	M+HCOOH-H	507,1508	1,1	###	11	+		+		3	137.0221 (0.9; -$C_{17}H_{22}O_9$), 227.0717 (4.1; -$C_{10}H_{16}O_9$), 241.0503 (9.7; -$C_{10}H_{18}O_8$), 257.0817 (100.0; -$C_9H_{14}O_8$), 281.0665 (6.6; -$C_{11}H_{14}O_5$), 299.0919 (0.5; -$C_7H_{12}O_7$), 401.1231 (0.4; -$C_3H_6O_4$)
172	(aloe-)emodin-galloyl-Hex	18,07	$C_{28}H_{24}O_{14}$	M-H	583,1093	0,9	9,3	17	+		+	2.0 ± 0.30	3	169.0131 (8.8; -$C_{21}H_{18}O_9$), 241.0856 (10.9; -$C_{13}H_{10}O_{11}$), 269.0451 (100.0; -$C_{13}H_{14}O_9$), 313.0563 (69.4; -$C_{15}H_{10}O_5$), 419.1134 (18.1; -$C_4H_4O_7$), 431.0964 (8.4; -$C_7H_4O_4$), 583.1077 (17.9)
173	*Unidentified*	18,17	$C_{30}H_{32}O_{13}$	M-H	599,1770	1,4	15,5	15			+		4	183.0295 (14.4; -$C_{22}H_{24}O_8$), 197.0447 (31.8; -$C_{21}H_{22}O_8$), 257.0815 (100.0; -$C_{15}H_{18}O_9$), 271.0964 (8.5; -$C_{14}H_{16}O_9$), 312.0475 (6.0; -$C_{17}H_{19}O_4$), 327.0717 (43.8; -$C_{16}H_{16}O_4$), 341.0874 (97.4; -$C_{15}H_{14}O_4$), 389.2194 (1.9; -$C_{30}H_{31}O_{13}$), 584.1526 (9.4; -CH_3), 599.176
174	aleosone-coumaroyl-Hex-2 (aloeresin-A)	18,22	$C_{28}H_{28}O_{11}$	M-H	539,1558	1,7	16,0	15			+		3	167.0342 (10.5; -$C_{20}H_{20}O_7$), 185.0608 (1.9; -$C_{16}H_{18}O_9$), 227.0708 (100.0; -$C_{14}H_{16}O_8$), 251.0551 (3.6; -$C_{16}H_{16}O_5$), 296.0532 (9.9; -$C_{15}H_{15}O_3$), 311.0769 (67.1; -$C_{14}H_{12}O_3$), 341.0661 (5.2; -$C_{10}H_{14}O_4$), 377.0648 (0.9; -$C_7H_{14}O_4$), 415.0632 (0.6; -$CH_{16}O_6$), 495.1294
175	maesopsin	18,27	$C_{15}H_{12}O_6$	M-H	287,0561	0,7	10,5	10	+		+		3	135.0438 (67.6; -$C_7H_4O_4$), 151.0019 (100.0; -$C_8H_8O_2$), 269.0425 (11.3; -H_2O), 287.0589 (31.5)
176	(aloe-)emodin-anthrone-galloyl-Hex-1	18,28	$C_{28}H_{26}O_{13}$	M-H	569,1300	-0,3	71,8	16	+				3	169.0126 (4.0; -$C_{21}H_{20}O_8$), 227.1653 (7.8; -$C_{15}H_2O_{10}$), 255.0661 (100.0; -$C_{13}H_{14}O_9$), 297.0759 (16.2; -$C_{11}H_{12}O_8$), 313.0561 (18.1; -$C_{15}H_{12}O_4$), 417.1191 (19.3; -$C_7H_4O_4$), 569.1293 (12.4)
177	cinnamoyl-galloyl-Hex-1	18,29	$C_{22}H_{22}O_{11}$	M-H	461,1089	1,5	3,6	12			+		3	123.0077 (7.1; -$C_{16}H_{18}O_8$), 151.0022 (14.8; -$C_{15}H_{18}O_7$), 169.0136 (53.7; -$C_{15}H_{16}O_6$), 189.0553 (12.5; -$C_{11}H_{12}O_8$), 211.0247 (40.1; -$C_{13}H_{14}O_5$), 253.0349 (24.9; -$C_{11}H_{12}O_4$), 313.0562 (58.4; -$C_9H_8O_2$), 401.0878 (11.2; -$C_2H_4O_2$), 461.1085 (100.0)
178	*Unidentified*	18,38	$C_{22}H_{22}O_9$	M+HCOOH-H	475,1245	1,7	5,7	12			+		4	249.0540 (7.6; -$C_7H_{14}O_8$), 267.0653 (100.0; -$C_7H_{12}O_7$), 429.1187 (0.6; -CH_2O_2)
179	*Unidentified*	18,51	$C_{34}H_{28}O_6$	M-H	531,1813	2,5	27,3	21	+		+		4	209.0601 (6.2; -$C_{20}H_{18}O_4$), 225.0553 (3.7; -$C_{20}H_{18}O_3$), 253.0504 (100.0; -$C_{19}H_{18}O_2$), 271.0604 (1.7; -$C_{19}H_{16}O$), 277.1218 (1.2; -$C_{15}H_{10}O_4$), 483.1454 (0.9; -$C_{34}H_{27}O_6$)
180	(iso-)rhapontigenin	18,56	$C_{15}H_{14}O_4$	M-H	257,0819	2,3	0,8	9	+	12.6 ± 0.79	+	10.7 ± 0.85	3	172.0524 (1.7; -$C_4H_5O_2$), 224.0475 (7.2; -CH_5O), 241.0502 (100.0; -CH_4), 257.0814 (31.0)
181	(aloe-)emodin-anthrone-malonyl-Hex-1	18,66	$C_{24}H_{24}O_{12}$	M-H	503,1195	0,7	24,2	13	+				3	211.0773 (0.6; -$C_{10}H_{12}O_{10}$), 255.0658 (100.0; -$C_9H_{12}O_8$), 297.0771 (2.3; -$C_7H_{10}O_7$), 417.1181 (2.4; -$C_3H_2O_3$), 459.1291 (9.1; -CO_2)
182	piceatannol-coumaroyl-Hex-3	18,72	$C_{29}H_{28}O_{11}$	M-H	551,1558	0,9	10,9	16	+		+		3	117.0332 (0.8; -$C_{21}H_{22}O_{10}$), 163.0393 (1.3; -$C_{20}H_{20}O_8$), 225.0547 (0.6; -$C_{15}H_{18}O_8$), 243.0653 (100.0; -$C_{15}H_{16}O_7$), 307.0813 (16.2; -$C_{14}H_{12}O_4$), 405.1177 (2.4; -$C_9H_6O_2$), 551.1564 (0.4)
183	dihydroxy-dimethoxy-stilbene-Hex-2	18,78	$C_{22}H_{26}O_9$	M+HCOOH-H	479,1558	0,8	11,4	10	+		+		3	256.0731 (17.6; -$C_8H_{15}O_7$), 271.0975 (100.0; -$C_7H_{12}O_7$)
184	emodin-malonyl-Hex-1	18,78	$C_{24}H_{22}O_{13}$	M-H	517,0987	0,9	3,1	14	+		+		3	225.0551 (2.1; -$C_{10}H_{12}O_{10}$), 237.0548 (1.6; -$C_9H_{12}O_{10}$), 255.0655 (1.5; -$C_9H_{10}O_9$), 269.0450 (100.0; -$C_9H_{12}O_8$), 282.0530 (3.4; -$C_8H_{11}O_8$), 293.0460 (8.2; -$C_7H_{12}O_8$), 311.0557 (2.0; -$C_7H_{10}O_7$), 431.0995 (0.5; -$C_3H_2O_3$), 473.1082 (9.4; -CO_2)
185	deoxyrhapontigenin-diHex-1	18,78	$C_{27}H_{34}O_{13}$	M+HCOOH-H	611,1981	1,0	20,9	11	+		+		3	161.0454 (5.9; -$C_{22}H_{26}O_{10}$), 241.0866 (100.0; -$C_{13}H_{22}O_{12}$), 309.0757 (9.7; -$C_{10}H_{22}O_{10}$), 323.0974 (23.1; -$C_{16}H_{16}O_5$), 361.1075 (15.3; -$C_6H_{18}O_{10}$), 403.1189 (36.2; -$C_4H_{16}O_9$)
186	(iso-)rhapontigenin-hydroxybenzoyl-Hex	18,86	$C_{28}H_{28}O_{11}$	M-H	539,1558	0,4	16,4	15	+	0.4 ± 0.06	+		3	137.0220 (6.8; -$C_{21}H_{22}O_8$), 224.0494 (1.1; -$C_{14}H_{19}O_8$), 241.0505 (11.3; -$C_{14}H_{18}O_7$), 257.0814 (50.0; -$C_{13}H_{14}O_7$), 281.0669 (100.0; -$C_{15}H_{14}O_4$), 291.0258 (1.3; -$C_4H_{24}O_{11}$), 313.0585 (0.9; -$C_{15}H_{14}O_2$), 413.0842 (1.9; -$H_{14}O_7$), 495.1311 (0.9; -C_2H_4O)
187	(iso-)rhapontigenin-Hex-3	18,93	$C_{21}H_{24}O_9$	M-H	419,1347	2,0	10,0	10	+				3	242.0586 (31.7; -$C_7H_{13}O_5$), 257.0820 (100.0; -$C_6H_{10}O_5$), 297.0804 (5.4; -$C_4H_{10}O_4$)
188	altechromone_A-coumaroyl-Hex	18,93	$C_{26}H_{26}O_{10}$	M-H	497,1453	1,4	17,1	14	+				3	145.0284 (11.8; -$C_{17}H_{20}O_8$), 189.0555 (100.0; -$C_{15}H_{16}O_7$), 307.0824 (32.3; -$C_{11}H_{10}O_3$)
189	(iso-)rhapontigenin-coumaroyl-Hex-1	18,93	$C_{30}H_{30}O_{11}$	M-H	565,1715	1,1	8,9	16	+	0.4 ± 0.11	+		3	145.0283 (20.2; -$C_{21}H_{24}O_9$), 163.0398 (7.4; -$C_{21}H_{22}O_8$), 205.0510 (3.1; -$C_{19}H_{20}O_7$), 242.0583 (26.6; -$C_{16}H_{19}O_7$), 257.0814 (87.7; -$C_{15}H_{16}O_7$), 307.0819 (100.0; -$C_{15}H_{14}O_4$), 565.1716 (5.7)
190	(iso-)rhapontigenin-feruloyl-Hex-1	19,03	$C_{31}H_{32}O_{12}$	M-H	595,1821	1,6	23,2	16			+		3	175.0385 (51.9; -$C_{19}H_{16}O_6$), 193.0501 (16.1; -$C_{19}H_{14}O_5$), 242.0572 (45.9; -$C_{15}H_{13}O_5$), 257.0804 (100.0; -$C_{14}H_{10}O_5$), 277.0713 (8.5; -$C_{15}H_{10}O_3$), 337.0934 (58.9; -$C_{13}H_6O$)
191	resveratrol-piceatannol-dimer (gnetuhainin-C)	19,08	$C_{28}H_{22}O_7$	M+HCOOH-H	515,1347	1,8	22,4	18			+		3	213.0558 (1.2; -$C_{16}H_{14}O_6$), 227.0706 (1.8; -$C_{15}H_{12}O_6$), 241.0502 (10.3; -$C_{15}H_{14}O_5$), 333.0771 (2.1; -$C_9H_{10}O_4$), 347.0922 (4.9; -$C_8H_8O_4$), 359.0922 (7.0; -$C_7H_8O_4$), 363.0866 (2.2; -$C_8H_8O_3$), 375.0874 (7.8; -$C_7H_8O_3$), 385.1078 (5.0; -$C_5H_6O_4$), 401.1399 (1.7; -C4H
192	di-coumaroyl-Hex-5	19,12	$C_{25}H_{28}O_9$	M-H	471,1660	1,7	5,0	12	+				3	145.0270 (34.8; -$C_{16}H_{22}O_7$), 163.0751 (12.9; -$C_{15}H_{16}O_7$), 224.0455 (3.1; -$C_{11}H_{19}O_6$), 247.0575 (5.1; -$C_5H_{20}O_9$), 307.0815 (100.0; -$C_{10}H_{12}O_2$), 471.1659 (47.9)
193	deoxyrhapontigenin-Hex-1	19,18	$C_{21}H_{24}O_8$	M+HCOOH-H	449,1453	1,7	5,4	10	+	138.6 ± 8.55	+	31.7 ± 2.08	3	161.0448 (0.0; -$C_{16}H_{16}O_5$), 199.0757 (0.1; -$C_9H_{14}O_8$), 209.0593 (0.0; -$C_8H_{16}O_8$), 225.0553 (2.4; -$C_8H_{16}O_7$), 241.0864 (100.0; -$C_7H_{12}O_7$), 265.0865 (0.1; -$C_5H_{12}O_7$), 283.0972 (0.1; -$C_5H_{10}O_6$), 403.1383 (0.0; -CH_2O_2)
194	resveratrol-coumaroyl-Hex-3	19,23	$C_{29}H_{28}O_{10}$	M-H	535,1609	0,9	15,8	16			+		3	117.0327 (4.9; -$C_{21}H_{22}O_9$), 145.0273 (29.2; -$C_{20}H_{22}O_8$), 163.0381 (12.1; -$C_{20}H_{20}O_7$), 227.0710 (100.0; -$C_{15}H_{16}O_7$), 307.0821 (93.8; -$C_{14}H_{12}O_3$), 535.1625 (6.4)
195	*Unidentified*	19,23	$C_{43}H_{34}O_{12}$	M-H	741,1977	1,3	31,2	27			+		4	241.0510 (15.1; -$C_{29}H_{24}O_8$), 361.0724 (25.1; -$C_{22}H_{20}O_6$), 373.0705 (28.5; -$C_{21}H_{20}O_6$), 483.1100 (35.3; -$C_{15}H_{14}O_4$), 499.1393 (29.2; -$C_{14}H_{10}O_4$), 619.1583 (66.8; -$C_{43}H_{33}O_{12}$), 631.1617 (76.5; -$C_6H_6O_2$), 741.1973 (100.0)
196	pinocembrine-Hex-5	19,24	$C_{21}H_{22}O_9$	M-H	417,1191	1,1	1,5	11	+				3	181.0643 (0.1; -$C_8H_{12}O_8$), 209.0606 (1.2; -$C_7H_{12}O_7$), 227.0710 (9.3; -$C_7H_{10}O_6$), 255.0659 (100.0; -$C_6H_{10}O_5$), 297.0767 (0.3; -$C_4H_8O_4$), 417.1189 (1.1)
197	torachrysone/dihydro-piceatannol-Hex-2	19,31	$C_{20}H_{24}O_9$	M-H	407,1347	1,2	4,2	9	+		+		3	215.0348 (0.3; -$C_8H_{16}O_5$), 230.0581 (9.0; -$C_7H_{13}O_5$), 245.0816 (100.0; -$C_6H_{10}O_5$), 269.0821 (0.1; -$C_4H_{10}O_5$), 287.0940 (0.1; -$C_4H_8O_4$), 407.1358 (0.1)
198	pinosylvin-Hex-2	19,36	$C_{20}H_{22}O_7$	M+HCOOH-H	419,1347	2,5	7,4	10	+		+		3	211.0761 (100.0; -$C_7H_{12}O_7$)
199	chrysophanol-Hex-1	19,39	$C_{21}H_{20}O_9$	M-H	415,1034	1,2	12,7	12	+	4.2 ± 0.82	+	17.4 ± 0.91	3	221.0600 (0.6; -$C_6H_{10}O_7$), 225.0579 (0.7; -$C_7H_{10}O_6$), 239.0706 (1.7; -$C_6H_8O_6$), 249.0561 (4.4; -$C_5H_{10}O_6$), 253.0504 (100.0; -$C_6H_{10}O_5$), 266.0577 (4.1; -$C_5H_9O_5$), 277.0507 (9.4; -$C_4H_{10}O_5$), 291.0653 (0.5; -$C_3H_8O_5$), 295.0616 (0.8; -$C_4H_8O_4$), 307.0622 (0.4; -C3H8
200	(iso-)rhapontigenin-sinapoyl-Hex-1	19,44	$C_{32}H_{34}O_{13}$	M-H	625,1926	1,6	27,5	16	+		+		3	190.0270 (15.9; -$C_{22}H_{27}O_9$), 205.0503 (90.0; -$C_{21}H_{24}O_9$), 223.0606 (28.8; -$C_{21}H_{22}O_8$), 241.0508 (20.3; -$C_{18}H_{24}O_9$), 257.0816 (100.0; -$C_{17}H_{20}O_9$), 367.1032 (71.8; -$C_{15}H_{14}O_4$), 625.1911 (5.9)
201	**apigenin-7-Glu**	19,51	$C_{21}H_{20}O_{10}$	M-H	431,0983	1,0	3,5	12	+	1.1 ± 0.17	+	3.5 ± 0.19	1	225.0554 (0.5; -$C_7H_{10}O_7$), 240.0424 (0.9; -$C_7H_{11}O_6$), 269.0452 (100.0; -$C_6H_{10}O_5$), 282.0529 (0.9; -$C_5H_9O_5$), 293.0454 (1.6; -$C_4H_{10}O_5$), 311.0560 (4.9; -$C_4H_8O_4$), 413.0877 (0.7; -H_2O), 431.0979 (49.9)
202	**pinocembroside**	19,59	$C_{21}H_{22}O_9$	M-H	417,1191	5,1	53,0	11	+				1	209.0604 (1.1; -$C_7H_{12}O_7$), 227.0712 (8.8; -$C_7H_{10}O_6$), 255.0660 (100.0; -$C_6H_{10}O_5$), 417.1189 (1.1)
203	(iso-)rhapontigenin-coumaroyl-Hex-2	19,67	$C_{30}H_{30}O_{11}$	M-H	565,1715	0,9	4,9	16	+	3.9 ± 0.60	+	3.0 ± 0.29	3	145.0277 (22.2; -$C_{21}H_{24}O_9$), 163.0388 (8.1; -$C_{21}H_{22}O_8$), 241.0504 (14.6; -$C_{16}H_{20}O_7$), 257.0815 (88.3; -$C_{15}H_{16}O_7$), 307.0821 (100.0; -$C_{15}H_{14}O_4$), 565.1711 (8.2)
204	*Unidentified*	19,80	$C_{15}H_{12}O_5$	M-H	271,0611	3,7	6,0	10			+		4	185.0608 (3.8; -$C_3H_2O_3$), 209.0603 (5.1; -CH_2O_3), 227.0708 (41.1; -CO_2), 253.0504 (100.0; -H_2O), 271.0612 (32.3)
205	chrysophanol-Hex-2	19,81	$C_{21}H_{20}O_9$	M-H	415,1034	1,3	5,6	12	+	2.5 ± 0.26	+	12.5 ± 0.73	3	249.0555 (4.7; -$C_5H_{10}O_6$), 253.0503 (100.0; -$C_6H_{10}O_5$), 266.0585 (3.6; -$C_5H_9O_5$), 277.0501 (9.9; -$C_4H_{10}O_5$)
206	resveratrol-dimer-Hex-2	19,86	$C_{34}H_{32}O_{11}$	M-H	615,1871	1,3	7,5	19	+		+		3	307.0976 (1.2; -$C_{15}H_{16}O_7$), 313.0576 (3.3; -$C_{21}H_{18}O_2$), 333.0762 (2.3; -$C_{14}H_{18}O_6$), 347.0914 (2.5; -$C_{13}H_{16}O_6$), 359.0928 (3.7; -$C_{12}H_{16}O_6$), 369.1110 (2.7; -$C_{10}H_{14}O_7$), 385.1462 (2.3; -$C_9H_{10}O_7$), 411.1238 (5.6; -$C_8H_{12}O_6$), 453.1341 (100.0; -$C_6H_{10}O_5$)
207	piceatannol-benzoyl-Hex	19,90	$C_{27}H_{26}O_{10}$	M-H	509,1453	1,8	6,3	15	+				3	201.0552 (1.0; -$C_{15}H_{16}O_7$), 225.0573 (0.6; -$C_{13}H_{16}O_7$), 243.0661 (100.0; -$C_{13}H_{14}O_6$)
208	(iso-)rhapontigenin-feruloyl-Hex-2	19,93	$C_{31}H_{32}O_{12}$	M-H	595,1821	1,0	11,9	16	+		+	2.0 ± 0.40	3	160.0153 (6.6; -$C_{22}H_{27}O_9$), 175.0390 (45.2; -$C_{21}H_{24}O_9$), 193.0498 (20.8; -$C_{21}H_{22}O_8$), 241.0499 (18.5; -$C_{17}H_{22}O_8$), 257.0809 (100.0; -$C_{16}H_{18}O_8$), 323.0768 (5.0; -$C_{16}H_{16}O_4$), 337.0923 (73.2; -$C_{15}H_{14}O_4$), 595.1804 (5.5)
209	deoxyrhapontigenin-galloyl-Hex-1	20,00	$C_{28}H_{28}O_{12}$	M-H	555,1508	1,4	1,5	15	+	6.8 ± 0.74	+	1.8 ± 0.22	3	125.0226 (1.7; -$C_{22}H_{22}O_9$), 151.0021 (1.4; -$C_{21}H_{24}O_8$), 169.0135 (17.8; -$C_{21}H_{22}O_7$), 211.0245 (1.5; -$C_{19}H_{20}O_6$), 241.0867 (21.6; -$C_{13}H_{14}O_9$), 253.0351 (2.7; -$C_{17}H_{18}O_5$), 271.0459 (0.7; -$C_{17}H_{16}O_4$), 283.0973 (0.5; -$C_{11}H_{12}O_8$), 295.0449 (0.6; -$C_{15}H_{16}O_4$), 313.0563
210	flavanol-piceatannol-dimer	20,01	$C_{29}H_{24}O_8$	M+HCOOH-H	545,1453	1,6	2,8	18			+	3.4 ± 0.43	3	213.0555 (0.9; -$C_{17}H_{16}O_7$), 241.0505 (8.5; -$C_{16}H_{16}O_6$), 344.0683 (1.0; -$C_9H_{13}O_5$), 362.0788 (4.3; -$C_9H_{11}O_4$), 377.1028 (1.4; -$C_8H_8O_4$), 389.1032 (2.3; -$C_7H_8O_4$), 415.1182 (4.1; -$C_5H_6O_4$), 431.1498 (1.2; -$C_4H_2O_4$), 441.0983 (0.8; -$C_4H_8O_3$), 457.1290 (1.1; -C3H4O
211	*Unidentified*	20,25	$C_{22}H_{24}O_{10}$	M-H	447,1296	0,6	20,9	11	+				4	225.0553 (3.4; -$C_8H_{14}O_7$), 241.0869 (31.2; -$C_7H_{10}O_7$), 257.0821 (6.9; -$C_7H_{10}O_6$), 265.0876 (1.3; -$C_5H_{10}O_7$), 270.0532 (4.6; -$C_7H_{13}O_5$), 285.0768 (100.0; -$C_6H_{10}O_5$), 385.1301 (0.8; -CH_2O_3)
212	rhapontigenin-coumaroyl-Hex-2	20,42	$C_{30}H_{30}O_{11}$	M-H	565,1715	1,7	4,1	16			+	2.1 ± 0.13	3	119.0486 (3.4; -$C_{22}H_{22}O_{10}$), 145.0273 (38.3; -$C_{21}H_{24}O_9$), 163.0384 (14.7; -$C_{21}H_{22}O_8$), 187.0398 (4.1; -$C_{19}H_{22}O_8$), 257.0809 (60.9; -$C_{15}H_{16}O_7$), 307.0815 (100.0; -$C_{15}H_{14}O_4$), 401.1253 (2.6; -$C_9H_8O_3$), 565.1701 (4.6)
213	resveratrol-dimer-Hex-3	20,43	$C_{34}H_{32}O_{11}$	M-H	615,1871	1,1	28,9	19	+				3	453.1349 (100.0; -$C_6H_{10}O_5$)
214	deoxyrhapontigenin-malonyl-Hex	20,44	$C_{24}H_{26}O_{11}$	M-H	489,1402	1,4	4,8	12	+	3.0 ± 0.31	+	1.2 ± 0.13	3	182.0726 (0.1; -$C_{11}H_{15}O_{10}$), 197.0600 (0.2; -$C_{11}H_{16}O_9$), 225.0554 (4.8; -$C_{10}H_{16}O_8$), 241.0866 (100.0; -$C_9H_{12}O_8$), 265.0870 (0.3; -$C_7H_{12}O_8$), 283.0964 (0.3; -$C_7H_{10}O_7$), 325.1069 (0.1; -$C_5H_8O_6$), 385.1292 (0.2; -$C_3H_4O_4$)
215	emodin-malonyl-Hex-2	20,66	$C_{24}H_{22}O_{13}$	M-H	517,0987	1,1	3,7	14	+	0.5 ± 0.17	+	0.5 ± 0.09	3	225.0556 (0.6; -$C_{10}H_{12}O_{10}$), 240.0429 (1.0; -$C_{10}H_{13}O_9$), 269.0452 (100.0; -$C_9H_{12}O_8$), 282.0535 (1.0; -$C_8H_{11}O_8$), 293.0453 (2.0; -$C_7H_{12}O_8$), 311.0558 (5.0; -$C_7H_{10}O_7$), 323.0558 (0.3; -$C_6H_{10}O_7$), 413.0872 (0.5; -$C_3H_4O_4$), 431.0982 (2.9; -$C_3H_2O_3$), 455.0973 (0.5; -
216	(aloe)-emodin-anthrone-Ac-Hex-1	20,70	$C_{23}H_{24}O_{10}$	M-H	459,1296	3,0	17,9	12	+				3	209.0593 (1.6; -$C_9H_{14}O_8$), 227.0699 (8.9; -$C_9H_{12}O_7$), 255.0655 (100.0; -$C_8H_{12}O_6$), 399.1075 (4.6; -$C_2H_4O_2$), 459.1295 (2.5)
217	deoxyrhapontigenin-Pent	20,72	$C_{20}H_{22}O_7$	M+HCOOH-H	419,1347	1,4	6,7	10	+	0.5 ± 0.15			3	225.0551 (1.7; -$C_7H_{14}O_6$), 241.0866 (100.0; -$C_6H_{10}O_6$), 373.1275 (0.2; -CH_2O_2)
218	rhapontigenin-feruloyl-Hex-3	20,72	$C_{31}H_{32}O_{12}$	M-H	595,1821	1,6	4,9	16			+	0.7 ± 0.13	3	134.0344 (6.0; -$C_{23}H_{25}O_{10}$), 160.0146 (12.7; -$C_{22}H_{27}O_9$), 175.0391 (100.0; -$C_{21}H_{24}O_9$), 193.0501 (53.1; -$C_{21}H_{22}O_8$), 217.0499 (5.8; -$C_{19}H_{22}O_8$), 241.0499 (17.0; -$C_{17}H_{22}O_8$), 257.0810 (73.1; -$C_{16}H_{18}O_8$), 322.0683 (28.8; -$C_{16}H_{17}O_4$), 337.0922 (89.3; -$C_{15}H_{14}O_4$), 4
219	(aloe-)emodin-anthrone-malonyl-Hex-2	20,77	$C_{24}H_{24}O_{12}$	M-H	503,1195	0,6	7,0	13	+				3	255.0657 (100.0; -$C_9H_{12}O_8$), 279.0661 (1.6; -$C_7H_{12}O_8$), 297.0764 (5.0; -$C_7H_{10}O_7$), 417.1191 (4.4; -$C_3H_2O_3$), 441.1197 (0.6; -CH_2O_3), 459.1291 (62.2; -CO_2)
220	torachrysone-Ac-Hex-2	20,89	$C_{22}H_{26}O_{10}$	M-H	449,1453	0,7	10,5	10			+		3	202.0629 (0.1; -$C_{10}H_{15}O_7$), 215.0345 (0.8; -$C_{10}H_{18}O_6$), 230.0583 (12.7; -$C_9H_{15}O_6$), 245.0816 (100.0; -$C_8H_{12}O_6$), 269.0808 (0.1; -$C_6H_{12}O_6$), 389.1190 (0.0; -$C_{22}H_{25}O_{10}$)
221	(aloe-)emodin-anthrone-malonyl-Hex(-CO2)-2	20,94	$C_{23}H_{24}O_{10}$	M-H	459,1296	1,3	2,2	12	+	2.2 ± 0.16	+		3	181.0648 (0.1; -$C_{10}H_{14}O_9$), 209.0606 (2.0; -$C_9H_{14}O_8$), 227.0712 (12.3; -$C_9H_{12}O_7$), 255.0660 (100.0; -$C_8H_{12}O_6$), 297.0766 (0.3; -$C_6H_{10}O_5$)
222	resveratrol-dimer-1	20,95	$C_{28}H_{22}O_6$	M+HCOOH-H	499,1398	1,4	6,5	18	+	3.3 ± 0.29	+	3.4 ± 0.16	3	333.0763 (3.0; -$C_9H_{10}O_3$), 347.0916 (11.0; -$C_8H_8O_3$), 359.0918 (9.4; -$C_7H_8O_3$), 385.1439 (2.9; -$C_4H_2O_4$), 411.1231 (4.0; -$C_3H_4O_3$), 435.1231 (4.9; -CH_4O_3), 453.1335 (100.0; -CH_2O_2)
223	torachrysone-malonyl-Hex(-CO2)-3	21,08	$C_{22}H_{26}O_{10}$	M-H	449,1453	1,4	7,7	10	+	5.1 ± 0.46	+	5.0 ± 0.29	3	202.0629 (0.1; -$C_{10}H_{15}O_7$), 215.0345 (0.8; -$C_{10}H_{18}O_6$), 230.0583 (12.7; -$C_9H_{15}O_6$), 245.0816 (100.0; -$C_8H_{12}O_6$), 269.0808 (0.1; -$C_6H_{12}O_6$), 389.1190 (0.0; -$C_{22}H_{25}O_{10}$)
224	chrysophanol-malonyl-Hex(-CO2)-2	21,09	$C_{23}H_{22}O_{10}$	M-H	457,1140	1,0	14,7	13	+		+		3	239.0714 (3.1; -$C_8H_{10}O_7$), 249.0559 (7.6; -$C_7H_{12}O_7$), 253.0507 (100.0; -$C_8H_{12}O_6$), 266.0589 (7.1; -$C_7H_{11}O_6$), 277.0506 (14.8; -$C_6H_{12}O_6$), 291.0652 (2.0; -$C_5H_{10}O_6$), 319.0620 (1.6; -$C_4H_{10}O_5$), 361.0730 (0.5; -$C_2H_8O_4$)
225	chrysophanol-galloyl-Hex	21,11	$C_{28}H_{24}O_{13}$	M-H	567,1144	0,6	4,3	17	+		+		3	125.0242 (1.3; -$C_{22}H_{18}O_{10}$), 169.0136 (18.0; -$C_{21}H_{18}O_8$), 211.0245 (3.5; -$C_{19}H_{16}O_7$), 241.0354 (9.6; -$C_{18}H_{14}O_6$), 253.0505 (49.5; -$C_{13}H_{14}O_9$), 271.0459 (5.7; -$C_{17}H_{12}O_5$), 313.0565 (100.0; -$C_{15}H_{10}O_4$), 567.1149 (4.9)
226	vitisinol-C	21,21	$C_{27}H_{24}O_5$	M-H	427,1550	0,5	12,4	16			+		2	265.0858 (46.5; -$C_{10}H_{10}O_2$), 307.0965 (41.7; -C_8H_8O), 333.1121 (22.7; -C_6H_6O), 385.1431 (18.5; -C_2H_2O), 427.1549 (100.0)
227	physcion-Hex-1 (rheochrysin)	21,24	$C_{21}H_{20}O_8$	M+HCOOH-H	445,1140	0,1	8,1	12	+		+	2.2 ± 0.38	3	240.0422 (6.7; -$C_8H_{13}O_6$), 251.0712 (0.9; -$C_6H_{10}O_7$), 268.0380 (2.9; -$C_7H_{13}O_5$), 279.0660 (2.4; -$C_5H_{10}O_6$), 283.0612 (100.0; -$C_6H_{10}O_5$), 292.0379 (3.9; -$C_5H_{13}O_5$), 296.0686 (4.1; -$C_5H_9O_5$), 307.0611 (5.3; -$C_4H_{10}O_5$)
228	*Unidentified*	21,28	$C_{45}H_{44}O_{19}$	M-H	887,2404	1,4	16,9	24	+				4	223.0757 (8.3; -$C_{30}H_{32}O_{17}$), 238.0632 (100.0; -$C_{30}H_{33}O_{16}$), 252.0785 (12.5; -$C_{29}H_{31}O_{16}$), 281.0814 (3.7; -$C_{28}H_{30}O_{15}$), 400.1162 (3.3; -$C_{24}H_{23}O_{11}$), 477.1333 (19.7; -$C_{15}H_{22}O_{13}$), 519.1427 (1.5; -$C_{13}H_{20}O_{12}$), 621.1768 (1.8; -$C_9H_{14}O_9$), 639.1865 (29.9; -$C_9H_{12}O_8$), 6
229	resveratrol-piceatannol-mixed-tetramer	21,33	$C_{38}H_{52}O_{25}$	M-H	907,2724	-2,0	###	13		0.8 ± 0.07	+	2.9 ± 0.59	3	237.0557 (1.3; -$C_{41}H_{34}O_9$), 255.0657 (24.8; -$C_{41}H_{32}O_8$), 331.0954 (3.4; -$C_{35}H_{28}O_8$), 347.0919 (24.5; -$C_{35}H_{28}O_7$), 359.0917 (25.3; -$C_{34}H_{28}O_7$), 411.1227 (4.0; -$C_{30}H_{24}O_7$), 416.1109 (7.1; -$C_{35}H_{23}O_3$), 453.1338 (59.5; -$C_{28}H_{22}O_6$), 465.1337 (17.1; -$C_{27}H_{22}O_6$), 489.1
230	piceatannol-cinnamoyl-Hex	21,37	$C_{29}H_{28}O_{10}$	M-H	535,1609	1,2	3,9	16	+		+		3	147.0422 (0.4; -$C_{20}H_{20}O_8$), 193.1249 (1.0; -$C_{17}H_{10}O_8$), 201.0547 (1.6; -$C_{17}H_{18}O_7$), 243.0659 (100.0; -$C_{15}H_{16}O_6$), 327.0932 (0.3; -$C_{29}H_{27}O_{10}$), 387.1074 (0.6; -$C_9H_8O_2$)
231	chrysophanol-Ac-Hex-3	21,45	$C_{23}H_{22}O_{10}$	M-H	457,1140	2,7	2,1	13	+	6.0 ± 0.52	+		3	221.0604 (0.8; -$C_8H_{12}O_8$), 225.0555 (1.0; -$C_9H_{12}O_7$), 239.0706 (1.7; -$C_8H_{10}O_7$), 253.0503 (100.0; -$C_8H_{12}O_6$), 266.0583 (5.2; -$C_7H_{11}O_6$), 277.0505 (11.4; -$C_6H_{12}O_6$), 295.0608 (0.4; -$C_6H_{10}O_5$), 307.0629 (0.2; -$C_5H_{10}O_5$), 319.0599 (0.2; -$C_4H_{10}O_5$)
232	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-1	21,45	$C_{48}H_{44}O_{24}$	M-H	#######	1,0	3,5	27			+	16.0 ± 0.72	3	225.0546 (0.7; -$C_{34}H_{34}O_{21}$), 239.0708 (1.4; -$C_{33}H_{32}O_{21}$), 253.0504 (100.0; -$C_{33}H_{34}O_{20}$), 266.0580 (4.0; -$C_{32}H_{33}O_{20}$), 277.0503 (11.1; -$C_{31}H_{34}O_{20}$), 295.0604 (1.2; -$C_{31}H_{32}O_{19}$), 319.0617 (0.3; -$C_{29}H_{32}O_{19}$), 457.1126 (0.1; -$C_{25}H_{22}O_{14}$)
233	chrysophanol-dianthrone-di(malonyl-Hex)-1	21,47	$C_{48}H_{46}O_{22}$	M-H	973,2407	1,6	13,5	26	+				3	222.0683 (8.8; -$C_{33}H_{35}O_{20}$), 223.0758 (9.1; -$C_{33}H_{34}O_{20}$), 238.0634 (100.0; -$C_{33}H_{35}O_{19}$), 252.0787 (13.0; -$C_{32}H_{33}O_{19}$), 281.0812 (4.4; -$C_{31}H_{32}O_{18}$), 293.0811 (2.5; -$C_{30}H_{32}O_{18}$), 443.1338 (3.7; -$C_{25}H_{22}O_{13}$), 447.1232 (1.9; -$C_{19}H_{26}O_{17}$), 477.1329 (18.9; -$C_{18}H_{24}O_{16}$), 885.2602 (10.4; -C_2O_4)
234	(aloe-)emodin-Hex-4	21,59	$C_{21}H_{20}O_{10}$	M-H	431,0983	1,4	2,9	12			+		3	269.0451 (100.0; -$C_6H_{10}O_5$), 282.0531 (16.1; -$C_5H_9O_5$), 311.0548 (2.4; -$C_4H_8O_4$), 431.0963 (1.3)
235	*Unidentified*	21,62	$C_{23}H_{26}O_{11}$	M-H	477,1402	1,2	10,3	11	+				4	226.0622 (3.0; -$C_9H_{15}O_8$), 241.0870 (100.0; -$C_8H_{12}O_8$), 269.0450 (1.0; -$C_8H_{16}O_6$)
236	physcion-anthrone-Hex-1	21,63	$C_{22}H_{24}O_9$	M-H	431,1347	4,2	###	11	+				3	254.0581 (17.3; -$C_7H_{13}O_5$), 269.0820 (100.0; -$C_6H_{10}O_5$), 311.0907 (1.8; -$C_4H_8O_4$), 416.1108 (1.2; -CH_3), 431.1345 (13.3)
237	*Unidentified*	21,72	$C_{29}H_{24}O_7$	M-H	483,1449	1,2	13,8	18	+		+		4	346.0832 (49.4; -$C_8H_9O_2$), 389.1022 (7.7; -C_6H_6O), 399.1241 (7.2; -$C_4H_4O_2$), 415.1542 (12.7; -C_3O_2), 441.1342 (9.0; -C_2H_2O), 483.1447 (100.0)
238	nataloe-emodin-8-Me-Ac-Hex	21,76	$C_{24}H_{26}O_{11}$	M-H	489,1402	0,7	24,7	12	+				4	227.0354 (0.6; -$C_{11}H_{18}O_7$), 242.0585 (4.3; -$C_{10}H_{15}O_7$), 257.0820 (9.2; -$C_9H_{12}O_7$), 270.0533 (6.3; -$C_9H_{15}O_6$), 285.0768 (100.0; -$C_8H_{12}O_6$), 325.0723 (0.8; -$C_6H_{12}O_5$)
239	trihydroxy-octadecadienoic-acid	21,81	$C_{18}H_{32}O_5$	M-H	327,2176	1,6	23,5	3	+				3	211.1341 (24.6; -$C_6H_{12}O_2$), 229.1451 (28.7; -$C_6H_{10}O$), 239.1276 (6.4; -$C_5H_{12}O$), 291.1955 (12.0; -H_4O_2), 309.2068 (5.8; -H_2O), 327.2176 (100.0)
240	chrysophanol-malonyl-Hex(-CO2)-4	21,82	$C_{23}H_{22}O_{10}$	M-H	457,1140	0,2	4,9	13	+	5.8 ± 0.36	+		3	221.0628 (1.0; -$C_8H_{12}O_8$), 225.0546 (1.2; -$C_9H_{12}O_7$), 239.0705 (1.9; -$C_8H_{10}O_7$), 249.0555 (5.3; -$C_7H_{12}O_7$), 253.0506 (100.0; -$C_8H_{12}O_6$), 266.0591 (4.6; -$C_7H_{11}O_6$), 277.0506 (10.6; -$C_6H_{12}O_6$), 319.0606 (0.2; -$C_4H_{10}O_5$)
241	*Unidentified*	21,82	$C_{46}H_{44}O_{22}$	M-H	947,2251	0,8	15,7	25	+		+	4.7 ± 1.40	4	225.0553 (0.8; -$C_{32}H_{34}O_{19}$), 239.0706 (1.3; -$C_{31}H_{32}O_{19}$), 249.0555 (4.2; -$C_{30}H_{34}O_{19}$), 253.0501 (100.0; -$C_{31}H_{34}O_{18}$), 266.0579 (4.1; -$C_{30}H_{33}O_{18}$), 277.0500 (13.4; -$C_{29}H_{34}O_{18}$), 283.0612 (3.0; -$C_{30}H_{32}O_{17}$), 295.0611 (2.0; -$C_{29}H_{32}O_{17}$), 319.0612 (0.5; -$C_{27}H_{32}O_{17}$)
242	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-2	21,82	$C_{48}H_{44}O_{24}$	M-H	#######	1,7	3,4	18			+	11.5 ± 1.15	3	221.0607 (0.4; -$C_{33}H_{34}O_{22}$), 225.0550 (0.8; -$C_{34}H_{34}O_{21}$), 239.0706 (1.3; -$C_{33}H_{32}O_{21}$), 249.0552 (4.2; -$C_{32}H_{34}O_{21}$), 253.0500 (100.0; -$C_{33}H_{34}O_{20}$), 266.0579 (4.2; -$C_{32}H_{33}O_{20}$), 277.0501 (11.3; -$C_{31}H_{34}O_{20}$), 295.0606 (1.4; -$C_{31}H_{32}O_{19}$), 307.0610 (0.2; -$C_{30}H_{32}O_{19}$),
243	physcion-Hex-2 (rheochrysin)	21,83	$C_{22}H_{22}O_{10}$	M-H	445,1140	1,2	16,2	25			+		2	240.0421 (6.7; -$C_8H_{13}O_6$), 279.0649 (5.1; -$C_5H_{10}O_6$), 283.0608 (100.0; -$C_6H_{10}O_5$), 307.0605 (3.0; -$C_4H_{10}O_5$)

244	(aloe-)emodin-Hex-5	21,97	$C_{22}H_{24}O_{9}$	M-H	431,1347	1,7	19,2	11	+				3	254.0583 (14.5; $-C_{7}H_{13}O_{5}$), 269.0816 (100.0; $-C_{6}H_{10}O_{5}$), 311.0926 (1.7; $-C_{4}H_{8}O_{4}$), 416.1109 (1.6; $-CH_{3}$), 431.1344 (9.1)
245	keampferol	22,03	$C_{15}H_{10}O_{6}$	M-H	285,0404	-0,6	8,8	11			+		2	211.0416 (0.2; $-C_{2}H_{2}O_{3}$), 229.0493 (0.3; $-C_{2}O_{2}$), 241.0510 (1.0; $-CO_{2}$), 257.0451 (1.0; $-CO$), 285.0407 (100.0)
246	chrysophanol-dianthrone-malonyl-diHex-1	22,04	$C_{45}H_{44}O_{19}$	M-H	887,2404	1,4	14,3	24	+				3	210.0678 (1.1; $-C_{31}H_{33}O_{17}$), 222.0684 (7.1; $-C_{30}H_{33}O_{17}$), 238.0632 (100.0; $-C_{30}H_{33}O_{16}$), 252.0786 (10.7; $-C_{29}H_{31}O_{16}$), 281.0810 (2.6; $-C_{28}H_{30}O_{15}$), 293.0804 (1.8; $-C_{27}H_{30}O_{15}$), 309.0765 (1.0; $-C_{27}H_{30}O_{14}$), 400.1153 (2.0; $-C_{24}H_{23}O_{11}$), 401.1227 (1.9; $-C_{24}H_{22}O_{11}$), 639.1864 (24.9; $-C_{9}H_{12}O_{8}$), 663.1849 (2.4; $-C_{7}H_{12}O_{8}$), 681.1967 (21.2; $-C_{7}H_{10}O_{7}$), 801.2392 (2.5; $-C_{3}H_{2}O_{3}$), 843.2495 (9.5; $-CO_{2}$)
247	chrysophanol-dianthrone-malonyl-diHex-2	22,24	$C_{45}H_{44}O_{19}$	M-H	887,2404	1,5	10,1	24	+				3	210.0678 (1.1; $-C_{31}H_{33}O_{17}$), 222.0684 (7.1; $-C_{30}H_{33}O_{17}$), 238.0632 (100.0; $-C_{30}H_{33}O_{16}$), 252.0786 (10.7; $-C_{29}H_{31}O_{16}$), 281.0810 (2.6; $-C_{28}H_{30}O_{15}$), 293.0804 (1.8; $-C_{27}H_{30}O_{15}$), 309.0765 (1.0; $-C_{27}H_{30}O_{14}$), 400.1153 (2.0; $-C_{24}H_{23}O_{11}$), 401.1227 (1.9; $-C_{24}H_{22}O_{11}$), 639.1864 (24.9; $-C_{9}H_{12}O_{8}$), 663.1849 (2.4; $-C_{7}H_{12}O_{8}$), 681.1967 (21.2; $-C_{7}H_{10}O_{7}$), 801.2392 (2.5; $-C_{3}H_{2}O_{3}$), 843.2495 (9.5; $-CO_{2}$)
248	resveratrol-trimer (mirabilol-B)	22,26	$C_{42}H_{34}O_{9}$	M-H	681,2130	2,6	10,5	11			+		3	227.0706 (29.7; $-C_{28}H_{22}O_{6}$), 347.0924 (17.1; $-C_{21}H_{18}O_{4}$), 359.0924 (26.5; $-C_{20}H_{18}O_{4}$), 435.1223 (8.4; $-C_{14}H_{14}O_{4}$), 453.1344 (100.0; $-C_{14}H_{12}O_{3}$)
249	stilbene-dimer_cassigarol-B	22,27	$C_{21}H_{16}O_{6}$	M-H	363,0874	3,2	11,7	26			+		2	321.0749 (1.7; $-C_{2}H_{2}O$), 348.0632 (72.3; $-CH_{3}$), 363.0869 (100.0)
250	deoxyrhapontigenin-caffeoyl-Hex-2	22,27	$C_{30}H_{30}O_{11}$	M-H	565,1715	1,1	57,2	16	+				3	147.0439 (0.3; $-C_{21}H_{22}O_{9}$), 185.0613 (0.5; $-C_{18}H_{20}O_{9}$), 227.0714 (100.0; $-C_{16}H_{18}O_{8}$), 241.0880 (0.2; $-C_{15}H_{16}O_{8}$), 269.0819 (0.4; $-C_{14}H_{16}O_{7}$), 311.0931 (0.1; $-C_{12}H_{14}O_{6}$), 371.1140 (0.4; $-C_{10}H_{10}O_{4}$), 519.1661 (8.7; $-CH_{2}O_{2}$)
251	chrysophanol-dianthrone-diHex	22,32	$C_{42}H_{42}O_{16}$	M-H	801,2400	1,5	15,4	22	+				3	222.0683 (11.5; $-C_{27}H_{31}O_{14}$), 238.0632 (100.0; $-C_{27}H_{31}O_{13}$), 252.0786 (13.2; $-C_{26}H_{29}O_{13}$), 281.0803 (4.7; $-C_{25}H_{28}O_{12}$), 293.0834 (1.8; $-C_{24}H_{28}O_{12}$), 309.0764 (2.1; $-C_{24}H_{28}O_{11}$), 458.1173 (2.0; $-C_{12}H_{23}O_{11}$), 477.1330 (9.0; $-C_{12}H_{20}O_{10}$), 639.1853 (33.9; $-C_{6}H_{10}O_{5}$),
252	chrysophanol-dianthrone-di(malonyl-Hex)-2	22,49	$C_{48}H_{46}O_{22}$	M-H	973,2407	1,5	9,1	26	+	1.0 ± 0.13			3	223.0756 (8.4; $-C_{33}H_{34}O_{20}$), 238.0629 (100.0; $-C_{33}H_{35}O_{19}$), 477.1325 (18.3; $-C_{18}H_{24}O_{16}$), 639.1861 (2.8; $-C_{12}H_{14}O_{11}$), 663.1859 (3.2; $-C_{10}H_{14}O_{11}$), 681.1960 (39.4; $-C_{10}H_{12}O_{10}$), 843.2469 (2.3; $-C_{48}H_{45}O_{22}$), 885.2603 (6.8; $-C_{2}O_{4}$)
253	deoxyrhapontigenin-hydroxybenzoyl-Hex	22,58	$C_{28}H_{28}O_{10}$	M-H	523,1609	1,7	21,5	15	+				3	241.0870 (94.0; $-C_{13}H_{14}O_{7}$), 281.0668 (100.0; $-C_{15}H_{14}O_{3}$), 523.1623 (5.6)
254	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-3	22,66	$C_{49}H_{48}O_{23}$	M-H	#######	2,3	14,0	26	+				3	223.0755 (8.3; $-C_{34}H_{36}O_{21}$), 238.0632 (100.0; $-C_{34}H_{37}O_{20}$), 252.0784 (14.4; $-C_{33}H_{35}O_{20}$), 268.0736 (87.4; $-C_{33}H_{35}O_{19}$), 281.0814 (7.8; $-C_{32}H_{34}O_{19}$), 311.0920 (2.2; $-C_{31}H_{32}O_{18}$), 323.0922 (1.4; $-C_{30}H_{32}O_{18}$), 442.1267 (3.8; $-C_{26}H_{25}O_{14}$), 489.1343 (2.1; $-C_{18}H_{26}O_{17}$) $C_{10}H_{12}O_{10}$), 753.2166 (1.4; $-C_{8}H_{10}O_{9}$), 831.2482 (0.5; $-C_{6}H_{4}O_{6}$), 873.2592 (4.8; $-C_{4}H_{2}O_{5}$), 915.2702 (12.2; $-C_{2}O_{4}$)
255	chrysophanol-dianthrone-malonyl-diHex-3	22,79	$C_{45}H_{44}O_{19}$	M-H	887,2404	1,1	53,1	24	+				3	210.0678 (1.1; $-C_{31}H_{33}O_{17}$), 222.0684 (7.1; $-C_{30}H_{33}O_{17}$), 238.0632 (100.0; $-C_{30}H_{33}O_{16}$), 252.0786 (10.7; $-C_{29}H_{31}O_{16}$), 281.0810 (2.6; $-C_{28}H_{30}O_{15}$), 293.0804 (1.8; $-C_{27}H_{30}O_{15}$), 309.0765 (1.0; $-C_{27}H_{30}O_{14}$), 400.1153 (2.0; $-C_{24}H_{23}O_{11}$), 401.1227 (1.9; $-C_{24}H_{22}O_{11}$), 639.1864 (24.9; $-C_{9}H_{12}O_{8}$), 663.1849 (2.4; $-C_{7}H_{12}O_{8}$), 681.1967 (21.2; $-C_{7}H_{10}O_{7}$), 801.2392 (2.5; $-C_{3}H_{2}O_{3}$), 843.2495 (9.5; $-CO_{2}$)
256	chrysophanol-dianthrone-malonyl-diHex-4	22,95	$C_{45}H_{44}O_{19}$	M-H	887,2404	-1,3	###	2	+	0.6 ± 0.11	+		3	210.0678 (1.1; $-C_{31}H_{33}O_{17}$), 222.0684 (7.1; $-C_{30}H_{33}O_{17}$), 238.0632 (100.0; $-C_{30}H_{33}O_{16}$), 252.0786 (10.7; $-C_{29}H_{31}O_{16}$), 281.0810 (2.6; $-C_{28}H_{30}O_{15}$), 293.0804 (1.8; $-C_{27}H_{30}O_{15}$), 309.0765 (1.0; $-C_{27}H_{30}O_{14}$), 400.1153 (2.0; $-C_{24}H_{23}O_{11}$), 401.1227 (1.9; $-C_{24}H_{22}O_{11}$), 639.1864 (24.9; $-C_{9}H_{12}O_{8}$), 663.1849 (2.4; $-C_{7}H_{12}O_{8}$), 681.1967 (21.2; $-C_{7}H_{10}O_{7}$), 801.2392 (2.5; $-C_{3}H_{2}O_{3}$), 843.2495 (9.5; $-CO_{2}$)
257	chrysophanol-anthrone-malonyl-Hex-1	23,09	$C_{24}H_{24}O_{11}$	M-H	487,1245	-0,5	4,5	13	+				3	240.0432 (7.8; $-C_{10}H_{15}O_{7}$), 268.0381 (2.0; $-C_{9}H_{15}O_{6}$), 279.0666 (4.9; $-C_{7}H_{12}O_{7}$), 281.0448 (2.6; $-C_{8}H_{14}O_{6}$), 283.0614 (100.0; $-C_{8}H_{12}O_{6}$), 292.0382 (3.3; $-C_{7}H_{15}O_{6}$), 296.0691 (2.9; $-C_{7}H_{11}O_{6}$), 307.0620 (6.1; $-C_{6}H_{12}O_{6}$), 321.0774 (0.5; $-C_{5}H_{10}O_{6}$), 325.0710 (0.5; -
258	resveratrol-dimer-2	23,10	$C_{28}H_{22}O_{6}$	M+HCOOH-H	499,1398	2,7	4,9	18	+	8.4 ± 0.61	+	9.4 ± 0.57	3	225.0557 (0.5; $-C_{15}H_{14}O_{5}$), 251.0712 (0.4; $-C_{13}H_{12}O_{5}$), 307.0974 (1.3; $-C_{10}H_{8}O_{4}$), 333.0768 (3.0; $-C_{9}H_{10}O_{3}$), 347.0919 (2.7; $-C_{8}H_{8}O_{3}$), 359.0923 (4.4; $-C_{7}H_{8}O_{3}$), 367.1336 (1.4; $-C_{4}H_{4}O_{5}$), 369.1131 (3.5; $-C_{5}H_{6}O_{4}$), 385.1444 (3.6; $-C_{4}H_{2}O_{4}$), 409.1444 (2.8; $-C_{2}H_{2}O$
259	octadiene-Hex-Hex-1	23,10	$C_{22}H_{38}O_{11}$	M+HCOOH-H	523,2396	2,2	13,5	4	+				3	183.1389 (100.0; $-C_{12}H_{20}O_{11}$), 293.0869 (18.3; $-C_{12}H_{22}O_{4}$)
260	chrysophanol-dianthrone-malonyl-diHex-5	23,12	$C_{45}H_{44}O_{19}$	M-H	887,2404	2,2	57,3	24	+				3	210.0686 (1.2; $-C_{31}H_{33}O_{17}$), 222.0681 (6.7; $-C_{30}H_{33}O_{17}$), 238.0629 (100.0; $-C_{30}H_{33}O_{16}$), 252.0785 (10.1; $-C_{29}H_{31}O_{16}$), 281.0824 (2.3; $-C_{28}H_{30}O_{15}$), 293.0816 (2.1; $-C_{27}H_{30}O_{15}$), 401.1218 (2.3; $-C_{17}H_{26}O_{16}$), 443.1335 (1.9; $-C_{22}H_{20}O_{10}$), 459.1219 (1.7; $-C_{15}H_{24}O_{14}$), 801.2388 (2.2; $-C_{3}H_{2}O_{3}$), 843.2499 (7.7; $-CO_{2}$)
261	trihydroxy-octadecanoic-acid	23,19	$C_{18}H_{34}O_{5}$	M-H	329,2333	3,4	25,4	2	+				3	171.1023 (7.0; $-C_{9}H_{18}O_{2}$), 211.1324 (17.9; $-C_{6}H_{14}O_{2}$), 229.1435 (26.0; $-C_{6}H_{12}O$), 329.2320 (100.0)
262	deoxyrhapontigenin-coumaroyl-Hex-1	23,20	$C_{30}H_{30}O_{10}$	M-H	549,1766	2,1	4,5	16	+		+		3	117.0317 (2.4; $-C_{22}H_{24}O_{9}$), 145.0274 (26.9; $-C_{21}H_{24}O_{8}$), 163.0388 (8.9; $-C_{21}H_{22}O_{7}$), 187.0391 (1.7; $-C_{19}H_{22}O_{7}$), 205.0505 (1.3; $-C_{19}H_{20}O_{6}$), 225.0555 (7.2; $-C_{16}H_{20}O_{7}$), 241.0862 (100.0; $-C_{15}H_{16}O_{7}$), 247.0594 (2.5; $-C_{17}H_{18}O_{5}$), 307.0817 (81.0; $-C_{15}H_{14}O_{3}$), 549.17
263	chrysophanol-dianthrone-di(malonyl-Hex)-3	23,30	$C_{48}H_{46}O_{22}$	M-H	973,2407	1,4	41,7	26	+				3	210.0680 (1.0; $-C_{34}H_{35}O_{20}$), 223.0750 (6.0; $-C_{33}H_{34}O_{20}$), 239.0690 (28.2; $-C_{33}H_{34}O_{19}$), 252.0782 (9.3; $-C_{32}H_{33}O_{19}$), 281.0814 (3.1; $-C_{31}H_{32}O_{18}$), 293.0815 (1.5; $-C_{30}H_{32}O_{18}$), 443.1332 (3.2; $-C_{25}H_{22}O_{13}$), 458.1155 (1.7; $-C_{18}H_{27}O_{17}$), 477.1332 (20.2; $-C_{18}H_{24}O_{16}$), 5 885.2605 (6.5; $-C_{2}O_{4}$)
264	*Unidentified*	23,31	$C_{29}H_{24}O_{7}$	M+HCOOH-H	529,1504	1,7	8,3	18	+		+		4	241.0496 (12.7; $-C_{16}H_{16}O_{5}$), 347.0919 (2.2; $-C_{9}H_{10}O_{4}$), 361.1070 (2.5; $-C_{8}H_{8}O_{4}$), 373.1081 (4.1; $-C_{7}H_{8}O_{4}$), 399.1230 (5.9; $-C_{5}H_{6}O_{4}$), 415.1539 (2.5; $-C_{4}H_{2}O_{4}$), 441.1339 (2.0; $-C_{3}H_{4}O_{3}$), 465.1326 (2.4; $-CH_{4}O_{3}$), 483.1441 (100.0; $-CH_{2}O_{2}$)
265	octadiene-Hex-Hex-2	23,35	$C_{22}H_{38}O_{11}$	M+HCOOH-H	523,2396	1,4	47,9	4	+				3	183.1389 (100.0; $-C_{12}H_{20}O_{11}$), 293.0869 (18.3; $-C_{12}H_{22}O_{4}$)
266	chrysophanol-dianthrone-malonyl-diHex-7	23,38	$C_{45}H_{44}O_{19}$	M-H	887,2404	1,8	25,4	24	+				3	210.0686 (1.2; $-C_{31}H_{33}O_{17}$), 222.0681 (6.7; $-C_{30}H_{33}O_{17}$), 238.0629 (100.0; $-C_{30}H_{33}O_{16}$), 252.0785 (10.1; $-C_{29}H_{31}O_{16}$), 281.0824 (2.3; $-C_{28}H_{30}O_{15}$), 293.0816 (2.1; $-C_{27}H_{30}O_{15}$), 401.1218 (2.3; $-C_{17}H_{26}O_{16}$), 443.1335 (1.9; $-C_{22}H_{20}O_{10}$), 459.1219 (1.7; $-C_{15}H_{24}O_{14}$), 801.2388 (2.2; $-C_{3}H_{2}O_{3}$), 843.2499 (7.7; $-CO_{2}$)
267	chrysophanol-anthrone-malonyl-Hex-4	23,45	$C_{24}H_{24}O_{11}$	M-H	487,1245	-0,6	8,0	13	+		+	4.4 ± 0.33	3	240.0429 (7.6; $-C_{10}H_{15}O_{7}$), 264.0436 (0.6; $-C_{8}H_{15}O_{7}$), 268.0381 (2.0; $-C_{9}H_{15}O_{6}$), 279.0667 (3.1; $-C_{7}H_{12}O_{7}$), 283.0614 (100.0; $-C_{8}H_{12}O_{6}$), 292.0377 (4.5; $-C_{7}H_{15}O_{6}$), 296.0686 (1.8; $-C_{7}H_{11}O_{6}$), 307.0615 (4.4; $-C_{6}H_{12}O_{6}$), 325.0731 (0.4; $-C_{6}H_{10}O_{5}$), 337.0716 (0.4; -
268	*Unidentified*	23,45	$C_{50}H_{48}O_{26}$	M-H	#######	1,5	2,0	18			+		4	240.0424 (3.7; $-C_{36}H_{39}O_{22}$), 268.0366 (0.8; $-C_{35}H_{39}O_{21}$), 279.0655 (2.7; $-C_{33}H_{36}O_{22}$), 281.0445 (1.3; $-C_{34}H_{38}O_{21}$), 283.0605 (100.0; $-C_{34}H_{36}O_{21}$), 296.0683 (2.9; $-C_{33}H_{35}O_{21}$), 307.0608 (4.9; $-C_{32}H_{36}O_{21}$), 325.0714 (0.9; $-C_{32}H_{34}O_{20}$), 349.0710 (0.2; $-C_{30}H_{34}O_{20}$), 4
269	physcion-Ac-Hex-3	23,46	$C_{24}H_{24}O_{11}$	M-H	487,1245	0,9	9,6	27		0.6 ± 0.11	+		3	240.0429 (7.6; $-C_{10}H_{15}O_{7}$), 264.0436 (0.6; $-C_{8}H_{15}O_{7}$), 268.0381 (2.0; $-C_{9}H_{15}O_{6}$), 279.0667 (3.1; $-C_{7}H_{12}O_{7}$), 283.0614 (100.0; $-C_{8}H_{12}O_{6}$), 292.0377 (4.5; $-C_{7}H_{15}O_{6}$), 296.0686 (1.8; $-C_{7}H_{11}O_{6}$), 307.0615 (4.4; $-C_{6}H_{12}O_{6}$), 325.0731 (0.4; $-C_{6}H_{10}O_{5}$), 337.0716 (0.4; -
270	physcion-anthrone-malonyl-Hex	23,53	$C_{25}H_{26}O_{12}$	M-H	517,1351	2,1	31,7	13	+				3	226.0624 (0.8; $-C_{11}H_{15}O_{9}$), 254.0582 (13.2; $-C_{10}H_{15}O_{8}$), 269.0815 (100.0; $-C_{9}H_{12}O_{8}$), 293.0821 (0.4; $-C_{7}H_{12}O_{8}$), 311.0925 (1.6; $-C_{7}H_{10}O_{7}$), 458.1212 (1.2; $-C_{2}H_{3}O_{2}$), 473.1460 (2.0; $-CO_{2}$)
271	deoxyrhapontigenin-feruloyl-Hex	23,56	$C_{31}H_{32}O_{11}$	M-H	579,1871	1,8	7,3	16	+				3	160.0151 (8.4; $-C_{22}H_{27}O_{8}$), 175.0394 (54.4; $-C_{21}H_{24}O_{8}$), 193.0513 (26.4; $-C_{21}H_{22}O_{7}$), 225.0554 (8.1; $-C_{17}H_{22}O_{8}$), 241.0863 (100.0; $-C_{16}H_{18}O_{8}$), 322.0698 (4.4; $-C_{16}H_{17}O_{3}$), 337.0932 (63.3; $-C_{15}H_{14}O_{3}$), 579.1859 (3.2)
272	chrysophanol-dianthrone-malonyl-diHex-8	23,56	$C_{45}H_{44}O_{19}$	M-H	887,2404	1,9	16,5	24		0.7 ± 0.08	+		3	210.0686 (1.2; $-C_{31}H_{33}O_{17}$), 222.0681 (6.7; $-C_{30}H_{33}O_{17}$), 238.0629 (100.0; $-C_{30}H_{33}O_{16}$), 252.0785 (10.1; $-C_{29}H_{31}O_{16}$), 281.0824 (2.3; $-C_{28}H_{30}O_{15}$), 293.0816 (2.1; $-C_{27}H_{30}O_{15}$), 401.1218 (2.3; $-C_{17}H_{26}O_{16}$), 443.1335 (1.9; $-C_{22}H_{20}O_{10}$), 459.1219 (1.7; $-C_{15}H_{24}O_{14}$), 801.2388 (2.2; $-C_{3}H_{2}O_{3}$), 843.2499 (7.7; $-CO_{2}$)
273	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-4	23,60	$C_{49}H_{48}O_{23}$	M-H	#######	1,6	25,8	26	+				3	223.0755 (8.3; $-C_{34}H_{36}O_{21}$), 238.0632 (100.0; $-C_{34}H_{37}O_{20}$), 252.0784 (14.4; $-C_{33}H_{35}O_{20}$), 268.0736 (87.4; $-C_{33}H_{35}O_{19}$), 281.0814 (7.8; $-C_{32}H_{34}O_{19}$), 311.0920 (2.2; $-C_{31}H_{32}O_{18}$), 323.0922 (1.4; $-C_{30}H_{32}O_{18}$), 442.1267 (3.8; $-C_{26}H_{25}O_{14}$), 489.1343 (2.1; $-C_{18}H_{26}O_{17}$) $C_{10}H_{12}O_{10}$), 753.2166 (1.4; $-C_{8}H_{10}O_{9}$), 831.2482 (0.5; $-C_{6}H_{4}O_{6}$), 873.2592 (4.8; $-C_{4}H_{2}O_{5}$), 915.2702 (12.2; $-C_{2}O_{4}$)
274	torachrysone-Ac-Hex-4	23,64	$C_{22}H_{26}O_{10}$	M-H	449,1453	0,8	48,1	24			+		3	215.0334 (0.6; $-C_{10}H_{18}O_{6}$), 230.0579 (11.9; $-C_{9}H_{15}O_{6}$), 245.0812 (100.0; $-C_{8}H_{12}O_{6}$)
275	octadiene-Hex-Hex-3	23,74	$C_{23}H_{40}O_{13}$	M-H	523,2396	0,9	14,1	10			+		3	183.1389 (100.0; $-C_{12}H_{20}O_{11}$), 293.0869 (18.3; $-C_{12}H_{22}O_{4}$)
276	*Unidentified*	23,84	$C_{31}H_{32}O_{12}$	M-H	595,1821	0,9	25,4	4			+		3	224.0460 (2.4; $-C_{17}H_{23}O_{9}$), 241.0498 (20.5; $-C_{17}H_{22}O_{8}$), 257.0811 (100.0; $-C_{16}H_{18}O_{8}$)
277	chrysophanol-dianthrone-malonyl-diHex-9	23,86	$C_{45}H_{44}O_{19}$	M-H	887,2404	2,0	16,2	24	+				3	238.0626 (100.0; $-C_{30}H_{33}O_{16}$), 252.0781 (19.7; $-C_{29}H_{31}O_{16}$), 476.1262 (15.2; $-C_{15}H_{23}O_{13}$), 639.1841 (26.5; $-C_{2}H_{16}O_{13}$), 801.2361 (2.4; $-C_{45}H_{43}O_{19}$), 843.2497 (12.1; $-CO_{2}$)
278	**deoxyrhapontigenin**	23,89	$C_{15}H_{14}O_{3}$	M-H	241,0870	3,6	0,6	9	+	13.4 ± 1.16	+	2.2 ± 0.21	1	156.0566 (0.2; $-C_{4}H_{5}O_{2}$), 182.0726 (1.0; $-C_{2}H_{3}O_{2}$), 199.0758 (1.8; $-C_{2}H_{2}O$), 209.0608 (0.6; $-CH_{4}O$), 225.0551 (48.5; $-CH_{4}$), 241.0862 (100.0)
279	chrysophanol-Ac-Hex-5	23,97	$C_{23}H_{22}O_{10}$	M-H	457,1140	2,2	5,0	9			+		3	240.0798 (4.6; $-C_{8}H_{9}O_{7}$), 249.0552 (5.0; $-C_{7}H_{12}O_{7}$), 253.0500 (100.0; $-C_{8}H_{12}O_{6}$), 266.0570 (5.3; $-C_{7}H_{11}O_{6}$), 277.0503 (13.8; $-C_{6}H_{12}O_{6}$)
280	chrysophanol-dianthrone-di(malonyl-Hex)-5	24,01	$C_{48}H_{46}O_{22}$	M-H	973,2407	1,6	23,7	26	+				3	222.0679 (9.2; $-C_{33}H_{35}O_{20}$), 238.0628 (100.0; $-C_{33}H_{35}O_{19}$), 251.0698 (8.4; $-C_{32}H_{34}O_{19}$), 442.1252 (5.5; $-C_{25}H_{23}O_{13}$), 458.1132 (5.0; $-C_{36}H_{19}O_{4}$), 663.1856 (5.3; $-C_{48}H_{45}O_{22}$), 681.1950 (33.9; $-C_{48}H_{45}O_{22}$), 885.2577 (6.5; $-C_{48}H_{45}O_{22}$)
281	pterostilbene-Ac-Hex	24,03	$C_{24}H_{28}O_{9}$	M-H	459,1660	2,4	17,8	11	+				3	240.0789 (27.4; $-C_{9}H_{15}O_{6}$), 255.1024 (100.0; $-C_{8}H_{12}O_{6}$)
282	*Unidentified*	24,07	$C_{18}H_{12}O_{8}$	M-H	355,0459	0,4	5,5	13			+		4	268.0381 (8.6; $-C_{3}H_{3}O_{3}$), 311.0558 (100.0; $-CO_{2}$), 355.0446 (28.9)
283	resveratrol-trimer	24,07	$C_{42}H_{32}O_{9}$	M-H	679,1973	3,0	23,6	27	+		+		3	225.0544 (4.6; $-C_{28}H_{22}O_{6}$), 319.0971 (18.2; $-C_{22}H_{16}O_{5}$), 347.0913 (23.8; $-C_{21}H_{16}O_{4}$), 359.0918 (35.8; $-C_{20}H_{16}O_{4}$), 451.1181 (4.8; $-C_{14}H_{12}O_{3}$), 573.1538 (20.9; $-C_{7}H_{6}O$), 585.1544 (12.1; $-C_{6}H_{6}O$), 637.1859 (14.1; $-C_{2}H_{2}O$), 661.1856 (25.7; $-H_{2}O$), 679.1963 (100.
284	chrysophanol-Ac-Hex-6	24,17	$C_{23}H_{22}O_{10}$	M-H	457,1140	0,6	31,7	27			+		3	240.0798 (4.6; $-C_{8}H_{9}O_{7}$), 249.0552 (5.0; $-C_{7}H_{12}O_{7}$), 253.0500 (100.0; $-C_{8}H_{12}O_{6}$), 266.0570 (5.3; $-C_{7}H_{11}O_{6}$), 277.0503 (13.8; $-C_{6}H_{12}O_{6}$)
285	deoxyrhapontigenin-coumaroyl-Hex-2	24,25	$C_{30}H_{30}O_{10}$	M-H	549,1766	0,1	9,7	13			+		3	145.0276 (42.8; $-C_{21}H_{24}O_{8}$), 163.0387 (20.0; $-C_{21}H_{22}O_{7}$), 187.0409 (5.9; $-C_{19}H_{22}O_{7}$), 225.0554 (7.7; $-C_{16}H_{20}O_{7}$), 241.0868 (58.5; $-C_{15}H_{16}O_{7}$), 307.0817 (100.0; $-C_{15}H_{14}O_{3}$), 549.1766 (3.2)
286	chrysophanol-dianthrone-di(malonyl-Hex)-6	24,25	$C_{48}H_{46}O_{22}$	M-H	973,2407	1,2	12,7	26	+	0.8 ± 0.11			3	238.0628 (100.0; $-C_{33}H_{35}O_{19}$), 252.0779 (10.2; $-C_{32}H_{33}O_{19}$), 442.1259 (5.9; $-C_{25}H_{23}O_{13}$), 477.1322 (16.9; $-C_{18}H_{24}O_{16}$), 663.1840 (4.1; $-C_{10}H_{14}O_{11}$), 681.1954 (41.3; $-C_{10}H_{12}O_{10}$), 843.2465 (2.2; $-C_{4}H_{2}O_{5}$), 885.2591 (5.7; $-C_{2}O_{4}$)
287	citronellyl-Hex-Hex-1	24,31	$C_{22}H_{40}O_{11}$	M+HCOOH-H	525,2552	2,1	54,8	3	+				3	185.1544 (100.0; $-C_{12}H_{20}O_{11}$)
288	*Unidentified*	24,39	$C_{50}H_{50}O_{21}$	M-H	985,2771	0,1	10,6	16			+		4	145.0274 (88.4; $-C_{41}H_{44}O_{19}$), 175.0393 (100.0; $-C_{40}H_{42}O_{18}$), 193.0502 (33.6; $-C_{40}H_{40}O_{17}$), 235.0605 (39.5; $-C_{38}H_{38}O_{16}$), 307.0815 (24.2; $-C_{35}H_{34}O_{14}$), 469.1345 (31.1; $-C_{29}H_{24}O_{9}$), 487.1457 (19.8; $-C_{29}H_{22}O_{8}$), 627.1712 (21.6; $-C_{19}H_{18}O_{7}$), 645.1818 (73.3; $-C_{19}H$
289	chrysophanol-dianthrone-malonyl-diHex-10	24,42	$C_{45}H_{44}O_{19}$	M-H	887,2404	-1,3	###	2	+	0.6 ± 0.11			3	210.0686 (1.2; $-C_{31}H_{33}O_{17}$), 222.0681 (6.7; $-C_{30}H_{33}O_{17}$), 238.0629 (100.0; $-C_{30}H_{33}O_{16}$), 252.0785 (10.1; $-C_{29}H_{31}O_{16}$), 281.0824 (2.3; $-C_{28}H_{30}O_{15}$), 293.0816 (2.1; $-C_{27}H_{30}O_{15}$), 401.1218 (2.3; $-C_{17}H_{26}O_{16}$), 443.1335 (1.9; $-C_{22}H_{20}O_{10}$), 459.1219 (1.7; $-C_{15}H_{24}O_{14}$), 801.2388 (2.2; $-C_{3}H_{2}O_{3}$), 843.2499 (7.7; $-CO_{2}$)
290	citronellyl-Hex-Hex-2	24,45	$C_{22}H_{40}O_{11}$	M+HCOOH-H	525,2552	1,5	27,3	26	+		+		3	185.1542 (100.0; $-C_{12}H_{20}O_{11}$)
291	chrysophanol-dianthrone-di(malonyl-Hex)-7	24,47	$C_{48}H_{46}O_{22}$	M-H	973,2407	1,8	6,6	26	+	1.5 ± 0.25			3	238.0630 (100.0; $-C_{33}H_{35}O_{19}$), 252.0780 (11.9; $-C_{32}H_{33}O_{19}$), 476.1253 (15.1; $-C_{18}H_{25}O_{16}$), 681.1957 (38.0; $-C_{10}H_{12}O_{10}$), 843.2494 (2.2; $-C_{4}H_{2}O_{5}$), 885.2598 (7.3; $-C_{2}O_{4}$)
292	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-5	24,66	$C_{49}H_{48}O_{23}$	M-H	#######	2,2	21,7	26	+				3	223.0755 (8.3; $-C_{34}H_{36}O_{21}$), 238.0632 (100.0; $-C_{34}H_{37}O_{20}$), 252.0784 (14.4; $-C_{33}H_{35}O_{20}$), 268.0736 (87.4; $-C_{33}H_{35}O_{19}$), 281.0814 (7.8; $-C_{32}H_{34}O_{19}$), 311.0920 (2.2; $-C_{31}H_{32}O_{18}$), 323.0922 (1.4; $-C_{30}H_{32}O_{18}$), 442.1267 (3.8; $-C_{26}H_{25}O_{14}$), 489.1343 (2.1; $-C_{18}H_{26}O_{17}$) $C_{10}H_{12}O_{10}$), 753.2166 (1.4; $-C_{8}H_{10}O_{9}$), 831.2482 (0.5; $-C_{6}H_{4}O_{6}$), 873.2592 (4.8; $-C_{4}H_{2}O_{5}$), 915.2702 (12.2; $-C_{2}O_{4}$)
293	citronellyl-Hex-Hex-3	24,69	$C_{22}H_{40}O_{11}$	M+HCOOH-H	525,2552	0,4	5,4	3	+		+		3	185.1543 (100.0; $-C_{12}H_{20}O_{11}$)
294	*Unidentified*	24,78	$C_{15}H_{10}O_{8}S$	M-H	349,0023	-0,1	13,1	3			+		4	269.0449 (100.0; $-O_{3}S$)
295	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-6	24,96	$C_{49}H_{48}O_{23}$	M-H	#######	2,3	23,2	26	+				3	223.0755 (8.3; $-C_{34}H_{36}O_{21}$), 238.0632 (100.0; $-C_{34}H_{37}O_{20}$), 252.0784 (14.4; $-C_{33}H_{35}O_{20}$), 268.0736 (87.4; $-C_{33}H_{35}O_{19}$), 281.0814 (7.8; $-C_{32}H_{34}O_{19}$), 311.0920 (2.2; $-C_{31}H_{32}O_{18}$), 323.0922 (1.4; $-C_{30}H_{32}O_{18}$), 442.1267 (3.8; $-C_{26}H_{25}O_{14}$), 489.1343 (2.1; $-C_{18}H_{26}O_{17}$) $C_{10}H_{12}O_{10}$), 753.2166 (1.4; $-C_{8}H_{10}O_{9}$), 831.2482 (0.5; $-C_{6}H_{4}O_{6}$), 873.2592 (4.8; $-C_{4}H_{2}O_{5}$), 915.2702 (12.2; $-C_{2}O_{4}$)
296	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-7	25,08	$C_{49}H_{48}O_{23}$	M-H	#######	1,9	38,4	26	+				3	223.0755 (8.3; $-C_{34}H_{36}O_{21}$), 238.0632 (100.0; $-C_{34}H_{37}O_{20}$), 252.0784 (14.4; $-C_{33}H_{35}O_{20}$), 268.0736 (87.4; $-C_{33}H_{35}O_{19}$), 281.0814 (7.8; $-C_{32}H_{34}O_{19}$), 311.0920 (2.2; $-C_{31}H_{32}O_{18}$), 323.0922 (1.4; $-C_{30}H_{32}O_{18}$), 442.1267 (3.8; $-C_{26}H_{25}O_{14}$), 489.1343 (2.1; $-C_{18}H_{26}O_{17}$) $C_{10}H_{12}O_{10}$), 753.2166 (1.4; $-C_{8}H_{10}O_{9}$), 831.2482 (0.5; $-C_{6}H_{4}O_{6}$), 873.2592 (4.8; $-C_{4}H_{2}O_{5}$), 915.2702 (12.2; $-C_{2}O_{4}$)
297	chrysophanol-dianthrone-di(malonyl-Hex)-8	25,45	$C_{48}H_{46}O_{22}$	M-H	973,2407	1,1	11,5	26	+				3	238.0630 (100.0; $-C_{33}H_{35}O_{19}$), 252.0780 (11.9; $-C_{32}H_{33}O_{19}$), 476.1253 (15.1; $-C_{18}H_{25}O_{16}$), 681.1957 (38.0; $-C_{10}H_{12}O_{10}$), 843.2494 (2.2; $-C_{4}H_{2}O_{5}$), 885.2598 (7.3; $-C_{2}O_{4}$)
298	chrysophanol-dianthrone-malonyl-diHex-11	25,51	$C_{45}H_{44}O_{19}$	M-H	887,2404	1,2	10,6	24	+		+		3	210.0686 (1.2; $-C_{31}H_{33}O_{17}$), 222.0681 (6.7; $-C_{30}H_{33}O_{17}$), 238.0629 (100.0; $-C_{30}H_{33}O_{16}$), 252.0785 (10.1; $-C_{29}H_{31}O_{16}$), 281.0824 (2.3; $-C_{28}H_{30}O_{15}$), 293.0816 (2.1; $-C_{27}H_{30}O_{15}$), 401.1218 (2.3; $-C_{17}H_{26}O_{16}$), 443.1335 (1.9; $-C_{22}H_{20}O_{10}$), 459.1219 (1.7; $-C_{15}H_{24}O_{14}$), 801.2388 (2.2; $-C_{3}H_{2}O_{3}$), 843.2499 (7.7; $-CO_{2}$)
299	*Unidentified*	25,55	$C_{16}H_{16}O_{5}$	M-H	287,0924	2,3	20,9	9	+				4	203.0702 (2.6; $-C_{4}H_{4}O_{2}$), 245.0823 (4.9; $-C_{2}H_{2}O$), 254.0583 (3.2; $-CH_{5}O$), 269.0813 (20.3; $-H_{2}O$), 272.0678 (14.9; $-CH_{3}$), 287.0916 (100.0)
300	physcion-dianthrone-di(malonyl-Hex)-2	25,58	$C_{50}H_{50}O_{24}$	M-H	#######	1,5	15,8	26	+				3	225.0550 (4.9; $-C_{36}H_{40}O_{21}$), 252.0789 (3.0; $-C_{34}H_{37}O_{21}$), 265.0497 (1.6; $-C_{34}H_{40}O_{20}$), 268.0735 (100.0; $-C_{34}H_{37}O_{20}$), 282.0894 (4.5; $-C_{33}H_{35}O_{20}$), 311.0922 (1.8; $-C_{32}H_{34}O_{19}$), 473.1453 (1.6; $-C_{26}H_{24}O_{14}$), 537.1545 (10.3; $-C_{18}H_{24}O_{16}$), 699.2052 (1.4; $-C_{12}H_{14}O_{11}$),
301	*Unidentified*	25,62	$C_{25}H_{32}O_{8}$	M+HCOOH-H	505,2079	1,4	23,2	10	+				4	225.0556 (3.2; $-C_{12}H_{24}O_{7}$), 241.0866 (100.0; $-C_{11}H_{20}O_{7}$)
302	chrysophanol-physcion-dianthrone-malonyl-diHex-1	25,82	$C_{46}H_{46}O_{20}$	M-H	917,2509	1,1	18,9	24	+				3	222.0680 (6.5; $-C_{31}H_{35}O_{18}$), 238.0631 (100.0; $-C_{31}H_{35}O_{17}$), 252.0785 (11.4; $-C_{30}H_{33}O_{17}$), 268.0735 (86.4; $-C_{30}H_{33}O_{16}$), 281.0809 (5.4; $-C_{29}H_{32}O_{16}$), 400.1150 (3.2; $-C_{25}H_{25}O_{12}$), 507.1431 (20.9; $-C_{15}H_{22}O_{13}$), 651.1857 (1.8; $-C_{9}H_{14}O_{9}$), 669.1967 (27.5; $-C_{9}H_{12}O_{8}$),
303	*Unidentified*	25,98	$C_{17}H_{12}O_{6}$	M-H	311,0561	1,5	28,0	24			+		4	268.0363 (8.5; $-C_{2}H_{3}O$), 311.0554 (100.0)
304	stilbene-dimer	26,09	$C_{28}H_{20}O_{6}$	M-H	451,1187	0,8	28,7	12			+		4	367.0950 (2.1; $-C_{4}H_{4}O_{2}$), 381.1132 (1.3; $-C_{3}H_{2}O_{2}$), 383.1320 (1.7; $-C_{28}H_{19}O_{6}$), 407.1287 (6.6; $-CO_{2}$), 409.1086 (10.2; $-C_{2}H_{2}O$), 451.1179 (100.0)
305	chrysophanol-dianthrone-malonyl-diHex-12	26,09	$C_{45}H_{44}O_{19}$	M-H	887,2404	1,3	21,6	24	+		+		3	210.0686 (1.2; $-C_{31}H_{33}O_{17}$), 222.0681 (6.7; $-C_{30}H_{33}O_{17}$), 238.0629 (100.0; $-C_{30}H_{33}O_{16}$), 252.0785 (10.1; $-C_{29}H_{31}O_{16}$), 281.0824 (2.3; $-C_{28}H_{30}O_{15}$), 293.0816 (2.1; $-C_{27}H_{30}O_{15}$), 401.1218 (2.3; $-C_{17}H_{26}O_{16}$), 443.1335 (1.9; $-C_{22}H_{20}O_{10}$), 459.1219 (1.7; $-C_{15}H_{24}O_{14}$), 801.2388 (2.2; $-C_{3}H_{2}O_{3}$), 843.2499 (7.7; $-CO_{2}$)
306	physcion-Ac-Hex-4	26,13	$C_{24}H_{24}O_{11}$	M-H	487,1245	-0,3	31,1	24			+		3	240.0413 (21.0; $-C_{10}H_{15}O_{7}$), 283.0599 (100.0; $-C_{8}H_{12}O_{6}$)
307	physcion	26,33	$C_{16}H_{12}O_{5}$	M-H	283,0611	1,0	10,7	11			+		3	240.0435 (19.1; $-C_{2}H_{3}O$), 268.0395 (4.5; $-CH_{3}$), 283.0606 (100.0)
308	(aloe-)emodin-chrysophanol-dianthrone-Hex	26,54	$C_{36}H_{32}O_{12}$	M-H	655,1821	1,4	2,6	11			+		3	254.0576 (83.1; $-C_{21}H_{21}O_{8}$), 416.1102 (100.0; $-C_{15}H_{11}O_{3}$)
309	chrysophanol-dianthrone-di(malonyl-Hex)-9	26,55	$C_{48}H_{46}O_{22}$	M-H	973,2407	1,7	10,2	26	+	0.9 ± 0.03	+		3	238.0627 (100.0; $-C_{33}H_{35}O_{19}$), 477.1322 (18.6; $-C_{18}H_{24}O_{16}$), 681.1939 (29.0; $-C_{10}H_{12}O_{10}$), 843.2467 (1.7; $-C_{4}H_{2}O_{5}$), 885.2568 (4.2; $-C_{2}O_{4}$)
310	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-8	26,71	$C_{49}H_{48}O_{23}$	M-H	#######	1,6	13,8	26	+				3	223.0755 (8.3; $-C_{34}H_{36}O_{21}$), 238.0632 (100.0; $-C_{34}H_{37}O_{20}$), 252.0784 (14.4; $-C_{33}H_{35}O_{20}$), 268.0736 (87.4; $-C_{33}H_{35}O_{19}$), 281.0814 (7.8; $-C_{32}H_{34}O_{19}$), 311.0920 (2.2; $-C_{31}H_{32}O_{18}$), 323.0922 (1.4; $-C_{30}H_{32}O_{18}$), 442.1267 (3.8; $-C_{26}H_{25}O_{14}$), 489.1343 (2.1; $-C_{18}H_{26}O_{17}$) $C_{10}H_{12}O_{10}$), 753.2166 (1.4; $-C_{8}H_{10}O_{9}$), 831.2482 (0.5; $-C_{6}H_{4}O_{6}$), 873.2592 (4.8; $-C_{4}H_{2}O_{5}$), 915.2702 (12.2; $-C_{2}O_{4}$)
311	megastigmene-diol-Pent-Hex-1	27,12	$C_{24}H_{42}O_{11}$	M+HCOOH-H	551,2709	1,2	22,8	4	+		+		4	211.1698 (100.0; $-C_{12}H_{20}O_{11}$)
312	chrysophanol-dianthrone-di(malonyl-Hex)-10	27,16	$C_{48}H_{46}O_{22}$	M-H	973,2407	0,9	26,6	26	+				3	238.0627 (100.0; $-C_{33}H_{35}O_{19}$), 477.1322 (18.6; $-C_{18}H_{24}O_{16}$), 681.1939 (29.0; $-C_{10}H_{12}O_{10}$), 843.2467 (1.7; $-C_{4}H_{2}O_{5}$), 885.2568 (4.2; $-C_{2}O_{4}$)
313	chrysophanol-physcion-dianthrone-di(malonyl-Hex)-9	27,52	$C_{49}H_{48}O_{23}$	M-H	#######	1,2	25,5	26	+				3	223.0755 (8.3; $-C_{34}H_{36}O_{21}$), 238.0632 (100.0; $-C_{34}H_{37}O_{20}$), 252.0784 (14.4; $-C_{33}H_{35}O_{20}$), 268.0736 (87.4; $-C_{33}H_{35}O_{19}$), 281.0814 (7.8; $-C_{32}H_{34}O_{19}$), 311.0920 (2.2; $-C_{31}H_{32}O_{18}$), 323.0922 (1.4; $-C_{30}H_{32}O_{18}$), 442.1267 (3.8; $-C_{26}H_{25}O_{14}$), 489.1343 (2.1; $-C_{18}H_{26}O_{17}$) $C_{10}H_{12}O_{10}$), 753.2166 (1.4; $-C_{8}H_{10}O_{9}$), 831.2482 (0.5; $-C_{6}H_{4}O_{6}$), 873.2592 (4.8; $-C_{4}H_{2}O_{5}$), 915.2702 (12.2; $-C_{2}O_{4}$)
314	megastigmene-diol-Pent-Hex-2	27,53	$C_{24}H_{42}O_{11}$	M+HCOOH-H	551,2709	0,8	25,9	4			+		4	211.1698 (100.0; $-C_{12}H_{20}O_{11}$)

315	physcion-dianthrone-di(malonyl-Hex)-3	27,81	$C_{50}H_{50}O_{24}$	M-H	#######	2,5	16,9	26	+					3	225.0550 (4.9; -$C_{36}H_{46}O_{21}$), 252.0789 (3.0; -$C_{34}H_{37}O_{21}$), 265.0497 (1.6; -$C_{34}H_{46}O_{20}$), 268.0735 (100.0; -$C_{34}H_{37}O_{20}$), 282.0894 (4.5; -$C_{33}H_{35}O_{20}$), 311.0922 (1.8; -$C_{32}H_{34}O_{19}$), 473.1453 (1.6; -$C_{26}H_{25}O_{14}$), 537.1545 (10.3; -$C_{18}H_{24}O_{14}$), 699.2052 (1.4; -$C_{12}H_{14}O_{11}$),
316	chrysophanol-emodin-dianthrone-Hex	28,92	$C_{36}H_{32}O_{12}$	M-H	655,1821	1,1	19,6	21	+					3	254.0576 (83.1; -$C_{21}H_{21}O_8$), 416.1102 (100.0; -$C_{15}H_{11}O_3$)
317	*Unidentified*	29,13	$C_{17}H_{12}O_6$	M-H	311,0561	-0,1	7,3	12				+		4	267.0674 (0.9; -CO_2), 283.0623 (0.9; -CO), 311.0565 (100.0)
318	chrysophanol-dianthrone-Hex-1	29,51	$C_{36}H_{32}O_{11}$	M-H	639,1871	0,1	15,6	21	+	0.3 ± 0.03				3	238.0634 (100.0; -$C_{21}H_{21}O_8$), 401.1224 (1.8; -$C_{15}H_{19}O_3$), 458.1152 (2.2; -$C_6H_{13}O_6$), 477.1323 (11.3; -$C_6H_{15}O_5$), 621.1768 (1.2; -H_2O), 639.1865 (12.4)
319	(aloe-)emodin-anthrone-1	29,58	$C_{15}H_{12}O_4$	M-H	255,0662	-0,2	11,8	10	+					3	255.0658 (100.0)
320	chrysophanol-dianthrone-Hex-2	29,63	$C_{36}H_{32}O_{11}$	M-H	639,1871	0,2	12,1	21	+	0.5 ± 0.14				3	222.0684 (3.2; -$C_{21}H_{21}O_9$), 238.0634 (100.0; -$C_{21}H_{21}O_8$), 252.0784 (3.7; -$C_{20}H_{13}O_8$), 401.1240 (1.6; -$C_{15}H_{19}O_3$), 458.1161 (1.8; -$C_6H_{13}O_6$), 476.1263 (12.9; -$C_6H_{11}O_5$), 621.1763 (1.3; -H_2O), 639.1870 (11.0)
321	chrysophanol-dianthrone-malonyl-Hex-1	29,72	$C_{39}H_{34}O_{14}$	M-H	725,1875	0,3	59,1	23	+	0.3 ± 0.04				3	238.0635 (50.5; -$C_{24}H_{23}O_{11}$), 477.1338 (100.0; -$C_6H_{12}O_8$), 519.1450 (2.1; -$C_7H_{19}O_7$), 639.1872 (10.4; -$C_3H_2O_3$), 681.1970 (19.9; -CO_2)
322	**emodin**	29,75	$C_{15}H_{10}O_5$	M-H	269,0455	0,9	3,2	11	+	0.8 ± 0.12	+	2.8 ± 0.11		1	225.0557 (2.1; -CO_2), 241.0507 (0.8; -CO), 269.0453 (100.0)
323	torachrysone	29,87	$C_{14}H_{14}O_4$	M-H	245,0819	0,0	11,2	8	+					3	215.0357 (2.7; -C_2H_6), 230.0591 (59.3; -CH_3), 245.0822 (100.0)
324	chrysophanol-dianthrone-malonyl-Hex-2	29,91	$C_{39}H_{34}O_{14}$	M-H	725,1875	-0,9	14,0	23	+	3.2 ± 0.26	+			3	238.0635 (88.2; -$C_{24}H_{23}O_{11}$), 477.1336 (100.0; -$C_6H_{12}O_8$), 639.1871 (8.7; -$C_3H_2O_3$), 681.1975 (20.4; -CO_2)
325	chrysophanol-physcion-dianthrone-malonyl-Hex-1	29,96	$C_{40}H_{36}O_{15}$	M-H	755,1981	-0,3	37,6	23	+					3	225.0558 (8.5; -$C_{26}H_{25}O_{12}$), 238.0638 (39.7; -$C_{25}H_{25}O_{12}$), 268.0739 (47.5; -$C_{24}H_{23}O_{11}$), 507.1446 (100.0; -$C_9H_{12}O_8$), 669.1970 (8.9; -$C_3H_2O_3$), 711.2110 (24.2; -CO_2)
326	chrysophanol-dianthrone-malonyl-Hex-3	29,98	$C_{39}H_{34}O_{14}$	M-H	725,1875	0,2	27,8	23	+			+		3	238.0635 (88.2; -$C_{24}H_{23}O_{11}$), 477.1336 (100.0; -$C_6H_{12}O_8$), 639.1871 (8.7; -$C_3H_2O_3$), 681.1975 (20.4; -CO_2)
327	chrysophanol-physcion-dianthrone-malonyl-Hex-2	30,11	$C_{40}H_{36}O_{15}$	M-H	755,1981	-0,7	18,4	23	+	1.2 ± 0.14	+			3	225.0558 (8.5; -$C_{26}H_{25}O_{12}$), 238.0638 (39.7; -$C_{25}H_{25}O_{12}$), 268.0739 (47.5; -$C_{24}H_{23}O_{11}$), 507.1446 (100.0; -$C_9H_{12}O_8$), 669.1970 (8.9; -$C_3H_2O_3$), 711.2110 (24.2; -CO_2)
328	18:3-LPC-1	30,16	$C_{27}H_{50}NO_9P$	M-H	562,3150	-0,2	33,5	4	+					2	224.0695 (8.8; -$C_{16}H_{37}NO_4P$), 277.1836 (2.0; -$C_{18}H_{24}NO_5P$), 277.2169 (100.0; -$C_9H_{20}NO_7P$), 502.2938 (12.2; -$C_{27}H_{49}NO_9P$)
329	chrysophanol-physcion-dianthrone-malonyl-Hex-3	30,31	$C_{40}H_{36}O_{15}$	M-H	755,1981	-0,9	30,2	23	+			+		3	225.0553 (2.5; -$C_{26}H_{25}O_{12}$), 238.0630 (43.1; -$C_{25}H_{25}O_{12}$), 252.0781 (3.2; -$C_{24}H_{23}O_{12}$), 268.0736 (46.5; -$C_{24}H_{23}O_{11}$), 281.0809 (1.4; -$C_{23}H_{22}O_{11}$), 463.1187 (1.8; -$C_{11}H_{16}O_8$), 492.1199 (12.7; -$C_{18}H_{15}O_8$), 507.1435 (100.0; -$C_9H_{12}O_8$), 549.1548 (1.9; -$C_7H_{16}O_7$), 65
330	18:2-DGMG-1	30,42	$C_{33}H_{58}O_{14}$	M+HCOOH-H	723,3808	-0,1	21,7	5	+			+		2	179.0556 (2.3; -$C_{26}H_{46}O_{10}$), 221.0661 (1.6; -$C_{26}H_{46}O_9$), 235.0824 (5.5; -$C_{25}H_{44}O_9$), 279.2325 (100.0; -$C_{16}H_{28}O_{14}$), 397.1347 (20.5; -$C_{19}H_{34}O_4$), 415.1456 (11.2; -$C_{19}H_{32}O_3$)
331	18:2-LPE-1	30,53	$C_{23}H_{44}NO_7P$	M-H	476,2782	-0,2	20,8	3	+					2	152.9953 (0.4; -$C_{20}H_{37}NO_2$), 196.0373 (6.1; -$C_{18}H_{32}O_2$), 214.0488 (1.9; -$C_{18}H_{30}O$), 279.2326 (100.0; -$C_5H_{12}NO_5P$), 415.2257 (0.6; -C_2H_7NO), 476.2784 (4.0)
332	18:2-LPC-1	30,61	$C_{27}H_{52}NO_9P$	M-H	564,3306	-0,3	11,9	3	+			+		2	152.9945 (0.3; -$C_{24}H_{45}NO_4$), 168.0400 (0.3; -$C_{19}H_{43}NO_5P$), 224.0692 (8.6; -$C_{20}H_{36}O_4$), 242.0811 (2.1; -$C_{20}H_{34}O_3$), 279.2328 (100.0; -$C_6H_{20}NO_7P$), 504.3094 (13.8; -$C_2H_4O_2$)
333	18:2-DGMG-2	30,65	$C_{33}H_{58}O_{14}$	M+HCOOH-H	723,3808	0,8	15,7	5	+			+		2	235.0826 (4.6; -$C_{25}H_{44}O_9$), 279.2327 (100.0; -$C_{16}H_{28}O_{14}$), 397.1357 (19.0; -$C_{34}H_{59}O_{16}$), 415.1476 (9.7; -$C_{34}H_{59}O_{16}$)
334	18:2-LPE-2	30,73	$C_{23}H_{44}NO_7P$	M-H	476,2782	0,0	10,7	3	+					2	152.9953 (0.4; -$C_{20}H_{37}NO_2$), 196.0373 (6.1; -$C_{18}H_{32}O_2$), 214.0488 (1.9; -$C_{18}H_{30}O$), 279.2326 (100.0; -$C_5H_{12}NO_5P$), 415.2257 (0.6; -C_2H_7NO), 476.2784 (4.0)
335	18:2-LPC-2	30,81	$C_{27}H_{52}NO_9P$	M-H	564,3306	0,4	9,6	3	+			+		2	152.9945 (0.3; -$C_{24}H_{45}NO_4$), 168.0400 (0.3; -$C_{19}H_{43}NO_5P$), 224.0692 (8.6; -$C_{20}H_{36}O_4$), 242.0811 (2.1; -$C_{20}H_{34}O_3$), 279.2328 (100.0; -$C_6H_{20}NO_7P$), 504.3094 (13.8; -$C_2H_4O_2$)
336	(aloe-)emodin-di-anthrone-1	30,87	$C_{30}H_{22}O_8$	M-H	509,1241	-0,1	9,7	20				+		3	254.0578 (100.0; -$C_{15}H_{11}O_4$)
337	18:1-DGMG-1	31,14	$C_{33}H_{60}O_{14}$	M+HCOOH-H	725,3965	-0,3	24,8	4	+					2	185.0448 (1.3; -$C_{26}H_{53}O_{11}$), 221.0680 (1.6; -$C_{26}H_{46}O_9$), 235.0824 (6.7; -$C_{25}H_{46}O_9$), 281.2489 (100.0; -$C_{16}H_{28}O_{14}$), 397.1363 (20.6; -$C_{19}H_{36}O_4$), 415.1469 (14.5; -$C_{19}H_{34}O_3$), 519.3138 (5.4; -$C_8H_{14}O_6$)
338	16:0-LPE-2	31,15	$C_{21}H_{44}NO_7P$	M-H	452,2782	0,4	24,0	1	+			+		2	196.0377 (7.6; -$C_{16}H_{31}O_2$), 255.2328 (100.0; -$C_5H_{12}NO_5P$), 452.2779 (7.2)
339	hydroxyoctadecadienoic-acid	31,20	$C_{18}H_{32}O_3$	M-H	295,2278	-1,1	2,6	3				+		3	171.1021 (8.0; -C_6H_{16}), 195.1397 (4.0; -$C_6H_{12}O$), 277.2166 (78.9; -H_2O), 295.2273 (100.0)
340	emodin-anthrone-2	31,23	$C_{30}H_{22}O_8$	M-H	509,1241	-0,1	9,7	20				+		3	225.0563 (3.7; -CO), 240.0419 (1.1; -CH), 253.0512 (100.0)
341	16:0-LPC-2	31,24	$C_{25}H_{52}NO_9P$	M-H	540,3306	1,1	5,1	1	+			+		2	224.0679 (12.5; -$C_{18}H_{36}O_4$), 242.0810 (1.9; -$C_{18}H_{34}O_3$), 255.2324 (100.0; -$C_6H_{20}NO_7P$), 391.2285 (1.1; -$C_6H_{17}O_6$), 480.3087 (29.4; -$C_2H_4O_2$)
342	18:1-DGMG-2	31,37	$C_{33}H_{60}O_{14}$	M+HCOOH-H	725,3965	-1,5	45,6	4	+			+		2	185.0448 (1.3; -$C_{26}H_{53}O_{11}$), 221.0680 (1.6; -$C_{26}H_{46}O_9$), 235.0824 (6.7; -$C_{25}H_{46}O_9$), 281.2489 (100.0; -$C_{16}H_{28}O_{14}$), 397.1363 (20.6; -$C_{19}H_{36}O_4$), 415.1469 (14.5; -$C_{19}H_{34}O_3$), 519.3138 (5.4; -$C_8H_{14}O_6$)
343	21:0-LPS	31,55	$C_{27}H_{54}NO_9P$	M-H	566,3463	-1,1	13,3	2	+			+		2	224.0694 (7.3; -$C_{20}H_{38}O_4$), 242.0799 (2.4; -$C_{20}H_{36}O_3$), 281.2483 (100.0; -$C_6H_{20}NO_7P$), 506.3250 (16.6; -$C_2H_4O_2$)
344	(aloe-)emodin-physcion-di-anthrone-1	32,02	$C_{31}H_{24}O_8$	M-H	523,1398	0,3	10,5	20	+					3	254.0576 (100.0; -$C_{16}H_{13}O_4$)
345	(aloe-)emodin-chrysophanol-di-anthrone-2	32,07	$C_{30}H_{22}O_7$	M-H	493,1292	1,4	12,2	20	+			+		3	254.0581 (100.0; -$C_{15}H_{11}O_3$)
346	(aloe-)emodin-physcion-di-anthrone-2	32,15	$C_{31}H_{24}O_8$	M-H	523,1398	2,1	4,6	20	+					3	254.0576 (100.0; -$C_{16}H_{13}O_4$)

Supplementary table S3 (część 1)

No.	Metabolites		
	Detected only in petioles of R. rhabarbarum	**Detected only in petioles of R. rhaponticum**	**Detected in petioles of both species**
1	Unidentified (1)	gentisoyl-Hex (7)	Unidentified (2)
2	**gallocatechin (8)**	saliciloyl-Hex-1 (9)	Unidentified (3)
3	Unidentified (10)	syringoyl-Hex-1 (12)	**beta-glucogallin (4)**
4	**Trp (11)**	Unidentified (16)	homocitrate-1 (5)
5	benzyl-Hex-Pent (18)	(epi-)catechin-sulfate (24)	homocitrate-2 (6)
6	coumaroyl-Hex-1 (19)	Unidentified (27)	syringoyl-Hex-2 (13)
7	catechin-trimer (procyanidin-C1) (20)	**procyanidin-B2 (28)**	**procyanidin-B1 (14)**
8	Unidentified (26)	(epi-)catechin-1 (32)	catechin (15)
9	feruloyl-Hex-2 (29)	**epi-catechin (33)**	**catechin (17)**
10	sinapoyl-Hex-1 (30)	sinapoyl-Hex-3 (34)	coumaroyl-Hex-2 (21)
11	sinapoyl-Hex-2 (31)	Unidentified (36)	eucomic_acid-1 (22)
12	methyl-butyl-Hex-Pent (35)	myrcetin-HexA (47)	eucomic_acid-2 (23)
13	piceatannol-Hex-3 (39)	Unidentified (50)	Unidentified (25)
14	procyanidin-B1 (40)	syringoyl-galloyl-Hex (52)	benzoyloxy-hydroxypropyl-HexA (37)
15	resveratrol-Hex-1 (resveratroloside) (42)	Unidentified (53)	hydroxyoctanoic-acid (38)
16	Unidentified (44)	Unidentified (57)	Unidentified (41)
17	lyoniresinol-Hex-1 (49)	Unidentified (58)	tetrahomocitrate (43)
18	Unidentified (54)	Unidentified (59)	vicenin-I (45)
19	Unidentified (61)	quercetin-Hex-dHex-1 (60)	myrcetin-Hex-dHex (46)
20	benzyl-Hex-Hex (64)	quercetin-HexA (65)	vicenin-III (48)
21	coumaroyl-galloyl-Hex-6 (67)	quercetin-dHex-1 (quercitrin) (77)	myrcetin-Hex (51)
22	Unidentified (68)	Unidentified (82)	galloyl-catechin-1 (55)
23	Unidentified (69)	Unidentified (91)	**vicenin-III (56)**
24	resveratrol-Hex-2 (71)	diferuloyl-Suc (helonioside-A) (95)	rutin (62)
25	feruloyl-galloyl-Hex (72)	aleosone-malonyl-Hex (96)	quercetin-hydroxy-succinyl-Hex (63)
26	Unidentified (73)	Unidentified (100)	quercetin-Hex-2 (isoquercetrin) (66)
27	quercetin-Pent-2 (avicularin) (75)	diferuloyl-Ac-Suc (helonioside-B) (108)	**rhapontin (70)**
28	syringaresinol-Hex (acanthoside-B) (76)	(aloe-)emodin-anthrone-Ac-Hex-3 (119)	kaempferol-Hex-dHex (nicotiflorin) (74)
29	tetrahydroxy-dimethylanthraquinone-1 (80)	(aloe-)emodin-dianthrone-malonyl-diHex-4 (125)	Unidentified (78)
30	eriodictyol-Hex (81)	Unidentified (141)	isorhamnetin-Hex-dHex (narcissin) (79)
31	(iso-)rhapontigenin-malonyl-Hex-1 (87)	Unidentified (144)	aloesone-Hex-2 (83)
32	methyl-hexyl-Hex-Pent (89)	Unidentified (145)	kaempferide-Hex (84)
33	dicoumaroyl-Hex-3 (97)	18:3-LPC-1 (152)	isorhamnetin-Hex (85)
34	Unidentified (98)	Unidentified (153)	tetrahydroxy-dimethylanthraquinone-2 (86)
35	dicoumaroyl-Hex-4 (101)	Unidentified (171)	**phloridzin (88)**
36	deoxyrhapontigenin-Hex-1 (102)	36:5-MGDG (177)	rhapontin (90)
37	deoxyrhapontigenin-Hex-2 (104)	Unidentified (178)	pinocembrine-Hex-4 (92)
38	Unidentified (105)		emodin-Hex-3 (93)
39	(aloe-)emodin-Hex-3 (93)		Unidentified (94)
40	Unidentified (114)		quercetin (99)
41	rhein-dianthrone-di(malonyl-Hex) (117)		(aloe-)emodin-anthrone-malonyl-Hex-1 (103)
42	(aloe-)emodin-anthrone-malonyl-Hex-3 (120)		emodin-malonyl-Hex-1 (106)
43	(aloe-)emodin-anthrone-2 (149)		torachrysone-Hex-1 (107)
44	(aloe-)emodin-dianthrone-malonyl-diHex-6 (128)		(aloe-)emodin-dianthrone-di(malonyl-Hex)-1 (109)
45	emodin-physcion-dianthrone-di(malonyl-Hex)-3 (135)		(aloe-)emodin-succinyl-Hex (111)
46	emodin-physcion-dianthrone-malonyl-Hex-1 (142)		**apigenin-7-Glu (112)**
47	18:2-LPE-1 (162)		pinocembrine-Hex-5 (113)
48	Unidentified (176)		torachrysone-Ac-Hex-1 (115)
49	Unidentified (179)		(aloe-)emodin-anthrone-malonyl-Hex-2 (116)

emodin-malonyl-Hex-2 (118)
torachrysone-Ac-Hex-2 (121)
nataloe-emodin-8-Me-Ac-Hex (123)
Unidentified (124)
emodin-physcion-dianthrone-di(malonyl-Hex)-1 (126)
(aloe-)emodin-dianthrone-malonyl-diHex-5 (127)
(aloe-)emodin-dianthrone-di(malonyl-Hex)-2 (129)
physcion-anthrone-malonyl-Hex (130)
physcion-Ac-Hex-3 (131)
(aloe-)emodin-dianthrone-malonyl-diHex-7 (132)
(aloe-)emodin-dianthrone-di(malonyl-Hex)-3 (133)
emodin-physcion-dianthrone-di(malonyl-Hex)-2 (134)
Unidentified (136)
emodin-dianthrone-malonyl-Hex-1 (137)
emodin-physcion-dianthrone-di(malonyl-Hex)-4 (138)
physcion-anthrone-Hex-4 (139)
emodin-dianthrone-malonyl-Hex-2 (140)
emodin-dianthrone-malonyl-Hex-3 (143)
emodin-dianthrone-Hex-6 (146)
emodin-dianthrone-malonyl-Hex-4 (147)
torachrysone (148)
(aloe-)emodin-anthrone-2 (149)
emodin-physcion-dianthrone-malonyl-Hex-3 (150)
emodin (151)
18:3-DGMG-2 (154)
emodin-dianthrone-1? (155)
18:3-LPC-2 (156)
18:3-LPE (157)
Unidentified (158)
emodin-dianthrone-2 (159)
18:2-LPC-1 (160)
18:3-MGMG-1 (161)
Unidentified (163)
18:2-LPC-2 (164)
ketostearic acid-1 (165)
18:2-LPE-2 (166)
hydroxy-hexadecanedioic-acid-1 (167)
16:0-DGMG (168)
(aloe-)emodin-physcion-dianthrone-1 (169)
18:2-MGMG (170)
(aloe-)emodin-physcion-dianthrone-2 (172)
16:0-LPC-2 (173)
16:0-LPE-2 (174)
21:0-LPS (175)

Metabolites with level 1 identification indicated in **bold**

Supplementary table S3 (część 2)

No.	Metabolites		
	Detected only in roots of R. rhabarbarum	**Detected only in roots of R. rhaponticum**	**Detected in roots of both species**
1	digalloyl-Hex-2 (12)	Unidentified (9)	Hex-Hex (1)
2	saliciloyl-Hex-1 (13)	beta-glucogallin-4 (10)	malate (2)
3	catechin-Hex-2 (20)	tyrosol-Hex-1 (11)	**citrate (3)**
4	Unidentified (21)	Unidentified (14)	**tyrosine (4)**
5	Unidentified (23)	Unidentified (16)	**beta-glucogallin (5)**
6	Unidentified (24)	Unidentified (18)	beta-glucogallin-2 (6)
7	**procyanidin-B3 (27)**	tyrosol-Hex-2 (19)	**gallate (7)**
8	Unidentified (32)	Unidentified (25)	beta-glucogallin-3 (8)
9	Unidentified (35)	Unidentified (26)	digalloyl-Hex-4 (15)
10	Unidentified (38)	galloyl-arbutin (29)	Trp (17)
11	benzoyl-Hex-Pent (40)	Unidentified (31)	**procyanidin-B1 (22)**
12	Unidentified (46)	digalloyl-Hex-8 (39)	digalloyl-Hex-5 (28)
13	Unidentified (52)	piceatannol/oxyresveratrol-diHex-1 (41)	**catechin (30)**
14	trigalloyl-Hex (57)	hydroxybenozyl-galloyl-Hex-1 (43)	digalloyl-Hex-7 (33)
15	Unidentified (58)	aloesone/cassiachromone-Hex-1 (45)	coumaroyl-Hex-3 (34)
16	Unidentified (59)	Unidentified (47)	resveratrol-diHex-1 (36)
17	Unidentified (60)	aloesol-Hex-1 (48)	coumaroyl-Hex-4 (37)
18	(epi-)catechin-(epi-)catechin-gallate-1 (61)	feruloyl-Hex-5 (53)	**procyanidin-B2 (42)**
19	Unidentified (65)	**epi-catechin (54)**	feruloyl-Hex-1 (44)
20	Unidentified (67)	Unidentified (56)	coumaroyl-Hex-5 (49)
21	Unidentified (72)	Unidentified (62)	Unidentified (50)
22	Unidentified (73)	hydroxybenozyl-galloyl-Hex-4 (74)	piceatannol/oxyresveratrol-diHex-2 (51)
23	naringenin-C-Hex-1 (80)	coumaroyl-galloyl-Hex-2 (77)	methyl-butyl-Hex-Pent (55)
24	Unidentified (87)	trihydroxyresveratrol (79)	benzoyloxy-hydroxypropyl-HexA (63)
25	digalloyl-procyanidin-B2-2 (92)	aloesone/cassiachromone-Hex-2 (81)	piceatannol-Hex-1 (64)
26	dicaffeoyl-Hex-1 (93)	hexyl-diHex-2 (83)	piceatannol-Hex-2 (66)
27	Unidentified (97)	Unidentified (85)	dihydrokaempferol-1 (68)
28	hydroxyphenyl-butanone-galloyl-Hex-1?_lindleyin-1 (102)	galloyl-(epi-)catechin-galloyl-(epi-)catechin-(epi-)catechin-1 (86)	resveratrol-Hex-1 (resveratroloside) (69)
29	pinocembrine-Hex-1 (103)	digalloyl-procyanidin-B2-1 (90)	(epi-)catechin-(epi-)catechin-gallate-2 (70)
30	naringenin-C-Hex-2 (106)	piceatannol/oxyresveratrol-malonyl-Hex (94)	(iso-)rhapontigenin-diHex-1 (71)
31	catechin-catechin-gallate-3 (109)	coumaroyl-galloyl-Hex-3 (100)	resveratrol-diHex-2 (75)
32	dihydrokaempferol-Pent (111)	galloyl-(epi-)catechin-galloyl-(epi-)catechin-(epi-)catechin-2 (101)	hexyl-diHex-1 (76)
33	coumaroyl-galloyl-Hex-4 (114)	Unidentified (104)	Unidentified (78)
34	Unidentified (120)	(iso-)rhapontigenin-diHex-4 (105)	Unidentified (82)
35	Unidentified (135)	cinnamoyl-Hex (107)	**astringin (84)**
36	tetrahydroxy-dimethylanthraquinone (139)	pinocembrine-Hex-2 (115)	Unidentified (88)
37	resveratrol-Hex-2 (141)	Unidentified (118)	polyflavanostilbene-A (89)
38	pinocembrine-Hex-4 (146)	Unidentified (122)	**polydatin (91)**
39	Unidentified (152)	Unidentified (124)	(iso-)rhapontigenin-diHex-2 (95)
40	deoxyrhapontigenin-diHex-2 (155)	quercetin-dHex-1 (quercitrin) (126)	piceatannol-galloyl-Hex-1 (96)
41	Unidentified (156)	Unidentified (127)	**rutin (98)**
42	resveratrol-dimer-Hex-1 (164)	quercetin-dHex-2 (quercitrin) (128)	galloyl-catechin-2 (99)
43	Unidentified (168)	malonyl-vitexin (130)	Unidentified (108)
44	ebracteatoside-D (170)	coumaroyl-galloyl-Hex-6 (132)	piceatannol-Pent (110)
45	(aloe-)emodin-anthrone-galloyl-Hex-1 (176)	Unidentified (133)	piceatannol-galloyl-Hex-2 (112)
46	(aloe-)emodin-anthrone-malonyl-Hex-1 (181)	Unidentified (137)	(iso-)rhapontigenin-Hex-1 (113)
47	(iso-)rhapontigenin-Hex-3 (187)	Unidentified (144)	**trans-piceatannol (116)**
48	altechromone_A-coumaroyl-Hex (188)	Unidentified (149)	coumaroyl-galloyl-Hex-5 (117)
49	di-coumaroyl-Hex-5 (192)	Unidentified (150)	resveratrol-galloyl-Hex-2 (119)

pinocembrine-Hex-5 (196)
pinocembroside (202)
piceatannol-benzoyl-Hex (207)
Unidentified (211)
resveratrol-dimer-Hex-3 (213)
(aloe)-emodin-anthrone-Ac-Hex-1 (216)
deoxyrhapontigenin-Pent (217)
(aloe-)emodin-anthrone-malonyl-Hex-2 (219)
Unidentified (228)
chrysophanol-dianthrone-di(malonyl-Hex)-1 (233)
Unidentified (235)
physcion-anthrone-Hex-1 (236)
nataloe-emodin-8-Me-Ac-Hex (238)
trihydroxy-octadecadienoic-acid (239)
(aloe-)emodin-Hex-5 (244)
chrysophanol-dianthrone-malonyl-diHex-1 (246)
chrysophanol-dianthrone-malonyl-diHex-2 (247)
deoxyrhapontigenin-caffeoyl-Hex-2 (250)
chrysophanol-dianthrone-diHex (251)
chrysophanol-dianthrone-di(malonyl-Hex)-2 (252)
deoxyrhapontigenin-hydroxybenzoyl-Hex (253)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-3 (254)
chrysophanol-dianthrone-malonyl-diHex-3 (255)
chrysophanol-anthrone-malonyl-Hex-1 (257)
octadiene-Hex-Hex-1 (259)
chrysophanol-dianthrone-malonyl-diHex-5 (260)
trihydroxy-octadecanoic-acid (261)
chrysophanol-dianthrone-di(malonyl-Hex)-3 (263)
octadiene-Hex-Hex-2 (265)
chrysophanol-dianthrone-malonyl-diHex-7 (266)
physcion-anthrone-malonyl-Hex (270)
deoxyrhapontigenin-feruloyl-Hex (271)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-4 (273)
chrysophanol-dianthrone-malonyl-diHex-9 (277)
chrysophanol-dianthrone-di(malonyl-Hex)-5 (280)
pterostilbene-Ac-Hex (281)
chrysophanol-dianthrone-di(malonyl-Hex)-6 (286)
citronellyl-Hex-Hex-1 (287)
chrysophanol-dianthrone-malonyl-diHex-10 (289)
chrysophanol-dianthrone-di(malonyl-Hex)-7 (291)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-5 (292)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-6 (295)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-7 (296)
chrysophanol-dianthrone-di(malonyl-Hex)-8 (297)
Unidentified (299)
physcion-dianthrone-di(malonyl-Hex)-2 (300)
Unidentified (301)
chrysophanol-physcion-dianthrone-malonyl-diHex-1 (302)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-8 (310)
chrysophanol-dianthrone-di(malonyl-Hex)-10 (312)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-9 (313)
Unidentified (151)
Unidentified (154)
resveratrol-dimer-diHex (157)
Unidentified (162)
piceatannol-dimer (cararosinol-D) (163)
Unidentified (165)
Unidentified (173)
aleosone-coumaroyl-Hex-2 (aloeresin-A) (174)
cinnamoyl-galloyl-Hex-1 (177)
Unidentified (178)
(iso-)rhapontigenin-feruloyl-Hex-1 (190)
resveratrol-piceatannol-dimer (gnetuhainin-C) (191)
resveratrol-coumaroyl-Hex-3 (194)
Unidentified (195)
Unidentified (204)
flavanol-piceatannol-dimer (210)
rhapontigenin-coumaroyl-Hex-2 (212)
rhapontigenin-feruloyl-Hex-3 (218)
torachrysone-Ac-Hex-2 (220)
vitisinol-C (226)
resveratrol-piceatannol-mixed-tetramer (229)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-1 (232)
(aloe-)emodin-Hex-4 (234)
chrysophanol-physcion-dianthrone-di(malonyl-Hex)-2 (242)
physcion-Hex-2 (rheochrysin) (243)
keampferol (245)
resveratrol-trimer (mirabilol-B) (248)
stilbene-dimer_cassigarol-B (249)
Unidentified (268)
physcion-Ac-Hex-3 (269)
chrysophanol-dianthrone-malonyl-diHex-8 (272)
torachrysone-Ac-Hex-4 (274)
octadiene-Hex-Hex-3 (275)
Unidentified (276)
chrysophanol-Ac-Hex-5 (279)
Unidentified (282)
chrysophanol-Ac-Hex-6 (284)
deoxyrhapontigenin-coumaroyl-Hex-2 (285)
Unidentified (288)
Unidentified (294)
Unidentified (303)
stilbene-dimer (304)
physcion-Ac-Hex-4 (306)
physcion (307)
(aloe-)emodin-chrysophanol-dianthrone-Hex (308)
megastigmene-diol-Pent-Hex-2 (314)
Unidentified (317)
(aloe-)emodin-di-anthrone-1 (336)
hydroxyoctadecadienoic-acid (339)
emodin-anthrone-2 (340)
rhapontin (121)
resveratrol-galloyl-Hex-3 (123)
resveratrol-Ac-Hex (125)
(iso-)rhapontigenin-galloyl-Hex-1 (129)
(iso-)rhapontigenin-Pent (131)
eriodictyol-Hex (134)
Unidentified (136)
aloesone/cassiachromone-Hex-3 (138)
(iso-)rhapontigenin-galloyl-Hex-2 (140)
(iso-)rhapontigenin-malonyl-Hex-1 (142)
digalloyl-procyanidin-B2-3 (143)
piceatannol-hydroxybenzoyl-Hex (145)
emodin-Hex-3 (147)
Unidentified (148)
dihydroxy-dimethoxy-stilbene-Hex-1 (153)
Unidentified (158)
piceatannol-coumaroyl-Hex-1 (159)
piceatannol-sinapoyl-Hex (160)
Unidentified (161)
(iso-)rhapontigenin-malonyl-Hex-2 (166)
piceatannol-coumaroyl-Hex-2 (167)
piceatannol-feruloyl-Hex (169)
(iso-)rhapontigenin-Ac-Hex (171)
(aloe-)emodin-galloyl-Hex (172)
maesopsin (175)
Unidentified (179)
(iso-)rhapontigenin (180)
piceatannol-coumaroyl-Hex-3 (182)
dihydroxy-dimethoxy-stilbene-Hex-2 (183)
emodin-malonyl-Hex-1 (184)
deoxyrhapontigenin-diHex-1 (185)
(iso-)rhapontigenin-hydroxybenzoyl-Hex (186)
(iso-)rhapontigenin-coumaroyl-Hex-1 (189)
deoxyrhapontigenin-Hex-1 (193)
torachrysone/dihydro-piceatannol-Hex-2 (197)
pinosylvin-Hex-2 (198)
chrysophanol-Hex-1 (199)
(iso-)rhapontigenin-sinapoyl-Hex-1 (200)
apigenin-7-Glu (201)
(iso-)rhapontigenin-coumaroyl-Hex-2 (203)
chrysophanol-Hex-2 (205)
resveratrol-dimer-Hex-2 (206)
(iso-)rhapontigenin-feruloyl-Hex-2 (208)
deoxyrhapontigenin-galloyl-Hex-1 (209)
deoxyrhapontigenin-malonyl-Hex (214)
emodin-malonyl-Hex-2 (215)
(aloe-)emodin-anthrone-malonyl-Hex(-CO2)-2 (221)
resveratrol-dimer-1 (222)
torachrysone-malonyl-Hex(-CO2)-3 (223)
chrysophanol-malonyl-Hex(-CO2)-2 (224)
chrysophanol-galloyl-Hex (225)

physcion-dianthrone-di(malonyl-Hex)-3 (315)
chrysophanol-emodin-dianthrone-Hex (316)
chrysophanol-dianthrone-Hex-1 (318)
(aloe-)emodin-anthrone-1 (319)
chrysophanol-dianthrone-Hex-2 (320)
chrysophanol-dianthrone-malonyl-Hex-1 (321)
torachrysone (323)
chrysophanol-physcion-dianthrone-malonyl-Hex-1 (325)
18:3-LPC-1 (328)
18:2-LPE-1 (331)
18:2-LPE-2 (334)
18:1-DGMG-1 (337)
(aloe-)emodin-physcion-di-anthrone-1 (344)
(aloe-)emodin-physcion-di-anthrone-2 (346)

physcion-Hex-1 (rheochrysin) (227)
piceatannol-cinnamoyl-Hex (230)
chrysophanol-Ac-Hex-3 (231)
Unidentified (237)
chrysophanol-malonyl-Hex(-CO2)-4 (240)
Unidentified (241)
chrysophanol-dianthrone-malonyl-diHex-4 (256)
resveratrol-dimer-2 (258)
deoxyrhapontigenin-coumaroyl-Hex-1 (262)
Unidentified (264)
chrysophanol-anthrone-malonyl-Hex-4 (267)
deoxyrhapontigenin (278)
resveratrol-trimer (283)
citronellyl-Hex-Hex-2 (290)
citronellyl-Hex-Hex-3 (293)
chrysophanol-dianthrone-malonyl-diHex-11 (298)
chrysophanol-dianthrone-malonyl-diHex-12 (305)
chrysophanol-dianthrone-di(malonyl-Hex)-9 (309)
megastigmene-diol-Pent-Hex-1 (311)
emodin (322)
chrysophanol-dianthrone-malonyl-Hex-2 (324)
chrysophanol-dianthrone-malonyl-Hex-3 (326)
chrysophanol-physcion-dianthrone-malonyl-Hex-2 (327)
chrysophanol-physcion-dianthrone-malonyl-Hex-3 (329)
18:2-DGMG-1 (330)
18:2-LPC-1 (332)
18:2-DGMG-2 (333)
18:2-LPC-2 (335)
16:0-LPE-2 (338)
16:0-LPC-2 (341)
18:1-DGMG-2 (342)
21:0-LPS (343)
(aloe-)emodin-chrysophanol-di-anthrone-2 (345)

Metabolites with level 1 identification indicated in **bold**

Supplementary materials S2

Rheum rhaponticum and Rheum rhabarbarum Extracts As Modulators of Endothelial Cell Inflammatory Response

Oleksandra Liudvytska [1,*], Michał B. Ponczek [1], Oskar Ciesielski [2,3], Justyna Krzyżanowska-Kowalczyk [4], Mariusz Kowalczyk [4], Aneta Balcerczyk [2] and Joanna Kolodziejczyk-Czepas [1]

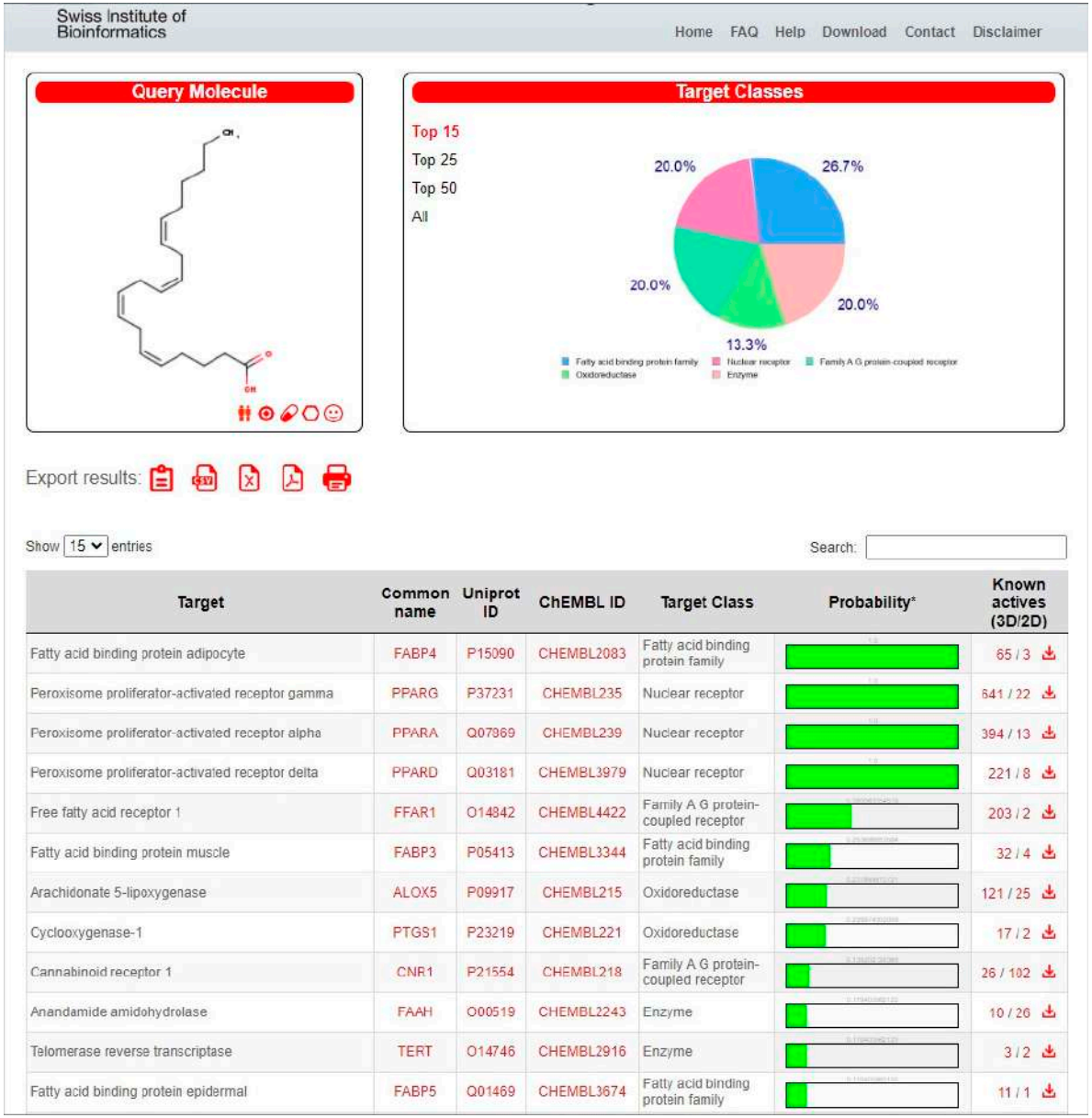
[1] Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland

[2] Department of Sociobiology and Epigenetics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland

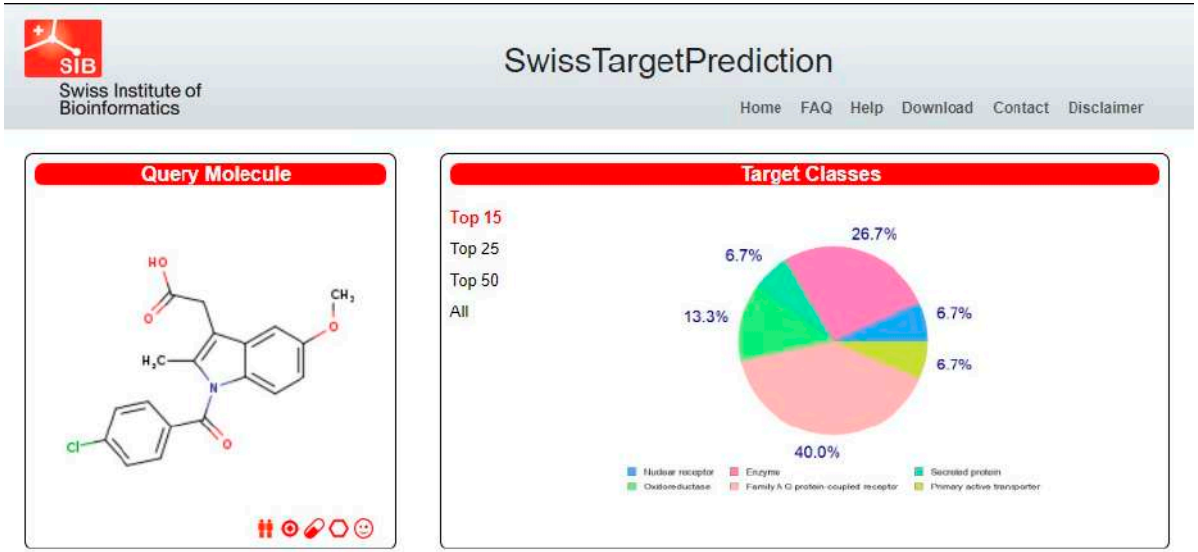
[3] The Bio-Med-Chem Doctoral School, University of Lodz and Lodz Institutes of the Polish Academy of Sciences, University of Lodz, Banacha 12/16, 90-237, Lodz, Poland

[4] Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland

***** Correspondence: oleksandra.liudvytska@biol.uni.lodz.pl; Tel.: +48-42-635-44-84



arachidonic acid (substrate for both enzymes)



Export results:

Show 15 entries Search:

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Androgen Receptor	AR	P10275	CHEMBL1871	Nuclear receptor		6 / 4
Aldose reductase	AKR1B1	P15121	CHEMBL1900	Enzyme		395 / 12
Interleukin-8	CXCL8	P10145	CHEMBL2157	Secreted protein		12 / 1
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		59 / 32
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		155 / 179
Aldo-keto-reductase family 1 member C3	AKR1C3	P42330	CHEMBL4681	Enzyme		172 / 55
G protein-coupled receptor 44	PTGDR2	Q9Y5Y4	CHEMBL5071	Family A G protein-coupled receptor		1115 / 341
Nitric oxide synthase, inducible *(by homology)*	NOS2	P35228	CHEMBL4481	Enzyme		2 / 1
P-glycoprotein 1	ABCB1	P08183	CHEMBL4302	Primary active transporter		1 / 20
Prostanoid DP receptor	PTGDR	Q13258	CHEMBL4427	Family A G protein-coupled receptor		148 / 90

Indomethacin (COX-2 inhibitor)

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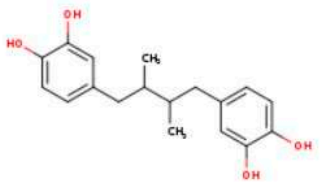
Query Molecule



Target Classes

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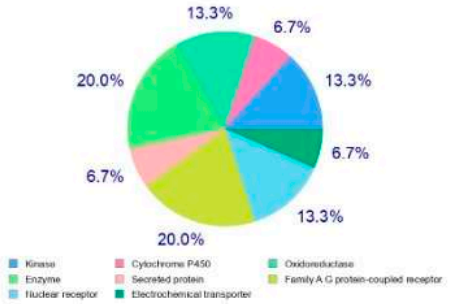
Top 25
Top 50
All



Export results:

Show 15 entries Search:

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Insulin-like growth factor I receptor	IGF1R	P08069	CHEMBL1957	Kinase		23 / 14
Cytochrome P450 19A1	CYP19A1	P11511	CHEMBL1978	Cytochrome P450		43 / 47
Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase		31 / 18
Arachidonate 15-lipoxygenase	ALOX15	P16050	CHEMBL2903	Enzyme		27 / 17
Transthyretin	TTR	P02766	CHEMBL3194	Secreted protein		2 / 2
Arachidonate 12-lipoxygenase	ALOX12	P18054	CHEMBL3687	Enzyme		10 / 9
Leukotriene B4 receptor 1	LTB4R	Q15722	CHEMBL3911	Family A G protein-coupled receptor		3 / 1
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		31 / 10
Estrogen receptor alpha	ESR1	P03372	CHEMBL206	Nuclear receptor		360 / 92

NDGA (COX-2 and 5-LOX inhibitor)

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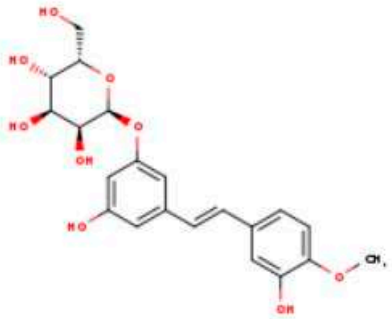
Query Molecule



Target Classes

Top 15
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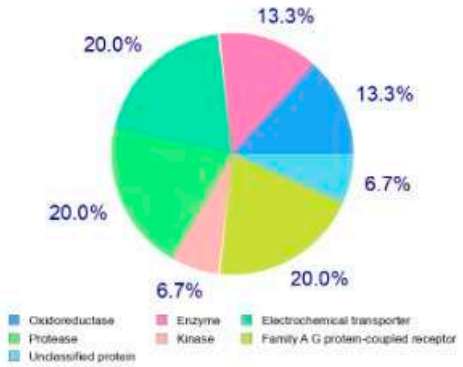
Top 50
All



Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		0 / 3
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		5 / 2
Hydroxycarboxylic acid receptor 2	HCAR2	Q8TDS4	CHEMBL3785	Family A G protein-coupled receptor		45 / 0

rhaponticin
(rhapontin)

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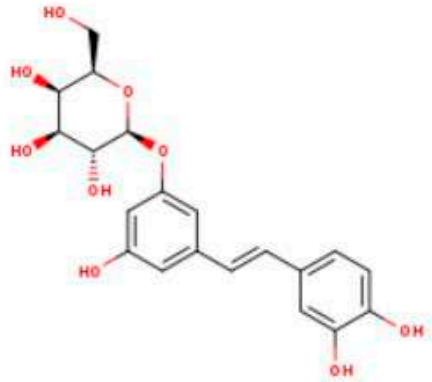
Query Molecule



Target Classes

Top 15
Top 25
Top 50
All

astringin

Export results:

Show 15 entries

Search: oxygen

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		2 / 3
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		1 / 3

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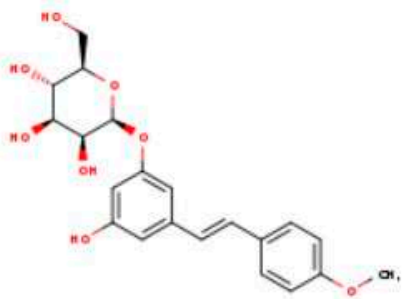
Query Molecule



Target Classes

Top 15
Top 25
Top 50
All

deoxyrhaponticin

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		0 / 4
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		1 / 3
Hydroxycarboxylic acid receptor 2	HCAR2	Q8TDS4	CHEMBL3785	Family A G protein-coupled receptor		20 / 0

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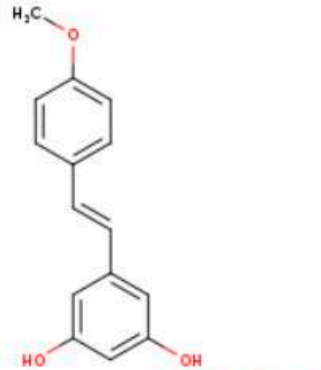
Query Molecule



Target Classes

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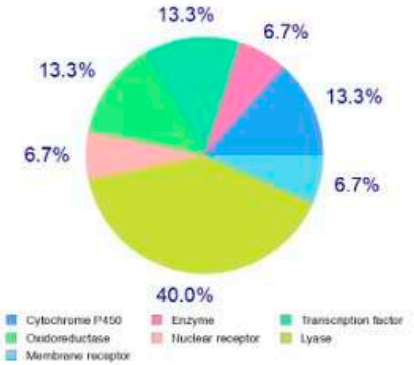
Top 50
All



deoxyrhapontigenin

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		20 / 29
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		33 / 42
Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase		83 / 23
11-beta-hydroxysteroid dehydrogenase 1	HSD11B1	P28845	CHEMBL4235	Enzyme		23 / 3

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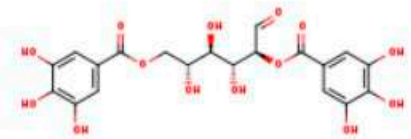
Query Molecule



Target Classes

Top 15
Top 25
Top 50
All

digalloyl glucoside

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Squalene monooxygenase *(by homology)*	SQLE	Q14534	CHEMBL3592	Enzyme		0 / 23
Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase		0 / 5
Hydroxycarboxylic acid receptor 2	HCAR2	Q8TDS4	CHEMBL3785	Family A G protein-coupled receptor		3 / 0
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		0 / 5

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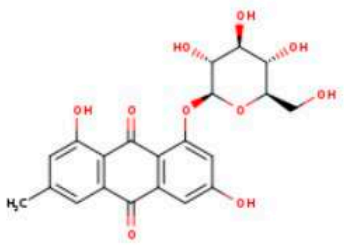
Query Molecule



Target Classes

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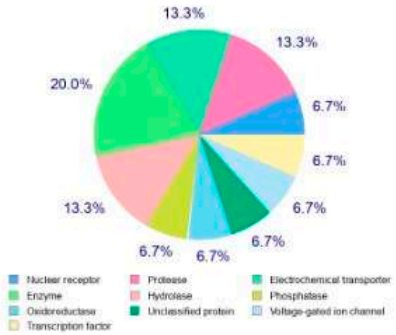
Top 50
All



emodin 8-O-glucoside

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cyclooxygenase-1 *(by homology)*	Ptgs1	P22437	CHEMBL2649	Enzyme		0 / 2
Cyclooxygenase-2 *(by homology)*	Ptgs2	Q05769	CHEMBL4321	Oxidoreductase		1 / 1

Showing 1 to 2 of 2 entries (filtered from 52 total entries)

Previous 1 Next

*Probability for the query molecule - assumed as bioactive - to have this protein as target.

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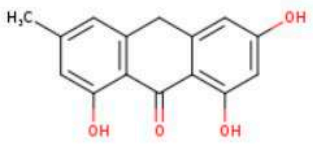
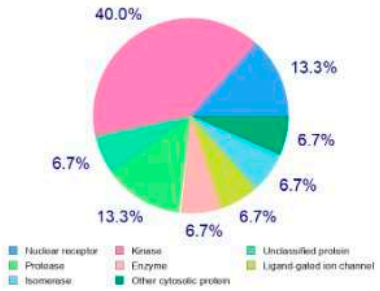
Query Molecule



Target Classes

Top 15
Top 25
Top 50
All



Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
11-beta-hydroxysteroid dehydrogenase 1	Hsd11b1	P50172	CHEMBL3910	Enzyme		12 / 0
Palmitoleoyl-protein carboxylesterase NOTUM *(by homology)*	Notum	Q8R116	CHEMBL3758064	Hydrolase		1 / 0
Arachidonate 5-lipoxygenase-activating protein *(by homology)*	Alox5ap	P30355	CHEMBL3414408	Other cytosolic protein		5 / 0
Hydroxycarboxylic acid receptor 2 *(by homology)*	Hcar2	Q9EP66	CHEMBL4420	Family A G protein-coupled receptor		2 / 0

Showing 1 to 4 of 4 entries (filtered from 100 total entries)

Previous 1 Next

*Probability for the query molecule - assumed as bioactive - to have this protein as target.

emodin anthrone

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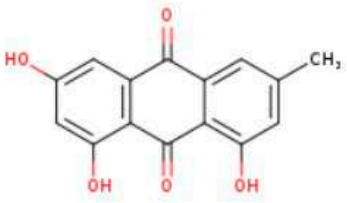
Query Molecule



Target Classes

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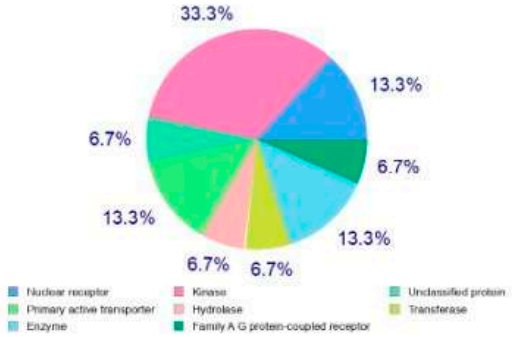
Top 50
All



Export results:

emodin anthrone

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cyclooxygenase-2 *(by homology)*	Ptgs2	Q05769	CHEMBL4321	Oxidoreductase		2 / 4
Cyclooxygenase-1 *(by homology)*	Ptgs1	P22437	CHEMBL2649	Enzyme		0 / 3
Arachidonate 5-lipoxygenase *(by homology)*	Alox5	P48999	CHEMBL5211	Oxidoreductase		4 / 5
Indoleamine 2,3-dioxygenase 1 *(by homology)*	Ido1	P28776	CHEMBL1075294	Enzyme		0 / 1
Arachidonate 12-lipoxygenase	Alox12	P39655	CHEMBL3225	Enzyme		9 / 3
Carboxylesterase 2 *(by homology)*	Ces2c	Q91WG0	CHEMBL2217	Enzyme		1 / 1

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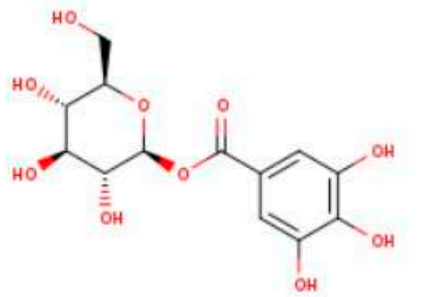
Query Molecule



Target Classes

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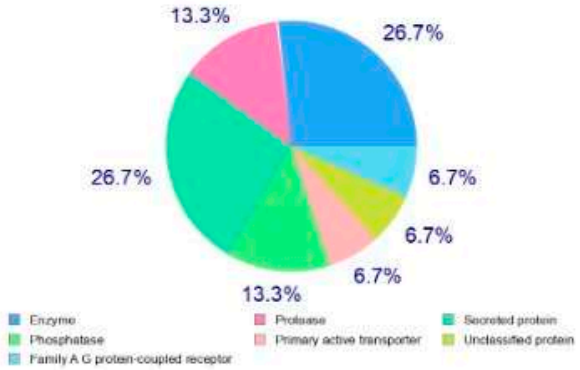
Top 50
All



glucagallin

Export results:

Show 15 entries

Search: oxygen

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Squalene monooxygenase *(by homology)*	SQLE	Q14534	CHEMBL3592	Enzyme		0 / 23
Indoleamine 2,3-dioxygenase	IDO1	P14902	CHEMBL4685	Enzyme		1 / 0
Arachidonate 12-lipoxygenase	ALOX12	P18054	CHEMBL3687	Enzyme		1 / 0

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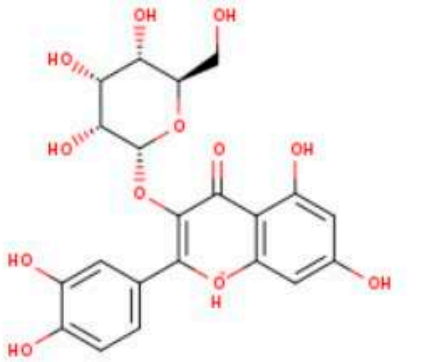
Query Molecule



Target Classes

Top 15
Top 25
Top 50
All

Isoquercitrin

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Squalene monooxygenase *(by homology)*	SQLE	Q14534	CHEMBL3592	Enzyme		2 / 0
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		0 / 2
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		1 / 1

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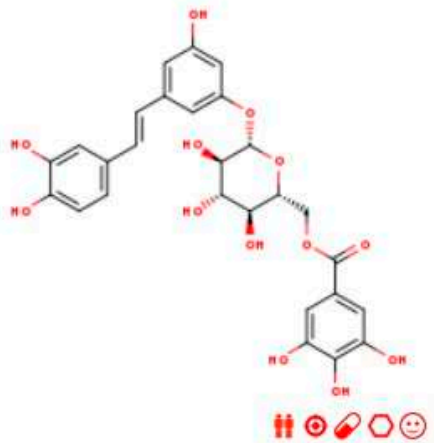
Query Molecule



Target Classes

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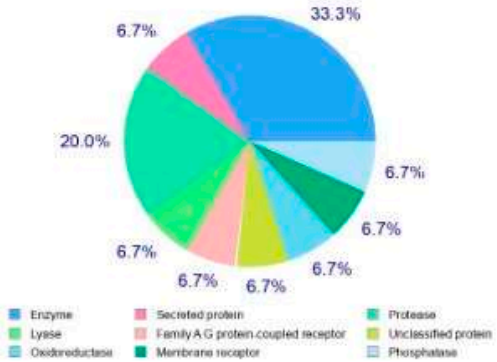

rhapontigenin-galloyl-glucopyranoside

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Squalene monooxygenase *(by homology)*	SQLE	Q14534	CHEMBL3592	Enzyme		0 / 14
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		1 / 2
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		2 / 5

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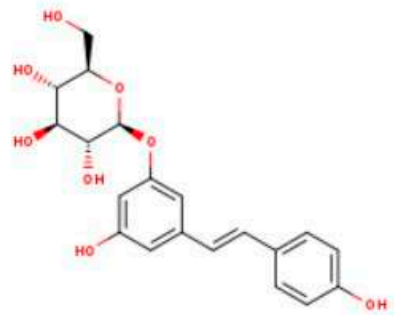
Query Molecule



Target Classes

Top 15
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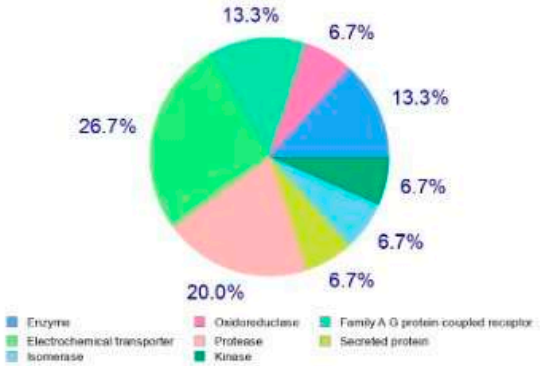
Top 50
All



piceid

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Hydroxycarboxylic acid receptor 2	HCAR2	Q8TDS4	CHEMBL3785	Family A G protein-coupled receptor		41 / 0
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		2 / 3
Glutamate carboxypeptidase II	FOLH1	Q04609	CHEMBL1892	Protease		4 / 0
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		1 / 3

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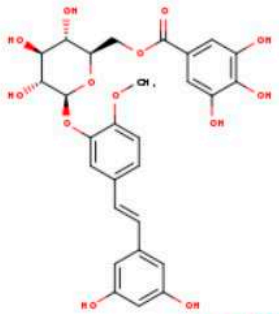
rhapontigenin-galloyl-glucopyranoside

Query Molecule



Target Classes

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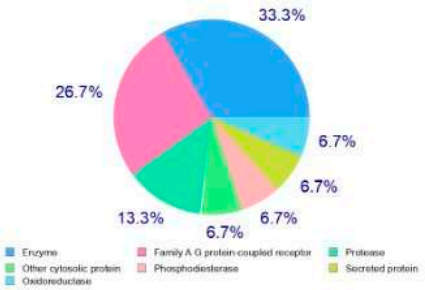
Top 50
All



Export results:

Show 15 entries

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Hydroxycarboxylic acid receptor 2	HCAR2	Q8TDS4	CHEMBL3785	Family A G protein-coupled receptor		62 / 0
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		7 / 9

Showing 1 to 3 of 3 entries (filtered from 100 total entries)

Previous 1 Next

*Probability for the query molecule - assumed as bioactive - to have this protein as target.

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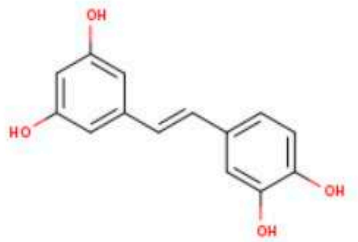
Query Molecule



Target Classes

Top 15
Top 25
Top 50
All

rhapontigenin

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		9 / 24
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		10 / 21
Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase		3 / 19
Arachidonate 15-lipoxygenase	ALOX15	P16050	CHEMBL2903	Enzyme		14 / 0
17-beta-hydroxysteroid dehydrogenase 14	HSD17B14	Q9BPX1	CHEMBL3712868	Enzyme		1 / 0

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Query Molecule

Target Classes

Top 15
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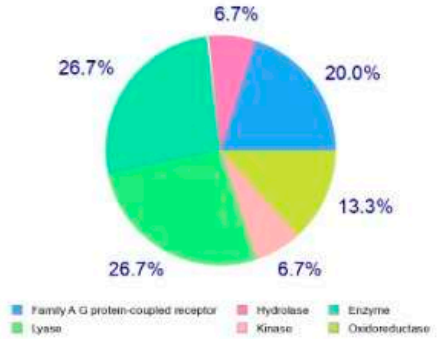
Top 50
All



rutin

Export results:

Show 15 entries

Search: oxy

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Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase		0 / 45
Squalene monooxygenase *(by homology)*	SQLE	Q14534	CHEMBL3592	Enzyme		2 / 0
Arachidonate 15-lipoxygenase	ALOX15	P16050	CHEMBL2903	Enzyme		0 / 3
Arachidonate 12-lipoxygenase	ALOX12	P18054	CHEMBL3687	Enzyme		0 / 2

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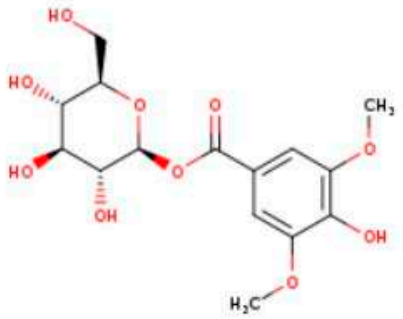
Query Molecule



Target Classes

Top 15
Top 25
Top 50
All

syringoyl 1-*O*-glucopyranoside

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Squalene monooxygenase *(by homology)*	SQLE	Q14534	CHEMBL3592	Enzyme		0 / 21
Arachidonate 12-lipoxygenase	ALOX12	P18054	CHEMBL3687	Enzyme		1 / 0
Glutamate carboxypeptidase II	FOLH1	Q04609	CHEMBL1892	Protease		15 / 0

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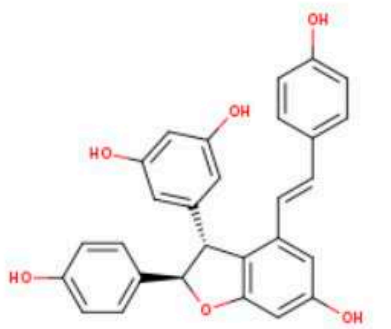
Query Molecule



Target Classes

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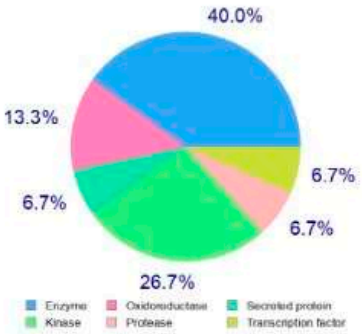

viniferin (resveratrol-dehydrodimer)

Export results:

Show 15 entries

Search: oxy

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase		6 / 6
Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase		7 / 6
Ubiquitin carboxyl-terminal hydrolase 4	USP4	Q13107	CHEMBL2406900	Enzyme		1 / 0
Ubiquitin carboxyl-terminal hydrolase 5	USP5	P45974	CHEMBL6158	Protease		1 / 0
11-beta-hydroxysteroid dehydrogenase 1	HSD11B1	P28845	CHEMBL4235	Enzyme		7 / 0

Figure S5. The estimation of the most probable macromolecular targets of rhubarb compounds. According to the tool description “The prediction is founded on a combination of 2D and 3D similarity with a library of 370000 known actives on more than 3000 proteins from three different species”. The webtool (http://www.swisstargetprediction.ch/ accessed on 15 October 2022) is described in the article: SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules, Daina A., Michielin O., Zoete V. Nucleic Acids Res. 2019;47(W1):W357-W364. doi: 10.1093/nar/gkz382. (2019).

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Effects of *Rheum rhaponticum* and *Rheum rhabarbarum* extracts on haemostatic activity of blood plasma components and endothelial cells *in vitro*

Oleksandra Liudvytska [a,*], Michał B. Ponczek [a], Justyna Krzyżanowska-Kowalczyk [c], Mariusz Kowalczyk [c], Aneta Balcerczyk [b], Joanna Kolodziejczyk-Czepas [a]

[a] Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236, Lodz, Poland
[b] Department of Oncobiology and Epigenetics, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236, Lodz, Poland
[c] Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8, 24-100, Puławy, Poland

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Rheum rhaponticum
Rheum rhabarbarum

ABSTRACT

Ethnopharmacological relevance: Traditional medicine recommends the use of *Rheum rhaponticum* L. and *R. rhabarbarum* L. to treat over thirty complaints, including disorders related to the cardiovascular system such as heartache, pains in the pericardium, epistaxis and other types of haemorrhage, blood purification as well as disorders of venous circulation.

Aim of the study: This work was dedicated to examining for the first time the effects of extracts from petioles and roots of *R. rhaponticum* and *R. rhabarbarum,* as well as two stilbene compounds (rhapontigenin and rhaponticin) on the haemostatic activity of endothelial cells and functionality of blood plasma components of the haemostatic system.

Materials and methods: The study was based on three main experimental modules, including the activity of proteins of the human blood plasma coagulation cascade and the fibrinolytic system as well as analyses of the haemostatic activity of human vascular endothelial cells. Additionally, interactions of the main components of the rhubarb extracts with crucial serine proteases of the coagulation cascade and fibrinolysis (*i.e.* thrombin, the coagulation factor Xa and plasmin) were analyzed *in silico*.

Results: The examined extracts displayed anticoagulant properties and significantly reduced the tissue factor-induced clotting of human blood plasma (by about 40%). Inhibitory effects of the tested extracts on thrombin and the coagulation factor Xa (FXa) were found as well. For the extracts, the IC_{50} was ranging from 20.26 to 48.11 μg/ml. Modulatory effects on the haemostatic response of endothelial cells, including the release of von Willebrand factor, tissue-type plasminogen activator and the plasminogen activator inhibitor-1, have been also found.

Conclusions: Our results indicated for the first time that the examined *Rheum* extracts influenced the haemostatic properties of blood plasma proteins and endothelial cells, with the prevalence of the anticoagulant action. The anticoagulant effect of the investigated extracts may be partly attributed to the inhibition of the FXa and thrombin activities, the key serine proteases of the blood coagulation cascade.

1. Introduction

For centuries *Rheum rhaponticum* L. (rhapontic rhubarb, syn. Siberian rhubarb) and *R. rhabarbarum* L. (garden rhubarb, syn. *R. undulatum* L.; according to www.theplantlist.org and http://www.worldfloraonline.org) have been in use in the traditional medicine of different regions of the world. Currently, the aforementioned rhubarb species are important medicinal plants in Asian, European, and other cultures. The pharmacological effects of rhubarb are immense. Ethnomedicinal data on external or internal use of preparations based on these plants include

* Corresponding author.
E-mail addresses: oleksandra.liudvytska@biol.uni.lodz.pl (O. Liudvytska), michal.ponczek@biol.uni.lodz.pl (M.B. Ponczek), jkrzyzanowska@iung.pulawy.pl (J. Krzyżanowska-Kowalczyk), mkowalczyk@iung.pulawy.pl (M. Kowalczyk), aneta.balcerczyk@biol.uni.lodz.pl (A. Balcerczyk), joanna.kolodziejczyk@biol.uni.lodz.pl (J. Kolodziejczyk-Czepas).

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over 30 recommendations, *e.g.* dysfunctions of gastrointestinal, pulmonary, circulatory, and reproductive systems as well as treatment of injuries (Kolodziejczyk-Czepas and Liudvytska, 2021). One of the oldest Polish books devoted to medicinal plants, *i.e.* "*Herbarz Polski*" (Marcin z Urzędowa/Marcin from Urzędów, 1595; available at https://www.wbc.poznan.pl/dlibra/doccontent?id=2636) recommended roots of *R. rhaponticum* to alleviate gastrointestinal pain, pulmonary system dysfunctions and disorders related to the reproductive system, including uterine and breast pains. *R. rhabarbarum* was recommended in the cases of gastrointestinal disorders as well as to cure diverse injuries, and as a purgative agent (Kolodziejczyk-Czepas and Liudvytska, 2021).

Herbarz Polski describes also the use of *R. rhaponticum* to treat heartache and pain in the pericardium and to stop spitting blood. *R. rhabarbarum* was used to treat epistaxis and other types of haemorrhage. However, the latter plant was recommended to cure venous circulation disorders and for blood purification as well, suggesting that it may also improve blood flow (Marcin z Urzędowa, 1595). On the other hand, in Chinese medicine, *R. rhabarbarum* has been administered to treat gastrointestinal haemorrhage (State Pharmacopoeia Committee, 2010).

Thus, results of ethnomedicinal surveys and historical resources suggest both pro-coagulant and blood flow improving effects of *R. rhaponticum* and *R. rhabarbarum*-derived preparations, without an indication of the predominant activity. Moreover, despite many ethnomedicinal recommendations, the use of preparations containing *R. rhaponticum* or *R. rhabarbarum* in contemporary medicine is limited. Due to an estrogenic activity, *R. rhaponticum* has been applied in the therapy of menopausal complaints (Kaszkin-Bettag et al., 2009). The *Rheum rhaponticum* extract ERr731® was the first rhapontic rhubarb-based medicinal product (Phytoestrol N) registered for the treatment of menopausal complaints (Chang et al., 2016). Different extracts of *R. rhaponticum* are also present in other herbal products and dietary supplements. However, literature reports provide very little data on the effects of extracts from these plants on functions of the haemostatic system, including endothelial cells and the activity of main components of the blood plasma coagulation cascade and the fibrinolytic system. Two papers presented vasorelaxant effects of stilbenes isolated from *R. rhabarbarum* (Oh et al., 2007; Yoo et al., 2007), and some information on the anti-thrombotic activity of the rhubarb-derived compounds combined with urokinase derives from the study on brain microvascular basement impairment in rats with thrombus-occluded cerebral ischemia (Li et al., 2010).

Therefore, the aim of our study was to recognise the effects of extracts isolated from petioles and roots of *R. rhaponticum* or *R. rhabarbarum* on components of the haemostatic system, particularly in the context of maintaining the haemostatic balance. Besides the extracts, the effects of rhapontigenin and its glycoside, rhaponiticin, two stilbenes abundantly occurring in the examined rhubarb species, were studied as well. The work design has been based on two main questions: 1) *Are the examined compounds/extracts able to influence the haemostatic properties of blood plasma proteins and endothelial cells?* 2) *Have the extracts and stilbenes anti-coagulant/anti-thrombotic properties or rather act as pro-coagulants?*

To obtain answers to the above questions, the study design employed different *in vitro* experimental models related to vascular physiology, *i.e.* human blood plasma and its haemostatic proteins (coagulation factors and enzymes of the fibrinolytic system) as well as vascular endothelial cells. Among the components of the blood plasma coagulation cascade, a special emphasis was put on two critical enzymes, *i.e.* the coagulation factor Xa (FXa) and thrombin (the coagulation factor IIa) (Fig. 1). The coagulation factor Xa (E.C.3.4.21.6) is one of serine proteases with a critical function in the blood coagulation cascade. As an activator of thrombin, the FXa controls generation of this enzyme, and in consequence, the thrombin-mediated fibrin clot formation (Fig. 1). It has been established that activation of one molecule of FX leads to the generation of 1000 molecules of thrombin (Ansell, 2007). In the blood coagulation cascade, thrombin (the coagulation factor IIa, EC 3.4.21.5) is an executive serine protease, responsible for the conversion of blood plasma

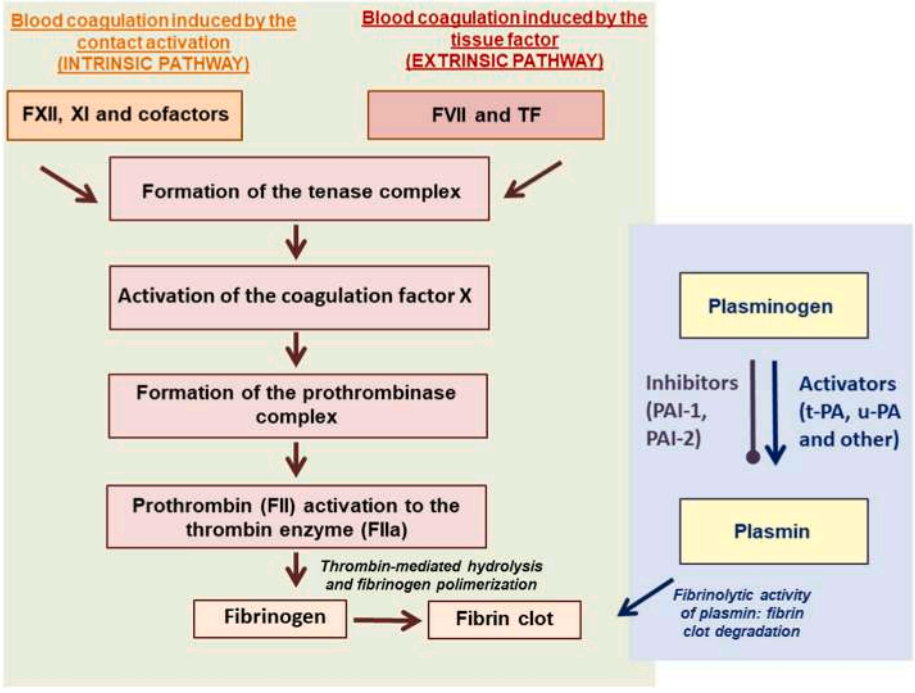


Fig. 1. A schematic representation of the main steps in the blood plasma coagulation cascade and fibrinolysis. Blood coagulation may be triggered through two diverse mechanisms, i.e. the extrinsic pathway and the intrinsic pathway. The extrinsic (tissue factor-dependent) pathway is the main physiological route of blood clotting activation. The tissue factor (TF) is expressed in the subendothelial tissues, however, after vascular injury, it becomes available to other proteins of the blood coagulation cascade. The intrinsic pathway is triggered by the autoactivation of the coagulation factor XII on negatively charged surfaces (the contact activation). Both pathways lead to the formation of the tenase and prothrombinase enzymatic complexes. The tenase complex is responsible for the activation of the coagulation factor X (FX) proenzyme into the FXa enzyme. The generated FXa is a key enzyme of the prothrombinase complex, activating the prothrombin zymogen to the thrombin enzyme (FIIa). The thrombin-catalyzed hydrolysis of blood plasma fibrinogen enables its polymerization and formation of the fibrin clot. The fibrinolytic system is responsible for fibrin degradation and controlling the thrombus size. Its functioning is based on a hydrolytic activity of plasmin, generated from the plasminogen zymogen by plasminogen activators (mainly by the tissue-type plasminogen activator (t-PA) and the urokinase-type plasminogen activator (u-PA)). The plasmin formation is controlled by the plasminogen activator inhibitors (mainly by the plasminogen activator inhibitor-1 (PAI-1)). The activity of plasmin is mainly inhibited by the α2-antiplasmin.

fibrinogen into an insoluble fibrin clot. In addition to the aforementioned function, thrombin plays also many other pro-coagulant and modulatory roles, including the activation of several components of blood plasma coagulation pathways (*e.g.* the coagulation factors V, VII, XI, and XIII) and stimulation of platelets (Al-Amer, 2022). For that reason, the FXa activity and the FXa-thrombin axis are so important molecular targets in research on anticoagulant and anti-thrombotic therapies.

2. Materials and methods

2.1. Chemicals

Chromogenic substrates for thrombin and plasmin, *i.e.* the Chromogenix S-2238 and S-2251 products, respectively, were purchased from Diapharma Group, Inc. (West Chester, USA). Fibrinogen (a physiological substrate for the thrombin enzyme) was isolated from human blood plasma, according to the procedure described by Doolittle et al. (1967). Plasminogen isolation from human plasma was carried out on the lysine-Sepharose gel (Sigma-Aldrich, St. Louis, USA), using the affinity chromatography technique (Deutsch and Mertz, 1970). Streptokinase (ENZ-315) was purchased from ProSpec-Tany Technogene Ltd. (Ness--Ziona, Israel), and the tissue-type plasminogen activator (t-PA, Actilyse®) was from the Boehringer Ingelheim International GmbH (Ingelheim am Rhein, Germany). Thrombin was purchased from Biomed (Lublin, Poland). Argatroban monohydrate, tris, sodium chloride, dimethyl sulfoxide (DMSO), and other non-specific reagents were from Sigma-Aldrich (St. Louis, USA). Kits for detection of the von Willebrand factor (ab108918), t-PA (ab190812), and PAI-1-t-PA complexes (ab269559) were purchased from Abcam (Cambridge, UK). MCDB-131 medium was from Life Technologies (Carlsbad, California, USA), fetal bovine serum (FBS) was purchased from EURx, Brazil, epidermal growth factor (EGF) was from Millipore (Burlington, USA), and L-glutamine from Invitrogen (Carlsbad, USA). General reagents for spectrophotometric assays (catechin, gallic acid, vanillin, SDS, Folin-Ciocalteu's phenol reagent, ferric chloride, sodium carbonate, potassium ferricyanide, and methanol LC-MS grade) were purchased from Merck (Darmstadt, Germany) and HCl was purchased from Avantor Performance Materials Poland S.A., formerly POCH (Polish Chemicals Reagents). Ultrapure water was prepared using a Milli-Q water purification system (Merck Millipore).

2.2. Plant material

Rheum rhaponticum L. underground (rhizomes and roots) and aerial parts (petioles) were obtained from The Botanical Garden of Maria Curie-Skłodowska University in Lublin, whereas analogous organs of *Rheum rhabarbarum* L. were received from The Botanical Garden of Łódź. Petioles were collected in early summer (in June 2019), whereas rhizomes were collected in late autumn (in October 2019). The voucher samples numbers were as follows: *R.rhaponticum*/OL/2019, *R.rhaponticum*/KR/2019, *R.rhabarbarum*/OL/2019, and *R.rhabarbarum*/KR/2019, for petiole and root material, respectively. The voucher specimen has been deposited at the Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation, Puławy, Poland.

2.3. Preparation of extracts from petioles and roots of rhubarbs

Plant material was cut into small pieces, dried at 35 °C, and finely milled in a laboratory grinder. Plant material was extracted twice with methanol, containing 0.1% formic acid, using an ultrasonic bath, at room temperature for 24 h, in the dark. The obtained crude extracts were filtered, and supernatants were trifle concentrated under reduced pressure and defatted with *n*-hexane using liquid-liquid extraction. Afterward, the specific metabolite fraction was prepared using *n*-butanol as an extraction solvent (Liudvytska et al., 2023).

All collected fractions (hexane, water, and butanol) were monitored for specific metabolites by the UHPLC-HR-MS analysis. Butanol fractions of petioles and roots were freeze-dried and used in the following research stages, LC-MS analyses, spectrophotometric estimations of some classes of the active constituents, and bioactivity assays.

2.4. Quantitative determinations of the total contents of selected classes of the active constituents

2.4.1. Estimation of total phenolic content

2.4.1.1. Analysis of total phenols content using Folin-Ciocalteu assay. Estimating the total phenolic content was based on the modified Folin-Ciocalteu method (Ainsworth and Gillespie, 2007). Briefly, 100 μl of each sample diluted with methanol was mixed with 200 μl of 10% (v/v) Folin-Ciocalteu reagent and incubated for 5 min. Afterward, 800 μl of 7.5% (w/v) Na_2CO_3 solution was added, and the mixture was incubated for 2 h in the dark. Following centrifugation at room temperature (21600×*g*, 15 min), the absorbance of samples was measured at 765 nm by a UV/visible spectrophotometer (Evolution 260 Bio, Thermo Scientific, Waltham, MA, USA). Gallic acid solutions (5–100 μg/mL) were used to prepare the calibration curve ($r^2 = 0.998$). The results were expressed as mg of gallic acid equivalents per g of the extract.

2.4.1.2. Analysis of total phenols with the ferricyanide/Prussian Blue method. The total phenolic content was also determined based on the modified Prussian Blue method (Işıl Berker et al., 2010; Margraf et al., 2015). Briefly, 10–50 μl of each sample of rhubarbs butanol extract (diluted to a final volume of 1 ml with methanol) was mixed with 6.3 ml of miliQ water, 200 μl of 1M HCl, 1.5 ml of 1% solution of $K_3[Fe(CN)_6]$, 500 μl of 1% SDS and finally with 500 μl of $FeCl_3$. Samples were then incubated for 30 min at room temperature. The absorbance was measured at 750 nm, and phenolic content was calculated as described in the previous paragraph.

2.4.2. Analysis of total condensed tannins content

The content of condensed tannins was determined based on their reaction with vanillin in the presence of HCl (Ofosu et al., 2021). Briefly, to 200 μl of rhubarbs butanol extracts (diluted appropriately with methanol), 1 ml of a mixture of vanillin reagent (1% vanillin in methanol and 8% HCl in methanol, 1:1) was added. Blanks were prepared by adding 4% HCl in methanol instead of 1% vanillin reagent to analogous extract samples. Samples and blanks were incubated for 20 min at 30 °C on a shaker (300 rpm). The absorbance was measured at 500 nm against blanks without the vanillin reagent with the same equipment as above. Catechin solutions (5–100 μg/mL) were used to prepare the calibration curve ($r^2 = 0.996$).

2.5. UHPLC HR-MS analyses

The phytochemical composition of the butanol extracts was established as described previously using high-resolution UHPLC-MS (Liudvytska et al., 2023). Automated extraction of the relevant feature data from the high-resolution LC-MS files was carried out after converting them to mzML format with ProteoWizard (Chambers et al., 2012). The "patRoon" R library (Helmus et al., 2021) was used for feature detection and grouping employing the OpenMS package (Röst et al., 2016). Data were componentized after the filtration of detected features based on intensity threshold (minimal intensity 2500 counts), reproducibility (within group peak area RSD not higher than 30%), and chromatographic peak shape (modality score higher than 0.3, symmetry score higher than 0.5). The resulting matrix was further cleaned up by keeping only features detected in the pooled QC samples and observed in at least 60% of replicates. Next, the matrix was normalized to the sample weight and processed using the ANOSIM function from the "vegan" R package.

The data was also processed by principal component analysis (PCA) after zero-centring and scaling to unit variance. All statistical analyses were carried out using R software version 4.2.

2.6. Blood plasma isolation and preparation of the examined samples

Plasma was isolated (2000×*g*, 15 min, 25 °C) from human buffy coats (Kolodziejczyk-Czepas et al., 2013), purchased from the Regional Centre of Blood Donation and Blood Treatment in Lodz, Poland. Blood, a source of buffy coats, was collected on a citrate/phosphate/dextrose (CPD) solution. The material was anonymized and commercially available. The study was carried out in accordance with the requirements of the Declaration of Helsinki. All experiments were approved by the committee on the Ethics of Research at the University of Lodz (15/KBBN-UŁ/III/2019).

Analyses of plasmin and thrombin activities were executed with the use of the working solutions of the isolated proteins, *i.e.* the plasminogen samples (0.18 mg/ml, in the 0.1 M Tris/HCl buffer; pH 7.4) and thrombin (0.75 U, in the 0.05 M tris-buffered saline (TBS), enriched with 0.025 M $CaCl_2$; pH 7.4). Plasma and the isolated plasminogen, thrombin and FXa samples were pre-incubated with the examined rhubarb extracts, stilbenes, or reference compounds for 15 min, at 37 °C.

2.7. Determination of the tissue factor-initiated coagulation of blood plasma (activity of the extrinsic coagulation pathway)

The extrinsic blood plasma coagulation pathway was activated by the Dia-PT reagent (Diagon, Budapest, Hungary), containing the tissue factor, lipids and calcium ions. Dia-PT is a commercial diagnostic reagent, that activates the tissue factor-dependent pathway of blood coagulation and is used for measurements of a well-known diagnostic parameter, *i.e.* the prothrombin time (PT) (Levy et al., 2014). The assay conditions were optimized to observe the fibrin clot formation in a prolonged time, allowing the analysis of the kinetic curve and recording alterations during the process of plasma clotting (Kolodziejczyk-Czepas et al., 2018). The experimental system was composed of 50 μl of human blood plasma (control or preincubated with the examined substances), 250 μl of the tris-buffered saline (0.05 M Tris, 0.9% NaCl, enriched with 0.025 M $CaCl_2$, pH 7.4), and 5 μl of the Dia-PT reagent (which was added to microplate wells immediately before starting measurements). Measurements were conducted at $\lambda = 360$ nm.

2.8. Measurements of thrombin activity (amidolytic and fibrinogen polymerization tests)

The amidolytic and proteolytic activities of thrombin were monitored kinetically, according to our previously established protocols (Kolodziejczyk-Czepas et al., 2017). The hydrolytic activity of thrombin in all samples was measured based on the maximal velocity of the reaction (the V_{max} parameter).

During the amidolytic assay, thrombin hydrolyzed the amide bond between arginine and *p*-nitroaniline (pNA) in the chromogenic substrate (Chromogenix S-2238™; H-D-Phe-Pip-Arg-*p*-nitroaniline dihydrochloride), releasing the chromophore. The reaction mixture included 40 μl of 3 mM chromogenic substrate S-2238 and 280 μl of thrombin (0.75 U, in 0.05 M TBS, pH 7.4). Absorbance changes were recorded at the wavelength of 415 nm.

Measurements of the proteolytic activity of thrombin (fibrinogen polymerization tests) were executed using the following mixture of the reagents: 100 μl of human fibrinogen (3 mg/ml, in 0.05 M TBS) and 200 μl of thrombin solution (0.75 U, in 0.05 M TBS, enriched with 0.025 M $CaCl_2$, pH 7.4). The wavelength for the fibrinogen polymerization tests was 360 nm.

2.9. Effects of the examined substances on the coagulation factor Xa

Evaluation of the effect on coagulation Factor Xa activity was carried out using the fluorometric Inhibitor Screening Assay Kit (Abcam, ab204712). The test was based on the ability of the coagulation factor Xa to cleave a synthetic substrate and release a fluorophore (*i.e.* 7-amino-4-methylcoumarin, AMC), which was quantified at the excitation/emission wavelengths of 350/450 nm. Following the experimental protocol provided by the manufacturer, a reference inhibitor was the GGACK (glutamyl-glycyl-argininechloromethylketone) dihydrochloride, appli ed at a concentration of 4 μg/ml.

2.10. Measurements of the amidolytic activity of plasmin generated after the streptokinase or t-PA-induced activation of human plasminogen

Plasminogen (0.18 mg/ml) was pre-incubated with the examined stilbene compounds or extracts for 15 min, at 37 °C. The generated plasmin activity was measured at $\lambda = 415$ nm, using the S-2251 substrate (H-D-Valyl-L-leucyl-L-lysine-*p*-nitroaniline dihydrochloride, Chromogenix). All reagents were applied into a 96-well microplate. Measurements of plasmin activity in experiments employing the streptokinase as a plasminogen activator were executed with the use of a kinetic mode. The reagent mixture contained 20 μl of plasminogen, 220 μl of 50 mM Tris/HCl buffer (pH 8.2), 10 μl of streptokinase solution (10,000 U/ml) and 30 μl of the S-2251 substrate (3 mM).

The t-PA-induced plasmin generation was measured using the "endpoint" protocol. The reaction mixture was composed of 10 μl plasminogen (0.18 mg/ml), 10 μl t-PA (Actilyse, 20 μg/ml), 20 μl of fibrinogen (3 mg/ml, in 0.05 M TBS, pH 7.4), 10 μl of thrombin (5 U/ml), 50 μl of the S-2251 substrate (3 mM). The samples were incubated at 37 °C for 15 min. Then, the reaction was blocked by adding 150 μl of 50% CH_3COOH.

2.11. Cell culture

HUVECs (Human Umbilical Vein Endothelial Cells) were cultured in MCDB-131 medium (Life Technologies, Carlsbad, California, U.S.), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies, Carlsbad, USA), 10 ng/ml of epidermal growth factor (EGF) (Millipore, Burlington, Massachusetts, U.S.) and 10 mM glutamine (Invitrogen; Carlsbad, California, U.S.). The cells were isolated from freshly collected umbilical cords, by collagenase type II digestion, according to Jaffe's protocol (Jaffe et al., 1973), and used for the experiments at passages 3–4. The Bioethics Commission at the University of Lodz approved the study protocol (decision No. 16 (III)/KBBN-UŁ/I/2021–22).

2.12. Evaluation of the haemostatic activity of the HUVECs

HUVECs were seeded onto 96-well plates (density of 10×10^3 cells/well). After 24 h, cells were treated (incubation 16 h) with the rhubarb extracts or stilbenes (rhapontigenin (RHPG) (CAS: 500-65-2, PhytoLab, Germany) and rhaponticin (RHPT) (CAS:156-58-8, Sigma, USA) and activated by thrombin (4 U/ml; 4 h). Cell culture supernatants were collected after incubation (−20 °C). Effects of the examined extracts and stilbenes on the haemostatic response of the HUVECs were established using commercial enzyme-linked immunosorbent assay (ELISA) kits provided by Abcam, *i.e.* the ab108918 kit for the Willebrand factor, the ab190812 kit for the t-PA, and the ab269559 product for the detection of PAI-1-t-PA complexes.

2.13. Molecular docking

Structures of proteins were downloaded from PDBe (https://www.ebi.ac.uk/pdbe/) and used as receptors to predict binding by blind docking to protease domains of thrombin (unliganded PDB ID: 3U69

chains L, H, liganded PDB ID: 1FPH.PDB, chins L. H, prothrombinase complex PDB ID: 7TPP chain E), FXa (PDB ID: 1XKB chain C, PDB ID: prothrombinase complex 7TPP chain B, plasmin (PDB ID: 3UIR chain A), and to the whole plasminogen (PDB ID: 4A5T) in Autodock Vina 1.1.2 (http://vina.scripps.edu/) (Trott and Olson, 2010). X-ray crystallographic structures 1FPH, 1XKB and cryogenic electron microscopy structure 7TPP chains were superposed in ChimeraX relative to 3U69 and the following cubic space coordinates were used for blind docking: centre x 14.987, y −12.021, z −13.130 and size z 54, y 56, z 58. The catalytic triad residues S525 (prothrombin) and S379 (FXa) were replaced by Ala to prevent (auto)proteolysis in 7TPP structure thus they were restored in the ChimeraX 1.5 program as serine residues (rotamer library Dunbrack) for proper interactions. Plasmin crystal structure 3UIR was centred in a box x −50, y 33, z −22 with edge dimensions 55 to cover the whole volume of protease domain. Plasminogen crystal structure 4A5T was inserted in a box to embrace the whole protein with coordinates: centre x, y, z −7.000, 30.000, −5.000 and size x, y, z 117, 75, 95. Hydrogen atoms were added, and all hetero atoms (HETATM) were removed from the protein structures by saving in UCSF Chimera 1.15 as new PDB files, which were converted to PDBQT format in ADT software (http://autodock.scripps.edu/resources/adt). Rhubarb compounds as ligands for docking were prepared as described previously (Liudvytska et al., 2023). Molecular illustrations and structural docking analyses were performed with UCSF ChimeraX (https://www.cgl.ucsf.edu/chimerax/) (Goddard et al., 2018; Pettersen et al., 2021) and UCSF Chimera 1.15 (http://www.cgl.ucsf.edu/chimera/) (Waterhouse et al., 2009) software, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, USA. LigPlot + v.2.2 (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/accessed on February 24, 2023) was used to generate the ligand-protein interaction diagrams (Wallace et al., 1995; Laskowski and Swindells, 2011).

2.14. Statistical analysis

The statistical analysis was performed using the STATISTICA 13.0 PL software (StatSoft INC, Tulsa, Oklahoma). The first step of statistical analysis was the elimination of the uncertain data by the Grubbs' tests (GraphPad Prism 5.01, San Diego, California). Next, the differences between groups were assessed by the non-parametric Wilcoxon test (for unpaired data) and the t-Student test was used for data with normal distribution. Both tests report a two-tailed p-value. A probability $p<0.05$ was considered statistically significant. All the values in this work are expressed as mean ± standard deviation (SD).

3. Results

3.1. Phytochemical profiling

In our previous work, we have shown that the investigated *Rheum* species differ in the contents of selected metabolites (Liudvytska et al., 2023). Unfortunately, a complete set of reference standards to quantify all compounds we detected in *Rheum* is unavailable, and chromatographic resolution often prevents the quantitation of low-abundance components using the universal detector approach applied previously. Therefore, we used high-resolution mass spectrometry data and statistical methodology to compare the investigated samples unbiasedly. Features from the HR-MS data were automatically extracted and grouped by the *m/z* and retention time [RT]. Subsequently, the data matrix of ion peak intensities referenced by the sample and the *m/z*-RT pairs was subjected to analysis of similarities.

As shown in Fig. 2, the plot of the Bray-Curtis dissimilarity ranks indicates that the differences in extracts composition between species are smaller than the dissimilarity within the species (R = 0.109 at p = 0.151). This result agrees with previous findings on the relative similarity of phytochemical composition between the two *Rheum* species. On the other hand, the composition of the extracts between petioles and root significantly differs (R = 0.999 at p = 0.003), even though within-group dissimilarities are minor. Since petioles and root extracts of the two investigated species were grouped, this result again indicates their phytochemical similarity. However, it can be presumed that due to differences in composition, the biological activity properties of extracts from petioles and roots may differ.

Chemical components associated with each type of extract were investigated using PCA. Fig. 3 shows the results for the first three components encompassing nearly 90% of the samples' variability. The petioles and root samples are differentiated across the first principal component (PC1), encompassing almost 50% of the samples' phytochemical composition variability. On the negative side of the scale, primary features highly contributing to PC1 included compounds characterized as the main root extracts components: chrysophanol-malonyl-Hex, resveratroloside, rhapontin, and astrignin (compounds nr. **240**, **69**, **121**, **84** from Table S2 in ref. (Liudvytska et al., 2023). In contrast, the highest positively correlated loadings had features observed in relatively high amounts in petioles, such as isoquercetin, homocitric acid, and 18:3-DGMG (gingerglycolipid A) (compounds nr: **66**, **6**, **154** from Table S1 in ref. (Liudvytska et al., 2023).

The second principal component (PC2), constituting 25.9% of samples variability, separates root extracts of *R. rhabarbarum* and *R. rhaponticum*. Among the positively correlated loadings associated with *R. rhabarbarum* root extracts, the highest contribution to PC2 had catechin, β-glucogallin, and torachrysone (compounds: **30**, **5** and **323** from Table S2 in ref (Liudvytska et al., 2023).), along with several unidentified metabolites. Primary negative loadings associated with *R. rhaponticum* roots belonged to metabolites identified as malonylated hexoside of emodin, hexoside of torachrysone, procyanidin B2, and malonylated vitexin (compounds nr. **184**, **197**, **42**, **130** from Table S2 ref. (Liudvytska et al., 2023), as well as a few unidentified features.

The third principal component (PC3) encompassed 13.4% of the variability and separated samples from petioles of both species. Features

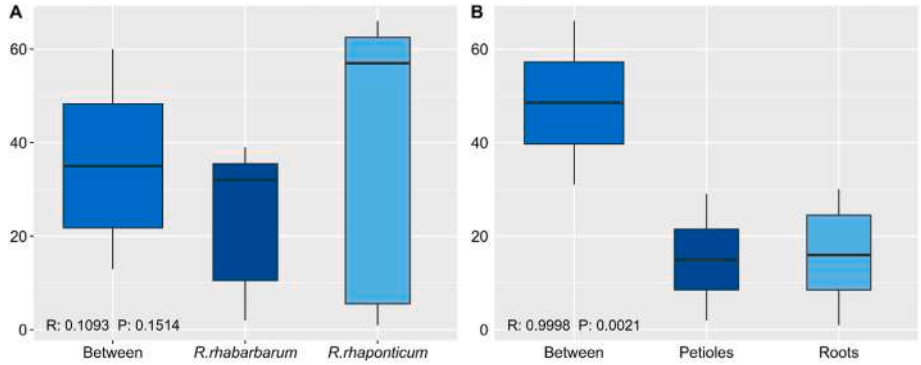


Fig. 2. The plot of Bray-Curtis dissimilarity ranks of the investigated extracts grouped by the originating species (A) and plant organ (B).

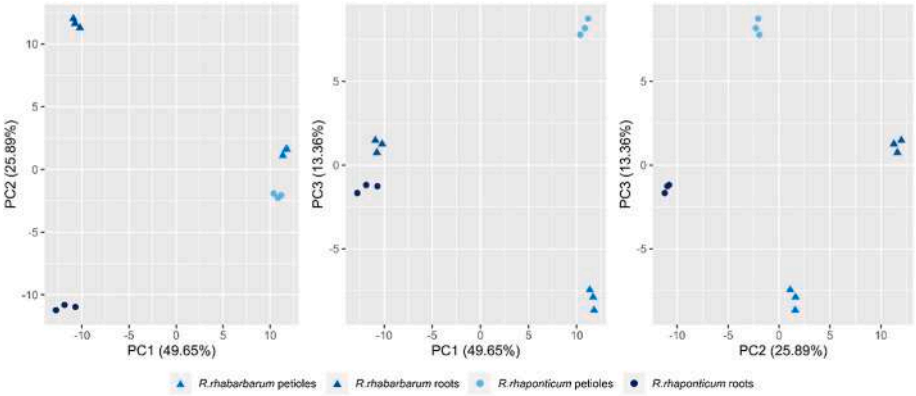


Fig. 3. PCA of the rhapontic rhubarbs' extracts phytochemical composition.

with the highest, positively correlated to PC3 loadings, could be associated with the petioles of *R. rhaponticum*. Among them were fragment ions from physcion-anthrone malonylated hexoside, an unidentified emodin-anthrone derivative, and several unidentified metabolites (compounds nr. **130**, **94**, **27** from Table S1 ref (Liudvytska et al., 2023).). Features with loadings negatively correlated to PC3 were associated with the petioles of *R. rhabarbarum.* They included a variable range of compounds: derivatives of phenolic acids (dicoumaroyl-hexoside, two malonylated dihexosides of anthraquinones: emodin-physcion-dianthrone, and rhein-dianthrone, aliphatic alcohol glycoside, (compounds **97**, **126**, **117** and **35** from Table S1 in ref (Liudvytska et al., 2023).) and a few other, less identified compounds. Nevertheless, the primary negative contributor to PC3 was a compound forming deprotonated ion at *m/z* 465.104 and RT of 14.69 min, which remained unidentified in our last publication (compound **54**, MS/MS spectrum in Fig. 4). Based on the characteristic loss of 162.05 Da (corresponding to the $C_6H_{10}O_5$ neutral fragment), we concluded that it is a hexoside. However, the loss of 152.01 Da (neutral fragment $C_7H_4O_4$) is more puzzling, assuming the base peak at *m/z* 303.05 (corresponding to $C_{15}H_{11}O_7$), possibly pentahydroxyflavone, maybe the aglycone. In that case, such a neutral loss could occur only by fragmenting the aglycone, likely through the fission of the C-ring. The resulting fragments (*m/z* 313. 01 and *m/z* 151.00) indicate saturated ring C and possible substitution with hexose in position C-3 as well as two hydroxyl groups substituents on ring B.

To our knowledge, only one aglycone matching that structural features was reported from the Polygonaceae family: dihydroquercetin, also known as taxifolin (Li et al., 1999). Previous semi-quantitative analyses detected this compound in the petioles of *R. rhabarbarum* (at 0.82 ± 0.16 mg/g d.w.). An analogous compound was detected but not quantified in the root extracts from the same species (Liudvytska et al., 2023). However, the ion *m/z* 465.103 was not observed at the corresponding retention time in either petioles or roots of *R. rhaponticum*. Therefore, taxifolin-3-*O*-hexoside may be a diagnostic feature of rhubarb products originating from *R. rhabarbarum*, although it is yet unclear whether it also occurs in other species of rhubarb. Preliminary UHPLC-MS analyses of identically prepared extracts from petioles of *R. officinale* and *R. tanguticum* did not indicate the occurrence of taxifolin hexoside (Fig. 4., right inlay). The presence of free taxifolin was reported from *R. ribes* (Çınar Ayan et al., 2021). Taxifolin glucoside was observed also in *R. rhaponticum* petioles extract (Raudsepp et al., 2019), however, the previous studies of the mentioned group concerning the phytochemical composition of petioles and root extracts of *R. rhaponticum* based on LC-MS analysis did not show the presence of both free taxifolin as well as its glycoside/glycosides in any of examined preparations (Püssa et al., 2009).

In addition to phytochemical characterization by UHPLC-MS analyses, we also carried out three classic spectrophotometric tests to gain a

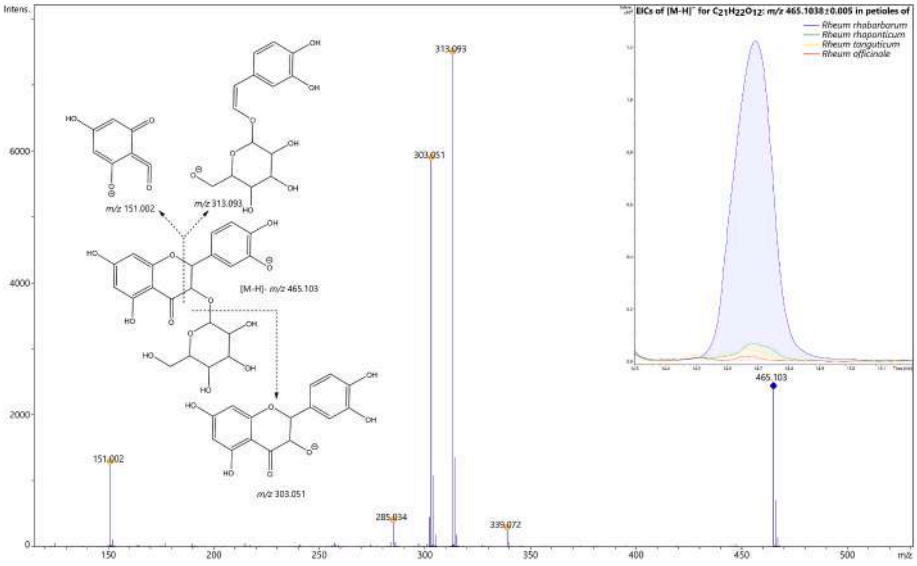


Fig. 4. MS/MS spectrum of the *m/z* 465.104 unidentified component at RT 14.69. Left inlay: proposed fragmentation pathway, right inlay: extracted ion chromatograms for *m/z* 465.1038 in the butanol extracts of petioles from four species of *Rheum*.

better insight into the composition of the extracts. As shown in Fig. 5, the total phenolic content was found to vary significantly between different organs (petioles *vs*. roots) than between the examined rhubarb species. Moreover, the presented results indicate that, compared to the Prussian Blue assay, the Folin-Ciocalteu assay indicated a higher value of total phenolic content in all examined rhubarb extracts. Folin-Ciocalteu results show petioles extract's total phenolic content (TPC) varying between 58.12 and 105.01 μg GA eq/mg, whereas root extracts had 337.11 and 335.35 μg GA eq/mg for *R. rhaponticum* and *R. rhabarbarum* respectively. Overall, the Prussian Blue assay results showed lower values for petioles extracts ranging from 35.56 μg GAeq/mg for petioles extract of *R. rhaponticum* to 68.95 μg GA eq/mg for petioles extract of *R. rhabarbarum*. In contrast, in the root extract of *R. rhabarbarum*, Prussian Blue TPC was at a similar level as Folin-Ciocalteu TPC (334.44 μg GAeq/mg). In the extract of *R. rhaponticum* roots, the Prussian Blue TPC assay gave an even higher result, 369.68 μg GA eq/mg. Overall, the results of both assays showed that lower values of TPC characterized the extracts of petioles, whereas their root counterparts were characterized by higher TPC, regardless of the method used. The condensed tannin content also varied but slightly less than TPC. The lowest content was observed for petioles of *R. rhaponticum* (amounting to 55.00 μg CA eq/mg). However, the remaining extracts had condensed tannin content at similar levels (161.94, 165.84, and 182.56 μg CA eq/mg for petioles and roots of *R. rhabarbarum*, and roots of *R. rhaponticum*, respectively).

3.2. Effect of the examined rhubarb extracts and stilbenes on the blood coagulation cascade

3.2.1. Effect of rhubarb extracts and stilbenes on the tissue factor-initiated coagulation of blood plasma

The test enabled the determination of the effects of the analyzed extracts and stilbenes (5 and 50 μg/ml) on the functionality of the extrinsic coagulation pathway (including the TF-dependent generation of FXa and thrombin) in whole blood plasma, under conditions similar to those occurring *in vivo*. All of the examined rhubarb extracts displayed anticoagulant properties and reduced blood plasma clotting (Fig. 6). Their efficiency was comparable, and the maximal reduction of blood plasma clotting attained about 40%. The RHPG did not influence the extrinsic coagulation pathway, however, for RHPT, a slight increase (by about 15–20%) of blood clotting rate was found. Argatroban, a reference anticoagulant drug, reduced the plasma clotting by about 60%, at a concentration of 5 μg/ml (Fig. 6). At a concentration of 50 μg/ml, argatroban completely inhibited the plasma clotting (data not presented).

3.2.2. Evaluation of the examined rhubarb extracts and stilbenes effects on the enzymatic activity of thrombin

Effects of extracts from *R. rhaponticum* and *R. rhabarbarum* and the stilbenes on thrombin activity were evaluated in two different assays, using a synthetic chromogen (Fig. 7) and fibrinogen, a physiological substrate for this enzyme (Fig. 8). Both the amidolytic assay and fibrinogen polymerization tests revealed the thrombin-inhibitory action of the examined plant extracts. The amidolytic activity tests demonstrated that extracts from the petioles of *R. rhaponticum* and *R. rhabarbarum* inhibited the thrombin enzyme with IC_{50} values of 48.11 and 35.67 μg/ml, respectively. At concentrations of 5 and 50 μg/ml, the statistical significance of the obtained results was $p<0.01$ and $p<0.001$, whereas at the concentration of 1 μg/ml, no statistically significant effects were found. For the extracts from the underground parts of *R. rhaponticum* and *R. rhabarbarum*, the thrombin IC_{50} values were established as 26.53 and 20.26 μg/ml, respectively. RHPG and RHPT displayed weak abilities to inhibit the amidolytic activity of thrombin, and their inhibitory efficiency did not exceed a 20% decrease in this enzyme activity.

Effects of the examined plant extracts and stilbenes on the proteolytic activity of thrombin were evaluated using two parameters, *i.e.* the maximal velocity (V_{max}) of fibrinogen polymerization and the maximal absorbance (A_{max}; $\lambda = 360$ nm), which is a marker of the fibrin clot density (Fig. 8A and B). The obtained results indicated that the extracts originating from roots of *R. rhaponticum* and *R. rhabarbarum* (1–50 μg/ml) were more efficient inhibitors of the hydrolytic activity of thrombin towards its physiological substrate, when compared to the inhibitory efficiency of the petiole extracts. At a concentration of 50 μg/ml, the root extracts reduced thrombin activity by >80%. For the petiole extracts, the inhibitory efficiency at the same concentration was 29% and 69%, for the *R. rhaponticum* and *R. rhabarbarum*, respectively. There was no inhibitory effect of RHPG and RHPT on the proteolytic activity of thrombin (Fig. 8A).

Results of the A_{max} measurements revealed that the fibrin clot density was significantly reduced by extracts from the underground parts of *R. rhaponticum* and *R. rhabarbarum,* at concentrations of 1–50 μg/ml (*$p<0.05$; **$p<0.01$, ***$p<0.001$). In the case of stilbenes and most of the samples treated with the petiole extracts, no effects on the fibrin clot density (the A_{max} parameter) were noted (Fig. 8B).

3.2.3. Effect of rhubarb extracts and stilbenes on the activity of the coagulation factor Xa

Analyses of the coagulation factor Xa (FXa) inhibitory efficiency of the examined extracts and stilbenes revealed that the extracts from the *R. rhaponticum* and *R. rhabarbarum* petioles and roots, as well as stilbenes, were able to significantly inhibit the activity of this serine protease (Fig. 9). Among the examined substances, the extracts from the underground parts of *R. rhaponticum* and *R. rhabarbarum* were the most efficient inhibitors of the FXa. At a concentration of 50 μg/ml, the activity of this enzyme was reduced by over 80%. The reference compound was GGACK, an irreversible inhibitor of serine proteases (incl. the FXa), used at a concentration of 4 μg/ml.

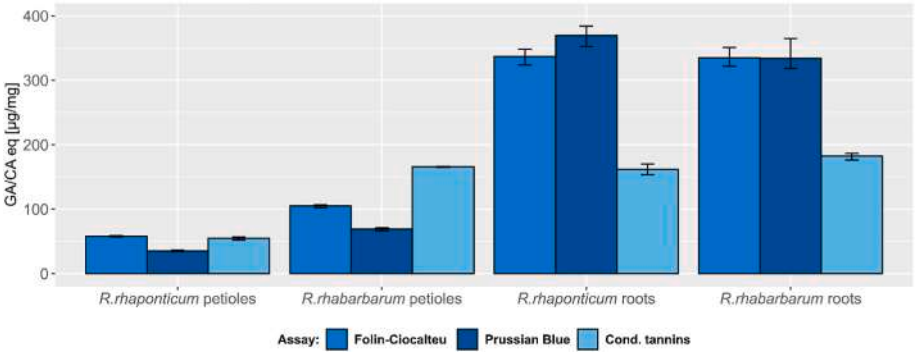


Fig. 5. Evaluation of total phenolic content and condensed tannins content in the rhubarbs' extracts (bars are mean value, error bars represent 95% confidence intervals).

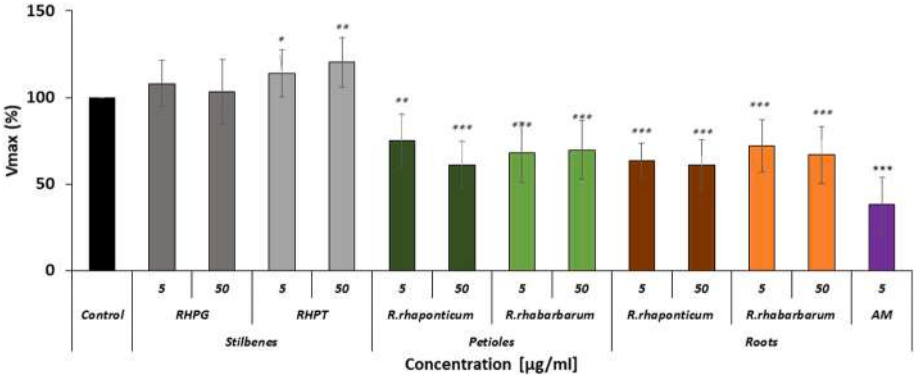


Fig. 6. Effects of *R. rhaponticum* and *R. rhabarbarum* extracts and stilbenes (RHPG and RHPT) on the tissue factor-activated coagulation of blood plasma. Effects of the examined substances on the activity of the extrinsic coagulation pathway in human blood plasma were established based on the maximal velocity of blood plasma clotting (the V_{max} parameter). The mean V_{max} value for the control samples was 0.45 ± 0.03 OD/min. AM - argatroban monohydrate, a reference compound (thrombin inhibitor). The clotting ability of control plasma (untreated with the examined substances) was assumed as 100%. The figure represents mean values ± SD; n = 11; *p<0.05; **p<0.01; ***p < 0.001.

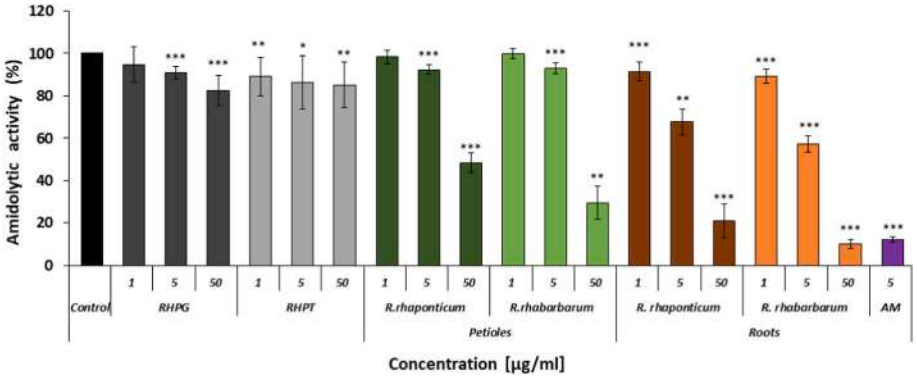


Fig. 7. Effects of *R. rhaponticum* and *R. rhabarbarum* extracts and stilbenes (RHPG and RHPT) on the amidolytic activity of thrombin. The enzyme activity was measured kinetically, using the chromogenic substrate S-2238. The mean V_{max} value for the control samples attained 0.141 ± 0.003 OD/min. AM - argatroban monohydrate, a reference compound (thrombin inhibitor). The figure represents mean values ± SD, n = 9. The activity of control thrombin (untreated with the examined substances) was assumed as 100%; *p<0.05; **p<0.01; ***p<0.001).

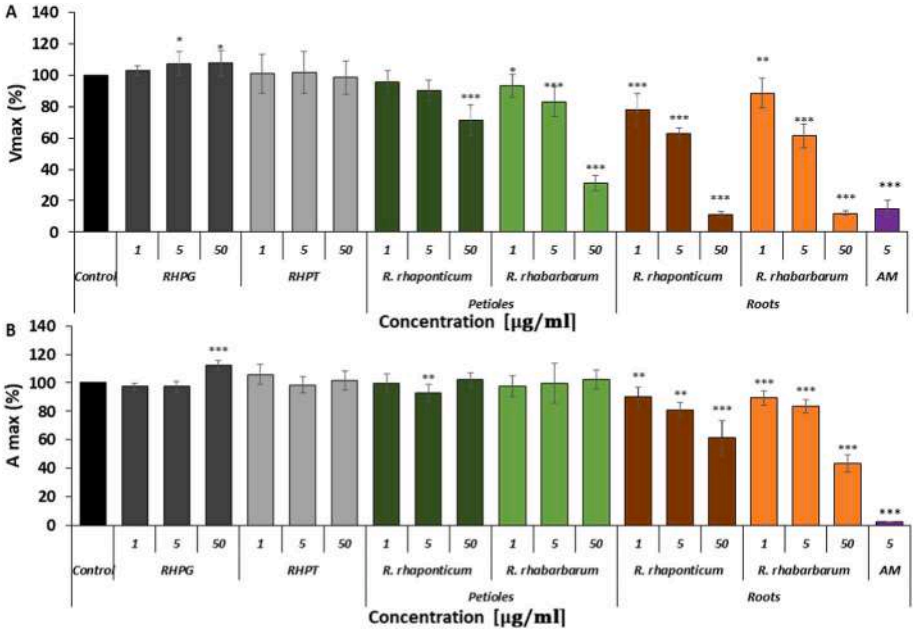


Fig. 8. Effects of *R. rhaponticum* and *R. rhabarbarum* extracts and stilbenes (RHPG and RHPT) on the proteolytic activity of thrombin (the fibrinogen polymerization test).
Experiments were carried out using human fibrinogen as a substrate for thrombin. The inhibitory efficiency of the examined extracts and stilbenes was estimated based on the maximal velocity of fibrinogen polymerization (the V_{max} parameter; panel A of the figure) and density of the formed fibrin clot (the A_{max} parameter; panel B of the figure). The mean V_{max} value for the control samples attained 0.814 ± 0.1 OD/min, and the mean A_{max} value for control samples was 1.344 ± 0.1. AM - argatroban monohydrate, a reference compound (thrombin inhibitor). The figure represents mean values ± SD, n = 9. The V_{max} of proteolytic activity and the A_{max} values recorded in samples containing the control/untreated thrombin were assumed as 100% (*p<0.05; **p<0.01; ***p<0.001).

3.3. The effect of rhubarb extracts and stilbenes on the activity of plasmin generated by different activators

To check whether the rhubarb extracts and stilbenes affect the process of plasmin generation, two diverse plasminogen activators were applied. In the first part of this experimental module, plasminogen (the proenzyme) was activated to the plasmin enzyme by streptokinase (SK), an exogenous activator of fibrinolysis, used as a thrombolytic drug. SK is a non-proteolytic activator of plasminogen, forming a complex with the zymogene in a 1:1 stoichiometric ratio. The plasminogen-SK complex is biologically active and capable of activating other plasminogen molecules to plasmin. To activate the zymogen (plasminogen) into an active enzyme, it was pre-incubated with streptokinase, and then, the activity of plasmin generated in this experimental system was measured. Analyses of the SK-mediated conversion of plasminogen to plasmin demonstrated that the examined substances are weak inhibitors of

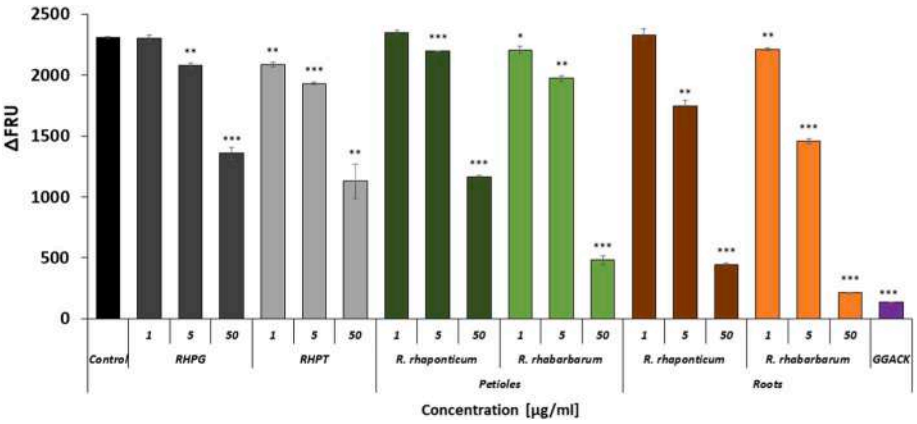


Fig. 9. Influence of extracts from petioles and roots of *R. rhaponticum* and *R. rhabarbarum* and the tested stilbenes (RHPG/RHPT) on the activity of the blood coagulation factor Xa. Results were obtained during a fluorometric assay ($\lambda_{ex/em}$ = 350/450 nm), n = 3. The figure represents mean values ± SD. The activity of control/untreated FXa was assumed as 100% (*p<0.05; **p<0.01; ***p<0.001). GGACK (Glu-Gly-Arg-chloromethyl ketone), a reference FXa inhibitor (4 μg/ml); n = 3.

plasmin generation, induced by this exogenous activator. The *R. rhabarbarum* root extract (1–5 μg/ml) slightly decreased the plasmin generation, maximally by about 10% (**p<0.01). RHPG (at a concentration of 50 μg/ml) also display an inhibitory effect in this assay (***p<0.001), but its inhibitory efficiency was about 20%. In most samples treated with RHPT, the *R. rhaponticum* petiole and root extracts or *R. rhabarbarum* petiole extract, no effects on the SK-mediated activation of the plasminogen zymogene were found (Fig. 10).

The second part of amidolytic tests involved the use of t-PA, which is the main physiological activator of plasminogen. Most of the examined substances did not influence the t-PA-induced activation of plasminogen to the plasmin enzyme. Only the extract from roots of *R. rhabarbarum* (50 μg/ml) and RHPG (1 μg/ml) reduced the t-PA-induced plasmin generation by about 10–15% (Fig. 11).

3.4. Influence of stilbenes and rhubarb extracts on haemostatic activity of endothelial cells

The modulatory properties of the endothelium are essential for maintaining blood fluidity and controlling both the coagulation and fibrinolytic processes. For the evaluation of the effects of the examined substances on the haemostatic properties of endothelial cells, an experimental model of the thrombin-stimulated HUVECs was used. The haemostatic activity of the HUVECs was monitored using three parameters: the von Willebrand factor (a marker of pro-coagulant response), t-PA (a marker of the fibrinolytic activity) and PAI-1/t-PA complexes (a marker of fibrinolysis-regulatory mechanisms) release. The vWF, t-PA and PAI-1/t-PA complexes levels were determined in cell culture medium, using the ELISA kits.

Based on our previous experiments on the cellular safety of the examined plant-derived substances (Liudvytska et al., 2023), their concentration ranges have been slightly modified in this part of the study. Due to a significant decrease of HUVECs viability in samples treated with RHPG and *R. rhabarbarum* root extracts at a concentration of 50 μg/ml, the maximal concentrations of these substances in experiments on HUVECs were established to attain 25 and 30 μg/ml, respectively. The action of the remaining extracts and RHPT was evaluated in the concentration range of 1–50 μg/ml.

3.4.1. Measurements of the pro-coagulant action of endothelial cells (release of von Willebrand factor)

At concentrations of 1–5 μg/ml, no statistically significant effects were found for most of the examined substances, except the *R. rhabarbarum* root extract. The thrombin-induced release of the vWF from HUVECs was partly inhibited by RHPG at a concentration of 25 μg/ml, RHPT (50 μg/ml), as well as extracts from the petioles of *R. rhaponticum* (50 μg/ml) and roots of *R. rhabarbarum* (at concentrations of 5 and 30 μg/ml). The *R. rhabarbarum* petiole and *R. rhaponticum* root extracts did not influence the vWF release from HUVECs. The most effective substance was RHPG, which reduced the procoagulant activity of HUVECs by about 50%, at a concentration of 25 μg/ml (Fig. 12).

3.4.2. Evaluation of the effect of stilbenes and rhubarb extracts on profibrinolytic potential of endothelium: determination of the t-PA release

The assay demonstrated that stilbenes and extracts at all of the tested concentrations decreased the level of the t-PA, released from the thrombin-stimulated HUVECs to the culture medium (Fig. 13). At the highest concentrations of the examined substances (*i.e.* 25, 30, and 50 μg/ml), about 50% inhibition of the t-PA release was observed (compared to the control HUVECs, simulated with thrombin in the absence of the examined plant substances).

3.4.3. Determination of the fibrinolysis-regulatory activity of endothelial cells, based on a level of PAI-1/t-PA complexes

The ELISA-based detection of the PAI-1/t-PA complexes in the cell culture medium revealed slight reducing effects of the examined stilbenes (Fig. 14). The *R. rhaponticum* petiole extracts (5–50 μg/ml) and *R. rhabarbarum* (1–50 μg/ml) had no effect on the PAI-1/t-PA complexes

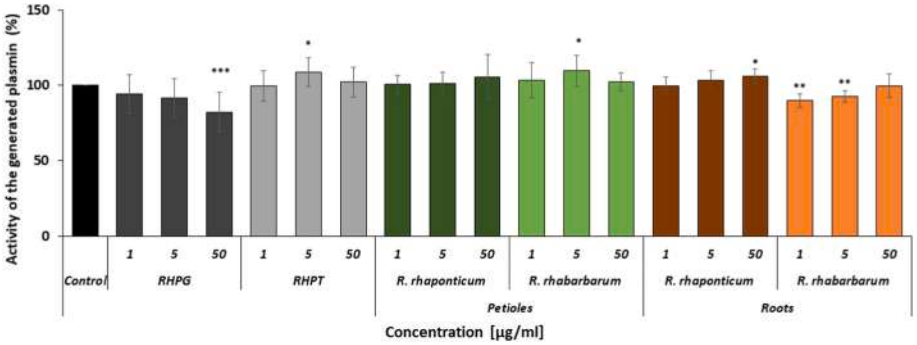


Fig. 10. Effects of the stilbenes (RHPG/RHPT) and extracts from *R. rhaponticum* and *R. rhabarbarum* on the streptokinase-induced conversion of plasminogen to plasmin. Plasmin generation was monitored kinetically and assessed by the amidolytic method, based on the hydrolysis of the S-2251 substrate. Absorbance changes were recorded at λ = 415; n = 9. The figure represents mean values (±SD). The activity of plasmin generated in the control samples was assumed as 100% (*p<0.05; **p<0.01; ***p<0.001).

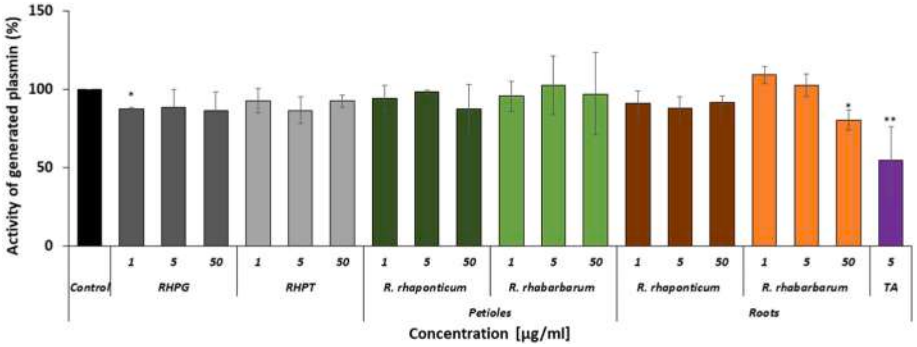


Fig. 11. Effects of the stilbenes (RHPG/RHPT) and extracts from *R. rhaponticum* and *R. rhabarbarum* on the t-PA-induced activation of plasminogen to the plasmin enzyme. The assay was carried out in an experimental model employing human plasminogen (the plasmin zymogen) and human fibrinogen (the activation surface). TA - tranexamic acid - a reference anti-fibrinolytic drug. The figure represents mean values (±SD); n = 9. The activity of plasmin generated in the control samples (untreated with the examined substances) was assumed as 100% (*p<0.05; **p<0.01).

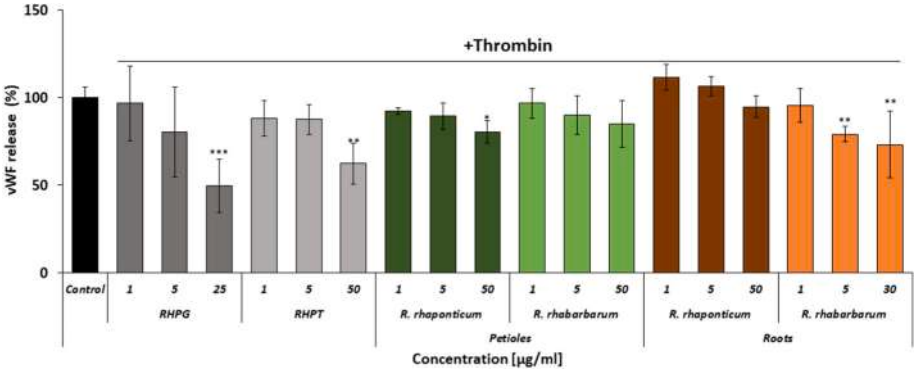


Fig. 12. Evaluation of the effect of stilbenes (RHPG/RHPT) and rhubarb extracts on the pro-coagulant response of endothelial cells, based on the release of von Willebrand factor. The vWF release from HUVECs into the cell culture medium was determined by the ELISA. The figure represents mean values ± SD; n = 4. The vWF release recorded for the control samples (HUVECs stimulated with thrombin, without presence of the examined substances) was assumed as 100%; *p<0.05; **p<0.01; ***p<0.001.

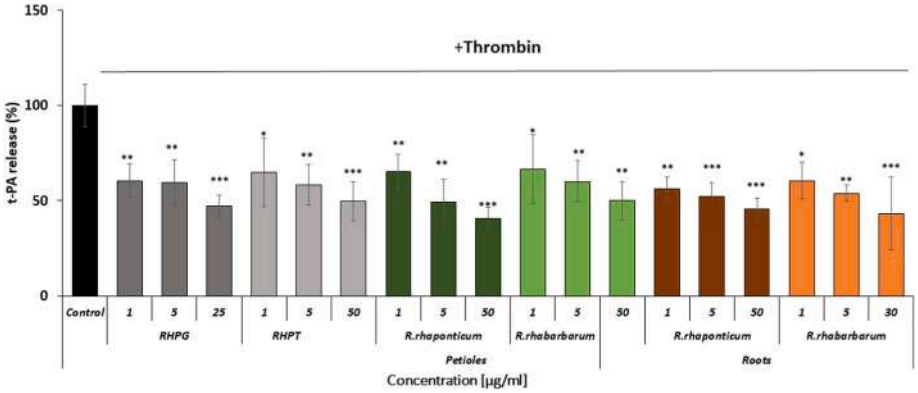


Fig. 13. Evaluation of the effect of stilbenes (RHPG/RHPT) and rhubarb extracts on the pro-fibrinolytic potential of endothelial cells, based on the t-PA release. The figure represents mean values ± SD; n = 4. The t-PA release recorded for the control samples (HUVECs stimulated with thrombin, without presence of the examined substances) was assumed as 100%; *p<0.05; **p<0.01; ***p<0.001.

level. In samples derived from HUVECs treated with *R. rhabarbarum* root extract at a concentration of 1 µg/ml, a decrease of PAI-1/t-PA complexes was found. However, in samples treated with the root extract from this rhubarb species at a concentration of 30 µg/ml, a 3-fold increase in the concentration of PAI-1/t-PA complexes was detected.

4. Molecular docking

The most abundant and well characterized organic chemical compounds of rhubarb extracts were used to examine computational molecular docking with structures of prothrombin, thrombin, FXa, plasminogen and plasmin. Many of them appeared to be potential inhibitors of studied enzymes of haemostasis. Some of them showed high affinity for the active site of thrombin and FXa, possibly blocking the catalytic triad from substrate access as a possible competitive inhibitor, in contrast to plasminogen and plasmin, where the most compounds bound at non-function-specific sites (Table 1). Due to the high content of rhaponticin, astringin, piceatannol-galloylglucoside and viniferin in the roots of both species (with a certain predominance of *R. rhabarbarum*) and FXa inhibitory effect (Fig. 8), the docking locations of these selected compounds to FXa were illustrated (Fig. 15).

According to visual inspection using UCSF Chimera, ChimeraX and LigPlot + v.2.2 analysis viniferin, astringin and piceatannol-galloylglucoside were bound in the catalytic triad of FXa near amino acid residues Ser, His and Asp, stabilized by polar interactions and hydrophobic effects (Fig. 15). The location of the rhaponticin was relative to them off side.

According to visual inspection using UCSF Chimera, ChimeraX and LigPlot + v.2.2 analysis rhaponticin molecule was bound in catalytic triad of thrombin and FXa near amino acid residues Ser, His and Asp,

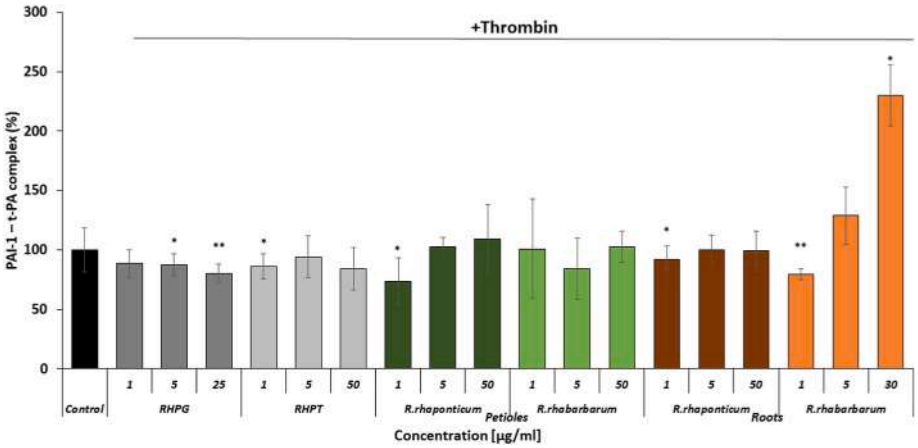


Fig. 14. Evaluation of the effect of stilbenes (RHPG/RHPT) and rhubarb extracts on the fibrinolysis-regulatory potential of endothelial cells, based on measurements of PAI-1/t-PA complexes level. The figure represents mean values ± SD; n = 4. The level of PAI-1/t-PA complexes detected in control samples (HUVECs stimulated with thrombin, without presence of the examined substances) was assumed as 100%; *$p<0.05$; **$p<0.01$; ***$p<0.001$.

stabilized by polar interactions and hydrophobic effects. Interactions via hydrogen bonds with the serine residue were clearly visible in all cases.

5. Discussion

The haemostatic system is an integral part of cardiovascular physiology. Its components are responsible not only for preventing blood loss after injury and removal of fibrin clots but also for maintaining the physiological haemostatic balance, blood fluidity, and vascular tone as well as for wound healing, tissue repair, and remodelling (Stassen, 2004; Versteeg et al., 2013; Hoffman, 2018; Draxler and Medcalf, 2015). Physiological balance within the vascular system depends on complex interactions between different components of blood plasma and blood cells as well as vasoactive and modulatory activity of the endothelium (van Hinsbergh, 2012; De Pablo-Moreno et al., 2022; Neubauer and Zieger, 2022). However, this equilibrium is very prone to alterations triggered by diverse endogenous and exogenous factors such as inflammation, diseases, weight gain, stress, or intoxication. The pathophysiology of civilization diseases, including cardiovascular diseases (CVDs), involves numerous pathways and reactions, resulting in the inflammation-associated impairment of regulatory properties of the blood vessel endothelium, oxidative stress, and alterations in the haemostatic balance (including enhancement of the pro-coagulant activity of blood plasma and impairment of fibrinolysis efficiency) (Nagareddy and Smyth, 2013; Wojtala et al., 2017; d'Alessandro et al., 2020).

The health-promoting and therapeutic properties of natural, plant-derived substances and extracts have gained scientific attention in recent decades. Results from numerous studies indicate that consuming a phytochemicals-rich diet and plant-based whole foods may be a promising strategy to prevent CVDs. Beneficial effects of plant-based preparations on cardiovascular health are mainly attributed to the presence of natural substances displaying antioxidant, anti-inflammatory, lipid-lowering, and anti-platelet properties (Bachheti et al., 2022; Zhao et al., 2017; Varadharaj et al., 2017). In addition, substances of plant origin are also investigated as potential anti-thrombotic agents, preventing or reducing the hypercoagulative conditions associated with many civilization diseases (Akram and Rashid, 2017; Benmeziane - Derradji and Aoun, 2022).

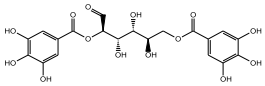
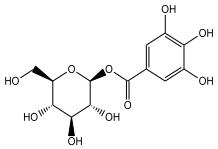
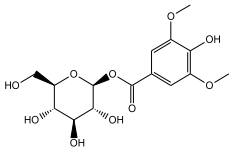
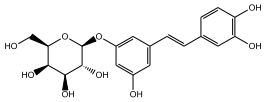
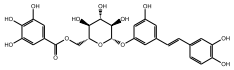
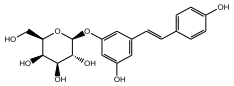
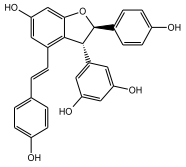
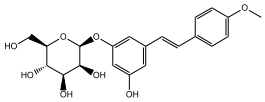
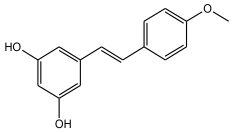
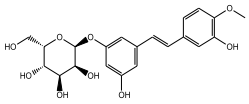
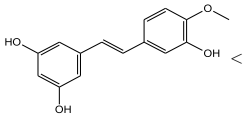
As commonly known edible plants, *R. rhaponticum* and *R. rhabarbarum* have been examined in the context of their biological activity and effects on human health. A growing number of data from historical sources, ethnomedicinal studies, phytochemical analyses, studies on biological activities, along with the presence of these species in contemporary herbal medicine have indicated that these plants may possess considerable potential for therapeutic use. Both *in vitro* works and *in vivo* studies on substances and extracts isolated from these plants revealed their phytoestrogenic, anticancer, antimicrobial and antiviral, hepatoprotective, and anti-obesity properties (Cao et al., 2017; Xiang et al., 2020; Mohtashami et al., 2021; Liudvytska and Kolodziejczyk-Czepas, 2022). According to those studies, the most important classes of biologically active metabolites are anthraquinones, anthrones, stilbenes, flavonoids, phenolic acids, cinnamic acid, butyrophenones, chromones, tannins (Cao et al., 2017). Some of these compounds, such as aloe-emodin (Dong et al., 2020), emodin (Dong et al., 2016), rhein (Zhou et al., 2015), chrysophanol (Xie et al., 2019), physcion (XunLi et al., 2019), resveratrol (Berman et al., 2017; Zhang et al., 2021), epigallocatechin gallate (Meng et al., 2019; Schneekenburger et al., 2019; Ciesielski et al., 2020) piceid (polydatin) (Du et al., 2013; Karami et al., 2022), rhaponticin (Sun et al., 2017; Kołodziejczyk-Czepas and Czepas, 2019) have extensive, well-documented biological properties, and include a wide range of pharmacological activities. In addition, some of the aforementioned compounds (rhein, emodin, chrysophanol) are characterized by low risk of toxicity and side effects, which suggests good application prospects (Cao et al., 2017). Especially compounds of the anthraquinone class or their semi-synthetic derivatives have been applied as drugs for medical applications, as laxatives, antimicrobial or anti-inflammatory agents (Malik and Müller, 2016).

The analyses we carried out on the constituents of the extracts from *R. rhabarbarum* and *R. rhaponticum* indicated similarity. However, it should be emphasized that there are significant differences in the qualitative and quantitative composition of examined rhubarb species demonstrated by LC-MS and spectrophotometric assays concerning, in particular, the examined organs, i.e., petioles and roots. This similarity is also reflected in the biological activities investigated in this work. In general, butanol root extracts of *R. rhaponticum* and *R. rhabarbarum* are characterized by greater diversity and contents of phenolic compounds than petioles extracts. Observed differences in total phenolic content, depending on the method used (the Folin-Ciocalteu or the Prussian Blue assay), particularly visible in the case of petioles analyses, may be a result of the matrix interferences. The presence of some classes of compounds, such as reducing sugars, AA, and organic acids, can affect the analyses by reducing the Folin-Ciocalteu reagent and thus skewing the results of TPC (Sánchez-Rangel et al., 2013).

The effects of *R. rhaponticum*, *R. rhabarbarum,* and other rhubarb species on blood physiology, including their influence on functions of different components of the haemostatic system, remain poorly elucidated. Our previous work provides promising results on the anti-inflammatory potential of the examined rhubarb extracts and stilbenes. The examined plant preparations acted at different levels of the

Table 1

Blind docking of the most abundant compounds found in *R. rhaponticum* and *R. rhabarbarum*. ΔG°_{bind} - molecular docking predicted standard free energy change of ligand binding to protein structures (PDB ID below). AS - bound in the area of the active site (HDS catalytic triad), EI, EII – bound near Exosite I or II, respectively, "-" bound outside of active sites and exosites.

		Pro(thrombin) ΔG°_{bind} (kcal·mol^{-1})			FXa ΔG°_{bind} (kcal·mol^{-1})		Plasmin(ogen) ΔG°_{bind} (kcal·mol^{-1})	
(No.)	Compound names and chemical structure	3U69	1FPH	7TPP E	1XKB C	7TPP B	3UIR A	4A5T
(1)	digalloyl glucoside	−8.4	−7.9	−7.7	−6.9	−6.5	−6.5	−7.2
		AS	AS	AS	AS	AS	–	–
(2)	glucogallin	−7.4	−7.7	−6.7	−6.8	−6.8	−6.5	−7.4
		AS	AS	AS	AS	–	–	–
(3)	syringoyl 1-*O*-glucopyranoside	−7.2	−6.8	−6.6	−7.0	−6.5	−6.2	−6.5
		–	–	–	–	–	–	–
(4)	**astringin**	−9.4	−7.7	−8.3	−7.2	−7.0	−7.3	−8.7
		AS	AS	–	AS	–	–	–
(5)	piceatannol-galloylglucoside	−9.3	−8.4	−9.7	−8.3	−7.3	−7.3	−9.1
		AS	AS	–	AS	–	–	–
(6)	piceid (polydatin; resveratrol 3-*O*-*β*-glucopyranoside)	−9.0	−7.6	−8.2	−7.9	−7.2	−7.8	−7.8
		AS	AS	–	AS	AS	–	–
(7)	viniferin (resveratrol-dehydrodimer)	−9.3	−8.9	−9.5	−9.0	−7.1	−8.0	−8.1
		AS	AS	–	AS	AS	–	–
(8)	deoxyrhaponticin	−7.8	−8.0	−8.3	−6.8	−7.1	−7.0	−7.1
		–	–	–	AS	–	–	–
(9)	**deoxyrhapontigenin**	−7.3	−6.3	−7.1	−6.5	−6.5	−6.4	−6.4
		EII-AS	EII-AS	–	AS	–	–	–
(10)	**rhaponticin (rhapontin)**	−8.6	−8.9	−8.2	−7.0	−7.1	−7.6	−8.2
		AS	AS	–	AS	hEI	–	–
(11)	Rhapontigenin	−7.2	−7.0	−8.7	−6.9	−6.7	−6.4	−6.8
		AS	AS	–	hEII	–	–	–

(*continued on next page*)

Table 1 (*continued*)

(No.)	Compound names and chemical structure	Pro(thrombin)ΔG°_{bind}(kcal·mol^{-1})			FXa ΔG°_{bind}(kcal·mol^{-1})		Plasmin(ogen) ΔG°_{bind} (kcal·mol^{-1})	
		3U69	1FPH	7TPP E	1XKB C	7TPP B	3UIR A	4A5T
(12)	rhapontigenin-galloyl-glucopyranoside	−9.0 –	−8.7 –	−8.8 –	−8.1 –	−7.8 –	−8.1 AS	−8.7 –
(13)	(epi)catechin	−8.0 AS	−7.4 –	−7.7 –	−7.1 –	−6.9 –	−6.9 AS	−7.4 –
(14)	isoquercitrin	−7.0 –	−7.2 –	−8.0 –	−7.1 –	−6.7 –	−6.1 AS	−9.2 –
(15)	rutin	−7.8 –	−9.8 –	−9.1 –	−8.6 –	−6.7 –	−6.0 AS	−9.0 –
(16)	emodin	−8.2 AS	−8.2 AS	−8.2 –	−7.3 –	−7.2 –	−6.9 AS	−8.0 –
(17)	emodin 8-*O*-glucoside	−9.6 –	−9.2 –	−8.6 –	−8.4 –	−7.3 –	−7.2 AS	−7.8 –
(18)	emodin anthrone	−7.8 AS	−6.5 AS	−8.3 –	−7.4 –	−6.8 –	−8.1 –	−7.8 –
(19)	chrysophanol 8-*O*-glucoside	−8.5 AS	−8.6 AS	−8.7 –	−8.0 AS	−8.1 AS	−8.1 AS	−8.9 –

endothelial cell response: modulated the pro-inflammatory gene expression, inhibited the COX-2 enzyme activity, suppressed the release of pro-inflammatory cytokines, and reduced the macrophage influx to endothelial cells (Liudvytska et al., 2023). Therefore, in this work, we continue studies on the biological activity of these extracts. To the best of our knowledge, this is the first study devoted to the possible effects of extracts from *R. rhaponticum* and *R. rhabarbarum* on the haemostatic response of endothelial cells and the haemostatic activity of blood plasma components. To obtain a comprehensive insight into the action of the examined *Rheum* extracts and stilbenes, the study has been composed of three main experimental modules, providing data on the effects of the examined plant extracts and stilbenes on the blood plasma coagulation cascade proteins (1), generation of plasmin (2), and the haemostatic activity of endothelial cells (3).

The extrinsic (the tissue factor-dependent) pathway is the main physiological route of blood clotting activation. For that reason, to determine the effects of the examined substances on the blood plasma coagulation cascade, an experimental model of human plasma stimulated by the tissue factor was applied in this study. The performed assays revealed that all of the examined rhubarb extracts influenced the extrinsic pathway of the blood plasma coagulation cascade, leading to a decrease in its clotting ability. The next steps of the study included

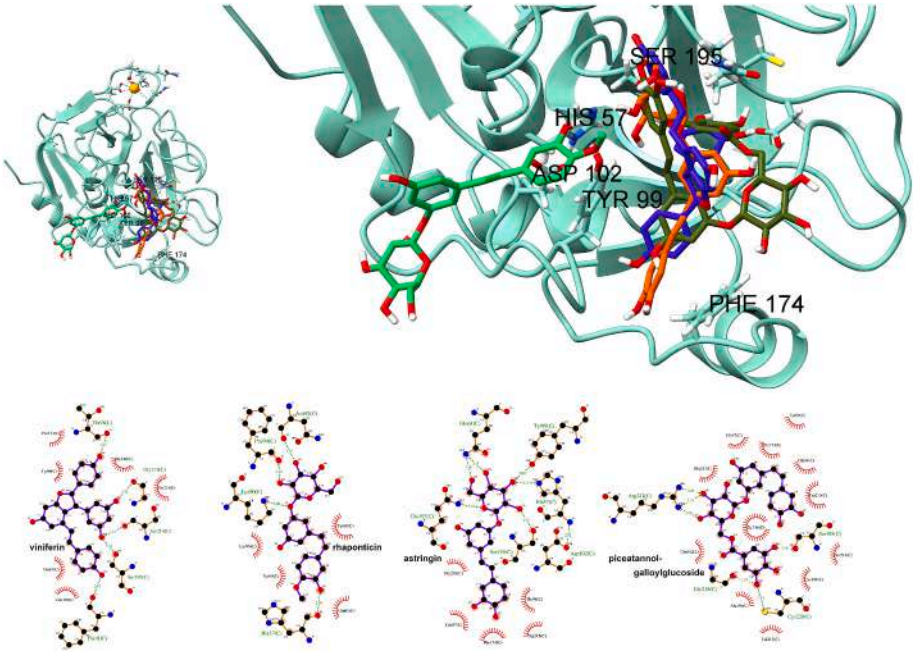


Fig. 15. Rhubarb stilbenes docked in near the active site of FXa. The top panels represent a 1XKB cartoon structure - green, with stick models of stilbenes: viniferin - navy, rhaponticin - green, astringin - orange and piceatannol-galloylglucoside - khaki, the top left side the whole protein molecule, top right the zoom to the active site. The bottom panels show LigPlot + v.2.2 analysis for 1XKB stilbene complexes. The residues of the catalytic triad are His57, Asp102 and Ser195. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

analyses of the examined extracts and stilbenes effects on two key serine proteases of this pathway, *i.e.* the coagulation factor Xa and thrombin. The *in vitro* studies were also supplemented by *in silico* mechanistic analyses of interactions of major components of the examined extracts with these enzymes. The FXa-thrombin axis is critical for the generation of thrombin, and for the fibrin clot formation from fibrinogen. Physiologically, the FXa and thrombin constitute checkpoints in the blood coagulation cascade, and therefore, they are a potential target for different modulators of blood plasma clotting (including anticoagulant drugs) (Palta et al., 2014; Adams and Bird, 2009). Evaluation of the effects of the examined rhubarb extracts on the enzymatic activity of FXa and thrombin revealed that these plant preparations were able to inhibit both of them at micromolar concentrations (Table 2).

Although the *in silico* prediction suggested stilbene affinity to thrombin, no anticoagulant effects were found for RHPTG and RHPT. Both of the stilbenes were inactive towards thrombin *in vitro*, and only RHPT inhibited the FXa activity (Table 2). On the other hand, extracts from *R. rhabarbarum* roots inhibited thrombin and FXa the most effectively, and such extract contained the highest content of RHPT, viniferin, astringin and piceatannol-galloylglucoside (the phytochemical profiling of the extracts was detailly presented in Liudvytska et al., 2023). The compounds acted well in the calculations of molecular docking, binding near the active site and interacting by hydrogen bonds with the key serine residue of the FXa catalytic triad (Fig. 15), however RHPT definitely was off side in relation to the others. The inhibitory efficiency of the examined extracts may be also a result of interactions of other plant metabolites with serine proteases or a synergistic action of their components. Interactions of the rhubarb extracts with other enzymes of the coagulation cascade, finally resulting in a common anticoagulant effect are also possible. Furthermore, the lack of anticoagulant effect in blood plasma samples preincubated with RHPG and RHPT may be also the result of their interaction with fibrinogen. Literature data suggest that polyphenols influence the fibrinogen structure or its ability to form the fibrin clot. It has been shown that some flavonoids (i.e. genistein, naringin, rutin, hesperidin, myricetin, and puerarin) decreased the α-helix structure of fibrinogen (Li et al., 2022).

Another important observation from the present study is the weak effect of the extracts on the generation of plasmin, a key serine protease of the fibrinolytic system. This fact is particularly important in the context of the search for selective, natural inhibitors of the coagulation cascade or fibrinolysis. It has been evidenced some of the trypsin-like proteases, including enzymes of the haemostatic system are prone to the inhibitory action of phytochemicals based on different carbon backbones (Weir et al., 1998; Bijak et al., 2014a,b; Liu et al., 2010; Cuccioloni et al., 2012; Kolodziejczyk-Czepas et al., 2017). However, structural and functional similarities within the family S1 (trypsin-like fold) proteases significantly hinder the identification of natural compounds, capable of precisely targeting the activity of individual enzymes. Many of the identified plant-derived compounds are able to inhibit both the enzymes of the coagulation cascade, fibrinolysis, and other serine proteases. For example, silybin was found to inhibit both thrombin, and urokinase at a similar level of efficiency (IC_{50} of 20.9 μM and 21.0 μM, respectively) (Jedinák et al., 2006). Rutin was able to inhibit plasmin (Mozzicafreddo et al., 2008), thrombin, and urokinase (Viskupicova et al., 2012). Inhibition of thrombin (Mozzicafreddo et al., 2006), plasmin (Mozzicafreddo et al., 2008), factor Xa (Bijak et al., 2014a), trypsin, and urokinase (Jedinák et al., 2006) by quercetin has been also described.

Table 2
Summary of the inhibitory efficiency (the IC_{50} values) calculated for the examined rhubarb extracts and stilbenes. IC_{50} - the half maximal inhibitory concentration.

Sample treated with:	Amidolytic activity of thrombin	Proteolytic acyivity of thrombin (fibrinogen polymerization)	FXa activity
	IC_{50} [μg/ml]		
RHPG	–	–	–
RHPT	–	–	41.05
R.rhaponticum petiole extract	48.11	–	45.61
R.rhabarbarum petiole extract	35.67	31.02	22.75
R.rhaponticum root extract	26.53	20.15	22.40
R.rhabarbarum root extract	20.26	21.03	20.31
Argatroban	2.63	0.045	–
GGACK[a]	–	–	0.39

[a] data provided by the kit manufacturer.

The endothelium plays a key role in the regulation of the haemostatic balance and blood flow, and its dysfunction may contribute to serious disorders at the systemic level, including thrombosis (Verhamme and Hoylaerts, 2006; Monteiro et al., 2019; Medina-Leyte et al., 2021). The examined rhubarb-derived substances decreased the reactivity of the thrombin-stimulated endothelial cells. A partial decrease of the von Willebrand factor indicated that some of the examined substances (*i.e.* RHPG, RHPT, *R. rhaponticum* petiole and *R. rhabarbarum* root extracts) reduce the procoagulant activity of endothelial cells, but the reduction of the t-PA level suggest that all of the investigated plants extracts influence the pro-fibrinolytic activity of endothelial cells as well. A very slight decrease in the PAI-1/t-PA complexes level, found in cells treated with the *R. rhabarbarum* root extract and stilbenes also demonstrated amelioration of the thrombin-induced activation of the HUVECs. On the other hand, at a concentration of 30 μg/ml, *R. rhabarbarum* root extract evidently increased the level of PAI-1/t-PA complexes, suggesting its dose-dependent effects and some stimulatory action on the cell response at higher concentrations. The PAI-1 is not only a regulator of plasmin formation and the intravascular fibrinolysis, but also an important modulator of extravascular proteolysis, cell migration and tissue remodelling. Its secretion is triggered by many pro-inflammatory mediators including thrombin, cytokines and various growth factors. After the release, PAI-1 reacts with the t-PA forming the PAI-1/t-PA complexes, or converts into a stable latent form (Sillen and Declerck, 2021). Quantification of the PAI-1/t-PA complexes is considered to reflect both the inhibitor level and reactivity of the blood vessel wall, especially under pathological conditions (Nordenhem et al., 2005).

In conclusion, this work provides the first data on the effects of rhubarb-derived substances on the activity of blood plasma haemostatic proteins and their inhibitory action towards thrombin and FXa, key enzymes of the blood coagulation cascade. Experiments on HUVECs revealed the modulatory effects of the examined extracts and stilbenes on the haemostatic response of these cells. Results derived from most assays in our study indicated comparable effectiveness of both the examined species. However, some differences between the inhibitory activity of the petiole and root extracts can be observed, especially in tests employing enzymes of the blood plasma coagulation cascade. In a context of modulation of the coagulation factors activity and blood plasma clotting, extracts from the roots were more efficient than the petiole extracts, while the examined stilbenes displayed considerably lower anticoagulant activity. Thus, the root extracts were the most efficient inhibitors of thrombin and the coagulation factor Xa activity (at concentrations of 50 or 5 μg/ml, respectively).

Our findings suggest that the examined rhubarb extracts display rather anti-coagulant than pro-coagulant properties, with a marginal effect on the activity of plasmin. The obtained results may be a background for further studies dedicated to the assessment of the physiological relevance of the observed anticoagulant and modulatory effects.

CRediT authorship contribution statement

Oleksandra Liudvytska: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Michał B. Ponczek:** Methodology, Software, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Justyna Krzyżanowska-Kowalczyk:** Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Mariusz Kowalczyk:** Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Aneta Balcerczyk:** Supervision. **Joanna Kolodziejczyk-Czepas:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro

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Corresponding Author:	Oleksandra Liudvytska, MSc Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz Łódź, POLAND
First Author:	Oleksandra Liudvytska, MSc
Order of Authors:	Oleksandra Liudvytska, MSc
	Magdalena Bandyszewska, MSc
	Tomasz Skirecki, PhD, DSc, Assoc. Prof.
	Justyna Krzyżanowska-Kowalczyk, PhD
	Mariusz Kowalczyk, PhD
	Joanna Kolodziejczyk-Czepas, PhD, DSc, Assoc. Prof
Abstract:	Rheum rhaponticum L. (rhapontic rhubarb) and Rheum rhabarbarum L. (garden rhubarb) are edible and medicinal rhubarb species long used in traditional medicine. This work was focused on the biological activity of extracts from petioles and roots of R. rhaponticum and R. rhabarbarum as well as rhapontigenin and rhaponticin, typical stilbenes present in these rhubarbs, in a context of blood physiology and cardiovascular health. Anti-inflammatory properties of the examined substances were evaluated in human peripheral blood mononuclear cells (PBMCs) and THP1-ASC-GFP inflammasome reporter cells. Due to the coexistence of inflammation and oxidative stress in cardiovascular diseases, the study design included also antioxidant assays. This part of the work involved the assessment of the protective efficiency of the examined substances against the peroxynitrite-triggered damage to human blood plasma components, including fibrinogen, a protein of critical importance for blood clotting and maintaining the haemostatic balance. Pre-incubation of PBMCs with the examined substances (1-50 mg/mL) considerably decreased the synthesis of prostaglandin E2 as well as the release of pro-inflammatory cytokines (IL-2 and TNF-α) and metalloproteinase-9. A reduced level of secreted apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) specks in the THP-1-ASC-GFP cells was also observed. The examined substances significantly diminished the extent of $ONOO^-$-induced oxidative modifications of blood plasma proteins and lipids and normalized, or even strengthened its antioxidant capacity. Furthermore, a reduction of oxidative damage to fibrinogen, including modifications of tyrosine and tryptophan residues along with the formation of protein aggregates was found.

Oleksandra Liudvytska
Dept. General Biochemistry
University of Lodz, Poland
90-236 Lodz, Pomorska 141/143
E-mail: oleksandra.liudvytska@biol.uni.lodz.pl

Dear Editor,

I am enclosing herewith the manuscript of our original paper entitled ” *Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro*”. I would be appreciated very much if you could consider this article for publication.

Rheum rhaponticum L. (rhapontic rhubarb) and *R. rhabarbarum* L. (garden rhubarb) are edible and medicinal plants, well known in many regions of the world. However, most studies on biological properties of extracts from the aforementioned rhubarb species have been related to their estrogenic properties. Nowadays, extracts from *R. rhaponticum* are key ingredients of numerous preparations (including dietary supplements), recommended to alleviate menopausal disorders. In contrast, effects of the rhubarb-derived substances on physiology of the cardiovascular system and blood components are poorly recognized.

Our study was conducted in a context of blood physiology and possible cardioprotective effects of the examined rhubarbs, and involved a comparative analysis of anti-inflammatory and antioxidant actions of two types of rhubarb extracts, *i.e.* the root/rhizome-derived extracts (which are a typical herbal material) and the petiole extracts (edible parts of rhubarb). Our previous, extensive phytochemical profiling of the examined rhubarb extracts revealed a presence over 150 and 300 compounds in the petioles and roots, respectively. In addition to this abundance of bioactive phytochemicals, an ability of the examined extracts to modulate functions of endothelial cells was also found. The present study has provided novel data on beneficial activity of these plant preparations in other experimental systems related to cardiovascular physiology. Furthermore, effects of two stilbenes (rhapontigenin and its glycoside, rhaponticin), typically found in these plants, were also studied.

A combination of biochemical/molecular biology tests, antioxidant assays, fluorescent microscope imaging and studies on protein structure modifications enabled a comprehensive insight into the biological activity of the examined plant extracts and stilbenes. Among blood plasma proteins, a special emphasis was put on fibrinogen, which is not only a key element of the blood coagulation cascade, but also a positive acute phase protein, a modulator of inflammation, and an important factor of many other physiological and pathological processes. Moreover, oxidative and nitrative modifications of fibrinogen (along with its functional abnormalities) have been detected in many diseases.

We have revealed the antioxidant and anti-inflammatory potential of rhubarb extracts and stilbenes, resulting in their protective effects on blood plasma components and cells under an inflammatory stimulation. The examined rhubarb extracts reduced the inflammatory response of human peripheral blood mononuclear cells and inflammasome formation in THP1-ASC-GFP cells. Furthermore, the examined substances significantly lowered the peroxynitrite-induced damage to blood plasma lipids and proteins. A significant reduction of oxidative damage to fibrinogen molecule (including 3-nitrotyrosine formation, modification of tryptophan residues and formation of protein aggregates) was also found.

Sincerely yours,
Oleksandra Liudvytska and Co-authors

Anti-inflammatory and antioxidant actions of extracts from *Rheum rhaponticum* and *Rheum rhabarbarum* in human blood plasma and cells *in vitro*

Oleksandra Liudvytska[1]*, Magdalena Bandyszewska[2], Tomasz Skirecki[2], Justyna Krzyżanowska-Kowalczyk[3], Mariusz Kowalczyk[3], Joanna Kolodziejczyk-Czepas[1]

[1] *Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland; oleksandra.liudvytska@biol.uni.lodz.pl (O.L.); joanna.kolodziejczyk@biol.uni.lodz.pl (J.K.-C.)*

[2] *Department of Translational Immunology and Experimental Intensive Care, Centre of Postgraduate Medical Education, Marymoncka 99/103, 01-813, Warsaw, Poland; magdalena.bandyszewska@cmkp.edu.pl (M.B.); tomasz.skirecki@cmkp.edu.pl (T.S.).*

[3] *Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland; jkrzyzanowska@iung.pulawy.pl (J.K.-K.), mkowalczyk@iung.pulawy.pl (M.K.)*

* Correspondence: oleksandra.liudvytska@biol.uni.lodz.pl

Abstract

Rheum rhaponticum L. (rhapontic rhubarb) and *Rheum rhabarbarum* L. (garden rhubarb) are edible and medicinal rhubarb species long used in traditional medicine. This work was focused on the biological activity of extracts from petioles and roots of *R. rhaponticum* and *R. rhabarbarum* as well as rhapontigenin and rhaponticin, typical stilbenes present in these rhubarbs, in a context of blood physiology and cardiovascular health. Anti-inflammatory properties of the examined substances were evaluated in human peripheral blood mononuclear cells (PBMCs) and THP1-ASC-GFP inflammasome reporter cells. Due to the coexistence of inflammation and oxidative stress in cardiovascular diseases, the study design included also antioxidant assays. This part of the work involved the assessment of the protective efficiency of the examined substances against the peroxynitrite-triggered damage to human blood plasma components, including fibrinogen, a protein of critical importance for blood clotting and maintaining the haemostatic balance.

Pre-incubation of PBMCs with the examined substances (1-50 μg/mL) considerably decreased the synthesis of prostaglandin E_2 as well as the release of pro-inflammatory cytokines (IL-2 and TNF-α) and metalloproteinase-9. A reduced level of secreted apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) specks in the THP-1-ASC-GFP cells was also observed. The examined substances significantly diminished the extent of $ONOO^-$-induced oxidative modifications of blood plasma proteins and lipids and normalized, or even strengthened its antioxidant capacity. Furthermore, a reduction of oxidative damage to fibrinogen, including modifications of tyrosine and tryptophan residues along with the formation of protein aggregates was found.

Keywords: *Rheum rhabarbarum, Rheum rhaponticum,* human plasma, inflammasome, oxidative stress, cardiovascular disease

Introduction

Numerous studies have confirmed the significant role of plant-derived dietary components in maintaining human health and preventing many diseases (Adefegha, 2018; Joshi et al., 2020; Koch, 2019). For the maintenance of cardiovascular health, diet is one of the most important modifiable factors to prevent disease. Therefore, bioactive components of dietary plants have gained a rising scientific interest as potentially valuable for disease prevention and treatment (Patel et al., 2017). Antioxidant properties of polyphenols were one of the first biological activities identified as factors contributing to the cardioprotective effects of plant-derived dietary components. Further research and in-depth analyses revealed the pleiotropic activities of different phytochemicals and their ability to modulate various biochemical pathways. Those findings revised a seemingly obvious link between antioxidant activity and disease-preventive effects. Nowadays, antioxidant properties of plant derived-substances are still considered to have a significant role in maintaining human health. However, other activities of plant metabolites (*i.e.,* anti-inflammatory, immunomodulatory, and antithrombotic effects) are also important (Tangney and Rasmussen, 2013; Kishimoto et al., 2013; Goszcz et al., 2017).

The *Rheum* L. genus includes edible and/or medicinal rhubarb species. The two edible rhubarb specimens examined in this study, *i.e., Rheum rhaponticum* L. (rhapontic rhubarb) and *Rheum rhabarbarum* L. (garden rhubarb), have been known both in traditional medicine and contemporary herbal therapies (Kolodziejczyk-Czepas and Liudvytska, 2021). Although the extracts originating from various rhubarb species have been extensively studied in the context of the treatment of many diseases, including infections (Lai et al., 2015), gastrointestinal disorders (Gao et al., 2021), pancreatitis (Hu et al., 2018), diabetes and obesity (Liudvytska and Kolodziejczyk-Czepas, 2022), and cancer (Jiang et al., 2021; Tan and Lu, 2022), the number of data on antioxidant and inflammatory effects of these plants is still limited. Moreover, contrary to other *Rheum* species such as *Rheum palmatum* L. and *Rheum officinale* Baillon having a well-documented therapeutic significance (European Medicines Agency, April 2023; European Union herbal monograph on *Rheum palmatum* L. and *Rheum officinale* Baillon, radix. EMA/HMPC/113700/2019), medicinal properties and biological effects of *R. rhaponticum* and *R. rhabarbarum* have been recognized, and described to a minor extent. Only the phytoestrogenic action of *R. rhaponticum* has been evidenced in clinical trials (Chang et al., 2016), whereas the effects of *R. rhaponticum* and *R. rhabarbarum* on blood components and cardiovascular physiology remain poorly elucidated.

Since oxidative stress plays an important role in cardiovascular disorders, and its occurrence accompanies both chronic or severe inflammation (Dubois-Deruy et al., 2020), the present work includes two experimental panels dedicated to evaluating the anti-inflammatory and antioxidant effects of extracts originating from petioles and roots of *R. rhaponticum* and *R. rhabarbarum* as well as two stilbenoid compounds, rhapontigenin, and rhaponticin. The study design involved a combination of different cellular experimental models, human blood plasma, and the isolated fibrinogen to provide a comprehensive insight into the biological activity of the rhubarb-derived extracts and stilbenes. Examinations of their effects on the inflammatory response of human peripheral blood mononuclear cells (PBMCs) were supported by additional experiments employing THP1-ASC-GFP, the inflammasome reporter cells. Many publications have evidenced and discussed the emerging role of the immune response and the formation of inflammasomes in cardiovascular diseases (Liao et al., 2022; van Hout et al., 2018; Olsen et al., 2022). For that reason, in the present work, the ability of rhubarb-derived phytochemicals to modulate the immune response and inflammasome formation in THP1-ASC-GFP cells was also evaluated. Antioxidant activities of rhubarb extracts and stilbenes were examined in human blood plasma exposed to oxidative stress induced by peroxynitrite, one of the primary oxidants involved in the pathophysiology of various cardiovascular disorders (Pacher and Szabó, 2006; Uppu et al., 2007) as well as in vascular aging (Van der Loo, 2000). Among blood plasma proteins, a particular emphasis was put on fibrinogen, a critical element of the blood coagulation cascade and a positive acute phase protein, a modulator of inflammation, and an essential factor of many other physiological and pathological processes (Mosesson,2005; Davalos and Akassoglou, 2012).

Materials and Methods

Chemicals

Peroxynitrite was synthesized according to a protocol of Pryor et al. (1995) (Pryor et al., 1995). Concanavalin A, streptomycin, Tris, ferric chloride, dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), tris, Trolox, Sigma Fast OPD substrate for peroxidase, 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB, Ellman's Reagent), sulphuric, thiobarbituric and trichloroacetic acids, as well as the human fibrinogen were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was from Merck (Darmstadt, Germany). Antibody for the enzyme-linked immunosorbent assay (ELISA)-based detection of 3-nitrotyrosine, *i.e.*, primary anti-3-nitrotyrosine antibody, biotinylated secondary antibody,

and Streptavidin/ HRP complex were purchased from Abcam (Cambridge, UK). BCA Protein Assay Kit for estimation of protein content in the examined samples was purchased from ThermoFisher Scientific (Waltham, MA, USA).

The fetal bovine serum (FBS), RPMI1640 and Lymphosep (diatrizoic acid dihydrate - 95.281 g/l, EDTA tetrasodium salt dihydrate - 0.231 g/l, sodium hydroxide pellets - 5.860 g/l, polysaccharose 400 - 57 g/l, water) were from BioWest (Nuaillé, France). Trypan blue solution was from BioRad (Hercules, CA, USA). The resazurin-based in vitro toxicology assay kit was from Sigma-Aldrich (St. Louis, MO, USA). Quantikine ELISA kits for detecting IL-2 and TNF-α were purchased from R&D Systems Inc. (Minneapolis, USA).

Plant material

Rhizomes, roots as well as petioles of *R. rhaponticum* L. were received from The Botanical Garden of Maria Curie-Skłodowska University in Lublin, whereas analogous organs of *R. rhabarbarum* L. were donated from The Botanical Garden of Łódź. The voucher samples (*R.rhaponticum*/OL/2019, *R.rhaponticum*/KR/2019, *R.rhabarbarum*/OL/2019, and *R.rhabarbarum*/KR/2019) have been deposited at the Department of Biochemistry and Crop Quality of the Institute.

Preparation of rhubarbs extracts

Rhubarb extracts were prepared according to procedures described in our earlier publications (Liudvytska et al., 2023a; Liudvytska et al., 2023b). Petioles and rhizomes were cut into small pieces, dried at 35°C, and finely milled in a laboratory grinder. Powdered plant material was extracted twice with methanol containing 0.1% formic acid, using an ultrasonic bath at room temperature for 24 h in the dark. Filtered extracts were concentrated to reduce the volume by rotary evaporation and next defatted with *n*-hexane using liquid-liquid extraction. The defatted extracts were rotary-evaporated to remove the organic solvent. The residues were suspended in Milli-Q water and subjected to *n*-butanol extraction. The obtained butanol extracts were rotary evaporated, re-suspended in Milli-Q water, and freeze-dried (Gamma 2–16 LSC, Christ). The phytochemical composition of the rhubarb extracts was carried out as described previously (Liudvytska et al., 2023a).

Biological material

Blood plasma and peripheral blood mononuclear cells (PBMCs) were isolated from commercially available human buffy coats. The anonymized material was purchased from the

Regional Centre of Blood Donation and Blood Treatment in Lodz, Poland. The study was executed following the requirements of the Declaration of Helsinki. All experiments were approved by the Committee on the Ethics of Research at the University of Lodz, Poland (15/KBBN-UŁ/III/2019). Blood plasma was isolated using a procedure based on centrifugation (2000 × *g*, 15 min, 25ºC) (Kolodziejczyk-Czepas et al., 2013).

Evaluation of the anti-inflammatory effects in PBMCs

Cell cultures

PBMCs were isolated from human buffy coat units by the density centrifugation technique, using the Lymphosep medium (BioWest, Nuaillé, France), according to our previously described protocol (Kozachok et al., 2018). Cell count and viability were evaluated in an automatic cell counter (BioRad, Hercules, CA, USA) based on the trypan blue staining (Strober, 2001). After the isolation, PBMCs (1.5×10^6, suspended in RPMI 1640 medium, containing 10% fetal calf serum and 0.1% of penicillin-streptomycin) were seeded onto a 96-well microplate (3.75×10^5 cells/well). The first step of the experimental procedure was a 1h-preincubation of cells with the examined extracts or stilbenes (added to the final concentrations of 1-50 µg/mL). After the pre-incubation (in a laboratory CO_2 incubator, at 37°C and 95% humidity), the PBMCs pro-inflammatory response was induced by adding the concanavalin (Con A) solution (the final conc. of 10 µg/mL). Then, the cells were cultured for 24 hours. The next day, microplates were centrifuged to obtain supernatants (cell culture medium) for further analyses. Anti-inflammatory effects of the examined extracts and stilbenes were evaluated based on measurements of interleukin-2 (IL-2), TNF-α, matrix metalloproteinase-9 (MMP-9), and prostaglandin E_2 (PGE_2) secretion from PBMCs. Concentrations of the aforementioned substances in cell culture medium were determined with commercial enzyme-linked immunosorbent assay (ELISA) kits, according to protocols provided by manufacturers.

Measurements of cytokine release and MMP-9 secretion

IL-2 and TNF-α were detected in cell culture medium supernatants. The cytokine release was quantified using the Quantikine kits for human cytokines (IL-2 - Catalog #: DT2050, TNF-α - Catalog #: DTA00D, respectively; R&D Systems Inc., Minneapolis, MN, USA), based on a sandwich-type ELISA.

MMP-9 release in the cells supernatant was quantified using a commercial kit (ELISA Kit, Catalog #: E0553h, EIAab Science INC, Wuhan, China) based on a sandwich-type ELISA.

Analysis of the arachidonic acid metabolism: measurements of the PGE_2 level

The cyclooxygenase (COX) -mediated metabolism of the arachidonic acid, leading to the generation of prostaglandin E_2 (PGE_2), was monitored using a commercial ELISA kit (Parameter Assay Kit, Catalog #: KGE004B, R&D Systems Inc., Minneapolis, MN, USA).

Cytotoxicity Assays

PBMCs were seeded onto 96-well microplates (3.75×10^5 cells/well) and incubated with the examined substances (1-50 μg/mL) for 24 hours, at 37°C, 5% of CO_2 concentration, and 95% humidity. PBMCs treated with 1% Triton-X100 were the reference samples, with no viable cells (0% of viability). Then, the resazurin solution was added (to the final concentration of 10%). Cell viability was determined after 4 hours, using a microplate spectrophotometer BMG Labtech SectroStarNano, at λ=600 nm, with 690 nm as a reference wavelength.

The trypan blue-based tests were executed according to the previously described protocol (Krzyzanowska-Kowalczyk et al., 2017). PBMCs suspensions (1.5×10^6 cells/mL, control/untreated and incubated with the examined substances at concentrations of 1-50 μg/mL) were mixed with the dye in a volume ratio of 1:1 and counted in the automatic cell counter (BioRad, Hercules, CA, USA).

Determination of anti-inflammatory effects of the examined extracts and stilbenes in the THP1-ASC-GFP cells

THP1-ASC-GFP cells were cultured in RPMI-1640 medium with 2 mM L-glutamine, 25 mM HEPES and phenol red (Gibco, Laboratories, Grand Island, N.Y.), supplemented with a 10% heat-inactivated FBS (30 min, at 56 °C) (EURx, Brasil) and 100 μg/mL Normocin™ (InvivoGen, San Diego, C.A.), Pen-Strep (100 U/mL-100 μg/mL) (Gibco, Laboratories, Grand Island, N.Y.). THP1-ASC-GFP cells were seeded onto black 96-well plates with clear bottoms at 30×10^3 cells/well density. After three hours, the growth medium was exchanged into the test medium RPMI-1640, without phenol red, but enriched with 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/mL-100 μg/mL). After 24 h, cells were treated for 16 h with the rhubarb extracts or stilbenes (rhapontigenin - RHPG, CAS: 500-65-2, PhytoLab, Germany, and rhaponticin - RHPT, CAS:156-58-8, Sigma-Aldrich, St. Louis, MO, USA), and then activated by the lipopolysaccharide - LPS (O55:B5, from *Escherichia coli*, Sigma-Aldrich, St. Louis, MO, USA; L6529; at the final conc. of 1 μg/mL,

for 3h). The formation of the ASC-GFP specks was analyzed using inverted fluorescent microscope (IX71/IX51 Olympus, JP) and cellSense software (Olympus).

Evaluation of antioxidant effects of the examined substances in blood plasma

Sample preparation

Stock solutions of the examined plant-derived substances were made in 30% DMSO and then diluted with 0.05 M Tris/HCl in a volume ratio of 1:1 to obtain working solutions. The final concentration of DMSO in the examined samples was 0.15%, and its effect on the measured parameters was excluded. Blood plasma samples were pre-incubated for 15 min at 37°C with the examined plant extracts, stilbenes, or a reference compound (Trolox) at 1-50 µg/mL concentrations and then exposed to oxidative stress induced by $ONOO^-$. To the samples undergoing the estimation of the ferric-reducing ability of blood plasma (the FRAP assay), $ONOO^-$ was added to the final concentration of 150 µM. In the remaining antioxidant tests, the final concentration of $ONOO^-$ was 100 µM. Plasma samples treated with $ONOO^-$ without the examined substances or reference compound were also prepared. Control plasma contained neither the examined substances nor $ONOO^-$.

Evaluation of blood protein oxidation/nitration and lipid peroxidation in blood plasma

Plasma lipid peroxidation was measured based on a level of the thiobarbituric acid-reactive substances (TBARS). The TBARS concentration was determined according to the method described previously by Wachowicz and Kustron (Wachowicz and Kustroń, 1992), and data calculations were based on the molar coefficient for the malondialdehyde (MDA, $1.56 \times 10^5\ M^{-1} \times cm^{-1}$, λ=532 nm). The immunodetection of 3-nitrotyrosine (3-NT) in plasma proteins was carried out according to our previously described competitive ELISA protocol (Olas et al., 2006). Results were expressed as equivalents of a 3-nitrotyrosine-containing protein standard per mg of blood plasma protein. Thiol groups in blood plasma protein were determined colorimetrically using Ellman's reagent (Rice-Evans et al., 1991).

Effects of the examined extracts and stilbenes on the non-enzymatic antioxidant capacity (NEAC) of blood plasma were evaluated based on its ferric-reducing ability (the FRAP assay), according to the previously described procedure (Kolodziejczyk-Czepas et al., 2014). Results were calculated from a standard curve and expressed as mmol/l of Fe^{2+} equivalents.

Immunodetection of 3-nitrotyrosine (3-NT) in human fibrinogen

Determination of 3-NT in human fibrinogen was carried out using the competitive ELISA. Human fibrinogen samples (2 mg/mL, in 0.05 M Tris/HCl, pH 7.4) were pre-incubated for 15 min with the rhubarb extracts or stilbenes (1-50 μg/mL). Then, the protein samples were exposed to 100 μM $ONOO^-$. The reference antioxidant was Trolox (1-50 μg/mL). The protein sample series contained fibrinogen exposed to $ONOO^-$ in the absence of the examined rhubarb extracts or stilbenes and the control sample (fibrinogen untreated with $ONOO^-$ or the examined plant-derived substances) as well. The quantification of 3-NT content in fibrinogen samples was based on a standard curve prepared from the 3-NT-containing protein standard.

Fluorometric analyses of the tryptophan oxidation in fibrinogen molecule

Determination of the $ONOO^-$-induced oxidation of tryptophan residues in fibrinogen molecules was based on the susceptibility of the indole group of this amino acid to the UV-induced excitation and energy emission (Lettieri-Barbato et al., 2013). The exposure of the native protein (*e.g.*, human fibrinogen) to $ONOO^-$-induced oxidative stress results in the oxidation and/or nitration of the tryptophan molecule, leading to a decline in its fluorescence. The examined protein samples (2 mg/mL, in 0.1 M Tris/HCl, pH 7.4; control fibrinogen and the $ONOO^-$-treated samples) were assayed using Fluoroscan Ascent microplate reader (Labsystem Inc.) in 96-well microplates. The excitation wavelength was 280 nm, and the emission wavelength was 340 nm.

SDS-PAGE-based detection of the $ONOO^-$-induced formation of protein aggregates

The $ONOO^-$-mediated modifications of fibrinogen structure include the formation of high molecular protein aggregates (HMW) that may be visualized using the 1D-electrophoresis. The exposure of human fibrinogen to $ONOO^-$ action was performed analogously to the protocol described in the *Immunodetection of 3-nitrotyrosine (3-NT) in the human fibrinogen* section. For the SDS-PAGE, the fibrinogen samples (2 mg/mL in 0.1 M Tris/HCl, pH 7.4) were mixed with reducing, 2× concentrated Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) in a volume ratio of 1:1, and heated for 5 min, at 95°C. The electrophoresis and protein visualization (using the Coomassie Brilliant Blue R250 dye; Thermo Scientific™, Waltham, MA USA) was conducted according to the previously described protocol (Marchelak et al., 2021). Samples were assayed in a Mini-PROTEAN Tetra Vertical Electrophoresis Cell (Bio-Rad, Hercules, CA, USA), using the 4-15% Mini-PROTEAN TGX Precast Gels (Bio-Rad, Hercules, CA, USA). Densitometric analyses of the obtained electrophoretic patterns (*i.e.*, SDS-PAGE gels,

visualized with the Coomassie Brilliant Blue R250) were performed using CLIQS Gel Image Analysis Software (TotalLab, Newcastle-Upon-Tyne, UK).

Statistical analysis

The statistical analysis was performed using the STATISTICA 13.0 PL software (StatSoft INC, Tulsa, Oklahoma). The first statistical analysis step was eliminating the uncertain data by the Grubbs' tests (GraphPad Prism 5.01, San Diego, California). Next, the differences between groups were assessed by the non-parametric Wilcoxon test (for unpaired data), and the t-Student test was used for data with normal distribution. The $p<0.05$ level was considered statistically significant. All the values in this work are expressed as mean ± standard deviation (±SD).

Results

Rhubarb extracts and stilbenes effects on the inflammatory response of human PBMCs

Determination of the IL-2 and TNF-α release

At 1-5 μg/mL concentrations, most of the examined plant substances decreased the IL-2 secretion by about 40-50%, except the *R. rhabarbarum* root extract, having a lower anti-inflammatory efficiency (Figure 1A). At a concentration of 50 μg/mL, the most effective inhibitor of IL-2 release was RHPG, which almost completely inhibited this cytokine release. At the same concentration (50 μg/mL), extracts from the petioles and roots of the examined rhubarb species (*R. rhaponticum* and *R. rhabarbarum)* displayed lower inhibitory effects, reducing the IL-2 release by about 70%.

The TNF-α release from PBMCs was also reduced by the examined rhubarb-derived extracts and stilbenes. However, this inhibitory effect (especially at a concentration range of 1-5 μg/mL) was weaker when compared to the IL-2 release inhibition. The most evident reduction of TNF-α secretion was found in PBMCs treated with RHPG (approx. 90% reduction of TNF-α release). RHPT was effective only at a 5 μg/mL concentration (**p<0.01). The petiole extracts were more effective than extracts from the roots. At 5-50 μg/mL concentrations, the petiole extracts from *R. rhaponticum* and *R. rhabarbarum* reduced the TNF-α release by 25 - 65% and 25 - 80%, respectively. Extract from the roots of *R. rhabarbarum* was ineffective at all of the examined concentrations. In contrast, *R. rhaponticum* root extract inhibited the TNF-α release at concentrations of 1 μg/mL (*p< 0.05) and 50 μg/mL (**p< 0.01) (Figure 1B).

Measurements of the MMP-9 release

Under inflammatory conditions, matrix metalloproteinase-9 (MMP-9) is one of the most important mediators of extracellular matrix degradation and leukocyte migration (Kim et al., 2012). Pre-incubation of the PBMCs with rhubarb extracts and stilbenes markedly diminished amounts of MMP-9 released into the cell milieu (*i.e.* cell culture medium) (Figure 2). A statistically significant reduction of MMP-9 secretion was found for all the examined substances, mainly in a full range of the tested concentrations (1-50 μg/mL), except the *R. rhabarbarum* petiole extract at a concentration of 1 μg/mL. Their efficiency in reducing MMP-9 release ranged from about 25% (at 1 μg/mL) to 75% (at 50 μg/mL). At a 50 μg/mL concentration, the most effective anti-inflammatory agents in this test were RHPG and *R. rhabarbarum* petiole extract.

Analysis of the arachidonic acid metabolism: measurements of the PGE_2 level

The examined rhubarb extracts and stilbenes inhibited the cyclooxygenase-catalyzed metabolism of the arachidonic acid, reducing the generated prostaglandin E_2 (Figure 3). While the most evident effects were found in PBMCs pre-incubated with RHPG (30-50% reduction of PGE_2 generation), its glycoside (RHPT) was ineffective at concentrations of 1-5 μg/mL, and a concentration of 50 μg/mL the PGE_2 generation was reduced by about 25%. Among the examined rhubarb extracts, the petiole-derived preparations were more effective than extracts originating from the roots. At a 50 μg/mL concentration, the *R. rhabarbarum* petiole extracts were the most effective, reducing the PGE_2 generation by over 60%.

Effects of the rhubarb extracts and stilbenes on the inflammasome formation

The experiment aimed to evaluate the ability of the examined rhubarb extracts and stilbenes to inhibit the formation of ASC specks in the LPS-stimulated inflammasome reporter cells. ASC specks were formed in THP1-ASC-GFP cells, a cell line derived from THP-1 human monocytic cells, stably expressing an ASC::GFP fusion protein under the control of the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) transcription factor. The exposure of THP1-ASC-GFP cells to LPS activates the fusion protein expression in these cells, which can be visualized throughout the cell cytoplasm (Moasses Ghafary et al., 2022). Upon activation of the PYD-containing NLRP3 inflammasome proteins the ASC::GFP oligomerize and form highly fluorescent puncta called specks.

The LPS-stimulated ASC specks were determined by fluorescence microscopy (Figure 4A). The microscopic imaging confirmed the anti-inflammatory potential of the examined extracts and stilbenes. At a concentration of 50 μg/mL, all of the examined substances displayed a significant (***$p<0.001$) inhibition (by approx. 45-65 %) of the ASC specks formation after the stimulation with LPS. The most evident reduction of speck generation was found in cells pre-incubated with stilbenes RHPG and RHPT (by 55- 80% and 15- 60%, respectively). The petiole and root rhubarb extracts were ineffective at 1 μg/mL concentrations. At a 5 μg/mL concentration, the root extracts from *R. rhaponticum* and *R. rhabarbarum* inhibited the ASC specks generation by approximately 50 and 40%, respectively (Figure 4B).

Effects of the examined extracts and stilbenes on PBMCs viability

The determination of cytotoxicity included two different assays. *i.e.*, the trypan blue excluding test and the resazurin-based assay. The trypan blue-based assay provided information on the cell membrane integrity in the presence of the examined substances, and the resazurin-

based test provided data on the metabolic activity of PBMCs. In both assays, no toxicity of the examined extracts and stilbenes towards the PBMCs was found, except the effect of RHPG in the trypan blue-based test, at a concentration of 50 μg/mL (Table 1).

Table 1. Determination of cellular safety of examined rhubarb extracts and stilbenes (RHPG and RHPT). The viability of PBMCs in control samples (untreated with the examined substances) was assumed as 100%; (n = 8-9), $^{**}p<0.01$.

The examined samples		Concentration [μg/mL]	% of cell viability based on the trypan blue excluding test	% of cell viability in the resazurin-based assay
Stilbenes	RHPG	1	97.34 ± 10.28	98.39 ± 10.68
		5	87.65 ± 6.39	90.90 ± 9.19
		50	66.53 ± 10.07**	70.40 ± 23.33
	RHPT	1	99.71 ± 6.33	107.58 ± 22.26
		5	99.39 ± 7.25	104.46 ± 26.95
		50	93.84 ± 10.12	97.48 ± 10.78
Extracts from petioles	*R. rhaponticum*	1	98.48 ± 6.33	95.97 ± 7.49
		5	99.25 ± 7.00	95.90 ± 9.54
		50	95.24 ± 8.40	105.78 ± 24.01
	R. rhabarbarum	1	98.08 ± 10.36	101.50 ± 8.59
		5	99.20 ± 8.83	99.03 ± 9.92
		50	94.44 ± 4.99	95.69 ± 13.93
Extracts from roots	*R. rhaponticum*	1	98.73 ± 9.89	94.67 ± 7.74
		5	97.09 ± 10.51	95.07 ± 9,50
		50	90.26 ± 9.97	96.87 ± 7.66
	R. rhabarbarum	1	97.14 ± 14.37	96.47 ± 10.72
		5	91.68 ± 14.03	97.21 ± 7.17
		50	82.88 ± 14.53	91.87 ± 8.87

Antioxidant actions of the examined rhubarb extracts and stilbenes in blood plasma

Antioxidant properties of the examined rhubarb extracts and stilbenes in human blood plasma were evaluated based on well-known biomarkers of oxidative stress, *i.e.* 3-nitrotyrosine

(3-NT), protein-SH groups, and TBARS. The oxidative stress was induced by $ONOO^-$, one of the primary oxidants generated within the cardiovascular system under pathological conditions. The examined substances displayed antioxidant properties and partly reduced the extent of oxidative and nitrative damage to blood plasma components (Table 2, Figure 5B).

The maximal reduction of protein tyrosine nitration attained over 50% at a concentration of 50 μg/mL. Among the tested substances, the most effective were RHPG and *R. rhaponticum* root extract. Also, stilbenes (at concentrations of 5-50 μg/mL) and rhubarb petiole and root extracts (at concentrations of 1-50 μg/mL) were able to partly prevent the oxidation of protein thiol groups. The TBARS assay revealed that the examined rhubarb extracts also displayed anti-lipoperoxidation effects. In this assay, the most effective antioxidants were RHPG and *R. rhaponticum* root extract, along with the *R. rhabarbarum* root extract (Table 2).

Furthermore, the exposure of blood plasma to $ONOO^-$ diminished its physiological non-enzymatic antioxidant capacity (measured in the FRAP assay). However, the presence of rhubarb extracts and stilbenes reduced this harmful effect and enhanced the reducing potential of blood plasma. At a concentration of 50 μg/mL, the examined stilbenes and root extracts markedly strengthened the antioxidant capacity of blood plasma (Figure 5A).

Table 2. Antioxidant activity of rhubarb extracts and stilbenes in human blood plasma exposed to the $ONOO^-$-triggered oxidative stress *in vitro*. The 3-NT and TBARS levels in samples treated with $ONOO^-$ without the examined extracts and stilbenes were assumed as 100% of nitration/oxidation; n = 8-9.

Examined samples		[μg/mL]	Thiobarbituric acid-reactive substances (%)	3-nitrotyrosine formation in plasma proteins (%)
Plasma + 100 μM $ONOO^-$ in the absence of the examined substances			100	100
Stilbenes	RHPG	1	89.46 ± 12.89 *	77.07 ± 9.57 **
		5	80.58 ± 7.71 *	64.41 ± 17.38 ***
		50	63.40 ± 4.66 **	42.50 ± 14.80 **
	RHPT	1	98.81 ± 13.21 *	88.00 ± 15.92 *
		5	88.75 ± 7.99 **	74.23 ± 21.62 **
		50	82.34 ± 9.32 **	46.87 ± 18.63 **
Extracts from petioles	*R. rhaponticum*	1	98.24 ± 6.7	74.82 ± 10.60 **
		5	98.01 ± 8.74	65.77 ± 18.30 **
		50	79.69 ± 12.13 **	48.01 ± 14.98 **
	R. rhabarbarum	1	99.18 ± 3.97	87.68 ± 10.65 **
		5	89.31 ± 10.87 *	76.44 ± 19.26 **
		50	69.84 ± 10.53 *	66.47 ± 18.24 **
Extracts from roots	*R. rhaponticum*	1	85.22 ± 7.25 *	77.14 ± 19.77 **
		5	80.28 ± 9.32 *	59.47 ± 11.70 **
		50	57.56 ± 12.65 *	35.61 ± 13.21 **
	R. rhabarbarum	1	78.81 ± 9.19 *	79.06 ± 27.54 *
		5	74.07 ± 10.34 *	63.40 ± 20.30 **
		50	58.73 ± 17.91 *	48.28 ± 21.98 **
	Trolox	1	92.98 ± 7.59	92.93 ± 8.97
		5	86.57 ± 9.66 **	78.70 ± 9.70 **

		50	62.30 ± 10.88 ***	46.17 ± 19.82 **

[#] The table represents mean values (±SD); *p<0.05, **p<0.01, ***p<0.001.

Protective effects against the oxidative and nitrative damage to fibrinogen

Analysis of the $ONOO^-$-induced formation of protein aggregates

The SDS-PAGE pattern of native fibrinogen is characterized by the presence of three bands corresponding to Aα (67 kDa), Bβ (56 kDa), and γ (48 kDa) polypeptide chains. The exposure of this protein to $ONOO^-$ (100 μM) led to its oxidative modification and formation of high molecular weight (HMW) protein aggregates, appearing as additional bands in the electrophoretic pattern of fibrinogen samples. Due to the molecular weight >120 kDa, the HMW aggregates located over the typically seen three-band pattern of fibrinogen. The formation of HMW protein aggregates was associated with a decrease in the intensity of bands corresponding to all of the fibrinogen chains, and the most evident loss of the band intensity was observed for the Aα chain, which is the most prone to oxidative modifications. The presence of the examined rhubarb extracts and stilbenes significantly diminished the damaging effect of $ONOO^-$ on fibrinogen structure and reduced the formation of HMW aggregates.

Nitration of tyrosine residues in fibrinogen

Exposure of fibrinogen to $ONOO^-$ resulted in the nitration of tyrosine residues in this protein molecule. The presence of rhubarb extracts or stilbenes protected fibrinogen against the $ONOO^-$-mediated nitrative damage and significantly reduced the content of 3-NT. At a 1 μg/mL concentration, all the examined rhubarb-derived extracts and stilbenes reduced the fibrinogen 3-NT content by at least 40%. The most effective antioxidant in this test was RHPG, capable of reducing tyrosine nitration by about 95%, at 5 and 50 μg/mL concentrations.

Determination of the $ONOO^-$-induced modifications of tryptophan residues in the fibrinogen molecule

The exposure to $ONOO^-$ induced a significant oxidation/nitration of tryptophan residues in the fibrinogen molecule (p<0.01), confirmed by a 40 % decrease in the fluorescence intensity of this amino acid. In fibrinogen samples treated with $ONOO^-$ in the presence of the examined substances (at the selected concentrations of 1-5 μg/mL), some reduction of the $ONOO^-$-induced modifications of tryptophan was observed. A statistically significant protective effect

was found for RHPG (1-5 μg/mL) and RHPT (50 μg/mL). Extracts from roots *R. rhaponticum* (5 μg/mL) and *R. rhabarbarum* (1-5 μg/mL) also partly reduced the oxidative modifications of tryptophan residues in the fibrinogen molecule.

Discussion

The etiology and pathophysiology of cardiovascular diseases (CVDs) are multifactorial. However, the role of some critical factors has been well established. The most important factors are dyslipidemia, chronic inflammation, associated oxidative stress, endothelial dysfunction, and alterations of the haemostatic balance, resulting in pro-thrombotic conditions (Tangney and Rasmussen, 2013). Our previous experiments provided promising data on the protective action of *R. rhaponticum,* and *R. rhabarbarum* extracts on human endothelial cells, including their haemostatic response (Liudvytska et al., 2023a and 2023b). In this work, we continued the examination of rhubarb potential in the context of other activities that may be relevant for the cardioprotective effects. The study design included the assessment of anti-inflammatory and antioxidant effects in various *in vitro* experimental systems related to the physiology of the vascular system, *i.e.*, blood plasma, isolated fibrinogen and blood cells.

Prior to this work, we extensively characterized the phytochemical composition of the garden and rhapontic rhubarbs using qualitative UHPLC-HR-MS analyses, *semi*-quantitative metabolite contents assay, and spectrophotometric assays (Liudvytska et al., 2023a; Liudvytska et al., 2023b). High-resolution mass spectrometry analyses revealed the presence of numerous bioactive compounds in accordance with previously published data (Ye et al., 2007; Püssa et al., 2009; Zhu et al., 2016; Liu et al., 2020; Dou et al., 2021; Yao et al., 2021). Anthraquinone-derived compounds were the main phytochemicals detected in both petioles and root extracts of both investigated species (Liudvytska et al., 2023a). However, from the most to the least abundant, only derivatives of tentatively identified emodin-anthrone, emodin, and physcion (methoxy-emodin) were observed in the petioles. In contrast, in root extracts, derivatives of chrysophanol were dominant, presumably indicating differences in biosynthetic pathways operating in these tissues. In the MS/MS fragmentation patterns, derivatives of aloe-emodin and emodin can be easily distinguished by the diagnostic ion at *m/z* 225.056, formed by the loss of CO_2 from the deprotonated emodin moiety. However, despite literature claims to the contrary (Liu et al. 2020), an ion at *m/z* 240.043 (the loss of COH) did not indicate an aloe-emodin derivative in our fragmentation conditions. Reference standards of aloe-emodin-8-glucopyranoside and emodin-8-glucopyranoside both produced this ion during CID. Also, the ion at *m/z* 225.056 is often of low intensity (typically 0.1 - 1.0%) and can be easily obscured by

the noise (Liudvytska et al., 2023a). Therefore, in cases of low-abundant compounds, correct identification is difficult. However, aloe-emodin and emodin have different putative biosynthetic pathways, with chrysophanol-anthrone being a precursor to the former, whereas emodin-anthrone serves as a precursor to the latter. Since the investigated petioles contained almost no chrysophanol-anthrone or chrysophanol but did contain significant amounts of emodin-anthrone, we presume that most (if not all) detected compounds were derived solely from emodin. In roots, however, if the diagnostic ion peak at *m/z* 225.056 is absent, both anthraquinones are equally probable as aglycone.

Interestingly, petioles of both rhapontic rhubarb species contained compounds structurally analogous to sennosides known from pharmaceutical rhubarbs but derived from emodin rather than rhein. In the investigated samples, dianthrones of emodin occurred in petioles as free aglycone and mono- and di- glycosides with varying degrees of malonylation. It is unclear whether these derivatives share the same pharmacological properties as sennosides. Furthermore, similar structures based on chrysophanol dianthrones and mixed chrysophanol-physcion dianthrones were also detected in roots. Therefore, it can be speculated that the synthesis of glucosylated dianthrones is universal in rhubarbs, differing between the species only in available anthraquinone substrates. It is worth noting that chrysophanol is just three oxidation steps away from rhein. These enzymatic activities and the extended ability to synthesize stilbenes are the principal difference between official rhubarbs (*R. palmatum* L., *R. offcinale* Baill., *R. tanguticum* Maxim.ex Balf) and unofficial rhubarbs.

Stilbenes are the other most significant group of Rheum's bioactive compounds, which in the investigated extracts were represented by various resveratrol, piceatannol, rhapontigenin, and deoxyrhapontigenin derivatives. These compounds mainly occur as mono-glycosides, which could be further esterified with malonic or gallic acid. However, significant contents of free stilbenes, particularly piceatannol, rhapontigenin and deoxyrhapontigenin, were observed in root extracts. It is worth noting that rhapontin (rhaponticin) is recognized as a marker compound of the official rhubarb's authenticity/genuine, and for detection of other types of rhubarb admixtures to species used as a purgative drug. Proper identification and quality control of medicine material is a very important issue to obtain the desired therapeutic healing effect. According to the regulation of Chinese Pharmacopeia, in the assay of official rhubarbs purity, rhapontin is prohibited to be detected in such material by thin-layer chromatography, however, unofficial rhubarbs which are often used to its adulterate or counterfeit, contains a very high amount of this metabolite (Xu et al., 2017).

According to our previous analyses, root extracts contain another relatively uncommon class of bioactive stilbene-derived compounds - resveratrol oligomers. We detected several peaks corresponding to free and glucosylated resveratrol dimers (Liudvytska et al., 2023a). These compounds are difficult to identify by mass spectrometry without the appropriate reference standards and produce similar fragmentation spectra upon CID. Therefore, we were unable to provide complete identification of the observed dimers. Furthermore, dimerization of resveratrol occurs by at least four regioisomeric modes, resulting in several challenging to resolve isomeric structures (Keylor et al. 2015). In addition to dimeric derivatives and their glycosides, we also observed a few trimers and tetramers of resveratrol. All these resveratrol metabolites point to the complexity of stilbene metabolism in rhubarbs. Even though the bioavailability of stilbenes can undoubtedly be a limiting factor, considering their very high contents and structural variability in the investigated extracts, they are very likely responsible for a large proportion of observed *in vitro* bioactivities.

Reactive oxygen species (ROS) are a natural by-product of many metabolic pathways and elements of vascular physiology and adaptive processes. Under physiological conditions, various non-enzymatic and enzymatic antioxidant mechanisms minimize the risk of accumulation of oxidants and their damaging activity. However, disruption of a balance between the generation of oxidants and the efficiency of antioxidant mechanisms occurs in many civilization diseases and promotes pro-oxidant processes (D'Oria et al., 2020). In the pathophysiology of CVDs, chronic inflammation and oxidative stress form a mutually stimulating continuum, leading to endothelial dysfunction and atherosclerosis progression. The generation of ROS in the cardiovascular system is a result of the activity of several oxidative enzymes, including NADPH oxidase, xanthine oxidase, cyclooxygenase, lipoxygenase, myeloperoxidase, cytochrome P450 monooxygenase, peroxidase and uncoupled nitric oxide synthase (NOS) (Senoner and Dichtl, 2019). Moreover, superoxide anion ($O_2^{\bullet -}$), generated in reactions catalyzed by most of the above enzymes can rapidly react with nitric oxide (NO) and form another oxidant and nitrative agent, *i.e.,* peroxynitrite ($ONOO^-$). The formation of $ONOO^-$ amplifies the risk of oxidative and nitrative damage to the vessel walls and blood components. Peroxynitrite can cross biological membranes and modify other molecules through direct reactions and reactions mediated by secondary radicals. It has been assessed that the diffusion distance for hydroxyl radical (one of the most reactive oxygen species) is about 10,000 times smaller than the peroxynitrite migration route (Pacher et al., 2007). The damaging activity of peroxynitrite in the cardiovascular system and its reactivity towards blood components have been well-evidenced, including reports dealing with its effects on blood cells (Wachowicz et

al., 2008) and proteins of the haemostatic system (Nowak and Wachowicz, 2002; Nielsen et al., 2004; Helms et al., 2017, Lancellotti et al., 2010). Moreover, in addition to alterations in the blood coagulation cascade, components of the fibrinolytic systems can be damaged during oxidative stress as well (Gugliucci, 2003; Nowak et al., 2014; Kolodziejczyk-Czepas et al., 2015).

The present work is the first study providing information on the ability of the rhubarb-derived extracts and stilbenes to prevent the $ONOO^-$-mediated oxidative damage to blood plasma components. The $ONOO^-$ concentrations (*i.e.*, 100 - 150 μM, depending on the test) added to the experimental systems of blood plasma and fibrinogen corresponded to the levels of this oxidant that are locally achievable *in vivo* under inflammatory conditions. The peroxynitrite production rate in specific compartments has been assessed to attain 50-100 μM per min (Szabó et al., 2007). It has been also established that a bolus addition of 250 μM $ONOO^-$ corresponds to its concentration of 1 μM $ONOO^-$, maintained for 7 min (Beckman et al., 1990). Furthermore, based on the literature data indicating that the detected blood plasma levels of most phytochemicals and their metabolites range from nanomoles to a few micromoles per liter (Manach et al., 2005), the examined extracts were tested at concentrations of 1-50 μg/mL, delivering nano- and micromolar concentrations of their biologically active ingredients in the examined samples.

Both the extracts and stilbenes (RHPG, in particular) effectively reduced the nitrative and oxidative modification of proteins and lipids and enhanced the antioxidant capacity of blood plasma. Furthermore, experiments employing the isolated fibrinogen revealed the protective action of the examined substances towards the oxidative stress-induced modifications of this protein, including the formation of protein aggregates, tyrosine nitration and modifications of tryptophan residues. The protective role of the examined substances against the oxidative stress-induced alterations in fibrinogen molecules may be crucial for maintaining the haemostatic balance. Fibrinogen functionality is critical for haemostasis. However, this protein is highly susceptible to oxidative and nitrative modifications, including peroxynitrite-mediated damage (Nowak et al., 2007, Ponczek et al., 2014; Martinez et al., 2013). 3-nitrotyrosine is not only a well-known biomarker of oxidative stress and a "peroxynitrite footprint" (Radi, 2018), but its occurrence can significantly affect the biological properties of such modified proteins. Even a very low level of tyrosine residue nitration in fibrinogen (~45-65 μmol nitrotyrosine/mol tyrosine) considerably increases fibrin generation and alters the clot structure (Vadseth et al., 2007). Nitrated fibrinogen has been considered a

biomarker of oxidative stress in venous thromboembolism (Martinez et al., 2012). Furthermore, the fibrinogen nitrotyrosination after ischemic stroke resulted in an impairment of thrombolysis and the promotion of neuronal death (III-Raga et al., 2015).

Besides the protective action towards the $ONOO^-$-induced damage, the examined rhubarb extracts and stilbenes were also efficient mitigators of inflammatory response. Our previous studies demonstrated that these rhubarb-derived preparations reduced a pro-inflammatory response of endothelial cells, partly due to the suppression of *COX2* and *ALOX5* genes, encoding the cyclooxygenase-2 and 5-lipoxygenase, the key enzymes of the arachidonic acid metabolism (Liudvytska et al., 2023a). Following these findings, we extend the study by analyzing of anti-inflammatory effects of the examined plant substances using experimental systems based on leukocytes, which are crucial elements of the immune response to pathogens and essential players in the pathophysiology of many disorders of the cardiovascular system. Both the rhubarb extracts and stilbenes effectively reduced the cytokine secretion. Analyses of the PGE_2 generation indicated the inhibition of the cyclooxygenase-mediated metabolism of the arachidonic acid. A reduction of the MMP-9 release by the examined extracts and stilbenes suggests that these substances may also decrease the risk of extracellular matrix (ECM) degradation.

We also provided evidence that both *R. rhaponticum* and *R. rhabarbarum* extracts and the examined stilbenes are potent inhibitors of the NLRP inflammasome assembly. Formation of the inflammasome by extracellular LPS is possible due to residual contamination by other bacterial products leading to oxidative stress which can be sensed by the NLRP3 inflammasome (Paik et al., 2021). Hence, our results support the anti-oxidative and anti-inflammatory properties of the rhubarb extracts.

Due to the co-existence of inflammation and oxidative stress, the ability of natural compounds to combat both of these factors is particularly desirable. In the case of *R. rhaponticum* and *R. rhabarbarum*, the literature evidence of the aforementioned activities of extracts from these plants is poor. For example, seven fractions isolated from *R. rhabarbarum* petioles were examined in a context of a lipoperoxidation action using a chromatography/malonaldehyde (GC/MA) antioxidant assay, indicating the 100% methanol-eluted fraction as the most effective one (Won Jang et al., 2018). In other work, tannins and gallic acid were identified as predominant phenolic scavengers of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radicals ($ABTS^{\bullet+}$) in *R. officinale* roots (Cai et al., 2004).

Some more information can be found in papers dealing with the biological activity of individual compounds identified in these plants. In our studies, RHPG, the stilbene aglycone,

was the most effective substance in most of the antioxidant and anti-inflammatory assays. The RHPT displayed lower, but also considerable biological activity. These observations are consistent with the literature data. RHPG and RHPT isolated from *R. rhabarbarum* were found to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and H_2O_2, and reduce the intracellular ROS activity in V79-4 cells (hamster lung cell line). RHPG was found to be more effective than its glycoside, RHPT. Examinations of the RHPG effects on intracellular changes under the H_2O_2-induced stress demonstrated an increased expression and activity of catalase. RGPG increased the phosphorylation of extracellular signal-regulated kinase (ERK), whereas the activity of activator protein 1 (AP-1), a redox-sensitive transcription factor, was significantly inhibited (Zhang et al., 2007). A reduction of ROS production in RAW 264.7 macrophages by six stilbenes (rhaponticin, rhapontigenin, isorhaponticin, deoxyrhaponticin, deoxyrhapontigenin, and resveratrol), isolated from *R. rhabarbarum* rhizome has been also described (Choi et al., 2014). In other work, both the RHPG and RHPT displayed anti-complementary actions (IC_{50} = 370 μM and 700 μM, respectively) in the classical pathway (Oh et al., 1998). The anti-inflammatory action of aloe-emodin has been found in an experimental model of macrophages as well (Hu et al., 2014).

Conclusions

In addition to our previous extensive phytochemical profiling of the examined rhubarb extracts and results on their ability to modulate the functions of endothelial cells (Liudvytska et al., 2023a, 2023b), the present study has revealed the beneficial activity of these plant preparations in other experimental systems related to cardiovascular physiology. This work provides novel data on the protective effects of *R. rhaponticum* and *R. rhabarbarum* extracts and stilbenes against the peroxynitrite-induced damage to blood plasma components and their ability to ameliorate the inflammation. Results from experiments in PBMCs and THP1-ASC-GFP cells revealed anti-inflammatory effects of the examined extracts and stilbenes at a level of cell response to inflammatory stimuli. Although a comparable anti-inflammatory and antioxidant effectiveness of both the examined species was observed in most assays in our study, some differences could be found between the activity of the petiole and root extracts. While the petiole extracts were slightly more effective mitigators of the inflammatory response of PBMCs, the root extracts were more efficient antioxidants and reducers of inflammasome formation in the THP1-ASC-GFP cells. A considerable antioxidant and anti-inflammatory potential of the examined stilbenes (RHPG, in particular) suggest that

the observed effects of rhubarb extracts may be partly attributed to the presence of their stilbenoid components.

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Legend to Figure 1. Effects of the examined rhubarb extracts and stilbenes on the cytokine release in the concanavalin A-stimulated PBMCs. The cytokine level was quantified using the ELISA kits. Panel A: results from measurements of IL-2 secretion into the cell culture medium. Panel B: results from measurements of TNF-α secretion. IND (indomethacin) - a reference compound (nonsteroidal anti-inflammatory drug - NSAID). The figure represents mean values (±SD); the cytokine level (pg/mL) detected in samples derived from the ConA-stimulated cells, untreated with the examined substances, was assumed as 100%; *p<0.05, **p<0.01, ***p<0.001; n = 4.

Legend to Figure 2. Inhibitory effects of the examined rhubarb extracts and stilbenes on the metalloproteinase-9 release from the concanavalin A-stimulated PBMCs. The MMP-9 was detected using the ELISA kit. DEX (dexamethasone) - a reference compound (an agonist of the glucocorticoid receptor, steroidal anti-inflammatory drug - SAID). The figure represents mean values (±SD); the MMP-9 level detected in cell culture medium from the ConA-stimulated cells, untreated with the examined substances, was assumed as 100%; *p<0.05, **p<0.01, ***p<0.001; n = 4.

Legend to Figure 3. Inhibitory effects of the examined rhubarb extracts and stilbenes on the prostaglandin E_2 synthesis in the concanavalin A-stimulated PBMCs. PGE_2 concentration in the cell culture medium was quantified using the ELISA kits. DEX (dexamethasone) - a reference compound (an agonist of the glucocorticoid receptor, steroidal anti-inflammatory drug - SAID)). The figure represents mean values (± SD); the PGE_2 level detected for the ConA-stimulated cells, untreated with the examined substances, was assumed as 100%; *p<0.05, **p<0.01, ***p<0.001; n = 4.

Legend to Figure 4. Evaluation of the inhibitory effects of the examined rhubarb extracts and stilbenes on the LPS-induced formation of ASC specks. The imaging was executed using a fluorescence microscope (Olympus, IX71/IX51). Panel A: live-cell imaging of THP1-ASC-GFP cells treated with examined substances (1-50 μg/mL) and stimulated with LPS (1 μg/mL; 3 h). The arrows point to the ASC specks. The scale bar corresponds to 20 μm. Panel B: The ASC specks number, measured in THP1-ASC-GFP cells treated as indicated above. The results represent mean values (±SD) from three independent measurements. The results represent average number of specks, based on their count in the field of view in relation to the visualised cells, recalculated for the cell count in the culture dish. $^{###}p$<0.001 for control THP1-ASC-GFP cells versus the LPS-stimulated cells (without the examined substances). The

**p<0.01 and ***p<0.001 values are related to the cells pre-incubated with the examined substances (1-50 μg/mL) and stimulated with the LPS, versus the LPS-stimulated cells in the absence of the examined substances.

Legend to Figure 5. Effects of the examined rhubarb extracts and stilbenes on the antioxidant capacity of blood plasma and the level of plasma protein thiol groups under the $ONOO^-$-induced oxidative stress. Panel A: effects on the non-enzymatic antioxidant capacity of plasma (measured by the FRAP). Panel B: effects on the level of -SH protein groups in human plasma. Results are presented as means ± SD (n = 14). Statistical significance: $^{\#}p$<0.05 and $^{\#\#\#}p$<0.001 for control plasma versus $ONOO^-$-treated plasma (without the examined substances); *p<0.05, **p<0.01, and ***p<0.001 for $ONOO^-$-treated plasma in the presence of the examined substances (1-50 μg/mL) versus $ONOO^-$-treated plasma in the absence of the examined substances.

Legend to Figure 6. Protective effects of the examined rhubarb extracts and stilbenes (RHPG and RHPT) towards the $ONOO^-$-induced changes in the human fibrinogen structure. The SDS-PAGE of human fibrinogen was conducted under reducing conditions, using 4-15% gradient gels. Panel A: the representative gels of four independent experiments. Panel B: relative intensities of the high molecular weight (HMW) aggregate bands, detected in the fibrinogen samples. The intensities of HMW aggregate bands in fibrinogen treated with $ONOO^-$ in the absence of the examined substances were assumed to be 100%. Densitometric analyses of the obtained gels were performed using the CLIQS Gel Image Analysis Software (TotalLab, Newcastle-Upon-Tyne, UK). The *R.cum* and *R.rum* abbrevitations correspond to the *R. rhaponticum* and *R. rhabarbarum* extracts, respectively. Results are presented as means ±SD (n=4). Statistical significance: *p<0.05, ** p<0.01, and ***p<0.001 for $ONOO^-$-treated fibrinogen in the presence of the examined substances (1, 5, 50 μg/mL) versus $ONOO^-$-treated fibrinogen in the absence of the examined rhubarb-derived substances.

Legend to Figure 7. **Protective effects of rhubarb extracts and stilbenes (RHPG and RHPT) towards the $ONOO^-$-induced formation of 3-NT in human fibrinogen.** The 3-NT level in fibrinogen treated with $ONOO^-$ without the examined substances was assumed to be 100% tyrosine nitration. Results are presented as means ± SD (n = 10). Statistical significance: ***p<0.001 for $ONOO^-$-treated fibrinogen in the presence of the examined substances (1, 5, 50 μg/mL) versus $ONOO^-$-treated fibrinogen in the absence of the examined rhubarb-derived substances.

Legend to Figure 8. Fluorometric determination of the effects of rhubarb extracts and stilbenes (RHPG and RHPT) on the $ONOO^-$-triggered modifications of tryptophan residues in human fibrinogen. The test was based on a decay of tryptophan fluorescence resulting from the $ONOO^-$-induced oxidation of fibrinogen samples and the examined substances ability to reduce this effect. Results are presented as means ±SD (n = 5). Statistical significance: ***p<0.001 for control fibrinogen versus $ONOO^-$-treated fibrinogen (without the examined plant-derived substances); *p<0.05, ** p<0.01, and *** p<0.001 for $ONOO^-$-treated fibrinogen in the presence of rhubarb extracts and stilbenes (1-50 μg/mL) versus $ONOO^-$-treated fibrinogen in the absence of the examined substances. Fluorescence was measured at λ_{ex} = 280 nm and λ_{em} = 340 nm.

Figure

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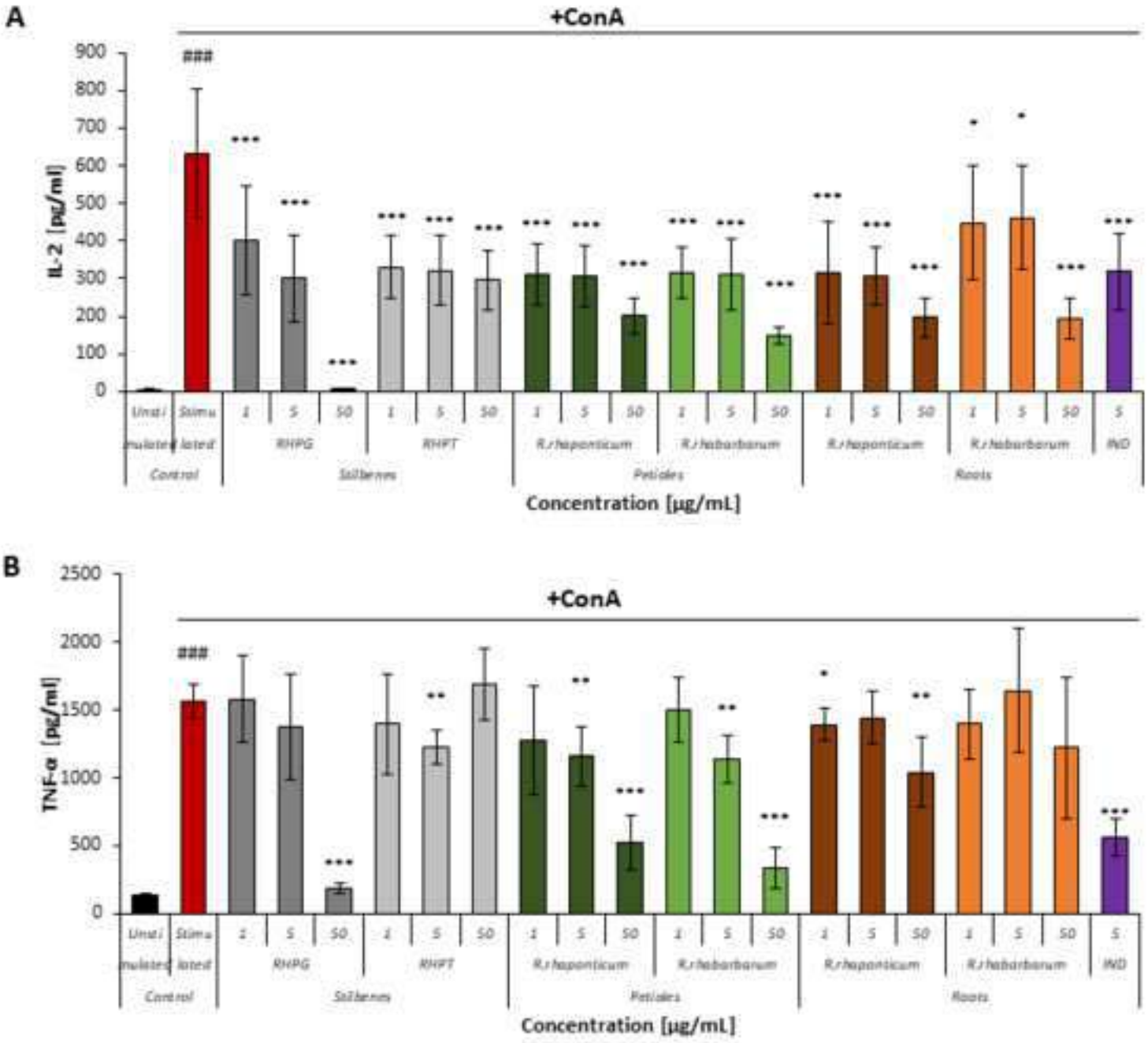

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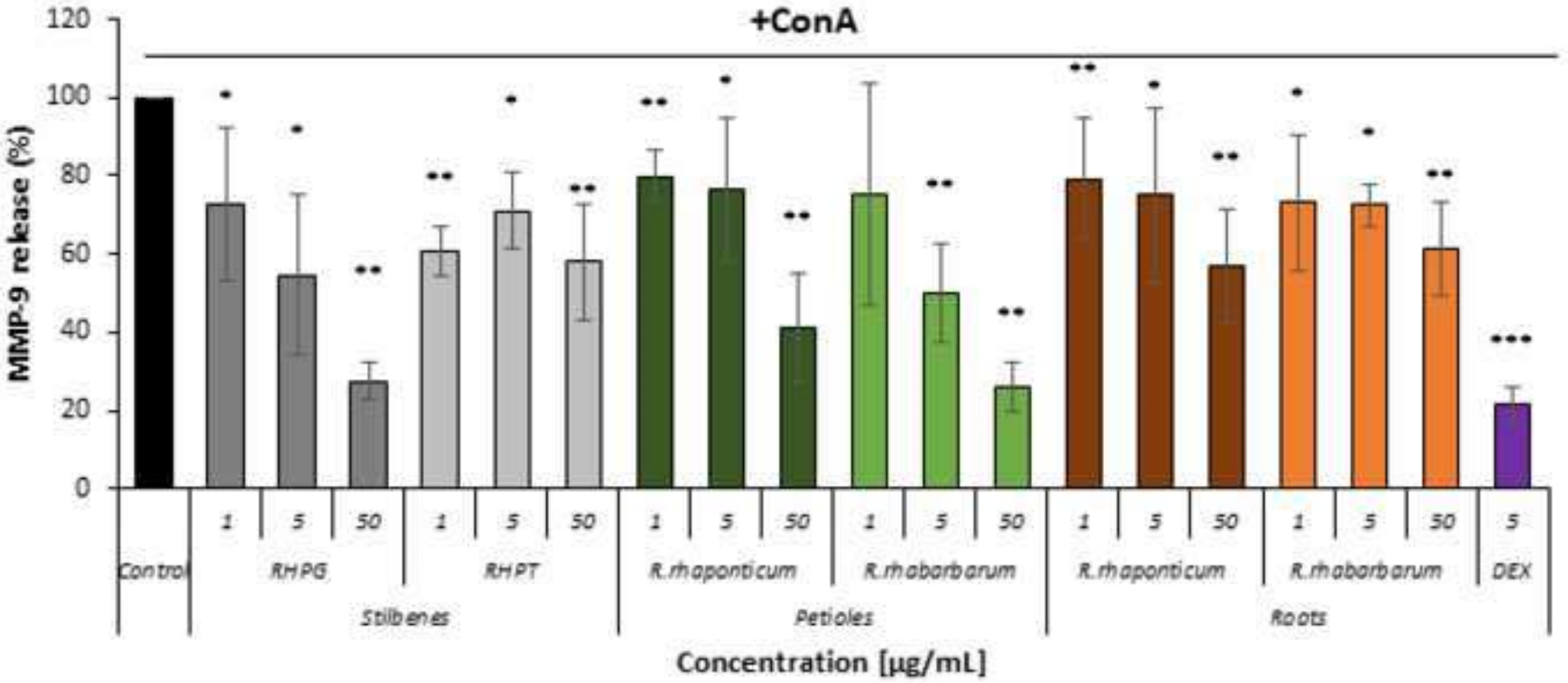

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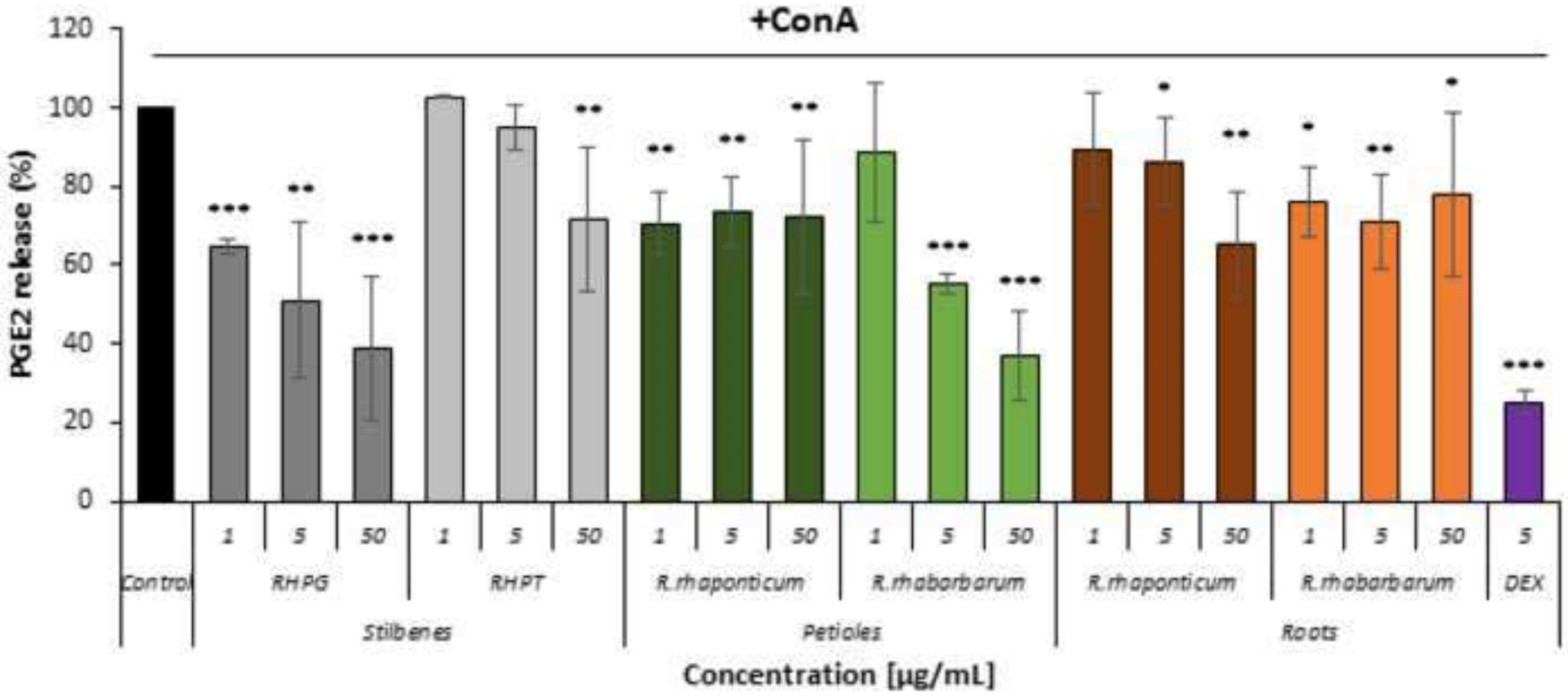

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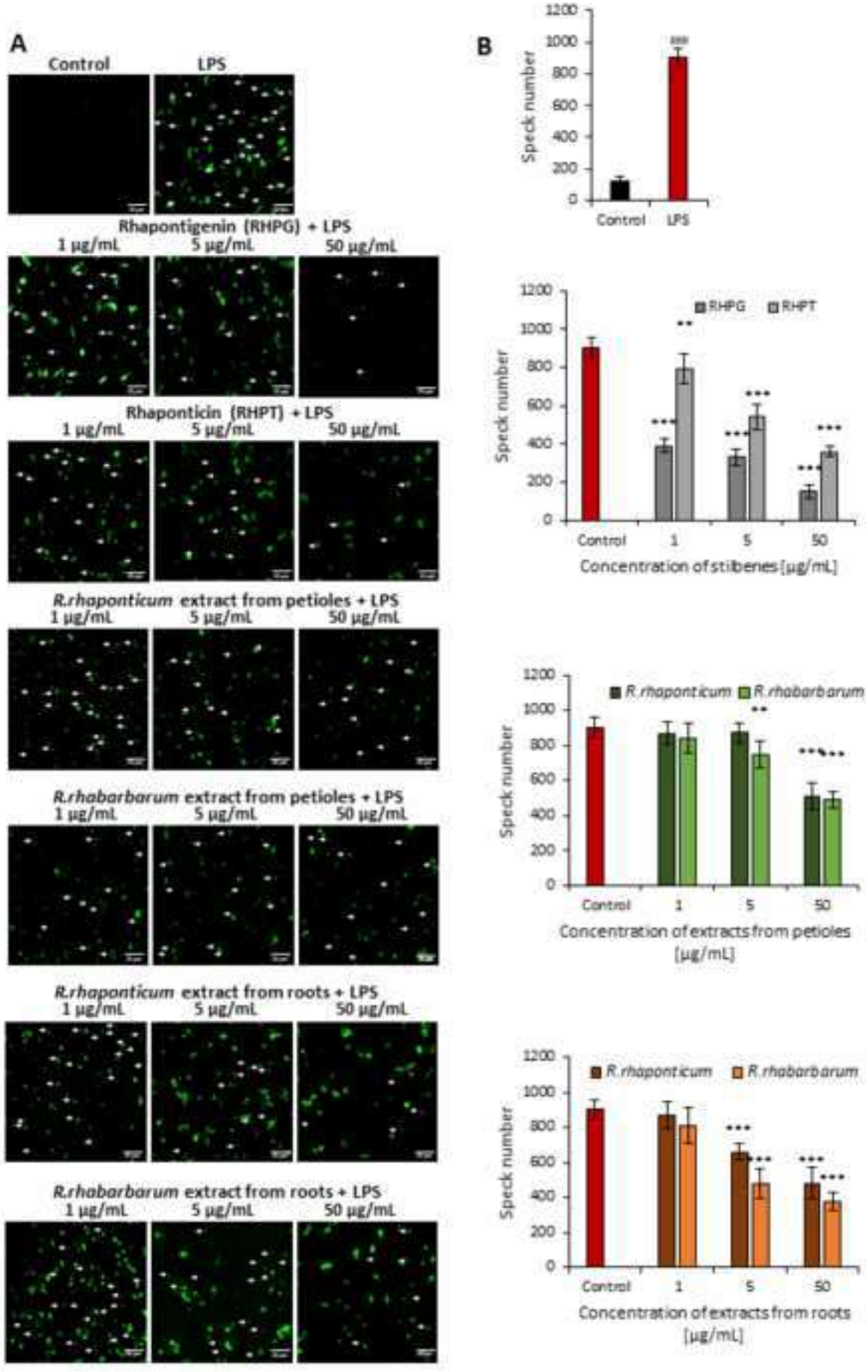

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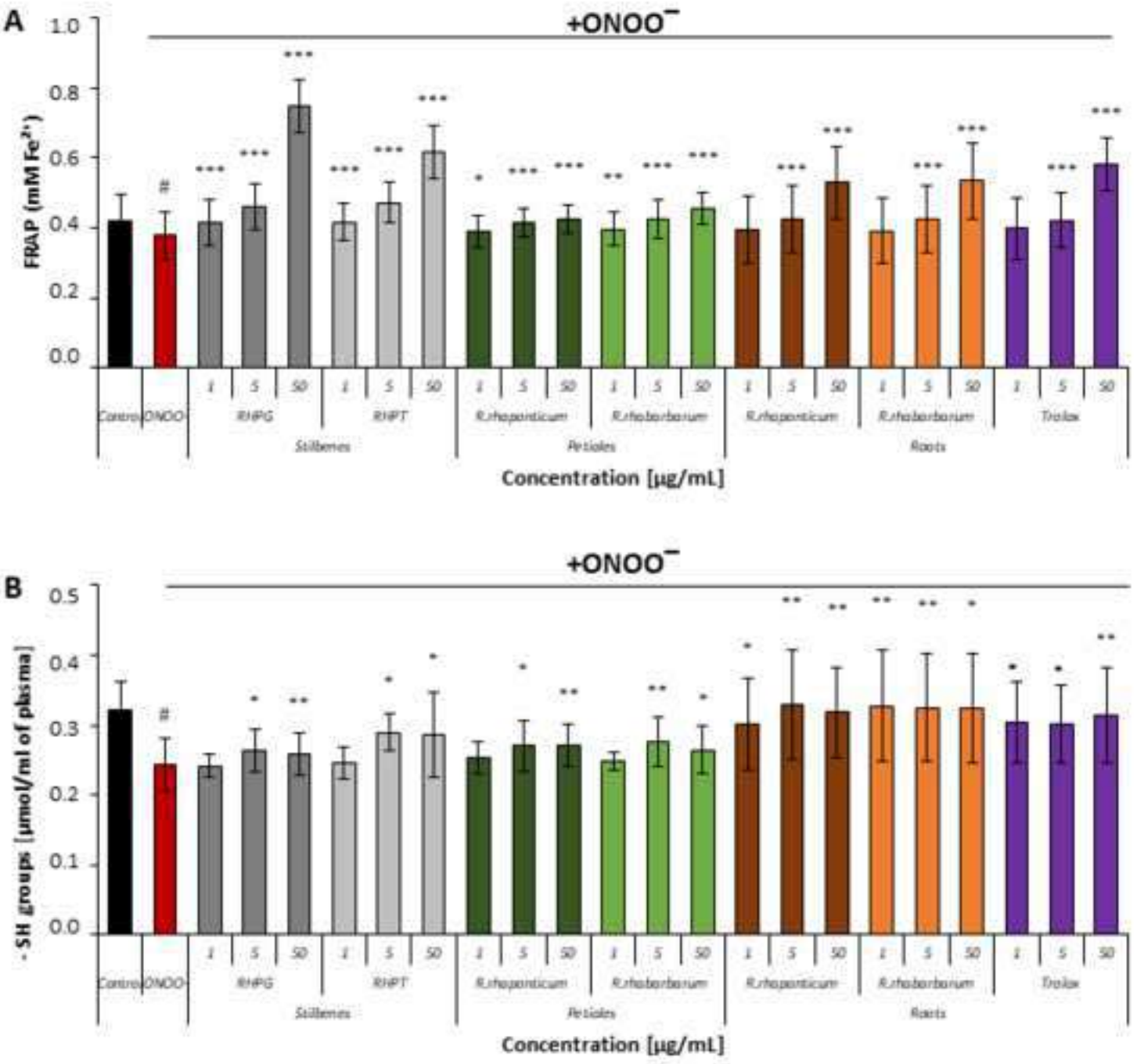

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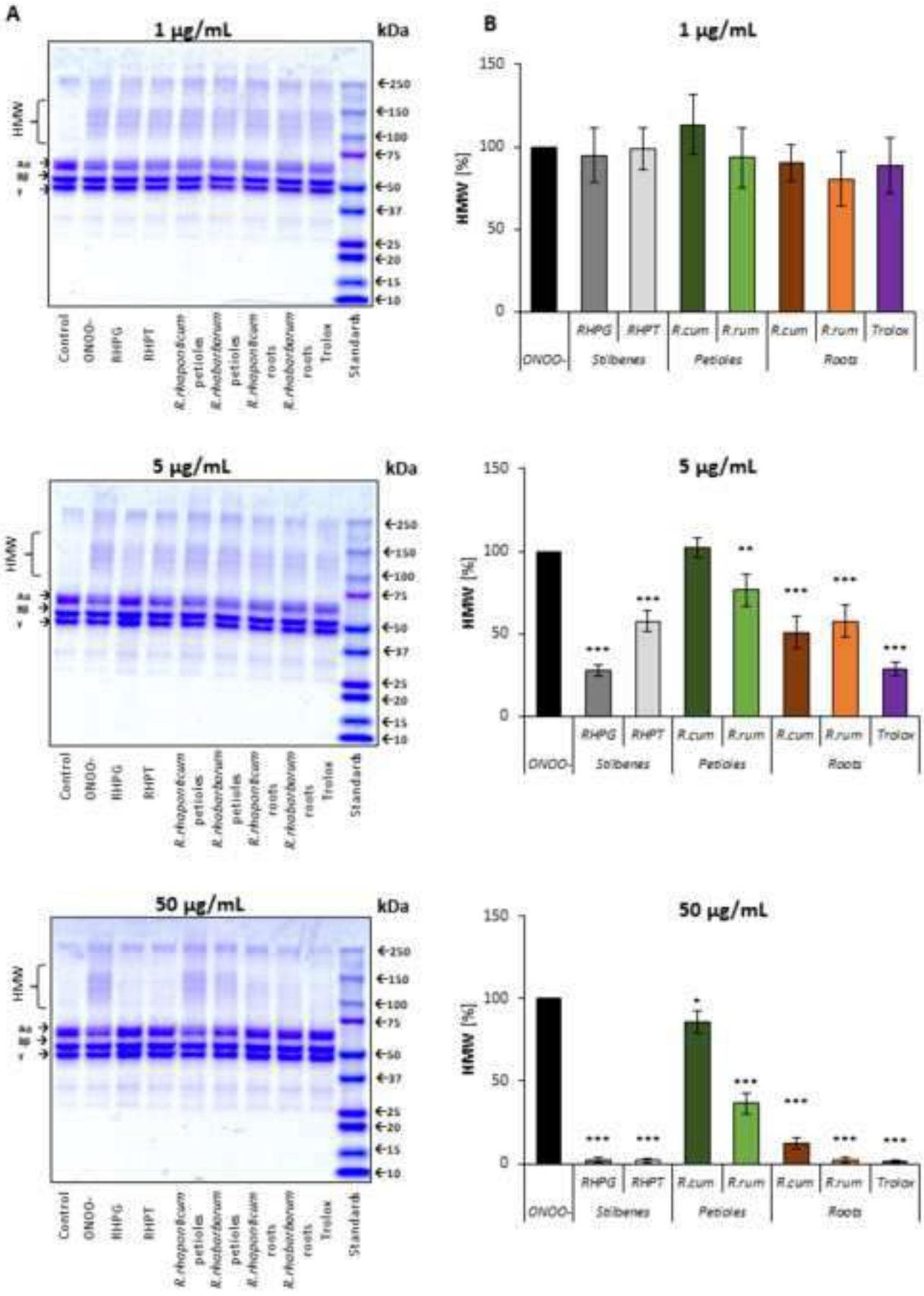

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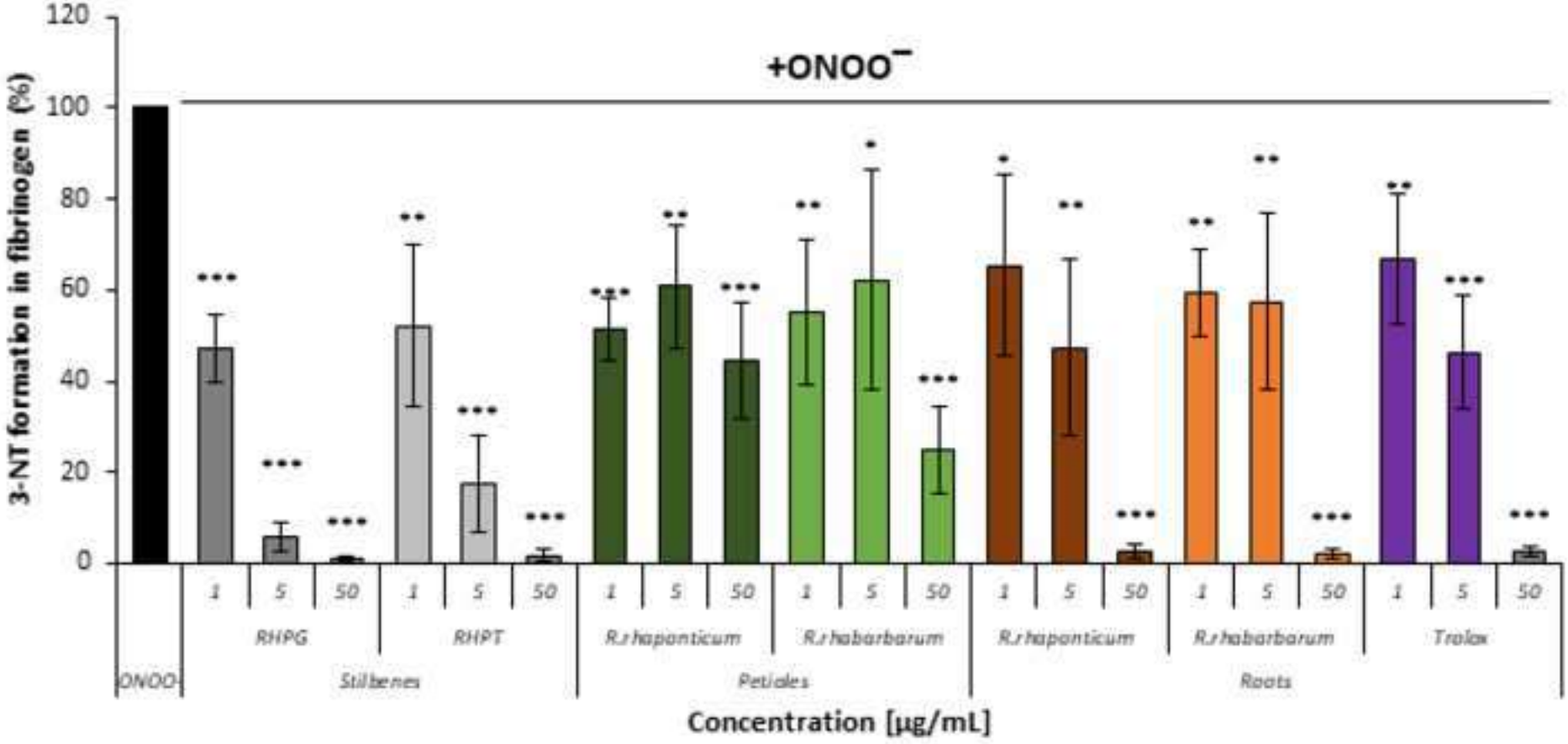

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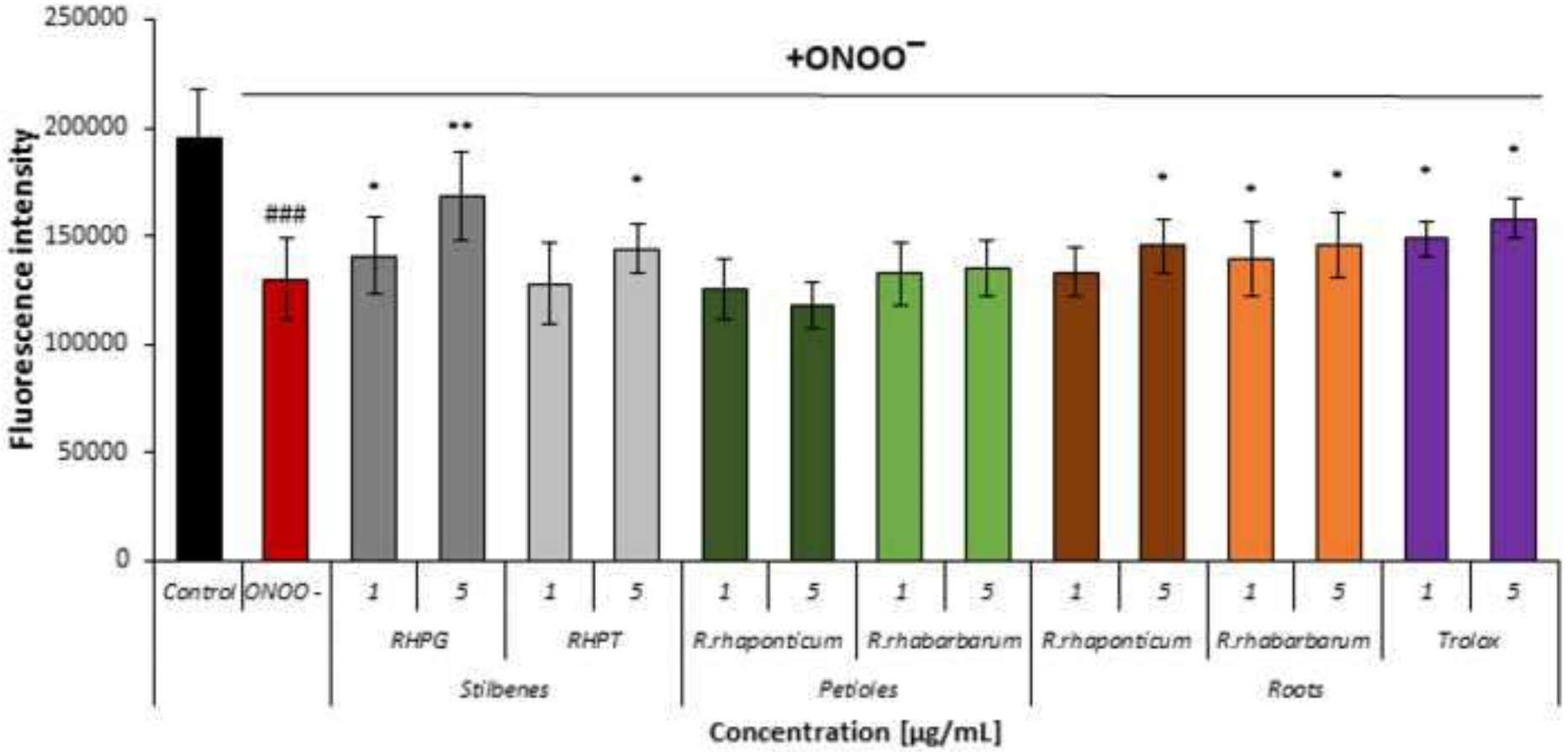

An original paper entitled: "Anti-inflammatory and antioxidant actions of extracts from *Rheum rhaponticum* and *Rheum rhabarbarum* in human blood plasma and cells *in vitro*"

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

	Oleksandra Liudvytska	**Magdalena Bandyszewska**	**Tomasz Skirecki**	Justyna Krzyżanowska-Kowalczyk	Mariusz Kowalczyk	Joanna Kolodziejczyk-Czepas
Having received fees for consulting.	No	No	No	No	No	No
Having received research funding.	No	No	No	No	No	No
Having been employed by a related company	No	No	No	No	No	No
Holding stocks or shares in a company which might be affected by the publication of your paper.	No	No	No	No	No	No
Having received funds reimbursing you for attending a related symposia, or talk.	No	No	No	No	No	No

On behalf of all co-authors
06.05.2023

Oleksandra Liudvytska (corresponding author)

Author Contributions.

An original paper entitled: "**Anti-inflammatory and antioxidant actions of extracts from *Rheum rhaponticum* and *Rheum rhabarbarum* in human blood plasma and cells *in vitro***"

	Oleksandra Liudvytska	Magdalena Bandyszewska	Tomasz Skirecki	Justyna Krzyżanowska-Kowalczyk	Mariusz Kowalczyk	Joanna Kolodziejczyk-Czepas
Term	X					X
Conceptualization	X					X
Methodology	X	X		X	X	X
Software	X					
Validation						X
Formal analysis	X	X	X	x	X	X
Investigation	X	X	X	X	X	
Resources						X
Data Curation	X					X
Writing - Original Draft	X			X	X	
Writing - Review & Editing	X	X	X	X	X	X
Visualization	X					
Supervision						X
Project administration						X
Funding acquisition						X

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Do: Oleksandra Liudvytska So, 06.05.2023 14:59

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Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro

Dear MSc Liudvytska,

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OŚWIADCZENIA WSPÓŁAUTORÓW PRAC

Praca przeglądowa

(I) Kołodziejczyk-Czepas, J., **Liudvytska, O.*** *Rheum rhaponticum and Rheum rhabarbarum: a review of phytochemistry, biological activities and therapeutic potential*. Phytochemistry Reviews (SPRINGER), 2021, 20, 589-607. DOI: 10.1007/s11101-020-09715-3.

Dr hab. Joanna Kołodziejczyk-Czepas, prof. UŁ
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: joanna.kolodziejczyk@biol.uni.lodz.pl

Łódź, 05.06.2023.

OŚWIADCZENIE

Oświadczam, że w pracy "***Kołodziejczyk-Czepas J., Liudvytska O. Rheum rhaponticum and Rheum rhabarbarum - a review of phytochemistry, biological activities and therapeutic potential. Phytochemistry Reviews 2021. 20:589-607. DOI: 10.1007/s11101-020-09715-3***"

mój udział polegał na współtworzeniu koncepcji i planu pracy, nadzorze merytorycznym nad przygotowaniem pracy, napisaniu rozdziału „*Phytochemical profile of R. rhaponticum and R. rhabarbarum*", wykonaniu tabeli 1 oraz 2, udziale w przygotowaniu finalnej wersji manuskryptu i jego modyfikacji w procesie publikacyjnym, a także w przygotowywaniu odpowiedzi na uwagi recenzentów.

Swój udział szacuję na 25%.

Joanna Kołodziejczyk-Czepas

Mgr Oleksandra Liudvytska

Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: oleksandra.liudvytska@biol.uni.lodz.pl

Łódź, 6.06.23

OŚWIADCZENIE

Oświadczam, że w pracy "***Kołodziejczyk-Czepas J., Liudvytska O. Rheum rhaponticum and Rheum rhabarbarum - a review of phytochemistry, biological activities and therapeutic potential. Phytochemistry Reviews 2021. 20:589-607. DOI: 10.1007/s11101-020-09715-3***"

mój udział polegał na: współtworzeniu koncepcji i planu pracy, zebraniu i analizie dostępnego piśmiennictwa, przygotowaniu pierwotnej wersji większości rozdziałów pracy (z wyjątkiem sekcji „*Phytochemical profile of R. rhaponticum and R. rhabarbarum*"), wraz z tabelami 3 i 4 oraz materiałami dodatkowymi (*supplementary materials*). Pełniłam ponadto rolę autora korespondencyjnego i uczestniczyłam w modyfikacji pracy oraz przygotowywaniu odpowiedzi na uwagi recenzentów.

Swój udział szacuję na 75%.

Oleksandra Kwiatkowska

Praca oryginalna

(II) Liudvytska O.*, Ponczek M.B., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kołodziejczyk-Czepas J. *Rheum rhaponticum and Rheum rhabarbarum extracts as modulators of endothelial cell inflammatory response*. Nutrients (MDPI), 2023; DOI: 10.3390/nu15040949.

Mgr Oleksandra Liudvytska
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: oleksandra.liudvytska@biol.uni.lodz.pl

Łódź, 6.06.23

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Rheum rhaponticum and Rheum rhabarbarum extracts as modulators of endothelial cell inflammatory response. Nutrients 2023, 15, 949. DOI: 10.3390/nu15040949***"

mój udział polegał na: współtworzeniu koncepcji i planu pracy, uczestniczeniu w izolacji komórek śródbłonka, przeprowadzeniu wszystkich zawartych w pracy oznaczeń dotyczących wpływu badanych ekstraktów i związków na żywotność i odpowiedź zapalną komórek śródbłonka, wykonaniu ich analiz statystycznych i uczestnictwie w interpretacji uzyskanych wyników. Przygotowałam pierwotną wersję części manuskryptu obejmujących wstęp, metodykę, opis wyników oraz ich dyskusję w zakresie dotyczącym wykonanych przeze mnie oznaczeń (rozdziały 1, 2.3-2.7, 2.9, 3.2-3.6, 4; wraz z graficzną ilustracją wyników: ryc. 2-7, tab. 1, 3).
Pełnię rolę autora korespondencyjnego.

Swój udział szacuję na 42%.

Oleksandra Liudvytska

Dr hab. Michał B. Ponczek
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: michał.ponczek@biol.uni.lodz.pl

Łódź, 12.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Rheum rhaponticum and Rheum rhabarbarum extracts as modulators of endothelial cell inflammatory response. Nutrients 2023, 15, 949. DOI: 10.3390/nu15040949***"

mój udział polegał na wykonaniu badań *in silico,* analizie i interpretacji uzyskanych z nich wyników oraz przygotowaniu części manuskryptu obejmujących metodykę i opis wyników w zakresie dotyczącym wykonanych przeze mnie badań (rozdział, 2.8 i 3.4 wraz z ich ilustracją graficzną: tab. 4, ryc. 8, *Supplementary materials S2*) i uczestnictwie w korekcie finalnej wersji manuskryptu.

Swój udział szacuję na 10%.

.....

Mgr Oskar Ciesielski
Katedra Biologii Nowotworów i Epigenetyki
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 10
email: oskar.ciesielski@biol.uni.lodz.pl

Łódź, 26 maja 2023

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Rheum rhaponticum and Rheum rhabarbarum extracts as modulators of endothelial cell inflammatory response. Nutrients 2023, 15, 949. DOI: 10.3390/nu15040949***"

mój udział polegał na izolacji komórek śródbłonka, a także uczestnictwie w optymalizacji warunków hodowli do analiz wpływu badanych ekstraktów i związków na żywotność i odpowiedź zapalną komórek śródbłonka.

Swój udział szacuję na 5%.

.....

Dr Justyna Krzyżanowska-Kowalczyk
Zakład Biochemii i Jakości Plonów
Instytut Uprawy Nawożenia i Gleboznawstwa – PIB
24-100 Puławy
tel. 81 4786 881
email: jkrzyzanowska@iung.pulawy.pl

Puławy, 05.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy: "**Liudvytska O., Ponczek M., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. *Rheum rhaponticum* and *Rheum rhabarbarum* extracts as modulators of endothelial cell inflammatory response. Nutrients 2023, 15, 949. DOI: 10.3390/nu15040949**"

mój wkład w powstanie tej pracy polegał na planowaniu i wykonaniu części eksperymentalnej dotyczącej analiz fitochemicznych oraz na udziale w przygotowaniu manuskryptu opisującego uzyskane wyniki (rozdziały 2.1, 2.2, 3.1, 4 wraz z graficzną ilustracją wyników: tab. 2, S1-S2 zawartymi w manuskrypcie oraz w *supplementary materials*). Uczestniczyłam w modyfikacji pracy oraz przygotowaniu odpowiedzi na uwagi recenzentów. Zoptymalizowałam procedury ekstrakcyjne i przygotowałam ekstrakty z ogonków liściowych oraz korzeni/karp *R. rhaponticum* oraz *R. rhabarbarum,* na których zostały przeprowadzone badania aktywności biologicznej, wybrałam związki oraz przygotowałam wzory strukturalne tych metabolitów do przeprowadzenia badań *in silico,* uczestniczyłam w wykonywaniu analiz jakościowych i pół-ilościowych za pomocą LC-HR-MS oraz w interpretacji i opracowaniu ich wyników.

Swój udział szacuję na 15%

J. Krzyżanowska-Kowalczyk

Dr Mariusz Kowalczyk
Zakład Biochemii i Jakości Plonów
Instytut Uprawy Nawożenia i Gleboznawstwa – PIB
24-100 Puławy
tel. 81 4786 886
email: mkowalczyk@iung.pulawy.pl

Puławy, 05.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy: "**Liudvytska O., Ponczek M., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. *Rheum rhaponticum* and *Rheum rhabarbarum* extracts as modulators of endothelial cell inflammatory response. Nutrients 2023, 15, 949. DOI: 10.3390/nu15040949**"

mój wkład w powstanie tej pracy polegał na planowaniu i wykonaniu części eksperymentalnej dotyczącej analiz fitochemicznych oraz na udziale w przygotowaniu manuskryptu opisującego uzyskane wyniki (rozdziały 2.1, 2.2, 3.1, 4 wraz z graficzną ilustracją wyników ryc. 1, S1-S4, tab. 2, S1-S3 zawartymi w manuskrypcie oraz w *supplementary materials*). Uczestniczyłem w modyfikacji i edycji pracy oraz przygotowaniu odpowiedzi na uwagi recenzentów. Opracowałem metody jakościowej i półilościowej analizy składu ekstraktów z ogonów i korzeni gatunków *Rheum* za pomocą LC-HR-MS, a następnie współuczestniczyłem w wykonaniu tych analiz. Brałem również udział w interpretacji uzyskanych wyników, jak też w ich opracowaniu na potrzeby publikacji.

Swój udział szacuję na 15%

.....

Dr hab. Aneta Balcerczyk, prof. UŁ
Katedra Biologii Nowotworów i Epigenetyki
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 76
email: aneta.balcerczyk@biol.uni.lodz.pl

Łódź, 26 maja 2023

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Rheum rhaponticum and Rheum rhabarbarum extracts as modulators of endothelial cell inflammatory response. Nutrients 2023, 15, 949. DOI: 10.3390/nu15040949***"

mój udział polegał na nadzorze merytorycznym nad optymalizacją warunków hodowli komórek i wykonaniem oznaczeń wpływu badanych substancji roślinnych na żywotność i odpowiedź zapalną komórek śródbłonka oraz uczestnictwie w analizie i dyskusji wyników, a także w przygotowaniu finalnej wersji manuskryptu.

Swój udział szacuję na 5%.

Aneta Balcerczyk

Dr hab. Joanna Kołodziejczyk-Czepas, prof. UŁ
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: joanna.kolodziejczyk@biol.uni.lodz.pl

Łódź, 05.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Ciesielski O., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Rheum rhaponticum and Rheum rhabarbarum extracts as modulators of endothelial cell inflammatory response. Nutrients 2023, 15, 949. DOI: 10.3390/nu15040949***"

mój udział polegał na współtworzeniu koncepcji i planu pracy, koordynowaniu badań, nadzorze merytorycznym nad wykonaniem oznaczeń dotyczących wpływu badanych ekstraktów i związków na żywotność i odpowiedź zapalną komórek śródbłonka i aktywność badanych enzymów, a także udziale w analizie i interpretacji wyników. Uczestniczyłam w przygotowaniu finalnej wersji manuskryptu i jego modyfikacji w procesie publikacyjnym oraz sporządzaniu odpowiedzi na uwagi recenzentów.
Pełnię funkcję kierownika projektu (NCN; 2018/31/B/NZ9/01238), w ramach którego wykonywano większość zawartych w pracy badań.

Swój udział szacuję na 8%.

Joanna Kołodziejczyk-Czepas

Praca oryginalna

(III) Liudvytska O.*, Ponczek M.B., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kołodziejczyk-Czepas J. *Effects of Rheum rhaponticum and Rheum rhabarbarum extracts on haemostatic activity of blood plasma components and endothelial cells in vitro.* Journal of Ethnopharmacology (ELSEVIER), 2023, DOI: 10.1016/j.jep.2023.116562;

Mgr Oleksandra Liudvytska
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: joanna.kolodziejczyk@biol.uni.lodz.pl

Łódź, 6.06.23

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Effects of Rheum rhaponticum and Rheum rhabarbarum extracts on haemostatic activity of blood plasma components and endothelial cells in vitro*" *Journal of Ethnopharmacology, 2023. DOI: 10.1016/j.jep.2023.116562***

mój udział polegał na: współtworzeniu koncepcji i planu pracy, uczestniczeniu w izolacji komórek śródbłonka, przeprowadzeniu wszystkich zawartych w pracy oznaczeń dotyczących wpływu badanych ekstraktów i związków na odpowiedź komórek śródbłonka i aktywność badanych białek, wykonaniu ich analiz statystycznych i uczestnictwie w interpretacji uzyskanych wyników. Przygotowałam pierwotną wersję części manuskryptu obejmujących metodykę, opis wyników oraz ich dyskusję w zakresie dotyczącym wykonanych przeze mnie oznaczeń (rozdziały 1; 2.1; 2.6 - 2.12; 2.14; 3.2 - 3.4; 5 wraz z graficzną ilustracją wyników: ryc. 6 - 14, tab. 2).
Pełniłam ponadto rolę autora korespondencyjnego i uczestniczyłam w modyfikacji manuskryptu oraz przygotowywaniu odpowiedzi na uwagi recenzentów.

Swój udział szacuję na 55%.

Oleksandra Liudvytska

Dr hab. Michał B. Ponczek
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: michał.ponczek@biol.uni.lodz.pl

Łódź, 02.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Effects of Rheum rhaponticum and Rheum rhabarbarum extracts on haemostatic activity of blood plasma components and endothelial cells in vitro. Journal of Ethnopharmacology, 2023. DOI: 10.1016/j.jep.2023.116562***

mój udział polegał na wykonaniu badań *in silico,* analizie i interpretacji uzyskanych z nich wyników oraz przygotowaniu części manuskryptu obejmujących metodykę i opis wyników w zakresie dotyczącym wykonanych przeze mnie badań (rozdziały 2.13. i 3. 5. oraz tabela 1) i uczestnictwie w korekcie finalnej wersji manuskryptu.

Swój udział szacuję na 10%.

.....

Dr Justyna Krzyżanowska-Kowalczyk
Zakład Biochemii i Jakości Plonów
Instytut Uprawy Nawożenia i Gleboznawstwa – PIB
24-100 Puławy
tel. 81 4786 881
email: jkrzyzanowska@iung.pulawy.pl

Puławy, 05.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy: **"Liudvytska O., Ponczek M., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Effects of *Rheum rhaponticum* and *Rheum rhabarbarum* extracts on haemostatic activity of blood plasma components and endothelial cells *in vitro*. Journal of Ethnopharmacology 2023, 315, 116562. DOI: 10.1016/j.jep.2023.116562"**

mój wkład w powstanie tej pracy polegał na planowaniu i wykonaniu części eksperymentalnej dotyczącej analiz fitochemicznych, na udziale w przygotowaniu części manuskryptu opisujących uzyskane wyniki (rozdziały 2.1. – 2.5., 3.1., 5) oraz modyfikacji i edycji pracy. Uczestniczyłam w wykonaniu spektrofotometrycznych oznaczeń ilościowych wybranych grup metabolitów swoistych, oraz interpretacji i opracowaniu wyników analiz.

Swój udział szacuję na 8%

J. Krzyżanowska-Kowalczyk

Dr Mariusz Kowalczyk
Zakład Biochemii i Jakości Plonów
Instytut Uprawy Nawożenia i Gleboznawstwa – PIB
24-100 Puławy
tel. 81 4786 886
email: mkowalczyk@iung.pulawy.pl

Puławy, 05.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy: **"Liudvytska O., Ponczek M., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Effects of *Rheum rhaponticum* and *Rheum rhabarbarum* extracts on haemostatic activity of blood plasma components and endothelial cells *in vitro*. Journal of Ethnopharmacology 2023, 315, 116562. DOI: 10.1016/j.jep.2023.116562"**

mój wkład w powstanie tej pracy polegał na planowaniu i wykonaniu części eksperymentalnej dotyczącej analiz fitochemicznych, na udziale w przygotowaniu manuskryptu opisującego uzyskane wyniki (rozdziały 2.1. – 2.5., 3.1., 5 wraz z graficzną ilustracją wyników ryc. 2 - 5) oraz modyfikacji i edycji pracy. Uczestniczyłem w wykonaniu spektrofotometrycznych oznaczeń ilościowych wybranych grup metabolitów swoistych, interpretacji i opracowaniu wyników analiz, oraz opracowałem i wykonałem statystyczne porównanie składu fitochemicznego badanych ekstraktów.

Swój udział szacuję na 12%

.....

Dr hab. Aneta Balcerczyk, prof. UŁ
Katedra Biologii Nowotworów i Epigenetyki
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 76
email: aneta.balcerczyk@biol.uni.lodz.pl

Łódź, 26 maja 2023

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Effects of Rheum rhaponticum and Rheum rhabarbarum extracts on haemostatic activity of blood plasma components and endothelial cells in vitro" Journal of Ethnopharmacology, 2023. DOI: 10.1016/j.jep.2023.116562***

mój udział polegał na nadzorze merytorycznym nad optymalizacją warunków hodowli komórek i wykonaniem oznaczeń wpływu badanych substancji roślinnych odpowiedź hemostatyczną śródbłonka.

Swój udział szacuję na 5%.

Aneta Balcerczyk

Dr hab. Joanna Kołodziejczyk-Czepas, prof. UŁ
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: joanna.kolodziejczyk@biol.uni.lodz.pl

Łódź, 05.06.2023.

OŚWIADCZENIE

Oświadczam, że w pracy "***Liudvytska O., Ponczek M., Krzyżanowska-Kowalczyk J., Kowalczyk M., Balcerczyk A., Kolodziejczyk-Czepas J. Effects of Rheum rhaponticum and Rheum rhabarbarum extracts on haemostatic activity of blood plasma components and endothelial cells in vitro" Journal of Ethnopharmacology, 2023. DOI: 10.1016/j.jep.2023.116562***

mój udział polegał na współtworzeniu koncepcji i planu pracy, koordynowaniu badań, a także na nadzorze merytorycznym nad wykonaniem oznaczeń wpływu ekstraktów oraz stilbenów na komórki śródbłonka, osocze i badane proteazy, a także udziale w analizie i interpretacji wyników. Przygotowałam rozdział „*Introduction*", rycinę 1 i uczestniczyłam w przygotowaniu finalnej wersji manuskryptu oraz jego modyfikacji w procesie publikacyjnym i przygotowywaniu odpowiedzi na uwagi recenzentów.
Pełnię funkcję kierownika projektu (NCN; 2018/31/B/NZ9/01238), w ramach którego wykonywano większość zawartych w pracy badań.

Swój udział szacuję na 10%.

Joanna Kołodziejczyk-Czepas

Praca oryginalna

(IV) Liudvytska O.*, Bandyszewska M., Skirecki T., Krzyżanowska-Kowalczyk J., Kowalczyk M., Kołodziejczyk-Czepas J. *Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro.* (praca złożona do redakcji)

Mgr Oleksandra Liudvytska
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: oleksandra.liudvytska@biol.uni.lodz.pl

Łódź, 6.06.23

OŚWIADCZENIE

Oświadczam, że w pracy ***"Liudvytska O., Bandyszewska M., Skirecki T., Krzyżanowska-Kowalczyk J., Kowalczyk M., Kolodziejczyk-Czepas J. Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro" (praca złożona do redakcji)***

mój udział polegał na: przeprowadzeniu zawartych w pracy oznaczeń dotyczących wpływu badanych ekstraktów i związków na jednojądrzaste komórki krwi obwodowej, osocze i fibrynogen oraz współwykonaniu oznaczeń odpowiedzi komórek THP1-ASC-GFP.

Wykonywałam analizy statystyczne i uczestniczyłam w interpretacji wyników uzyskanych w wyżej wymienionych pracach badawczych. Przygotowałam pierwotną wersję części manuskryptu obejmujących wstęp, metodykę, opis wyników oraz ich dyskusję w zakresie dotyczącym wykonanych przeze mnie oznaczeń (rozdziały Introduction; Materials and Methods: *Chemicals, Biological material, Evaluation of the anti-inflammatory effects in PBMCs: Cell cultures, Measurements of cytokine release and MMP-9 secretion, Analysis of the arachidonic acid metabolism: measurements of the PGE_2 level, Cytotoxicity Assays, Determination of anti-inflammatory effects of the examined extracts and stilbenes in the THP1-ASC-GFP cells, Evaluation of antioxidant effects of the examined substances in blood plasma: Sample preparation, Evaluation of blood protein oxidation/nitration and lipid peroxidation in blood plasma, Immunodetection of 3-nitrotyrosine (3-NT) in human fibrinogen, Fluorometric analyses of the tryptophan oxidation in fibrinogen molecule, SDS-PAGE-based detection of the $ONOO^-$-induced formation of protein aggregates;* Statistical analysis; Results: *Rhubarb extracts and stilbenes effects on the inflammatory response of human PBMCs: Determination of the IL-2 and TNF-α release, Measurements of the MMP-9 release, Analysis of the arachidonic acid metabolism: measurements of the PGE_2 level, Effects of the rhubarb extracts and stilbenes on the inflammasome formation, Effects of the examined extracts and stilbenes on PBMCs viability, Antioxidant actions of the examined rhubarb extracts and stilbenes in blood plasma, Protective effects against the oxidative and nitrative damage to fibrinogen: Analysis of the $ONOO^-$-induced formation of protein aggregates, Nitration of tyrosine residues in fibrinogen, Determination of the $ONOO^-$-induced modifications of tryptophan residues in the fibrinogen molecule;* Discussion; wraz z graficzną ilustracją wyników: ryc. 1 - 8, tab 1, 2). Pełnię rolę autora korespondencyjnego.

Swój udział szacuję na 74%.

Oleksandra Liudvytska

Mgr Magdalena Bandyszewska
Zakład Immunologii Translacyjnej
i Eksperymentalnej Intensywnej Terapii
Centrum Medyczne Kształcenia Podyplomowego
ul. Marymoncka 99/103,
01-813 Warszawa
tel: 22 5693 830
email: magdalena.bandyszewska@cmkp.edu.pl

Warszawa, 1.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy ***"Liudvytska O., Bandyszewska M., Skirecki T., Krzyżanowska-Kowalczyk J., Kowalczyk M., Kolodziejczyk-Czepas J. Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro" (praca złożona do redakcji)***

mój udział polegał współwykonaniu oznaczeń dotyczących wpływu badanych ekstraktów i związków na odpowiedź komórek THP1-ASC-GFP oraz uczestnictwie w interpretacji i dyskusji wyników uzyskanych w wyżej wymienionych doświadczeń.

Swój udział szacuję na 5%.

Magdalena Bandyszewska

Dr hab. Tomasz Skirecki, prof. CMKP
Zakład Immunologii Translacyjnej
i Eksperymentalnej Intensywnej Terapii
Centrum Medyczne Kształcenia Podyplomowego
ul. Marymoncka 99/103,
01-813 Warszawa
tel: 22 5693 830
email: tomasz.skirecki@cmkp.edu.pl

Warszawa, 23/05/2023

OŚWIADCZENIE

Oświadczam, że w pracy ***"Liudvytska O., Bandyszewska M., Skirecki T., Krzyżanowska-Kowalczyk J., Kowalczyk M., Kolodziejczyk-Czepas J. Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro" (praca złożona do redakcji)***

mój udział polegał na nadzorze merytorycznym nad przeprowadzeniem oznaczeń dotyczących wpływu badanych ekstraktów i związków na odpowiedź komórek THP1-ASC-GFP, uczestnictwie w interpretacji i dyskusji wyników uzyskanych w wyżej wymienionych pracach badawczych oraz w przygotowaniu finalnej wersji manuskryptu.

Swój udział szacuję na 5%.

Kierownik
Zakładu Immunologii Translacyjnej
i Eksperymentalnej Intensywnej Terapii
dr hab. n. med. Tomasz Skirecki, prof. CMKP

Dr Justyna Krzyżanowska-Kowalczyk
Zakład Biochemii i Jakości Plonów
Instytut Uprawy Nawożenia i Gleboznawstwa – PIB
24-100 Puławy
tel. 81 4786 881
email: jkrzyzanowska@iung.pulawy.pl

Puławy, 05.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy: **"Liudvytska O., Bandyszewska M., Skirecki T., Krzyżanowska-Kowalczyk J., Kowalczyk M., Kolodziejczyk-Czepas J. Anti-inflammatory and antioxidant actions of extracts from *Rheum rhaponticum* and *Rheum rhabarbarum* in human blood plasma and cells *in vitro*" (praca złożona do redakcji)**

mój wkład w powstanie tej pracy polegał na przygotowaniu rozdziałów manuskryptu związanych z opisem profilu fitochemicznego ekstraktów: *Plant Material*, *Preparation of rhubarbs extracts* oraz części sekcji *Discussion* dotyczących aspektów fitochemicznych, a także modyfikacji i edycji pracy.

Swój udział szacuję na 3%

J. Krzyżanowska-Kowalczyk

Dr Mariusz Kowalczyk
Zakład Biochemii i Jakości Plonów
Instytut Uprawy Nawożenia i Gleboznawstwa – PIB
24-100 Puławy
tel. 81 4786 886
email: mkowalczyk@iung.pulawy.pl

Puławy, 05.06.2023

OŚWIADCZENIE

Oświadczam, że w pracy: **"Liudvytska O., Bandyszewska M., Skirecki T., Krzyżanowska-Kowalczyk J., Kowalczyk M., Kolodziejczyk-Czepas J. Anti-inflammatory and antioxidant actions of extracts from *Rheum rhaponticum* and *Rheum rhabarbarum* in human blood plasma and cells *in vitro*" (praca złożona do redakcji)**

mój wkład w powstanie tej pracy polegał na przygotowaniu rozdziałów manuskryptu związanych z opisem profilu fitochemicznego ekstraktów: *Plant Material*, oraz części sekcji *Discussion* dotyczących aspektów fitochemicznych, a także modyfikacji i edycji pracy.

Swój udział szacuję na 3%

M. Kowalczyk

Dr hab. Joanna Kołodziejczyk-Czepas, prof. UŁ
Katedra Biochemii Ogólnej
Uniwersytet Łódzki
ul. Pomorska 141/143
90-236 Łódź
tel. 42 635 44 83
email: joanna.kolodziejczyk@biol.uni.lodz.pl

Łódź, 05.06.2023.

OŚWIADCZENIE

Oświadczam, że w pracy ***"Liudvytska O., Bandyszewska M., Skirecki T., Krzyżanowska-Kowalczyk J., Kowalczyk M., Kolodziejczyk-Czepas J. Anti-inflammatory and antioxidant actions of extracts from Rheum rhaponticum and Rheum rhabarbarum in human blood plasma and cells in vitro" (praca złożona do redakcji)***

mój udział polegał na współtworzeniu koncepcji i planu pracy, koordynowaniu badań, optymalizacji warunków doświadczeń z wykorzystaniem ludzkich komórek jednojądrzastych krwi obwodowej, a także na nadzorze merytorycznym nad wykonaniem oznaczeń obejmujących komórki jednojądrzaste krwi obwodowej, osocze i fibrynogen. Uczestniczyłam w analizach i interpretacji wyników oraz przygotowaniu finalnej wersji manuskryptu.
Pełnię funkcję kierownika projektu (NCN; 2018/31/B/NZ9/01238), w ramach którego wykonywano większość zawartych w pracy badań.

Swój udział szacuję na 10%.

Joanna Kołodziejczyk-Czepas