Uniwersytet Medyczny w Białymstoku

DZIEDZINA: nauki medyczne i nauki o zdrowiu

DYSCYPLINA: nauki farmaceutyczne

ROZPRAWA DOKTORSKA

Analiza fitochemiczna oraz ocena aktywności biologicznej wybranych gatunków z rodzaju *Potentilla* L. *sensu lato*

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Rozprawa doktorska została zrealizowana w ramach kształcenia w Szkole Doktorskiej UMB

Białystok, 2023

Pragnę złożyć podziękowania dr hab. Michałowi Tomczykowi za przekazaną wiedzę, wyrozumiałość, cierpliwość i nieocenioną pomoc w procesie przygotowywania niniejszej rozprawy.

Chciałbym również podziękować wszystkim pracownikom i doktorantom Zakładu Farmakognozji za wszelkie wsparcie, bezcenne uwagi, dyskusje i serdeczność.

Ostatnie słowa dedykuję najważniejszym osobom w moim życiu, moim Rodzicom, za ich bezgraniczną wiarę, zaufanie i nieocenioną pomoc w każdej chwili mojego życia, dzięki czemu doszedłem w swoim życiu do miejsca, o którym marzyłem.

Spis treści

Rozdział 1. Wykaz publikacji będących podstawą rozprawy doktorskiej

Publikacja 1

Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Anticancer potential of acetone extracts from selected *Potentilla* species against human colorectal cancer cells. *Frontiers in Pharmacology*. 2022; 13, 1027315.
DOI: 10.3389/fphar.2022.1027315
IF: 5,988, punktacja MEiN: 100 pkt
cytowania Web of Science/SCOPUS = 1/1

Publikacja 2

Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Phytochemical profiling of extracts from rare *Potentilla* species and evaluation of their anticancer potential. *International Journal of Molecular Sciences*. 2023; 24, 4836.
DOI: 10.3390/ijms24054836
IF: 6,208, punktacja MEiN: 140 pkt
cytowania Web of Science/SCOPUS = 0/0

Publikacja 3

Augustynowicz D., Latte, KP., Tomczyk, M. Recent phytochemical and pharmacological advances in the genus *Potentilla* L. *sensu lato*—An update covering the period from 2009 to 2020. *Journal of Ethnopharmacology*. 2021; 266, 113412.
DOI: 10.1016/j.jep.2020.113412
IF: 5,195, punktacja MEiN: 140 pkt
cytowania Web of Science/SCOPUS = 12/13

Sumaryczne dane naukometryczne publikacji 1–3 włączonych do jednotematycznego cyklu prac:
Sumaryczny wskaźnik oddziaływania Impact Factor (IF): 17,391
Sumaryczna liczba punktów MEiN: 380 pkt

Rozdział 2. Wprowadzenie

Rodzaj *Potentilla* L. *sensu lato* (*s. l.*), pięciornik (syn. *Dasiphora* RAFINESQUE, *Penthaphylloides* DUHAMEL, *Tormentilla* L.) jest jednym z rodzajów należących do plemienia *Potentilleae*, należącego do rodziny różowatych (Rosaceae). Nazwa rodzaju pochodzi od łacińskiego słowa *potentia* oznaczającego potęgę oraz siłę [1]. Rodzaj pięciornik jest jednym z najbardziej różnorodnych rodzajów roślin na półkuli północnej składający się z około 500 gatunków. Molekularne badania filogenetyczne pozwoliły na wyodrębnienie z rodzaju *Potentilla* kilku linii ewolucyjnych uważanych aktualnie za oddzielne rodzaje takich jak *Argentina*, *Dasiphora*, *Drymocallis*, *Sibbaldia* [2]. We florze Polski występuje w stanie dzikim ponad 30 gatunków z rodzaju *Potentilla* oraz kilka gatunków zaliczanych jako efemerofity oraz antropofity [3].

Rycina 1. *Potentilla erecta* (L.) Raeusch, pięciornik kurze ziele

Rodzaj pięciornik zawiera gatunki roślin jednorocznych, dwurocznych, bylin i małych krzewów. Przedstawicieli rodzaju charakteryzują pędy w pełni wyprostowane, podnoszące się lub płożące z dłoniastymi lub pierzastymi liśćmi złożonymi przytwierdzonymi krótkim ogonkiem lub bezpośrednio do głównej łodygi. Okres kwitnienia przypada na okres od maja do października. Obupłciowe kwiaty występują pojedynczo lub w kwiatostanach o pięciodziałowym kieliszku i kielichu, zazwyczaj cztero- lub pięciopłatkowe o zabarwieniu żółtym, rzadziej białym lub różowym. Owoce suche niepękające typu niełupka lub orzeszek [4, 5]. Na Rycinie 1 przedstawiony został jeden z gatunków wybranych do badań, pięciornik kurze ziele [*Potentilla erecta* (L.) Raeusch].

Systematyka roślin początkowo była oparta wyłącznie na cechach morfologicznych i anatomicznych. We współczesnej klasyfikacji roślin obok wymienionych badań coraz większą rolę odgrywa chemofenetyka, opierająca się na klasyfikacji gatunków w danym taksonie na podstawie składu chemicznego, bez wyjaśniania związków filogenetycznych [6]. Użycie nowoczesnych technik analitycznych umożliwia wykrywanie wyspecjalizowanych metabolitów wtórnych, nawet w śladowych ilościach, pogłębiając wiedzę o ich zawartości w obrębie tego samego rodzaju, plemienia czy rodziny botanicznej.

Najwcześniejsze doniesienia wskazujące na stosowanie lecznicze gatunków zaliczanych do rodzaju *Potentilla* L. – pięciornik, pochodzą z czasów starożytnych. Pliniusz Starszy (23 – 79 n.e.)

zalecał wykorzystanie pięciorników w chorobach skóry [7]. W czasach nowożytnych zastosowanie wybranych gatunków z rodzaju pięciornik zostało udokumentowane w książkach zielarskich wskazujących na ich zastosowanie w leczeniu bólu zębów, stanów zapalnych jamy ustnej i gardła, chorób skóry i ran, oraz wewnętrznie przeciwko biegunkom, czerwonce, chorobach zapalnych jelit, dnie moczanowej oraz w leczeniu nieokreślonych chorób nowotworowych [5, 8]. Najnowsze doniesienia z Europy wschodniej wskazują również na działanie wyciągów z pięciornika białego (*Potentilla alba* L.) jako komplementarnego składnika terapii chorób tarczycy [9]. Dostępne badania naukowe przeprowadzone w warunkach *in vitro, in vivo* oraz niewielka ilość przeprowadzonych badań klinicznych potwierdzają w większości jedynie działania znane z tradycyjnego stosowania.

Szczegółowy przegląd dostępnej literatury wskazał na obecność, w dotychczas przebadanych gatunkach zaliczanych do rodzaju *Potentilla s. l.,* głównie związków o charakterze polifenoli w tym garbników, flawonoidów, kwasów fenolowych, jak również związków triterpenowych. Jednocześnie duża różnorodność gatunkowa i trudności w oznaczeniach taksonomicznych uniemożliwiła szczegółowe przebadanie większości z nich zarówno pod względem fitochemicznym jak i oceny ich aktywności biologicznej.

Biorąc pod uwagę fakt, że brak jest doniesień na temat obecności związków czynnych o charakterze polifenolowym w wybranych do badań w ramach rozprawy doktorskiej surowcach roślinnych, przeprowadzenie analizy fitochemicznej z użyciem części nadziemnych oraz podziemnych szesnastu nieprzebadanych do tej pory lub słabo zbadanych gatunków z rodzaju pięciornik stanowi istotny wkład w określenie zależności chemofenetycznych między gatunkami z rodzaju *Potentilla s. l.* oraz w rodzinie różowatych (Rosaceae). Jednocześnie przeprowadzenie badań biologicznych dotyczących wpływu badanych gatunków na prawidłowe oraz nowotworowe linie komórkowe w obrębie przewodu pokarmowego, pozwoli na określenie potencjalnego wykorzystania ich w bezpiecznej i skutecznej fitoterapii jako bogate źródło związków biologicznie aktywnych, w szczególności o charakterze polifenolowym, o szerokim zakresie działania farmakologicznego. Tym samym, mogą stać się podstawą do zaproponowania i opracowania nowych preparatów roślinnych o charakterze polifenolowym wykorzystywanych w profilaktyce zdrowotnej.

Rozdział 3. Cel pracy

Celem podjętej tematyki badawczej pracy doktorskiej była charakterystyka składu chemicznego nadziemnych oraz podziemnych części wybranych gatunków z rodzaju *Potentilla* L. *s. l.*, a także ocena ich aktywności biologicznej.

Szczegółowe cele badawcze dotyczyły:

1. Otrzymania wyciągów wodno-acetonowych z wybranych gatunków z rodzaju *Potentilla* L. i następnie przeprowadzenie szczegółowej charakterystyki składników czynnych w oparciu o:

    a. zastosowanie ekstrakcji z zastosowaniem ultradźwięków oraz wstępne oczyszczenie z matrycy lipofilowej z zastosowaniem techniki frakcjonowania ciecz-ciecz (LLE) (**publikacja I** oraz **II**).
    b. oznaczenia ilościowej, całkowitej zawartości wybranych grup związków (polifenoli, kwasów fenolowych, garbników, proantocyjanidyn, flawonoidów) wykorzystując metody spektrofotometryczne (UV-Vis) (**publikacja I** oraz **II**).
    c. zoptymalizowanie warunków rozdzielenia chromatograficznego dopasowując parametry faz ruchomej i stacjonarnej, w tym warunki aparaturowe celem opracowania metody analitycznej do oceny jakościowej zawartości związków o charakterze polifenolowym z wykorzystaniem techniki chromatografii cieczowej sprzężonej ze spektrometrią masową (LC-PDA-HRMS) (**publikacja I** oraz **II**).

2. Oceny aktywności biologicznej w modelu *in vitro*, w tym wpływu na przeżywalność (MTT) i proliferację (BrdU) oraz cytotoksyczności wobec ludzkich komórek raka okrężnicy LS180 oraz prawidłowych komórek nabłonkowych okrężnicy CCD841 CoN w obecności wybranych ekstraktów z gatunków z rodzaju *Potentilla* L. (**publikacja I** oraz **II**).

3. Zestawienia i opisania w formie przeglądowej literatury dotyczącej składu fitochemicznego, etnofarmakologii oraz oceny potencjału biologicznego wyciągów, frakcji oraz czystych związków otrzymanych z gatunków z rodzaju *Potentilla* L. w latach 2009 - 2020 (**publikacja III**).

Rozdział 4. Realizacja celów naukowych, zwięzłe omówienie materiałów i metod badawczych, wyniki badań, podsumowanie i dyskusja będąca odniesieniem do publikacji stanowiących rozprawę z podaniem perspektyw dalszego rozwoju tematu

Badania eksperymentalne przeprowadzone w trakcie wykonywania rozprawy doktorskiej nie wymagały zgody Komisji Bioetycznej.

Rozdział 4.1. Materiał do badań

Materiał roślinny do badań stanowiły części nadziemne oraz podziemne wybranych gatunków z rodzaju *Potentilla* L. zebranych z ich naturalnego środowiska na terenach Puszczy Knyszyńskiej (województwo podlaskie, Polska; GPS 53°15′6″N 23°27′58″E) w okresie czerwiec-lipiec w latach 2017-2019 lub zebranych z roślin uprawnych na terenie ogrodu farmakognostycznego Uniwersytetu Medycznego w Białymstoku w latach 2016-2020. Dokładna lista gatunków wykorzystanych w badaniach oraz numery okazów zielnikowych zostały wyszczególnione w Tabeli 1. Próbki zebranego materiału roślinnego zostały wysuszone w cieniu i przewiewie, a następnie zidentyfikowane na podstawie dostępnej literatury botanicznej (**Publikacja I** i **II**) [10, 11]. Przygotowanym okazom zielnikowym zostały nadane numery wskazane w Tabeli 1. Materiał zielnikowy jest przechowywany w *Herbarium* Zakładu Farmakognozji Uniwersytetu Medycznego w Białymstoku.

Tabela 1. Lista gatunków wykorzystanych w badaniach z rodzaju pięciornik, numery okazów zielnikowych oraz wydajność ekstrakcji.

Próbka	Łacińska nazwa gatunkowa	Polska nazwa gatunkowa	Numer okazu zielnikowego	Materiał roślinny[1]	Wydajność ekstrakcji[2] (%)
PAL7	*Potentilla alba* L.	pięciornik biały	PAL-17039	z	12,4
PAL7r				k	11,2
PAR7	*Potentilla argentea* L.	pięciornik srebrny	PAR-02009	z	20,6
PAU7	*Potentilla aurea* L.	pięciornik złoty	PAU-20045	z	32,8
PER7	*Potentilla erecta* (L.) Raeusch	pięciornik kurze ziele	PER-06016	z	17,8
PER7r				k	15,7
PFR7	*Potentilla fruticosa* L.	pięciornik krzewiasty	PFR-06018	l	36,6
PGR7	*Potentilla grandiflora* L.	pięciornik wielkokwiatowy	PGR-06020	z	17,0
PHY7	*Potentilla hyparctica* Malte	pięciornik arktyczny	PHY-20046	z	26,5
PME7	*Potentilla megalantha* Takeda	pięciornik poziomkowaty	PME-18043	z	34,1
PNE7	*Potentilla nepalensis* Hook.	pięciornik nepalski	PNE-06023	z	33,4
PN7	*Potentilla norvegica* L.	pięciornik norweski	PNO-08024	z	24,8
PPE7	*Potentilla pensylvanica* L.	pięciornik pensylwański	PPS-08025	z	22,4
PPU7	*Potentilla pulcherrima* L.	pięciornik piękny	PPU-18044	z	28,0
PRE7	*Potentilla recta* L.	pięciornik wyprostowany	PRE-06019	z	20,8
PRI7	*Potentilla rigoi* Th. Wolf	brak polskiej nazwy	PRI-20047	z	30,6
PRU7	*Potentilla rupestris* L.	pięciornik skalny	PRU-06021	z	30,2
PTH7	*Potentilla thuringiaca* Bernh.	pięciornik turyngijski	PTH-06022	z	22,8

[1] z – części nadziemne, l – liście, k – części podziemne; [2] wydajność ekstrakcji oczyszczonej frakcji

Rozdział 4.2. Przygotowanie ekstraktów do badań

Każdorazowo surowce roślinne pozyskane z wybranych gatunków z rodzaju pięciornik (2 g każdy) zostały sproszkowane, a następnie poddane trzykrotnej ekstrakcji wspomaganej ultradźwiękami (sonifikacja) przy użyciu łaźni ultradźwiękowej Sonic-5 w kontrolowanej temperaturze (40°C ± 2°C) przy użyciu 150 mL 70% wodnego roztworu acetonu. Połączone ekstrakty zostały odparowane przy użyciu rotacyjnej wyparki próżniowej w kontrolowanej temperaturze (40°C ± 2°C). Pozostałości zostały następnie strącone w wodzie i pozostawione w chłodni na 24 godziny, następnie po odwirowaniu i przefiltrowaniu zostały poddane wyczerpującemu procesowi frakcjonowania ciecz-ciecz (LLE) uzyskując frakcje chloroformowe (10 x 20 mL) oraz oczyszczone ekstrakty wodno-acetonowe. Wszystkie ekstrakty zostały następnie

zamrożone, zliofilizowane i zważone (**publikacja I** i **II**). Otrzymane ekstrakty oraz wydajność ekstrakcji zostały przedstawione w Tabeli 1.

Rozdział 4.3. Realizacja celów naukowych

Rozdział 4.3.1. Oznaczenia całkowitej zawartości polifenoli, garbników, proantocyjanidyn, flawonoidów i kwasów fenolowych w ekstraktach metodą spektrofotometryczną

Pierwszy etap ogólnej charakterystyki fitochemicznej obejmował analizę ilościową całkowitej zawartości polifenoli, garbników, proantocyjanidyn, flawonoidów i kwasów fenolowych z wykorzystaniem wybranych metod spektrofotometrycznych. Uzyskane wyniki analiz zostały przedstawione w Tabeli 2.

Tabela 2. Całkowita zawartość polifenoli (TPC), garbników (TTC), proantocyjanidyn (TPrC), flawonoidów (TFC) oraz kwasów fenolowych (TPAC) w wyciągach z wybranych gatunków z rodzaju *Potentilla*.

Próbka	TPC (mg GAE/g ekstraktu)[A]	TTC (mg GAE/g ekstraktu)[A]	TPrC (mg CE/g ekstraktu)[B]	TFC (mg RE/g ekstraktu)[C]	TPAC (mg CAE/g ekstraktu)[D]
PAL7	159,9 ± 1,8	84,9 ± 1,4	21,3 ± 0,1	71,9 ± 1,4	124,2 ± 1,2
PAL7r	268,6 ± 6,9	237,6 ± 5,7	72,6 ± 2,5	15,0 ± 0,3	221,1 ± 7,0
PAR7	339,7 ± 5,3	246,9 ± 4,6	7,0 ± 0,1	56,8 ± 1 ,0	79,0 ± 0,9
PAU7	148,4 ± 2,3	129,2 ± 2,0	3,4 ± 0,1	59,7 ± 1,3	44,2 ± 1,4
PER7	201,2 ± 4,3	169,2 ± 7,0	2,1 ± 0,1	54,9 ± 0,4	59,9 ± 1,3
PER7r	326,3 ± 3,5	269,8 ± 2,4	61,6 ± 1,1	11,0 ± 0,1	263,5 ± 7,5
PFR7	240,1 ± 6,1	178,7 ± 5,5	53,6 ± 0,9	94,6 ± 2,4	197,8 ± 6,2
PGR7	228,4 ± 3,4	156,5 ± 3,7	3,8 ± 0,1	47,6 ± 0,4	58,6 ± 0,3
PHY7	199,2 ± 1,7	178,2 ± 3,9	1,6 ± 0,1	113,3 ± 1,5	44,0 ± 1,1
PME7	195,3 ± 4,4	168,5 ± 3,6	13,1 ± 0,4	84,6 ± 0,1	80,8 ± 2,0
PNE7	188,8 ± 2,5	163,5 ± 0,5	1,1 ± 0,1	66,5 ± 2,5	33,4 ± 0,3
PN7	332,1 ± 1,4	252,3 ± 1,7	1,1 ± 0,1	38,1 ± 0,8	92,8 ± 1,0
PPE7	218,9 ± 1,8	196,0 ± 3,1	0,2 ± 0,1	108,2 ± 0,5	50,5 ± 0,5
PPU7	151,5 ± 2,4	135,9 ± 2,4	5,5 ± 0,1	64,9 ± 0,6	50,2 ± 2,1
PRE7	257,7 ± 3,0	170,5 ± 2,9	2,7 ± 0,1	43,4 ± 0,9	75,2 ± 1,2
PRI7	212,2 ± 5,5	170,5 ± 4,4	5,6 ± 0,6	84,4 ± 0,7	58,1 ± 1,7
PRU7	304,1 ± 2,5	209,4 ± 2,6	1,1 ± 0,1	47,7 ± 0,7	55,5 ± 0,6
PTH7	149,8 ± 2,3	132,6 ± 2,3	4,9 ± 0,1	76,4 ± 1,6	58,8 ± 2,5

[A] – wyrażone jako ekwiwalent kwasu galusowego; [B] – wyrażone jako ekwiwalent katechiny; [C] – wyrażone jako ekwiwalent rutyny; [D] – wyrażone jako ekwiwalent kwasu kawowego; wyniki wyrażone jako średnia i odchylenie standardowe z minimum 3 pomiarów.

Całkowita zawartość związków polifenolowych w ekstraktach wahała się od 148,4 mg GAE/g dla pięciornika złotego (**PAU7**) do 339,7 mg GAE/g dla pięciornika srebrnego (**PAR7**), a natomiast całkowita zawartość garbników we wszystkich badanych ekstraktach mieściła się w granicach od 84,9 mg GAE/g dla pięciornika białego (**PAL7**) do 269,8 mg GAE/g dla pięciornika kurze ziele (**PER7r**). W oparciu o uzyskane wyniki zawartość proantocyjanidyn była najwyższa w ekstraktach uzyskanych z części podziemnych, a mianowicie **PAL7r** (72,6 mg CE/g) i **PER7r** (61,6 mg CE/g) oraz w liściach pięciornika krzewiastego **PFR7** (53,6 mg CE/g). Ocena ilościowej analizy związków flawonoidowych wykazała wysoką ich zawartość w pięciorniku arktycznym **PHY7** (113,3 mg RE/g) oraz pięciorniku pensylwańskim **PPE7** (108,2 mg RE/g). Natomiast najwyższą całkowitą zawartością kwasów fenolowych charakteryzowały się ekstrakty **PAL7r** (221,1 mg CAE/g) a także **PER7r** (263,5 mg CAE/g) (**publikacja I** oraz **II**).

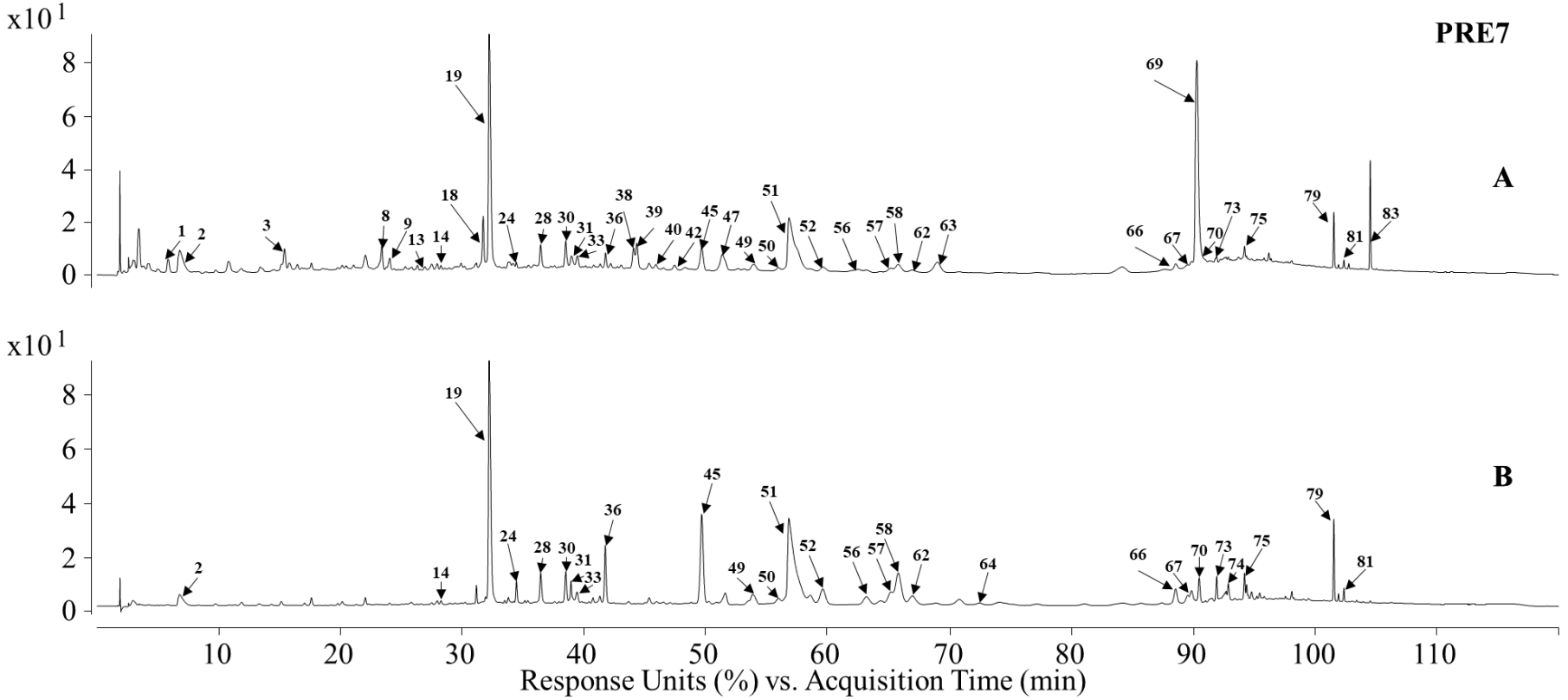
Rozdział 4.3.2. Analiza jakościowa wybranych ekstraktów techniką LC-PDA-HRMS

Ekstrakty wodno-acetonowe uzyskane z części nadziemnych oraz podziemnych wybranych do badań gatunków zbadano jakościowo przy wykorzystaniu techniki chromatografii cieczowej sprzężonej z detektorem z matrycą fotodiodową (PDA) oraz z wysokorozdzielczym detektorem

masowym (HRMS). Przeprowadzona została optymalizacja rozdzielenia chromatograficznego składników matrycy roślinnej. Zastosowana kolumna chromatograficzna ze modyfikowaną krzemionką C18 pozwoliła na skuteczne rozdzielenie metabolitów wtórnych przez zwiększenie ich stopnia retencji. Rozdział został przeprowadzony przy użyciu chromatografu cieczowego Agilent 1260 Infinity LC system na kolumnie analitycznej KINETEX XB-C18 (150 x 2,1 mm, wymiary cząstek 1,7 μm) z wykorzystaniem gradientu fazy ruchomej składającej się z ultra czystej wody (A) oraz acetonitrylu (B) z dodatkiem 0,2 % kwasu mrówkowego. Oznaczenie zostało wykonane przy zastosowaniu następującego programu gradientu: 0–3 min 1% B; 3–35 min, 1-12% B; 35–80 min, 12% B; 80–113 min, 12-45% B; przedłużonego o 7 min. równoważenie kolumny. Przepływ wynosił 0,2 mL/min, a temperaturę utrzymywano na poziomie 35°C ± 0,8. Rejestracja widm UV prowadzona była w zakresie 190-540 nm, a chromatogramów przy 280 i 360 nm. Detekcja masowa (MS) została przeprowadzona za pomocą wysokorozdzielczego spektrometru masowego Agilent 6230 LC/TOF, wyposażonego w źródło jonizacji typu elektrorozpylenie (ESI) z ogniskowaniem termicznym dla pozytywnego i negatywnego trybu jonizacji. Zastosowane zostały następujące parametry: przepływ gazu 11 L/min, temperatura gazu 350°C, ciśnienie nebulizatora 60 psi, napięcie na kapilarze 2500 i 4500 V, odpowiednio dla trybów jonizacji ujemnej i dodatniej oraz eksperymenty z fragmentorem przy 60, 180 i 320 V. Dane były rejestrowane w zakresie 120–3000 m/z. (**publikacja I**).

Rozdział 4.3.3. Analiza profili fitochemicznych ekstraktów techniką LC-PDA-HRMS

W celu identyfikacji związków występujących w badanych gatunkach wszystkie ekstrakty wodno-acetonowe zostały poddane profilowaniu z wykorzystaniem opracowanej nowej metody analitycznej (**publikacja I** oraz **II**). Szczegółowe wyniki analiz chromatograficznych zostały przestawione w Tabeli 3 oraz w **suplemencie do publikacji I** (Ryciny 1-6) oraz **suplemencie do publikacji II** (Ryciny S1-S12). Numery pików z analiz na chromatogramach zostały oznaczone jako P1 – numer piku w **Publikacji I** dla ekstraktów **PAL7**, **PAR7**, **PGR7**, **PN7**, **PRE7** i **PRU7**, oraz P2 – numer piku w **Publikacji II** dla reszty ekstraktów. Reprezentatywny chromatogram dla ekstraktu **PRE7** został zaprezentowany na Rycinie 2. Przeprowadzona została optymalizacja rozdziału związków obecnych w matrycy, co pozwoliło na wykazanie obecności 217 metabolitów wtórnych o charakterze polifenoli, w tym garbników, flawonoidów oraz kwasów fenolowych. Niemal wszystkie ekstrakty charakteryzowały się obecnością głównie garbników hydrolizujących zaliczanych do grupy elagotanin oraz galotanin, reprezentowanych przez agrimoninę (pik chromatograficzny w P1 jako związek **69** / pik chromatograficzny w P2 jako związek **162**), pedunkulaginę α i β (P1 – związki **3**, **8** / P2 – związki **6**, **18**), izomery lewigatyny (P1 – związki **39**, **40**, **47** / P2 – związki **84**, **109**, **114**, **124**, **128**) oraz produktów ich degradacji, takich jak kwas elagowy (P1 – związek **51**/ P2 – związek **135**) oraz kwas brewifolinokarboksylowy (P1 – związek **19** / P2 – związek **46**). Ponadto, analiza LC-PDA-HRMS ekstraktów **PAL7** oraz **PAL7r** otrzymanych z odpowiednio części nadziemnych oraz podziemnych pięciornika białego wykazała przewagę w nich garbników skondensowanych, wśród których dominowały procyjanidyny di- oraz trimeryczne, w tym procyjanidyny typu B - procyjanidyna B1 (P1 – związek **12/** P2 – związek **25**), procyjanidyna B2 (P1 – związek **20** / P2 – związek **47**), oraz procyjanidyna C1 (P1 – związek **37**/ P2 – związek **94**). Ekstrakty były również bogatym źródłem kwasów fenolowych, w tym pochodnych kwasu kawowego, kumarowego, syryngowego oraz galusowego. Wszystkie ekstrakty przygotowane z części zielnych zawierały pochodną fenoloamidową, N^1, N^5, N^{10}-trikumaroilospermidynę (P1 – związek **83** / P2 – związek **197**). Ponadto, ekstrakty z części nadziemnych charakteryzowały się wysoką zawartością związków flawonoidowych, pochodnych kwercetyny, kemferolu, izoramnetyny, apigeniny, trycyny, oraz naryngeniny. Wśród nich tylirozyd (3-*O*-(6′′-*O*-*trans*-*p*-kumaroilo)-glukozyd kemferolu, P1 – związek **79** / P2 – związek **190**), astragalina (3-*O*-glukozyd kemferolu, P1 – związek **66** / P2 – związek **155**), rutyna (3-*O*-rutynozyd kwercetyny, P1 – związek **56** / P2 – związek **138**) oraz hiperozyd (3-*O*-galaktozyd kwercetyny, P1 – związek **57** / P2 – związek **139**) są charakterystycznymi związkami dla rodzaju pięciornik.



Rycina 2. Chromatogram LC-MS przykładowego ekstraktu wodno-acetonowego uzyskanego z części nadziemnych pięciornika wyprostowanego (**PRE7**) w widmie UV 280 nm (A) oraz 360 nm (B). Oznaczenia zidentyfikowanych metabolitów wtórnych zgodne z Tabelą 3.

Tabela 3. Dane MS oraz UV-Vis związków zidentyfikowanych w ekstraktach wodno-acetonowych z wybranych gatunków z rodzaju *Potentilla* metodą chromatografii cieczowej z detekcją UV-Vis oraz wysokorozdzielczą spektrometrią mas LC-PDA-HRMS.

Nr		Nazwa związku	Rt	λ maks	Obserwowany jon[1]	Δ^2	Wzór	Fragmentacja w jonizacji		Obecność w ekstraktach																	
P1	P2		[min]	[nm]		[ppm]	sumaryczny	Negatywnej	Pozytywnej	PAL7	PAL7r	PAR7	PAU7	PER7	PER7r	PFR7	PGR7	PHY7	PME7	PNE7	PN7	PPE7	PPU7	PRE7	PRI7	PRU7	PTH7
1	1	kwas galusowy[W]	5,72	270	169,01335	-3,02	$C_7H_6O_5$	**169**, 125				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	2	kwas 2-pirono-4,6-dikarboksylowy	6,82	316	182,99292	-2,56	$C_7H_4O_6$	366, **183**, 139	**185**			+	+	+	+	+	+		+	+	+	+	+	+	+	+	+
	3	kwas bis-HHDP-glukuronowy	11,2	255*sh*	799,06359	0,27	$C_{34}H_{24}O_{23}$	**799**, 497, 301											+						+		
	4	niezidentyfikowany związek polifenolowy	13,25	310	281,02976	-1,48	$C_{12}H_{10}O_8$	**281**, 237	**283**, 191, 163					+					+	+		+					
	5	izomer kwasu kawoiloglukuronowego	15,26	298, 326	371,06060	-1,98	$C_{15}H_{16}O_{11}$	**371**, 209, 191										+	+				+				+
3	6	pedunkulagina α lub β[W]	15,35	260*sh*	783,06839	-0,48	$C_{34}H_{24}O_{22}$	**783**, 481, 301				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4		niezidentyfikowany związek polifenolowy	15,7	280	337,11359	-1,03	$C_{13}H_{22}O_{10}$	**337**, 193, 125		+																	
	7	trimeryczna procyjanidyna typu B	15,9	278	865,19739	-1,98	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 291		+																
	8	bis-HHDP-glukoza	16,58	260*sh*	783,06730	-1,29	$C_{34}H_{24}O_{22}$	**783**, 481, 301													+		+				
	9	izomer kwasu *O*-kawoiloglukuronowego	17,75	310*sh*, 326	371,06162	-1,12	$C_{15}H_{16}O_{11}$	**371**, 209, 191						+				+	+				+	+			+
5	10	kwas 5-*O*-kawoilochinowy[W]	20,09	295sh, 325	353,08747	-0,96	$C_{16}H_{18}O_9$	**353**, 191, 179	355, **163**	+		+					+				+		+		+		
	11	izomer *O*-heks katechiny lub epikatechiny	20,35	278	451,12458	-1,55	$C_{21}H_{24}O_{11}$	**451**, 289, 245	**291**		+					+											
	12	izomer kwasu *O*-p-kumaroiloglukarowego	20,42	312	355,06630	-1,84	$C_{15}H_{16}O_{10}$	**355**, 209, 191, 147															+	+			+
	13	pent kwasu dihydroksybenzoesowego	21,9	280	285,06146	-0,55	$C_{12}H_{14}O_8$	**285**, 152																+			+
	14	*O*-glu estru metylowego kwasu galusowego	22,01	268	345,08239	-1,59	$C_{14}H_{18}O_{10}$	**345**, 183, 168	**185**																+		+
	15	izomer kwasu *O*-kawoiloglukuronowego	22,05	300, 326	371,06085	-3,3	$C_{15}H_{16}O_{11}$	**371**, 209, 191										+	+				+	+			
6	16	galoilo-HHDP-glukoza	22,26	250*sh*	633,07245	-0,24	$C_{27}H_{22}O_{18}$	**633**, 301										+		+						+	
7		niezidentyfikowany związek polifenolowy	22,45	276	345,11788	-2,82	$C_{15}H_{22}O_9$	**345**, 299, 161																		+	
	17	pochodna kwasu *p*-kumarowego	23,12	312	355,06611	-2,36	$C_{15}H_{16}O_{10}$	**355**, 209, 191, 147															+	+			+
8	18	pedunkulagina α lub β[W]	23,3	260*sh*	783,06805	-0,82	$C_{34}H_{24}O_{22}$	**783**, 481, 301	**303**			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	19	*O*-heks dimerycznej procyjanidyny typu B	23,36	280	739,18619	-2,06	$C_{36}H_{36}O_{17}$	**739**, 451, 289	**741**, 579, 291		+																
	20	kwas digaloilo-HHDP-glukuronowy	23,89	274	801,07970	1,57	$C_{34}H_{26}O_{23}$	**801**, 633, 301, 169					+												+		
9	21	galoilo-HHDP-glukoza	24,13	280*sh*	633,07357	0,4	$C_{27}H_{22}O_{18}$	**633**, 481, 301					+		+	+	+	+	+	+	+	+	+	+	+		+
10		izomer kwasu *p*-kumaroilochinowego	24,41	308	337,09247	-0,92	$C_{16}H_{18}O_8$	**337**, 191, 163	**339**, 147	+																	

	22	izomer *O*-heks katechiny lub epikatechiny	24,45	280	451,12352	-2,38	$C_{21}H_{24}O_{11}$	**451**, 289	453, **291**		+						+														
	23	izomer *O*-heks katechiny lub epikatechiny	25,22	280	451,12257	-4,09	$C_{21}H_{24}O_{11}$	**451**, 289, 271	453, **291**		+																				
11		izomer kwasu *p*-kumaroilochinowego	24,41	308	337,09247	-0,92	$C_{16}H_{18}O_8$	**337**, 191, 163	**339**, 147	+																						
	24	tetrameryczna procyjanidyna typu A	25,6	280	1151,24372	-2,22	$C_{60}H_{48}O_{24}$	**1151**, 863, 575, 289	**1153**, 865, 577, 291		+																				
12	25	procyjanidyna B1[W]	25,9	280	577,13480	-0,55	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 289, 257		+	+	+		+			+				+								
	26	izomer kwasu *O*-feruloiloglukarowego	25,95	282, 326	385,07702	-2,26	$C_{16}H_{18}O_{11}$	**385**, 209, 191, 147										+				+	+							
	27	procyjanidyna B3[W]	26,25	280	577,13426	-1,28	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 289, 257						+	+		+					+		+		+			
13	28	katechina[W]	27,05	280	289,07096	-2,33	$C_{15}H_{14}O_6$	**289**, 245	**291**, 139		+	+	+	+	+	+		+				+	+	+	+		+			
	29	kwas kawowy[W]	27,55	292, 320*sh*	179,03455	-2,54	$C_9H_8O_4$	**179**, 135	**181**											+										
	30	izomer kwasu *O*-feruloiloglukarowego	27,98	300*sh*, 318	385,07654	-2,63	$C_{16}H_{18}O_{11}$	**385**, 209, 191, 147										+				+	+							
	31	digaloilo-pentoza	28,02	278	453,06751	0,08	$C_{19}H_{18}O_{13}$	**453**, 301						+						+										
14	32	kwas 3-*O*-kawoilochinowy[W]	28,35	295*sh*, 326	353,08691	-2,82	$C_{16}H_{18}O_9$	**353**, 191	355, **163**	+						+	+				+		+	+						
15	33	digaloilo-HHDP-glukoza	28,56	275	785,08401	-0,87	$C_{34}H_{26}O_{22}$	**785**, 301, 275								+											+	+		
	34	procyjanidyna C2	28,87	280	865,19788	-0,51	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 289		+				+	+		+											+	
	35	digaloilo-glukoza	29,32	276	483,07714	-2,2	$C_{20}H_{20}O_{14}$	**483**, 169, 125								+														
16		izomer kwasu feruloilochinowego	29,86	295*sh*, 325	367,10365	0,61	$C_{17}H_{20}O_9$			+							+													
	36	digaloilo-glukoza	29,98	278	483,07745	-1,11	$C_{20}H_{20}O_{14}$	**483**, 169, 125					+			+		+		+		+							
	37	izomer lewigatyny E	30,2	274	1265,13990	1,26	$C_{54}H_{42}O_{36}$	**1265**, 632, 301							+														+
	38	kwas metylogaloilojabłkowy	30,77	278	299,04042	-1,91	$C_{12}H_{12}O_9$	**299**, 183, 168, 133																				+		
	39	*O*-heks-*O*-deoksyheks-heks kwercetyny	30,9	254, 354	771,19840	-0,79	$C_{33}H_{40}O_{21}$	**771**, 609, 462, 299	**773**, 611, 465, 303				+																	
17		izomer kwasu kawoilochinowego	30,91	295*sh*, 325	353,08779	-0,08	$C_{16}H_{20}O_9$	**353**, 191, 179	355, **163**	+																				
	40	izomer lewigatyny E	31,07	275*sh*	1265,13669	-1,27	$C_{54}H_{42}O_{36}$	**1265**, 632, 301										+												
	41	izomer *O*-heks katechiny lub epikatechiny	31,43	280	451,12343	-2,47	$C_{21}H_{24}O_{11}$	**451**, 289	**453**, 291		+																			
	42	trimeryczna procyjanidyna typu B	31,54	280	865,19810	0,01	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 291		+					+		+											
18	43	galoilo-HHDP-glukoza	31,73	272	633,07313	-0,04	$C_{27}H_{22}O_{18}$	**633**, 481, 301				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	44	*O*-heks-*O*-heks-pent kwercetyny	31,82	256, 354	757,18267	-0,82	$C_{32}H_{38}O_{21}$	**757**, 462, 299	**759**, 627, 465, 303				+																	
	45	trimeryczna procyjanidyna typu B	32,01	280	865,19703	-1,1	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 291		+				+															
19	46	kwas brewifolinokarboksylowy	32,32	278, 360	291,01408	-1,94	$C_{13}H_8O_8$	**291**, 247	**293**			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	47	procyjanidyna B2[W]	33,71	278	577,13502	-0,04	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 291, 139	+	+																			
	48	galoilo-HHDP-glukoza	33,8	256, 342	633,07336	-0,48	$C_{27}H_{22}O_{18}$	**633**, 481, 301																			+			+

21	49	pochodna kwasu elagowego	33,84	280*sh*	898,13120	2,12	$C_{36}H_{35}O_{27}$	**898**, 783, 633, 301				+				+	+															
	50	izomer kwasu brewifolinokarboksylowego	34,01	284*sh*, 342	291,01448	-0,94	$C_{13}H_8O_8$	**291**, 247										+													+	
	51	*O*-heks-*O*-heks-pent kwercetyny	34,03	254, 342	757,18241	-0,85	$C_{32}H_{38}O_{21}$	**757**, 595, 462, 299	**759**, 597, 465, 303																		+					
22		pochodna kwasu elagowego	34,12	245*sh*, 325*sh*	632,06474	-1,85	$C_{27}H_{21}O_{18}$	632, 463, **301**								+																
	52	izomer lewigatyny E	34,19	245*sh*, 325*sh*	1265,13618	-1,68	$C_{54}H_{42}O_{36}$	**1265**, 632, 463, 301									+															
23	53	*O*-heks-*O*-uro pochodna kwercetyny	34,2	254, 346	639,11995	-0,44	$C_{27}H_{28}O_{18}$	**639**, 463, 300	**641**, 479, 303				+			+							+							+	
	54	tetrameryczna procyjanidyna typu A	34,46	280	1151,24437	-1,65	$C_{60}H_{48}O_{24}$	**1151**, 863, 575, 289	**1153**, 865, 577, 287		+																					
24	55	*O*-di-uro pochodna kwercetyny	34,5	256, 352	653,09909	-0,32	$C_{27}H_{26}O_{19}$	**653**, 447, 301	**655**, 479, 303			+		+		+	+	+						+	+				+	
	56	tetrameryczna procyjanidyna typu B	34,54	280	1153,26132	-0,53	$C_{60}H_{50}O_{24}$	**1153**, 576, 289	**1155**, 867, 577, 289						+																
25	57	izomer kwasu *p*-kumaroilochinowego	35,14	312	337,09286	0,02	$C_{16}H_{18}O_8$	**337**, 191	**339**, 147	+								+						+						
	58	*O*-heks-*O*-heks kwercetyna	35,15	256, 352	625,14017	-1,24	$C_{27}H_{30}O_{17}$	**625**, 462, 299	**627**, 465, 303				+								+		+			+		+			
26	59	*O*-heks-*O*-uro pochodna kwercetyny	35,4	254, 346	639,12105	-2,16	$C_{27}H_{28}O_{18}$	**639**, 463, 301	**641**, 465, 303			+	+						+		+		+					+	+		
	60	digaloilo-HHDP-glukoza	35,47	276	785,08456	-0,06	$C_{34}H_{26}O_{22}$	**785**, 615, 301, 169								+										+				
27	61	epikatechina[W]	35,74	280	289,07181	0,6	$C_{15}H_{14}O_6$	**289**, 245	**291**, 139	+	+				+															
	62	*O*-heks-*O*-uro pochodna kemferolu	36	264, 338	623,12581	0,57	$C_{27}H_{28}O_{17}$	**623**, 284	**625**, 463, 287					+																
	63	*O*-heks-*O*-heks-deoksyheks kwercetyna	36,03	256, 348	771,19893	-0,45	$C_{33}H_{40}O_{21}$	**771**, 462, 299	**773**, 627, 465, 303												+					+				
	64	trimeryczna procyjanidyna typu B	36,07	280	865,19912	0,04	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 291						+	+														
28	65	kwas 2-kawoiloizocytrynowy	36,32	300*sh*, 328	353,05058	-2,24	$C_{15}H_{14}O_{10}$	**353**, 191, 179, 173, 155												+	+	+		+	+	+	+	+		
	66	dimeryczna procyjanidyna typu B	36,35	280	577,13479	-0,45	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 291						+	+														
29		izomer kwasu *p*-kumaroilochinowego	36,52	312	337,09314	-0,26	$C_{16}H_{18}O_8$	**337**, 163	**339**, 147	+																					
	67	*O*-heks-deoksyheks-*O*-uro pochodna kemferolu	37,05	266, 346	769,18282	-0,4	$C_{33}H_{38}O_{21}$	**769**, 284	**771**, 625, 463, 287					+																
	68	dimeryczna elagotanina	37,55	270	1569,15737	-1,82	$C_{68}H_{50}O_{44}$	**1569**, 784, 469, 301																							+	
	69	dilakton kwasu walonowego	37,6	255*sh*, 362	469,00441	-0,32	$C_{21}H_{10}O_{13}$	**469**, 425, 301											+			+									
	70	pochodna kwasu elagowego	37,76	268, 342	741,18713	-0,79	$C_{32}H_{38}O_{20}$	**741**, 579, 446, 301																			+				

	71	trimeryczna procyjanidyna typu A	37,81	280	863,18110	-2,08	$C_{45}H_{36}O_{18}$	**863**, 711, 573, 411, 289	**865**, 575, 287		+																
	72	*O*-heks-deoksyheks-deoksyheks-*O*-uro pochodna kemferolu	37,86	266, 346	915,24077	-0,13	$C_{39}H_{48}O_{25}$	**915**, 285	**917**, 771, 625, 463, 287					+													
	73	*O*-galoilo- *O*-heks kwasu elagowego	38,3	250, 374	615,06204	-1,43	$C_{27}H_{20}O_{17}$	**615**, 463, 301							+			+				+	+				+
	74	*O*-uro pochodna kwercetyny	38,4	254, 350	725,11985	-0,75	$C_{30}H_{30}O_{21}$	**725**, 505, 300	**727**, 479, 303				+										+				+
30	75	kwas kawoilojabłkowy	38,54	298, 326	295,04504	-2,45	$C_{13}H_{12}O_{8}$	591, 179, **133**	**135**	+							+				+	+	+	+			+
	76	*O*-metylogaloilo-galoilo-glukoza	38,85	270	497,09317	-1,17	$C_{21}H_{22}O_{14}$	**497**, 345, 183, 169																	+		+
	77	dilakton kwasu sangwisorbowego	38,88	255*sh*, 362	469,00439	0,38	$C_{21}H_{10}O_{13}$	**469**, 425, 301											+								
31	78	*O*-di-uro pochodna kemferolu	38,92	265, 350	637,10483	0,37	$C_{27}H_{26}O_{18}$	**637**, 461, 285	**639**, 463, 287			+		+				+					+	+			
	79	*O*-(malonylo-heks)-*O*-heks kwercetyna	39,08	256, 354	711,14146	-0,09	$C_{30}H_{32}O_{20}$	**711**, 667, 462, 299	**713**, 551, 465, 303				+					+							+		
32	80	trigaloilo-glukoza	39,1	276	635,08854	-0,04	$C_{27}H_{24}O_{18}$	635, 465, 313, **169**								+						+			+	+	+
	81	*O*-heks-*O*-heks kemferolu	39,31	262, 348	609,14565	-1,26	$C_{27}H_{30}O_{16}$	**609**, 446, 283	**611**, 449, 287													+					+
	82	*O*-uro pochodna kwasu elagowego	39,4	252, 362	477,03029	-1,24	$C_{20}H_{14}O_{14}$	**477**, 301							+												
33	83	brewifolina	39,6	275, 350	247,02448	-1,92	$C_{12}H_{8}O_{6}$	**247**, 191	**249**			+		+		+	+		+	+	+	+		+		+	+
	84	izomer lewigatyny	39,77	255	1567,14302	-0,99	$C_{68}H_{48}O_{44}$	1567, **783**, 633, 301																	+		+
	85	*O*-deoksyheks-*O*-deoksyheks-heks kwercetyny	39,8	256, 354	755,20392	-0,22	$C_{33}H_{40}O_{20}$	**755**, 609, 446, 299	**757**, 611, 449, 303				+														
	86	trigaloilo-glukoza	40,05	276	635,08863	-0,5	$C_{27}H_{24}O_{18}$	635, 465, 313, **169**								+											
	87	*O*-heks-*O*-heks-pent izoramnetyny	40,1	254, 352	771,19887	-0,64	$C_{33}H_{40}O_{21}$	**771**, 476, 315, 300	**773**, 641, 479, 317																+		
	88	*O*-galoilo dimeryczna procyjanidyna typu B	40,28	278	729,14551	-0,59	$C_{37}H_{30}O_{16}$	**729**, 577, 559, 289, 169	**731**, 289							+											
	89	pochodna kwasu syryngowego	40,44	280	313,05569	-2,4	$C_{13}H_{14}O_{9}$	**313**, 197, 182																	+		
34	90	tetrameryczna procyjanidyna typu A	40,52	280	863,18180	-0,75	$C_{45}H_{36}O_{18}$	**863**, 573, 411, 289	**865**, 287	+	+																
	91	*O*-heks-*O*-uro pochodna izoramnetyny	40,67	266, 348	653,13595	0,25	$C_{28}H_{30}O_{18}$	**653**, 477, 314	**655**, 479, 317																		+
	92	*C*-di-heks apigeniny	40,75	270, 332	593,15106	-0,09	$C_{27}H_{30}O_{15}$	**593**, 473, 383, 353	**595**, 439, 355, 325				+										+				+
	93	tetrameryczna procyjanidyna typu B	40,8	280	1153,25158	-0,3	$C_{60}H_{50}O_{24}$	**1153**, 863, 576, 289	**1155**, 865, 577, 289						+												
35		*O*-heks kwasu elagowego	41,2	252, 365	463,05162	-0,79	$C_{20}H_{16}O_{13}$	**463**, 301													+					+	

	95	*O*-uro pochodna kwasu elagowego	41,47	252, 360	477,03021	-0,63	$C_{20}H_{14}O_{14}$	**477**, 301							+														
	96	tetrameryczna procyjanidyna typu A	41,51	280	1151,24448	-1,56	$C_{60}H_{48}O_{24}$	**1151**, 863, 575, 289	**1153**, 865, 577, 287		+																			
	97	*O*-heks kwasu elagowego	41,64	252, 362	463,05127	-0,58	$C_{20}H_{16}O_{13}$	**463**, 301						+	+			+	+		+	+		+		+			
36	98	*O*-di-uro pochodna izoramnetyny	41,8	254, 352	667,11526	0,2	$C_{28}H_{28}O_{19}$	1335, **667**, 491, 315	**669**, 493, 317			+		+			+	+						+	+			+	
37	94	procyjanidyna C1[W]	41,88	280	865,19784	-0,81	$C_{45}H_{38}O_{18}$	**865**, 577, 289	**867**, 579, 291	+	+																		
	99	*O*-deoksyheks-*O*-heks-pent kwercetyny	42,35	254, 352	741,18832	-0,66	$C_{32}H_{38}O_{20}$	**741**, 446, 299	**743**, 611, 449, 303				+																
	100	*O*-heksoso-*O*-uro pochodna izoramnetyny	42,43	254, 352	653,13560	-0,3	$C_{28}H_{30}O_{18}$	**653**, 477, 315	**655**, 479, 317									+											
	101	*O*-heks kwasu elagowego	42,7	250, 370	463,05107	-1,61	$C_{20}H_{16}O_{13}$	**463**, 301						+			+				+			+					
	102	kwas agrimonowy A lub B	43,11	270*sh*	1103,08618	0,64	$C_{43}H_{32}O_{31}$	**1103**, 935, 783, 301, 169						+								+							+
	103	tetragaloilo-glukoza	43,13	278	787,10004	-0,98	$C_{34}H_{28}O_{22}$	**787**, 465, 169							+														
	104	*O*-deoksyheks-heks-*O*-deoksyheks kemferolu	43,16	266, 346	739,20805	-0,74	$C_{33}H_{40}O_{19}$	**739**, 593, 430, 283	**741**, 595, 433, 287				+																+
	105	*O*-heks-*O*-heks kwercetyny	43,17	264, 344	625,14038	-1,14	$C_{27}H_{30}O_{17}$	**625**, 463, 300	**627**, 465, 303															+					
	106	izomer *O*-heks katechiny lub epikatechiny	43,3	278	451,12511	0,25	$C_{21}H_{24}O_{11}$	**451**, 289	**289**								+												
	107	dimeryczna procyjanidyna typu B	43,6	280	577,13537	-0,24	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 287		+						+												
38	108	galoilo-bis-HHDP-glukoza	44,2	255	935,08057	-0,13	$C_{41}H_{28}O_{26}$	**935**, 633, 467, 301				+	+	+		+			+	+	+	+		+	+	+	+	
39	109	izomer lewigatyny	44,6	255	1567,14331	-0,8	$C_{68}H_{48}O_{44}$	1567, **783**, 633, 301				+	+	+	+	+	+	+			+		+	+	+	+	+	
	110	tetrameryczna procyjanidyna typu A	45,03	280	1151,24657	0,26	$C_{60}H_{48}O_{24}$	**1151**, 863, 575, 289	**1153**, 865, 577, 289		+																		
	111	*O*-heks-*O*-heks kwercetyny	45,39	254, 346	625,14019	-1,3	$C_{27}H_{30}O_{17}$	**625**, 463, 300	**627**, 465, 303															+					
	112	HHDP-NHTP-glukoza	45,56	254	933,06390	-0,43	$C_{41}H_{26}O_{26}$	**933**, 631, 466, 301						+					+	+									
	113	*O*-di-uro pochodna kemferolu	45,86	266, 336	637,10484	-0,55	$C_{27}H_{26}O_{18}$	**637**, 461, 285	**639**, 463, 287				+																
40	114	izomer lewigatyny	45,99	255	1567,14487	0,19	$C_{68}H_{48}O_{44}$	1567, **783**, 301								+	+							+	+				
	115	pentameryczna procyjanidyna typu B	46,13	278	1441,32708	1,23	$C_{75}H_{62}O_{30}$	**1441**, 1153, 863, 575, 289	**1443**, 1155, 865, 577, 289						+														
	116	*O*-heks-*O*-uro pochodna kwercetyny	46,5	264, 340*sh*	639,12041	0,17	$C_{27}H_{28}O_{18}$	**639**, 463, 301	**641**, 465, 303															+					
	117	*O*-heks-*O*-heks-deoksyheks kemferolu	46,63	264, 350*sh*	755,20300	-0,67	$C_{33}H_{40}O_{20}$	**755**, 593, 447, 285	**757**, 595, 449, 287									+				+							+
41		*O*-heks-*O*-deoksyheks kwercetyny	46,98	255, 352	609,14615	-0,73	$C_{27}H_{30}O_{16}$	**609**, 446, 299	**611**, 499, 303											+									
42	118	galoilo-bis-HHDP-glukoza	47,7	275*sh*	935,07950	-0,36	$C_{41}H_{28}O_{26}$	**935**, 633, 467, 301				+							+	+	+	+		+		+	+	
43		*O*-heks-deoksyheks-pent kwercetyny	47,9	255, 355	741,18912	-0,64	$C_{32}H_{38}O_{20}$	741, 447, **300**	743, 611, 465, **303**	+																			

44		*O*-deoksyheks-*O*-heks-deoksyheks kwercetyny	48,66	256, 356	755,20326	-0,67	$C_{33}H_{40}O_{20}$	**755**, 609, 446, 299	**757**, 611, 449, 303									+												
	119	*C*-heks-*C*-pent apigeniny	49,2	268, 340	563,13970	-1,45	$C_{26}H_{28}O_{14}$	**563**, 519, 473, 443, 383, 353	**565**, 379, 355, 325																					+
45	120	*O*-heks-pent kwercetyny	50,08	255, 352	595,13009	0,61	$C_{26}H_{28}O_{16}$	**595**, 300	597, 465, **303**	+					+			+			+			+					
46	121	*O*-heks-pent kwercetyny	50,95	256, 354	595,12982	-0,59	$C_{26}H_{28}O_{16}$	**595**, 300	**597**, 465, 303	+		+			+						+						+		
	122	*O*-deoksyheks-*O*-uro pochodna kwercetyny	51,17	254, 352	623,12438	-1,13	$C_{27}H_{28}O_{17}$	**623**, 301	**625**, 479, 303															+					
	123	kwas feruloiloizocytrynowy	51,46	284, 326	367,06617	-2,08	$C_{16}H_{16}O_{10}$	**367**, 173											+		+								+
47	124	izomer lewigatyny	51,56	255	1567,14348	-0,69	$C_{68}H_{48}O_{44}$	1567, **783**, 301			+	+		+	+		+			+		+	+		+	+
	125	*O*-heks-heks izoramnetyny	51,65	270, 350	639,15591	-1,2	$C_{28}H_{32}O_{17}$	**639**, 314, 300	641, 479, **317**								+	+	+		+	+		+			+
	126	*O*-deoksyheks-heks kwercetyny	52,15	256, 354	609,11075	-0,28	$C_{27}H_{30}O_{16}$	**609**, 300	**611**, 449, 303			+																
48		*O*-pent-*O*-uro pochodna kwercetyny	52,3	255, 354	609,11075	-1,11	$C_{26}H_{26}O_{17}$	609, **301**	611, **479**, 303		+																+	
	127	*O*-galoilo-heks kwercetyny	52,7	264, 352	615,09845	-1,15	$C_{28}H_{24}O_{16}$	**615**, 463, 300, 169	**617**, 303						+													
	128	izomer lewigatyny	52,76	255	1567,14432	-0,15	$C_{68}H_{48}O_{44}$	1567, **783**, 301						+			+					+	+				+
	129	*O*-pent-heks kwercetyny	53,41	256, 354	595,12983	-1,16	$C_{26}H_{28}O_{16}$	**595**, 300	**597**, 435, 303																+			
49	130	*O*-metylo *O*-uro pochodna kwasu elagowego	54,1	254, 360	491,04680	0,22	$C_{21}H_{16}O_{14}$	**491**, 315, 299,9			+			+		+		+		+		+	+	+	+			+
	131	trigaloilo-HHDP-glukoza	54,3	270	937,09372	-1,14	$C_{41}H_{30}O_{26}$	**937**, 783, 468, 301, 169				+						+								+		
50	132	*O*-pent kwasu elagowego	56,35	252, 360	433,04120	-0,54	$C_{19}H_{14}O_{12}$	**463**, 301			+			+					+	+					+			+
	133	trigaloilo-HHDP-glukoza	56,57	278	937,09504	-0,77	$C_{41}H_{30}O_{26}$	**937**, 468, 301																		+			+
	134	*O*-galoilo-heks kwercetyny	57,03	264, 354	615,09882	-1,5	$C_{28}H_{24}O_{16}$	**615**, 463, 300, 169	**617**, 303						+													
51	135	kwas elagowy[W]	57,7	254, 370	300,99864	-0,98	$C_{14}H_6O_8$	**301**, 275	**303**		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
52	136	izokwercytryna (3-*O*-glu kwercetyny)[W]	59,8	254, 354	463,08790	-0,94	$C_{21}H_{20}O_{12}$	**463**, 300, 271	**465**, 303						+	+		+		+	+	+	+			+
53		niezidentyfikowany związek polifenolowy	59,8	290	435,09238	-2,77	$C_{20}H_{20}O_{11}$	871, **435**, 285, 151																			+	
54		*O*-metylo *O*-pent kwasu elagowego	60,4	252, 362	447,05600	-1,85	$C_{20}H_{16}O_{12}$	**447**, 301																			+	
55	137	tetragaloilo-glukoza	62	278	787,09906	-1,22	$C_{34}H_{28}O_{22}$	**787**, 465, 169							+												+	+
56	138	rutyna (3-*O*-rut kwercetyny)[W]	63,7	256, 354	609,14556	-0,6	$C_{27}H_{30}O_{16}$	**609**, 300, 271	**611**, 465, 303						+	+			+	+		+		+	+		
57	139	hiperozyd (3-*O*-gal kwercetyny)[W]	64,13	255, 355	463,08829	-0,76	$C_{21}H_{20}O_{12}$	**463**, 300	**465**, 303	+		+	+			+	+	+		+		+	+	+	+	+	+	+
58		3-*O*-gluc kwercetyny[W]	64,83	255, 355	477,06649	-1,73	$C_{21}H_{20}O_{13}$	**477**, 300	**479**, 303		+															+		+	
59	140	*O*-heks-pent kemferolu	65,75	266, 348	579,13529	-0,02	$C_{26}H_{28}O_{15}$	**579**, 284	581, 449, **287**	+									+									
60	141	*O*-uro pochodna kwercetyny	66,03	256, 354	477,06730	-0,23	$C_{21}H_{18}O_{13}$	**477**, 301	**479**, 303					+		+	+	+		+		+	+					+
61		*O*-heks-pent kemferolu	66,85	265, 350	579,13520	-0,41	$C_{26}H_{28}O_{15}$	577, **284**	581, **449**, 287	+																		
62	142	*O*-heks kemferolu	67,4	252, 350	447,09290	-1,32	$C_{21}H_{20}O_{11}$	**447**, 284	**449**, 287			+				+							+	+				
	143	*O*-uro pochodna kemferolu	68,88	254, 348	461,07221	-1,04	$C_{21}H_{18}O_{12}$	**461**, 285	**463**, 287			+																
63	144	galoilo-bis-HHDP-glukoza	69,66	260*sh*	935,07940	-0,31	$C_{41}H_{28}O_{26}$	**935**, 467, 301			+	+		+	+	+	+	+	+	+	+	+	+	+	+		+

	145	*O*-pent-*O*-pent-uro pochodna kwercetyny	71,48	254, 352	739,17255	-0,32	$C_{32}H_{36}O_{20}$	**739**, 300	**741**, 609, 433, 303										+								
	146	dimeryczna elagotanina	72,47	270	1871,16610	-0,21	$C_{82}H_{56}O_{52}$	1871, 1265, **935**, 783, 301																	+		
	147	*O*-deoksyheks-heks kemferolu	72,86	266, 345*sh*	579,13479	-1,17	$C_{26}H_{28}O_{15}$	**579**, 284	**581**, 449, 287																+		
64		*O*-heks-pent izoramnetyny	73,99	255, 352	609,14611	0,2	$C_{27}H_{30}O_{16}$	**609**, 314, 271	611, 479, **317**	+														+			
	148	*O*-deoksyheks-deoksyheks-*O*-heks kemferolu	74,17	264, 346	739,20843	-0,33	$C_{33}H_{40}O_{19}$	**739**, 593, 284	**741**, 595, 449, 287					+													
65	149	awikularyna (3-*O*-ara kwercetyny)[W]	85,55	254*sh*, 350	433,07623	-3,28	$C_{20}H_{18}O_{11}$	**433**, 300	435, **303**							+										+	
	150	*O*-pent-heks izoramnetyny	86,15	254, 354	609,14566	-1,28	$C_{27}H_{30}O_{16}$	**609**, 314, 300	**611**, 479, 317																+		
	151	dimeryczna elagotanina	87,1	250sh	1869,14746	-1,81	$C_{82}H_{54}O_{52}$	1869, **934**, 783, 301																	+		+
	152	*O*-pent-deoksyheks kwercetyny	87,15	256, 352	579,13374	-3,01	$C_{26}H_{28}O_{15}$	**579**, 300	**581**, 435, 303							+											
	153	*O*-deoksyheks-*O*-heks kemferolu	88,6	264, 346	593,15030	-1,35	$C_{27}H_{30}O_{15}$	**593**, 447, 284	**595**, 449, 287											+			+		+		
	154	pochodna kwercetyny	88,75	256, 350	607,12966	-1,56	$C_{27}H_{28}O_{16}$	**607**, 300	**609**, 303																		+
66	155	astragalina (3-*O*-glu kemferolu)[W]	88,8	264, 350	447,09299	-0,34	$C_{21}H_{20}O_{11}$	**447**, 284	**449**, 287	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	156	*O*-deoksyheks-heks kwercetyny	89,1	256, 348	609,14510	-1,89	$C_{27}H_{30}O_{16}$	**609**, 300	**611**, 448, 303							+											
67	157	3-*O*-gluc kemferolu[W]	89,25	265, 350	461,07183	-1,38	$C_{21}H_{18}O_{12}$	**461**, 285	**463**, 287			+						+		+		+		+		+	
	158	*O*-deoksyheks-deoksyheks-*O*-heks izoramnetyny	89,33	254, 352	769,21962	-0,11	$C_{34}H_{42}O_{20}$	**769**, 315	**771**, 625, 479, 317					+													
	159	*O*-uro pochodna kemferolu	89,6	268, 342*sh*	461,07133	-2,38	$C_{21}H_{18}O_{12}$	**461**, 285	**463**, 287							+						+					
	160	*O*-deoksyheks-*O*-heks-deoksyheks kemferolu	89,7	266, 348	737,19349	0,28	$C_{33}H_{38}O_{19}$	**737**, 593, 284	**739**, 593, 433, 287																		+
68	161	*O*-heks apigeniny	90,1	266, 336	431,09795	-1,23	$C_{21}H_{20}O_{10}$	**431**, 268	**433**, 271								+						+				+
69	162	agrimonina[W]	90,3	250*sh*	1869,14917	-0,89	$C_{82}H_{54}O_{52}$	1870, 1085, **934**, 783, 301				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
70	163	*O*-metylo-*O*-pent kwasu elagowego	90,45	280*sh*, 365	447,05603	-1,61	$C_{20}H_{16}O_{12}$	**447**, 315, 301				+							+		+			+			
	164	pochodna kemferolu	90,61	264, 348	723,17755	0,1	$C_{32}H_{36}O_{19}$	**723**, 621, 579, 284	**725**, 593, 287										+								
	165	pochodna kemferolu	91,08	266, 348	635,16048	-2,02	$C_{29}H_{32}O_{16}$	**635**, 284	**637**, 287																		+
	166	*O*-uro pochodna apigeniny	91,35	266, 336	445,07769	0,26	$C_{21}H_{18}O_{11}$	891, **445**, 269	**447**, 271				+														
	167	*O*-deoksyheks-heks izoramnetyny	91,43	254, 352	623,16185	0,37	$C_{28}H_{32}O_{16}$	**623**, 314	**625**, 479, 317											+					+		
71	169	3-*O*-glu izoramnetyny[W]	91,43	254, 350	477,10350	-0,23	$C_{22}H_{22}O_{12}$	**477**, 314, 300	**479**, 317	+										+							
72	168	pentagaloilo-glukoza	91,68	280	939,11106	-0,58	$C_{41}H_{32}O_{26}$	**939**, 769, 469, 169								+									+	+	
	170	pochodna kemferolu	91,94	266, 350	737,19229	-1,6	$C_{33}H_{38}O_{19}$	**737**, 284	**739**, 593, 287																		+
73	171	*O*-deoksyheks-heks *O*-pent izoramnetyny	92,02	254*sh*, 355	753,18765	-0,65	$C_{33}H_{38}O_{20}$	**753**, 314	755, 623, **317**										+					+			

74	172	*O*-uro pochodna izoramnetyny	92,79	254, 354	491,08194	-1,69	$C_{22}H_{20}O_{13}$	**491**, 315, 300	**493**, 317			+						+		+			+	+			
	173	*O*-pent-*O*-deoksyheks-*O*-uro pochodna izoramnetyny	92,93	254, 354	767,20286	-1,49	$C_{34}H_{40}O_{20}$	**767**, 621, 314	**769**, 623, 493, 317																		+
	174	*O*-uro pochodna chryzoeriolu	93,7	266*sh*, 346	475,08764	-0,91	$C_{22}H_{20}O_{12}$	951, **475**, 299	**477**, 301				+														
75		3,3'-di-*O*-metylo 4-*O*-ksyl kwasu elagowego[W]	94,14	245, 370	461,07148	-1,26	$C_{21}H_{18}O_{12}$	**461**, 328, 297	**463**, 331												+			+			
	175	*O*-acetylheks kemferolu	94,62	264, 346	489,10342	-1,01	$C_{23}H_{22}O_{12}$	**489**, 284	**491**, 287													+	+		+		+
	176	pochodna kemferolu	94,8	266, 348	591,13497	-0,5	$C_{27}H_{28}O_{15}$	**591**, 284	**593**, 287																		+
76	177	*O*-uro pochodna kwercetyny	94,87	266*sh*, 360	477,06702	-0,36	$C_{21}H_{18}O_{13}$	**477**, 301	**479**, 303				+	+				+			+		+				
77	178	*O*-malonyloheks kemferolu	94,97	266*sh*, 348	533,09266	-1,62	$C_{24}H_{22}O_{14}$	**533**, 284	**535**, 287								+						+				
	179	*O*-galoilodeoksyheks izoramnetyny	95,2	270, 348	629,11322	-2,41	$C_{29}H_{26}O_{16}$	**629**, 314, 299, 169	**631**, 317							+											
	180	*O*-heks naryngeniny	95,52	276*sh*, 362	433,11345	-1,15	$C_{21}H_{22}O_{10}$	**433**, 271	435, **273**													+					
	181	pochodna izoramnetyny	95,83	254, 352	621,14502	-1,78	$C_{28}H_{30}O_{16}$	**621**, 314, 300	**623**, 317																		+
	182	*O*-acetylheks izoramnetyny	95,96	254, 352	519,11432	-0,23	$C_{24}H_{22}O_{13}$	**519**, 314, 299	**521**, 317											+					+		+
	183	akacetyna	96,26	254	283,06188	0,6	$C_{16}H_{12}O_{5}$	**283**, 268	**285**, 242										+								
	184	*O*-acetylheks apigeniny	97,3	266, 326	473,10900	-0,77	$C_{23}H_{22}O_{11}$	**473**, 269															+				
78	185	apigenina[W]	98,1	268, 338	269,04538	-1,93	$C_{15}H_{10}O_{5}$	**269**	**271**								+						+				
	186	*O*-deoksyheks-deoksyheks-*O*-heks kwercetyny	98,43	266, 346	753,22397	-0,86	$C_{34}H_{42}O_{19}$	**753**, 299	**755**, 609, 463, 301					+													
	187	*O*-heks izoramnetyny	98,9	256, 356	477,10244	-3,05	$C_{22}H_{22}O_{12}$	**477**, 314, 299	**479**, 317							+											
	188	*O*-heks izoramnetyny	99,21	256, 354	477,10227	-2,92	$C_{22}H_{22}O_{12}$	**477**, 314, 271	**479**, 317							+											
	189	*O*-deoksyheks-deoksyheks-*O*-heks trycyny	99,5	254, 354	783,23498	-0,36	$C_{35}H_{44}O_{20}$	**783**, 329	**785**, 639, 493, 331					+													
79	190	*trans*-tylirozyd[W]	101,41	268, 315	593,13011	0,27	$C_{30}H_{26}O_{13}$	**593**, 284	**595**, 287	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
	191	*O*-pent izoramnetyny	101,5	258, 354	447,09386	-1,61	$C_{21}H_{20}O_{11}$	**447**, 315, 271	**449**, 317							+											
80	192	pochodna kemferolu	101,87	268, 330	623,13981	-0,98	$C_{31}H_{28}O_{14}$	**623**, 284	**625**, 287	+			+	+				+	+			+	+	+	+	+	+
81	193	*cis*-tylirozyd	102,37	268, 315	593,12995	-0,3	$C_{30}H_{26}O_{13}$	**593**, 284	**595**, 287			+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
82		niezidentyfikowany związek polifenolowy	102,54	280	445,18621	-1,13	$C_{24}H_{30}O_{8}$	**445**, 385		+																+	
	194	*O*-deoksyheks izoramnetyny	102,65	256, 350	461,10762	-2,95	$C_{22}H_{22}O_{11}$	**461**, 314, 271	**463**, 317, 274							+											
	195	*O*-uro pochodna trycyny	103,04	254*sh*, 352	505,09843	-0,58	$C_{23}H_{22}O_{13}$	**505**, 329	**507**, 331, 316					+													
	196	pochodna izoramnetyny	103,4	256, 350	593,14977	-2,29	$C_{27}H_{30}O_{15}$	**593**, 314, 299	**595**, 317							+											
83	197	N^1, N^5, N^{10}-trikumaroilospermidyna	104,47	295, 310*sh*	582,26028	-0,48	$C_{34}H_{37}N_{3}O_{6}$	**582**, 462, 342, 285	**584**, 438, 292, 147	+		+	+	+			+	+	+	+	+	+	+	+	+	+	+
	198	pochodna kwasu elagowego	111,73	350*sh*, 362	422,99970	0,41	$C_{20}H_{8}O_{11}$	**423**, 343, 269							+			+									

P1 – numer piku w **Publikacji I**; P2 – numer piku w **Publikacji II**;[1] dokładna masa jonu [M-H]$^-$ zarejestrowana w jonizacji negatywnej; [2] - błąd różnicy w masie wyznaczonej doświadczalnie i teoretycznie; *sh* – wartość na stoku piku; pogrubienie – najbardziej intensywny jon; (W) - substancja wzorcowa; HHDP – grupa heksaahydroksydifenylowa; NHTP – grupa nonahydroksytrifenylowa; pent – pentoza; heks – heksoza; deoksyheks – deoksyheksoza; uro – kwas uronowy; ara – arabinoza; ksyl – ksyloza; glu – glukoza; gal – galaktoza; gluc – kwas glukuronowy; rut – rutozyd

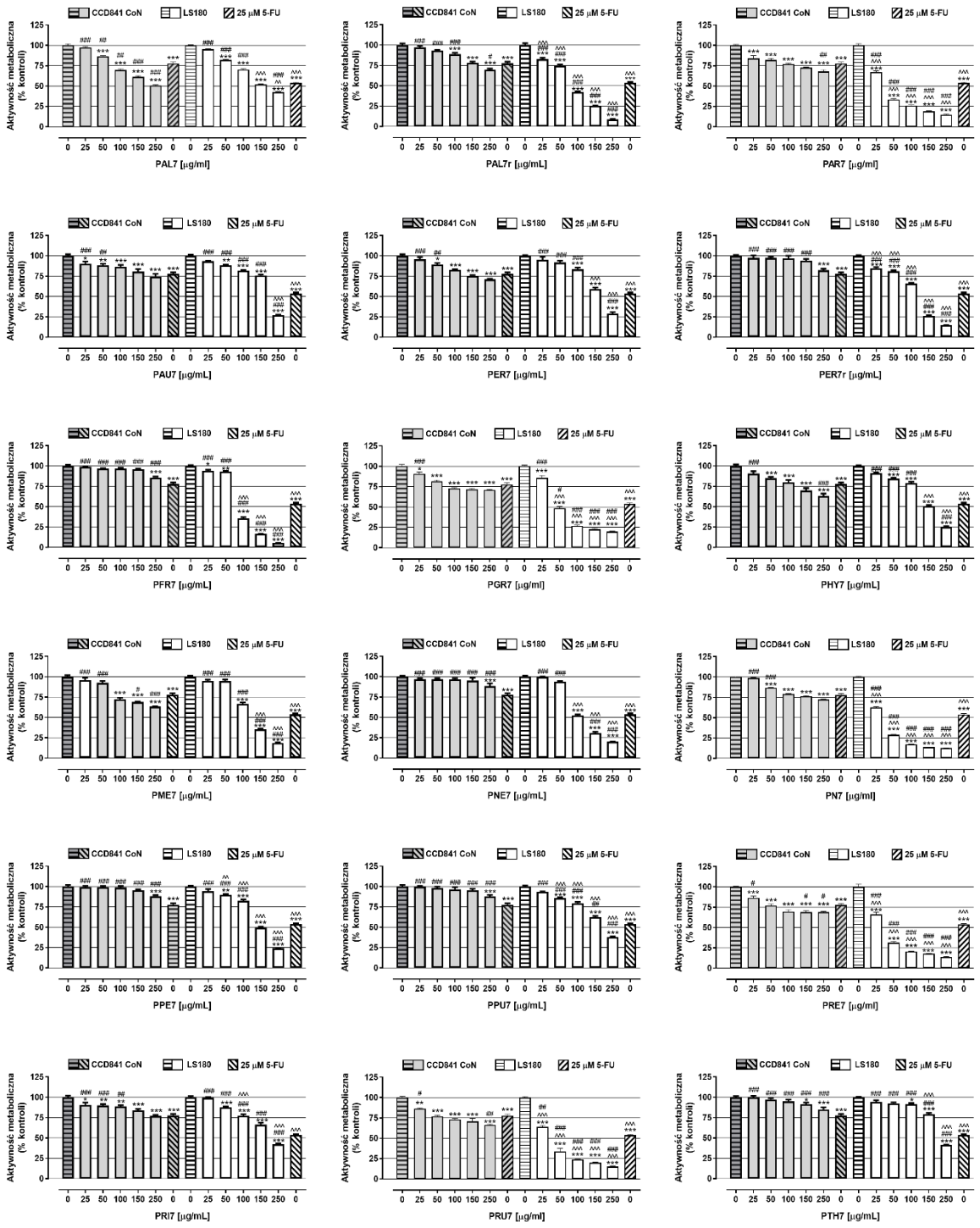
Rozdział 4.3.4. Ocena aktywności biologicznej ekstraktów z wybranych gatunków *Potentilla* L. w modelu *in vitro*

Nowotwory układu pokarmowego są zróżnicowaną grupą jednostek chorobowych, stanowiąc do 20% wszystkich przypadków stwierdzonych nowotworów. Wśród nich najczęściej diagnozowane są nowotwory jelita grubego, stanowiące w 2020 roku 13% wszystkich zdiagnozowanych nowotworów w Polsce [12]. Substancje pochodzenia roślinnego, takie jak winkrystyna, winblastyna, paklitaksel były jednymi z pierwszych substancji wykorzystywanych do leczenia chorób nowotworowych. Według badań wynika, że leki pochodzenia naturalnego oprócz potencjalnego działania przeciwnowotworowego, mogą być również stosowane z powodzeniem jako terapia komplementarna do chemioterapii oraz radioterapii łagodząc wywołane działania niepożądane [13].

Badania aktywności biologicznej zostały przeprowadzone na komórkach gruczolakoraka jelita grubego (LS180) i komórkach nabłonkowych okrężnicy (CCD841 CoN). Pierwszy etap obejmował ocenę wpływu wybranych ekstraktów wodno-acetonowych na przeżywalność obu linii komórkowych przy użyciu MTT (bromek 3-(4,5-dimetylotiazol-2-yl)- 2,5-difenylotetrazoliowy) według metody opisanej przez Carmichaela z modyfikacjami (**publikacja I** i **II**) [14]. Dla każdej próbki została oceniona przeżywalność komórek nowotworowych i prawidłowych poddanych 48 h inkubacji z medium (próba kontrolna), 25 μM 5-FU (5-fluorouracyl) lub ekstraktami w zakresie stężeń 25-250 μg/mL. Wyniki analiz zostały przestawione w Tabeli 4 oraz na Rycinie 3. Zaobserwowano, że wszystkie badane ekstrakty zmniejszały żywotność zarówno komórek normalnych, jak i nowotworowych w sposób zależny od dawki, jednakże komórki gruczolakoraka LS180 były bardziej wrażliwe na badane ekstrakty. Najsilniejsze działanie wobec komórek rakowych zostało stwierdzone dla ekstraktu z pięciornika srebrnego (**PAR7**) (IC_{50} = 38 μg/mL), pięciornika norweskiego (**PN7**) (IC_{50} = 32 μg/mL), pięciornika wyprostowanego (**PRE7**) (IC_{50} = 35 μg/mL) oraz pięciornika skalnego (**PRU7**) (IC_{50} = 36 μg/mL) osiągając skuteczność zbliżoną do 5-FU (IC_{50} = 31 μg/mL). Z drugiej strony, najsilniejszy wpływ na żywotność komórek prawidłowych został stwierdzony dla pięciornika białego (**PAL7**) oraz pięciornika poziomkowatego (**PME7**), jednakże znacznie słabszy w porównaniu do kontroli pozytywnej.

Tabela 4. Wartość IC_{50} przeżywalności komórek gruczolakoraka jelita grubego LS180 i komórek nabłonkowych okrężnicy CCD841 CoN traktowanych przez 48 h ekstraktami wodno-acetonowymi pozyskanymi z gatunków z rodzaju *Potentilla* oraz 5-fluorouracylem (5-FU).

Próbka	LS180			CCD841 CoN		
	IC_{50} (μg/mL)	Przedział ufności	R^2	IC_{50} (μg/mL)	Przedział ufności	R^2
PAL7	182	169-196	0,983	233	209-261	0,971
PAL7r	82	77-87	0,98	496	396-623	0,908
PAR7	38	32-44	0,974	1134	575-2235	0,902
PAU7	192	180-206	0,92	1575	536-4632	0,672
PER7	176	166–186	0,957	672	474–952	0,891
PER7r	110	101-120	0,943	523	326-839	0,595
PFR7	89	85-92	0,989	707	450-1113	0,737
PGR7	58	50-67	0,957	982	498-1938	0,89
PHY7	156	146-167	0,952	489	334-717	0,838
PME7	128	122-133	0,983	380	291-495	0,87
PNE7	112	106-118	0,977	1795	329-9800	0,365
PN7	32	28-37	0,981	757	459-1248	0,903
PPE7	158	150-167	0,966	620	367-1047	0,663
PPU7	197	185-210	0,967	865	359-2081	0,531
PRE7	35	30-42	0,969	918	449-1879	0,882
PRI7	213	200-228	0,968	2402	788-7326	0,717
PRU7	36	30-42	0,974	846	481-1489	0,916
PTH7	225	215-236	0,956	969	443-2119	0,643
5-FU	31	28-33	0,977	113	81-157	0,884

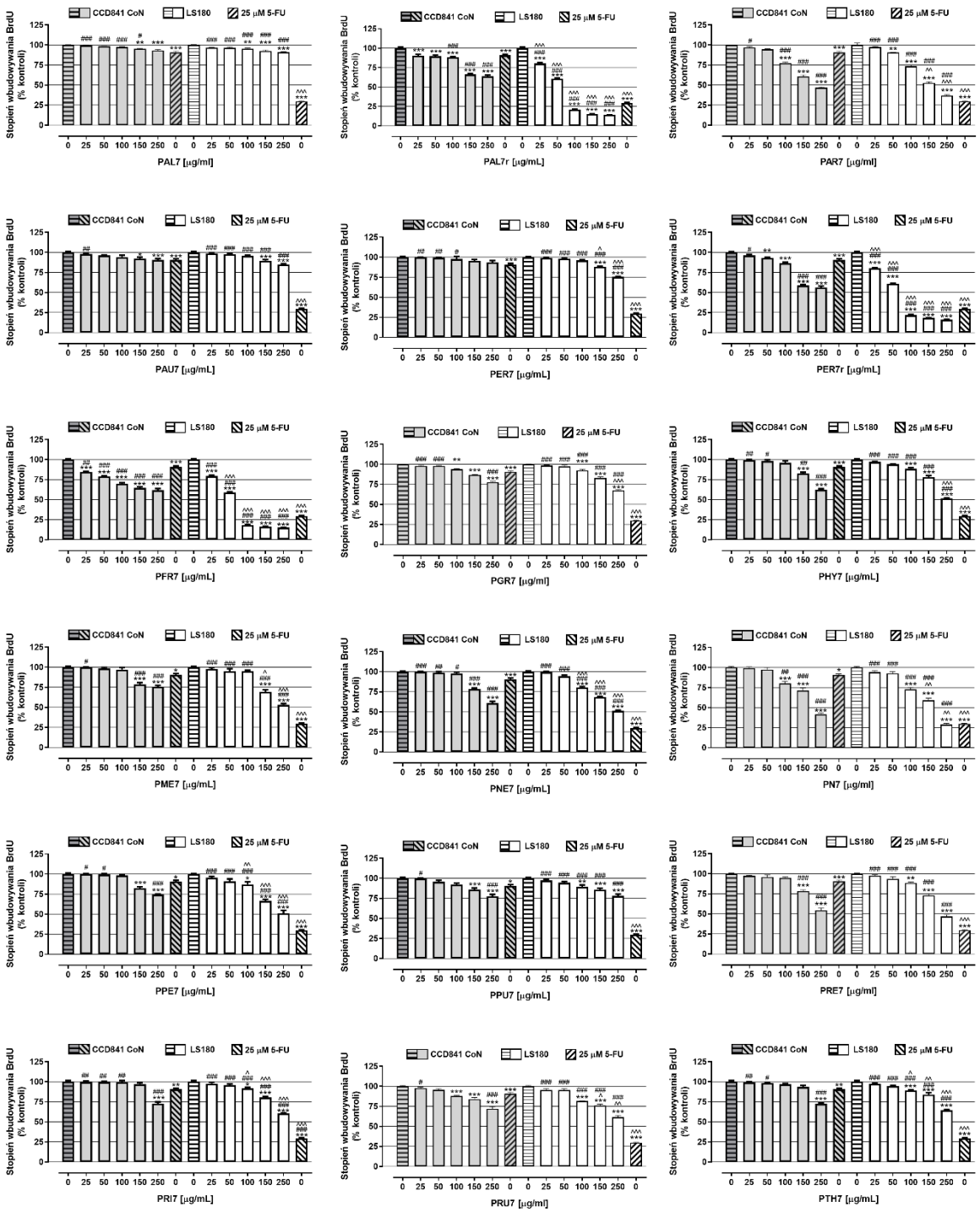


Rycina 3. Wpływ ekstraktów wodno-acetonowych pozyskanych z gatunków z rodzaju *Potentilla* na przeżywalność komórek gruczolakoraka jelita grubego LS180 i komórek nabłonkowych okrężnicy CCD841 CoN po 48 h inkubacji. Komórki były traktowane medium (próba kontrolna) oraz ekstraktami w zakresie stężeń 25-250 μg/mL lub 25 μM 5-FU (próba pozytywna). Przedstawiono średnie wartości procentowe ± odchylenie standardowe z co najmniej 5 niezależnych doświadczeń. *p <0.05; **p < 0.01; ***p < 0.001 w stosunku do próby kontrolnej, #p < 0.05; ##p < 0.01; ###p < 0.001 w stosunku do próby pozytywnej, ^p < 0,05; ^^p < 0,01; ^^^p < 0,001 komórki nowotworowe traktowane ekstraktem lub 5-FU w stosunku do komórek prawidłowych traktowanych ekstraktem lub 5-FU w odpowiednim stężeniu.

Kolejny etap badań nad aktywnością biologiczną obejmował ocenę wpływu wybranych ekstraktów na proliferację obu linii komórkowych przy wykorzystaniu testu badającego ilościowo syntezę DNA przez inkorporację BrdU (bromodeoksyurydyny) do DNA dzielącej się komórki w trakcie fazy S. Wyniki badania zostały przestawione w Tabeli 5 oraz na Rycinie 4. Wszystkie badane ekstrakty hamowały syntezę DNA w komórkach gruczolakoraka LS180 w sposób zależny od dawki. Najwyższą aktywność została wykazana dla ekstraktów z **PAL7r**, **PER7r** oraz **PFR7**, które w zakresie stężeń 100 – 250 µg/mL redukowały syntezę DNA o około 80%. Wspomniane ekstrakty wykazały również najniższe wartości IC_{50} (odpowiednio 52 µg/mL, 54 µg/mL oraz 50 µg/mL), jednakże były one znacznie niższe niż wartość IC_{50} dla 5-FU (15 µg/mL). Z drugiej strony, wszystkie ekstrakty znacznie słabiej wpływały na proliferację komórek prawidłowych, osiągając wartości IC_{50} znacznie wyższe niż 5-FU (**publikacja I** i **II**).

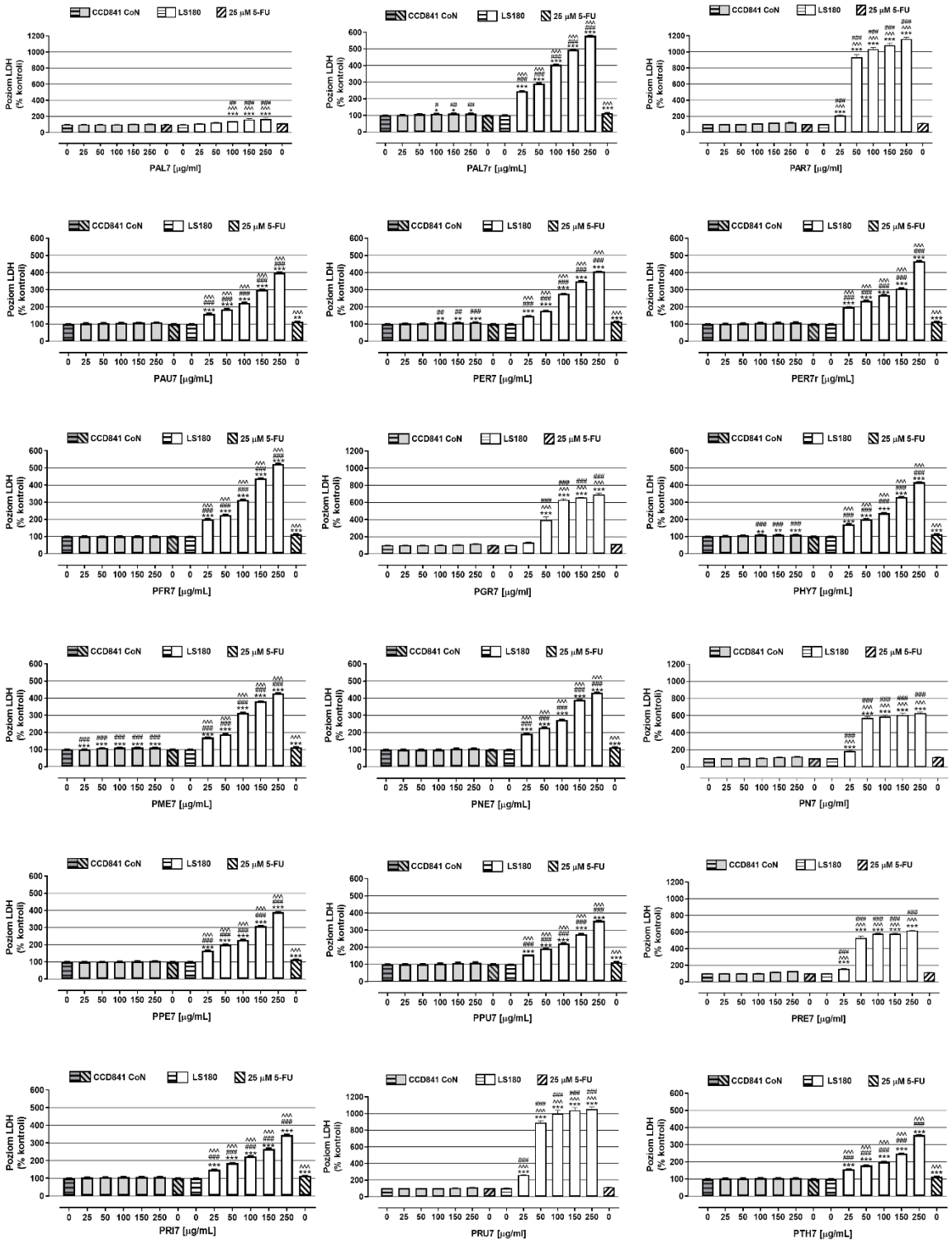
Tabela 5. Wartość IC_{50} proliferacji komórek gruczolakoraka jelita grubego LS180 i komórek nabłonkowych okrężnicy CCD841 CoN traktowanych przez 48 h ekstraktami wodno-acetonowymi pozyskanymi z gatunków z rodzaju *Potentilla* lub 5-fluorouracylem (5-FU).

Próbka	LS180			CCD841 CoN		
	IC_{50} (µg/mL)	**Przedział ufności**	**R^2**	**IC_{50} (µg/mL)**	**Przedział ufności**	**R^2**
PAL7	12008	2096-68805	0,752	4164	1759-9859	0,867
PAL7r	52	41-64	0,917	412	351-483	0,841
PAR7	174	165-183	0,982	217	203-231	0,977
PAU7	1495	1311-1704	0,871	2058	1626-2604	0,542
PER7	1001	847-1183	0,845	3705	2368-5796	0,336
PER7r	54	44-66	0,925	337	281-405	0,856
PFR7	50	40-62	0,916	282	244-327	0,809
PGR7	372	338-409	0,968	570	488-666	0,965
PHY7	425	350-516	0,843	631	495-804	0,765
PME7	417	325-536	0,774	837	661-1061	0,740
PNE7	343	298-395	0,915	586	451-763	0,748
PN7	169	159-179	0,974	217	202-233	0,958
PPE7	343	283-414	0,850	911	728-1140	0,768
PPU7	881	761-1019	0,836	937	803-1093	0,846
PRE7	237	223-251	0,966	268	248-289	0,943
PRI7	542	452-649	0,848	1230	837-1806	0,553
PRU7	360	311-416	0,95	538	425-681	0,926
PTH7	606	521-704	0,876	1039	791-1364	0,693
5-FU	15	13-16	0,956	94	80-111	0,933



Rycina 4. Wpływ ekstraktów wodno-acetonowych pozyskanych z gatunków z rodzaju *Potentilla* na proliferację komórek gruczolakoraka jelita grubego LS180 i komórek nabłonkowych okrężnicy CCD841 CoN po 48 h inkubacji. Komórki były traktowane medium (próba kontrolna) oraz ekstraktami w zakresie stężeń 25-250 μg/mL lub 25 μM 5-FU (próba pozytywna). Przedstawiono średnie wartości procentowe ± odchylenie standardowe z co najmniej 4 niezależnych doświadczeń. *p <0.05; **p < 0.01; ***p < 0.001 w stosunku do próby kontrolnej, #p < 0.05; ##p < 0.01; ###p < 0.001 w stosunku do próby pozytywnej, ^p < 0,05; ^^p < 0,01; ^^^p < 0,001 komórki nowotworowe traktowane ekstraktem lub 5-FU w stosunku do komórek prawidłowych traktowanych ekstraktem lub 5-FU w odpowiednim stężeniu.

Ocena cytotoksyczności ekstraktów została przeprowadzona przy użyciu testu LDH (dehydrogenaza mleczanowa), wewnątrzkomórkowego enzymu uwalnianego z komórki do medium na skutek dezintegracji błon komórkowych [14] Wyniki testu LDH zostały zaprezentowane na Rycinie 5. Większość z badanych ekstraktów nie wykazywała działania cytotoksycznego na komórki prawidłowe CCD841 CoN. **PME**7 w zakresie stężeń 25-250 µg/mL oraz **PAL7r**, **PER7** oraz **PHY7** w zakresie stężeń 100-250 µg/mL wykazywały działanie cytotoksyczne na komórki nabłonkowe, lecz w najwyższym badanym stężeniu zwiększały poziom LDH w porównaniu do kontroli negatywnej o około 11%, porównywalnie do 5-FU (13,4%). Wszystkie ekstrakty w całym zakresie stężeń wykazały zależny od dawki efekt cytotoksyczny na komórki nowotworowe LS180. Najsilniejsze działanie cytotoksyczne wykazały ekstrakty z **PAR7** oraz **PRU7**, które w stężeniu 250 µg/mL zwiększyły stężenie pozakomórkowego LDH w komórkach gruczolakoraka o odpowiednio 1062 i 956%. Ponadto, cytotoksyczność ekstraktów ściśle korelowała z zawartością garbników, szczególnie w tych ekstraktach z przewagą garbników hydrolizujących (**publikacja I** i **II**).



Rycina 5. Cytotoksyczność ekstraktów wodno-acetonowych pozyskanych z gatunków z rodzaju *Potentilla* na wobec komórek gruczolakoraka jelita grubego LS180 i komórek nabłonkowych okrężnicy CCD841 CoN po 48 h inkubacji. Komórki były traktowane medium (próba kontrolna) oraz ekstraktami w zakresie stężeń 25-250 μg/mL lub 25 μM 5-FU (próba pozytywna). Przedstawiono średnie wartości procentowe ± odchylenie standardowe z co najmniej 3 niezależnych doświadczeń. *p <0,05; **p < 0,01; ***p < 0,001 w stosunku do próby kontrolnej, #p < 0,05; ##p < 0,01; ###p < 0,001 w stosunku do próby pozytywnej, ^^^p < 0,001 komórki nowotworowe traktowane ekstraktem lub 5-FU w stosunku do komórek prawidłowych traktowanych ekstraktem lub 5-FU w odpowiednim stężeniu.

Rozdział 4.3.5. Ocena aktualnego stanu wiedzy dotyczącego postępu w zakresie fitochemii oraz badań biologicznych w rodzaju *Potentilla* L. *sensu lato*

Ostatni krok, uzupełniający moją rozprawę doktorską, obejmował opublikowanie szczegółowego opracowania danych dotyczących badań fitochemicznych oraz badań biologicznych w rodzaju *Potentilla s. l.* uwzględniających lata od 2009 roku. Pomimo, że w dostępnej literaturze opublikowana została jedna praca przeglądowa [5], która podjęła próbę usystematyzowanego przeglądu danych na temat badań składu fitochemicznego oraz aktywności biologicznej, obejmującej badania *in vitro*, *in vivo* oraz badania kliniczne, to zakres obejmował prace opublikowane do roku 2008. Wstępna analiza danych literaturowych opublikowanych w roku 2009 oraz w latach późniejszych wskazała na duży postęp naukowy w obu rozważanych zakresach. Mój zamysł był podstawą do stworzenia koncepcji pracy przeglądowej podejmującej temat usystematyzowania dostępnej wiedzy w latach 2009-2020 z zakresu etnofarmakologii oraz stale rosnącej liczbie opublikowanych zagadnień z zakresu badań fitochemicznych oraz badań biologicznych w obrębie rodzaju *Potentilla* L. W pierwszym etapie zostały zestawione aktualne doniesienia dotyczące zastosowania roślin oraz otrzymanych z nich preparatów w medycynie tradycyjnej. Kolejny krok obejmował podjęcie problemu usystematyzowania postępów badań nad obecnością i badaniami metabolitów wtórnych w szczególności uwzględniających dane strukturalne. W okresie 2009-2020 zostało zidentyfikowanych w co najmniej 40 gatunkach 173 metabolitów wtórnych dla części podziemnych oraz co najmniej 226 metabolitów wtórnych dla części nadziemnych. Następnie przygotowane zestawienie badań biologicznych w obrębie gatunków z rodzaju *Potentilla s. l.*, uwzględniło szereg badań w modelach *in vitro*, *in vivo* oraz bardzo nielicznych badań klinicznych, opisując właściwości przeciwcukrzycowe, przeciwdrobnoustrojowe, przeciwzapalne, anty-oksydacyjne, przeciwnowotworowe oraz hepatoprotekcyjne. Dopełnieniem pracy przeglądowej było zestawienie danych dotyczących bezpieczeństwa stosowania oraz toksyczności preparatów otrzymanych z roślin z badanego rodzaju (**publikacja III**).

Rozdział 4.3.6. Analiza statystyczna otrzymanych wyników

Analiza jakościowa LC-PDA-HRMS została przeprowadzona z wykorzystaniem oprogramowania Mass Hunter Qualitative Analysis 10.0. Analiza statystyczna została przeprowadzona z użyciem jednoczynnikowej analizy wariancji ANOVA z testem post hoc Tukeya i statystykami kolumnowymi. Obliczenia parametrów regresji, wartości IC_{50} oraz odchyleń standardowych zostały obliczone stosując oprogramowanie GraphPad Prism 5 (**publikacja I** i **II**).

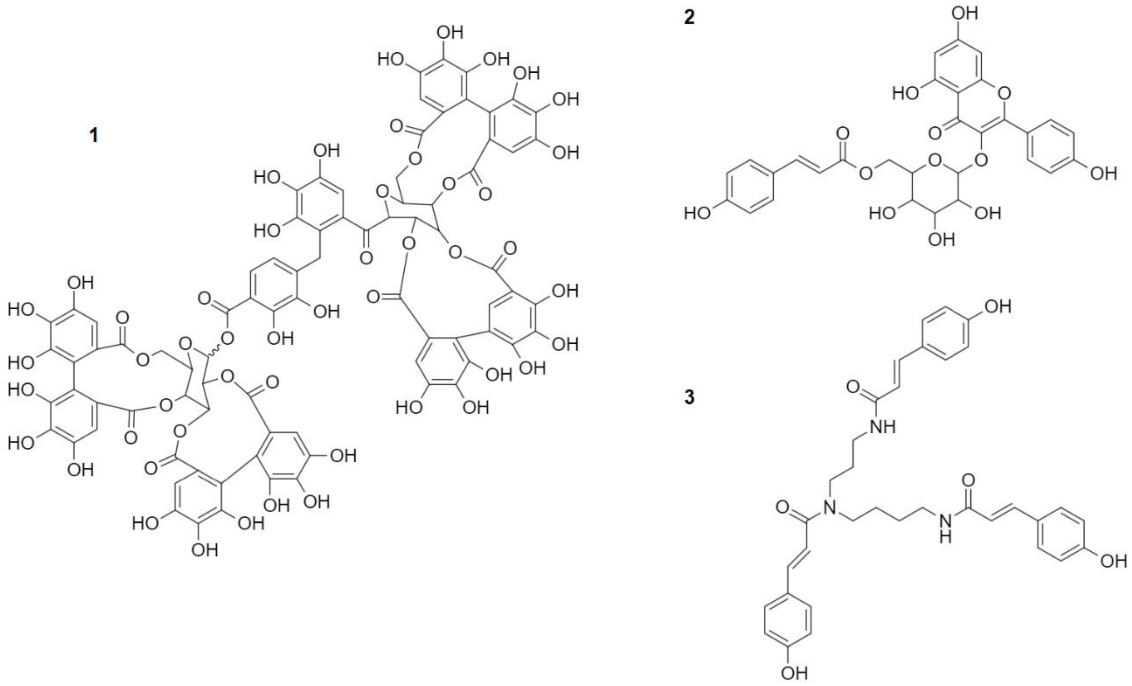
Rozdział 4.4 Dyskusja

Gatunki z rodzaju *Potentilla* L. (Rosaceae) występują powszechnie we florze Polski. Surowce z tego rodzaju charakteryzują się bogatą zawartością związków o charakterze polifenoli, w szczególności garbników hydrolizujących oraz skondensowanych. Ze względu na zawartość tej grupy związków preparaty uzyskane z części nadziemnych i podziemnych pięciorników były tradycyjnie wykorzystywane w lekach ziołowych stosowanych zewnętrznie w celu leczenia stanów zapalnych jamy ustnej i gardła, niewielkich uszkodzeń skóry, łagodzenia dolegliwości towarzyszących żylakom odbytu oraz wewnętrznie zapierająco, przeciwbakteryjnie, przeciw-zapalnie oraz hamująco na krwawienia z przewodu pokarmowego [15, 16]. Farmakopea Polska XII zawiera dwie monografie, tj. Tormentillae rhizoma oraz Tormentillae tinctura, opisujące aspekty jakościowe kłącza pięciornika (*Potentilla erecta* (L.) Raeusch) oraz przygotowywanej z niego nalewki [17]. Bezpieczeństwo przewlekłego stosowania surowców farmakopealnych oraz preparatów z gatunków z rodzaju *Potentilla* L. zostało potwierdzone w szeregu badań, zarówno w modelu *in vitro* jak i *in vivo* [18-20].

Biorąc pod uwagę fakt, że brak jest doniesień na temat obecności związków czynnych o charakterze polifenolowym w wybranych surowcach, tradycyjne stosowanie surowców w obrębie przewodu pokarmowego, ich bezpieczeństwo stosowania oraz brak dostępnych badań dotyczących potencjału chemoprewencyjnego podjęte zostały przekrojowe badania fitochemiczne oraz próba

oceny aktywności biologicznej w stosunku do linii komórek nowotworowych jelita grubego oraz komórek prawidłowych nabłonka okrężnicy.

W pierwszym etapie przeprowadzone analizy spektrofotometryczne oceny całkowitej zawartości polifenoli, garbników, flawonoidów oraz kwasów fenolowych wykazały, iż we wszystkich ekstraktach wodno-acetonowych garbniki stanowiły dominującą grupę związków. Ocena całkowitej zawartości proantocyjanidyn pozwoliła na określenie przewagi garbników hydrolizujących we wszystkich ekstraktach z wyjątkiem ekstraktów otrzymanych z części nadziemnych oraz podziemnych pięciornika białego (**PAL7** oraz **PAL7r**). Szczegółowa analiza z wykorzystaniem opracowanej metody analitycznej wykorzystującej technikę LC-PDA-HRMS pozwoliła na wstępną jakościową identyfikację 217 metabolitów wtórnych. Dominujące związki zostały przedstawione na Rycinie 6. W częściach podziemnych analizowanych gatunków dominującą grupą związków były garbniki skondensowane oraz hydrolizujące, jednakże w przypadku **PAL7r** stwierdzona została obecność jedynie garbników skondensowanych. W częściach nadziemnych dominowały również związki polifenolowe należące do garbników hydrolizujących i skondensowanych, jak oraz flawonoidów i kwasów fenolowych. Mając na uwadze duże rozpowszechnienie monomerycznych oraz olimerycznych elagotanin w rodzinie Rosaceae oraz dużą różnorodność gatunkową w rodzaju *Potentilla s. l.* można wskazać w obrębie tej grupy fitochemicznej na dominujące związki w tym agrimoninę, pedunkulaginę oraz izomery lewigatyny, które pełnią rolę przynależności chemofenetycznej. Wśród związków flawonoidowych za kolejne markery należy uznać pochodne kemferolu: tylirozyd, astragalinę, a także pochodne kwercetyny: rutynę oraz hiperozyd, które zostały stwierdzone również w innych gatunkach z rodzaju *Potentilla*, m.in. *P. indica*, *P. atrosanguinea* oraz *P. reptans* [21-23]. Warto nadmienić, iż po raz pierwszy w rodzaju *Potentilla* została przeze mnie stwierdzona N-acylowana pochodna spermidyny – N^1, N^5, N^{10}-trikumaroilospermidyna [24].



Rycina 6. Wzory strukturalne dominujących związków:
1. agrimonina,
2. tylirozyd,
3. N^1, N^5, N^{10}-trikumaroilospermidyna

Uzyskane w toku badań ekstrakty zostały poddane ocenie aktywności biologicznej w modelu eksperymentalnym *in vitro*. Ocena obejmowała zbadanie wpływu ekstraktów na przeżywalność, proliferację oraz cytotoksyczności wobec zarówno linii nowotworu jelita grubego (LS180) oraz

komórek prawidłowych nabłonka jelita grubego (CCD841 CoN). Przeprowadzona ocena żywotności wykazała, że wszystkie badane ekstrakty w sposób zależny od dawki zmniejszały żywotność zarówno komórek normalnych, jak i nowotworowych, jednakże zaobserwowany efekt był znacznie silniejszy w przypadku komórek nowotworowych. 4 spośród 18 testowanych ekstraktów, a mianowicie z pięciornika srebrnego (**PAR7**), pięciornika norweskiego (**PN7**), pięciornika wyprostowanego (**PRE7**) i pięciornika skalnego (**PRU7**) wpływało na żywotność komórek LS180 w sposób porównywalny do kontroli pozytywnej. Otrzymane wyniki korelują z wysoką całkowitą zawartością związków garbnikowych. Dominująca w tych ekstraktach agrimonina może wpływać znacząco na metabolizm energetyczny komórek nowotworowych poprzez hamowanie szlaku sygnałowego PI3K-AKT-mTOR, skutkując zahamowaniem procesów glikolizy beztlenowej. Ponadto, wykazano, że agrimonina wpływa hamująco na aktywację czynnika Nrf2 (jądrowy czynnik transkrypcyjny pochodzenia erytroidalnego typu 2), prowadząc do zwiększenia wewnątrzkomórkowego poziomu reaktywnych form tlenu (ROS) skutkując uszkodzeniem mitochondriów, w konsekwencji promując apoptozę w badanych liniach komórkowych raka trzustki PANC-1 oraz CFPAC-1 [25].

Na zahamowanie proliferacji obu linii komórkowych najsilniej wpływały ekstrakty z pięciornika białego (**PAL7r**), pięciornika kurze ziele (**PER7r**) oraz pięciornika krzewiastego (**PFR7**), jednakże efekt w przypadku prawidłowych komórek był słabszy. Obserwowany efekt koreluje z wysoką zawartością proantocyjanidyn w wymienionych ekstraktach. Aktualne doniesienia potwierdzają silny efekt antyproliferacyjny garbników skondensowanych poprzez supresje komórek w fazie G1 i G2 cyklu oraz znaczącą indukcję procesu apoptozy [26, 27]. Ponadto, za obserwowany efekt może być również odpowiedzialna frakcja flawonoidowa, w szczególności obecny w niej tylirozyd, który posiada właściwości hamujące aktywność topoizomerazy I oraz II [28].

Aktualne, jednak nieliczne doniesienia dotyczące pochodnych kumaroilospermidyny wskazują na ich silne właściwości przeciwnowotworowe. Wcześniejsze badania wykazały, iż dikumaroilospermidyna silnie indukuje wewnątrzpochodny szlak apoptozy w ludzkich komórek raka wątroby (HepG2) poprzez zwiększoną aktywację kaspazy-3, kaspazy-9, cytochromu c oraz białka Bax, jednocześnie obniżając aktywność białka Bcl-2 [29]. Na obserwowany efekt antyproliferacyjny może mieć również wpływ kwas elagowy, który na modelu linii komórkowej raka jelita grubego HCT 116 wpływał hamująco na ekspresję genów zaangażowanych w szlaki sygnałowe związane z białkiem p53, PI3K-Akt, kinazą białkową aktywowaną mitogenem (MAPK) oraz TGF-β [30].

Przeprowadzona w ramach eksperymentu ocena aktywności cytotoksycznej wykazała brak lub niewielki wpływ ekstraktów na komórki prawidłowe CCD841 CoN. Z drugiej strony wszystkie ekstrakty wykazały silne działanie cytotoksyczne już w najniższym zastosowanym stężeniu (25 μg/mL) wobec komórek nowotworowych, zwiększając przepuszczalność błon komórkowych skutkując podwyższeniem stężenia LDH w macierzy pozakomórkowej. Najsilniejszy efekt został stwierdzony dla **PAR7** oraz **PRU7**, a efekt jest silnie związany z wysoką zawartością garbników hydrolizujących. Aktualne doniesienia naukowe sugerują, że garbniki hydrolizujące, a w szczególności agrimonina, odpowiedzialne są za inhibicję aktywowanych wapniem kanałów chlorkowych prowadząc do tworzenia się selektywnych porów na powierzchni błon komórkowych. Ponadto, agrimonina wpływa na przepuszczalność błon mitochondrialnych, prowadząc w konsekwencji do zmniejszenia produkcji energii i aktywacji wewnętrznego szlaku apoptozy [31, 32].

Rozdział 5. Wnioski

- Wybrane do badań w ramach pracy doktorskiej gatunki roślin z rodzaju *Potentilla* L. charakteryzuje obecność w nich związków polifenolowych, takich jak agrimonina, tylirozyd oraz po raz pierwszy opisany w rodzaju związek - N^1, N^5, N^{10}-trikumaroilospermidyna. Powtarzalność występowania tych związków może stanowić ważny marker chemofenetyczny dla rodzaju *Potentilla*. Przypuszcza się również, że mogą one warunkować wysoką aktywność biologiczną opisywanych preparatów w szczególności wobec linii ludzkich komórek gruczolakoraka okrężnicy.
- Opracowana nowa metoda analityczna umożliwia efektywne rozdzielenie chromatograficzne dużej liczby metabolitów wtórnych obecnych w analizowanych ekstraktach, dając podstawy do wykorzystania jej w ocenie fitochemicznej surowców oraz preparatów roślinnych.
- Wymagane jest dalsze przeprowadzenie analiz izolacyjnych oraz ilościowych związków obecnych w gatunkach z rodzaju *Potentilla*, w celu wskazania najaktywniejszych związków oraz zaplanowania bardziej złożonych badań w modelach *in vitro* oraz *in vivo*, w celu poznania potencjalnych mechanizmów molekularnych działania biologicznego, w szczególności obejmujących możliwy wpływ metabolitów po ich biotransformacji przez florę jelitową.
- Rozwinięcie badań biologicznych i farmakologicznych nad badanymi gatunkami może być podstawą do stworzenia roślinnych preparatów leczniczych o działaniu chemoprewencyjnym w przypadku chorób przewodu pokarmowego.

Rozdział 6. Zastosowane skróty

5FU	5-fluorouracyl
$[M-H]^-$	jon uzyskany w trybie jonizacji ujemnej
ANOVA	jednoczynnikowa analiza wariancji
BrdU	test proliferacji z wykorzystaniem bromodeoksyurydyny
CCD841 CoN	linia ludzkich prawidłowych komórek nabłonkowych okrężnicy
CAE/g	ekwiwalent kwasu kawowego
CE/g	ekwiwalent katechiny
CFPAC-1	linia ludzkich komórek gruczolakoraka trzustki
ESI	źródło jonizacji uzyskanym przez elektrorozpylenie
GAE/g	ekwiwalent kwasu galusowego
HCT 116	linia ludzkich komórek raka nabłonkowego okrężnicy
HepG2	linia ludzkich komórek raka wątroby
IC_{50}	połowa maksymalnego stężenia hamującego
LC-PDA-HRMS	chromatografia cieczowa sprzężona z detektorami fotodiodowym i wysokorozdzielczym detektorem masowym
LDH	test cytotoksyczności z zastosowaniem pomiaru dehydrogenazy mleczanowej
LLE	frakcjonowanie ciecz-ciecz
LS180	linia ludzkich komórek gruczolakoraka okrężnicy
MAPK	kinaza białkowa aktywowana mitogenem
MTT	test przeżywalności z wykorzystaniem bromku 3-(4,5-dimetylotiazol-2-yl)-2,5-difenylotetrazoliowego
Nrf2	jądrowy czynnik transkrypcyjny pochodzenia erytroidalnego typu 2
PAL7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla alba*
PAL7r	ekstrakt wodno-acetonowy z części podziemnych z *Potentilla alba*
PANC-1	linia ludzkich komórek raka nabłonkowatego trzustki
PAR7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla argentea*
PAU7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla aurea*
PER7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla erecta*
PER7r	ekstrakt wodno-acetonowy z części podziemnych z *Potentilla erecta*
PFR7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla fruticosa*
PGR7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla grandiflora*
PHY7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla hyparctica*
PME7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla megalantha*
PNE7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla nepalensis*
PN7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla norvegica*
PPE7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla pensylvanica*
PPU7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla pulcherrima*
PRE7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla recta*
PRI7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla rigoi*
PRU7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla rupestris*
PTH7	ekstrakt wodno-acetonowy z części nadziemnych z *Potentilla thuringiaca*
RE/g	ekwiwalent rutyny
ROS	reaktywne formy tlenu
s. l.	*sensu lato*, w szerokim znaczeniu
TFC	całkowita zawartość flawonoidów
TPAC	całkowita zawartość kwasów fenolowych
TPC	całkowita zawartość związków polifenolowych
TPrC	całkowita zawartość proantocyjanidyn
TTC	całkowita zawartość związków garbnikowych
UV-Vis	zakres światła ultrafioletowego i widzialnego

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Rozdział 8. Streszczenie w języku polskim

Rodzaj *Potentilla* L. *sensu lato*, pięciornik (syn. *Dasiphora* RAFINESQUE, *Penthaphylloides* DUHAMEL, *Tormentilla* L.) jest jednym najbardziej różnorodnych rodzajów składający się z około 300 do 500 gatunków, należacy do rodziny różowatych (Rosaceae). Preparaty uzyskane z części nadziemnych i podziemnych pięciorników są tradycyjnie wykorzystywane w lekach ziołowych stosowanych zewnętrznie w celu leczenia stanów zapalnych jamy ustnej i gardła, niewielkich uszkodzeń skóry oraz wewnętrznie zapierająco, przeciwbakteryjnie, przeciwzapalnie oraz hamująco na krwawienia z przewodu pokarmowego. Bardzo nieliczne doniesienia na temat oceny składu chemicznego wybranych gatunków oraz aktywności biologicznej skłoniły do przeprowadzenia analizy 18 ekstraktów wodno-acetonowych z wybranych gatunków z rodzaju *Potentilla*. Spektrofotometryczne oznaczenia ilościowe zostały przeprowadzone wybranych grup związków, w tym całkowitej zawartości polifenoli (TPC), garbników (TTC), proantocyjanidyn (TPrC), flawonoidów (TFC) oraz kwasów fenolowych (TPAC). Uzyskane wyniki wskazują, że wszystkie ekstrakty stanowią bogate źródło związków o charakterze polifenoli, w tym szczególnie związków o charakterze garbnikowym. Szczegółowa analiza jakościowa profili fitochemicznych przy użyciu chromatografii cieczowej (LC-PDA-HRMS) wykazała obecność 217 związków, w tym agrimoniny, pedunkulaginy, kwasu elagowego, astragaliny, tilirozydu, kwasu brewifolino-karboksylowego oraz N^1, N^5, N^{10}-trikumaroilospermidyny. Kolejny etap obejmował ocenę wpływu ekstraktów na przeżywalność, proliferację oraz cytotoksyczność ekstraktów wobec linii komórkowej ludzkiego nabłonka okrężnicy CCD841 CoN i linii komórkowej ludzkiego gruczolakoraka okrężnicy LS180, przy użyciu odpowiednio testu MTT, BrdU oraz LDH. Żywotność oraz zdolność do proliferacji obu linii komórkowych zmniejszyła się pod wpływem wszystkich badanych ekstraktów, lecz wyraźnie silniejszy efekt zaobserwowano w komórkach raka okrężnicy. Badanie cytotoksyczności wykazało, że 14 z 18 ekstraktów nie wykazywało właściwości cytotoksycznych wobec komórek prawidłowych, przy jednoczesnym znacznym uszkodzeniu błon komórkowych komórek nowotworowych, a obserwowany efekt był zależny od dawki. Przeprowadzone badania wykazały, że wszystkie zbadane gatunki z rodzaju *Potentilla sensu lato* mogą służyć jako podstawa do opracowania nowych skutecznych i bezpiecznych preparatów o działaniu chemoprewencyjnym dla osób zagrożonych bądź chorujących na raka jelita grubego, jednakże są wymagane dodatkowe badania dotyczące wpływu flory jelitowej na działanie surowca oraz w celu potwierdzenia bezpieczeństwa stosowania.

Rozdział 9. Streszczenie w języku angielskim/Summary

The genus *Potentilla* L. *sensu lato*, cinquefoil (syn. *Dasiphora* RAFINESQUE, *Penthaphylloides* DUHAMEL, *Tormentilla* L.) is one of the most diverse genera, consisting of about 300 to 500 species, belonging to the Rosaceae family. Preparations obtained from the above-ground and underground parts of cinquefoil are traditionally used as herbal medicines used externally to treat inflammation of the mouth and throat, minor skin damage, and internally as an antidiarrheal, antibacterial, anti-inflammatory and inhibitory agent for gastrointestinal bleeding. Very few reports on assessing the chemical composition of rarely selected species and their biological activity led to the analysis of 18 aqueous acetone extracts from selected species from the genus *Potentilla*. Spectrophotometric quantitative determination of selected groups of compounds, including total content of polyphenols (TPC), tannins (TTC), proanthocyanidins (TPrC), flavonoids (TFC) and phenolic acids (TPAC), were conducted. The obtained results indicate that all extracts are a rich source of polyphenol compounds, especially tannins. Detailed qualitative analysis of phytochemical profiles using liquid chromatography (LC-PDA-HRMS) showed the presence of 217 compounds, including agrimoniin, pedunculagin, ellagic acid, astragalin, tiliroside, brevifolincarboxylic acid and N^1, N^5, N^{10}-tricoumaroyl spermidine. The next step of experiments involved evaluating the effect of the extracts on the survival, proliferation and cytotoxicity of the extracts against the CCD841 CoN human colon epithelial cell line and the LS180 human colon adenocarcinoma cell line, using the MTT, BrdU and LDH assays, respectively. The viability and proliferation capacity of both cell lines decreased under the influence of all tested extracts, but a markedly stronger effect was observed in colon cancer cells. The cytotoxicity study showed that 14 out of 18 extracts did not show cytotoxic properties against normal cells, while significantly damaging the cell membranes of cancer cells, and the observed effect was dose-dependent. The conducted research showed that all tested species of the genus *Potentilla sensu lato* could be used as the basis for the development of new effective and safe preparations with chemopreventive effects for people threatened by or suffering from colorectal cancer, however, additional research is required to uncover the impact of the intestinal flora on the effect of the extract and to evaluate their safety.

Rozdział 10. Kopie publikacji wchodzących w cykl rozprawy doktorskiej

Publikacja 1

Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Anticancer potential of acetone extracts from selected *Potentilla* species against human colorectal cancer cells. *Frontiers in Pharmacology*. 2022; 13, 1027315. DOI: 10.3389/fphar.2022.1027315.

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 29 September 2022
DOI 10.3389/fphar.2022.1027315

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 24 August 2022
ACCEPTED 13 September 2022
PUBLISHED 29 September 2022

CITATION
Augustynowicz D, Lemieszek MK, Strawa JW, Wiater A and Tomczyk M (2022), Anticancer potential of acetone extracts from selected Potentilla species against human colorectal cancer cells. *Front. Pharmacol.* 13:1027315. doi: 10.3389/fphar.2022.1027315

Anticancer potential of acetone extracts from selected *Potentilla* species against human colorectal cancer cells

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Cinquefoils have been widely used in local folk medicine in Europe and Asia to manage various gastrointestinal inflammations and/or infections, certain forms of cancer, thyroid gland disorders, and wound healing. In the present paper, acetone extracts from aerial parts of selected *Potentilla* species, namely *P. alba* (PAL7), *P. argentea* (PAR7), *P. grandiflora* (PGR7), *P. norvegica* (PN7), *P. recta* (PRE7), and the closely related *Drymocalis rupestris* (syn. *P. rupestris*) (PRU7), were analysed for their cytotoxicity and antiproliferative activities against human colon adenocarcinoma cell line LS180 and human colon epithelial cell line CCD841 CoN. Moreover, quantitative assessments of the total polyphenolic (TPC), total tannin (TTC), total proanthocyanidins (TPrC), total flavonoid (TFC), and total phenolic acid (TPAC) were conducted. The analysis of secondary metabolite composition was carried out by LC-PDA-HRMS. The highest TPC and TTC were found in PAR7 (339.72 and 246.92 mg gallic acid equivalents (GAE)/g extract, respectively) and PN7 (332.11 and 252.3 mg GAE/g extract, respectively). The highest TPrC, TFC, and TPAC levels were found for PAL7 (21.28 mg catechin equivalents (CAT)/g extract, 71.85 mg rutin equivalents (RE)/g extract, and 124.18 mg caffeic acid equivalents (CAE)/g extract, respectively). LC-PDA-HRMS analysis revealed the presence of 83 compounds, including brevifolincarboxylic acid, ellagic acid, pedunculagin, agrimoniin, chlorogenic acid, astragalin, and tiliroside. Moreover, the presence of tri-coumaroyl spermidine was demonstrated for the first time in the genus *Potentilla*. Results of the MTT assay revealed that all tested extracts decreased the viability of both cell lines; however, a markedly stronger effect was observed in the colon cancer cells. The highest selectivity was demonstrated by PAR7, which effectively inhibited the metabolic activity of LS180 cells (IC_{50} = 38 µg/ml), while at the same time causing the lowest unwanted effects in CCD841 CoN cells (IC_{50} = 1,134 µg/ml). BrdU assay revealed a significant decrease in DNA synthesis in both examined cell lines in response to all investigated extracts. It should be emphasized that the tested extracts had a stronger effect on colon cancer cells than normal colon cells, and the most significant antiproliferative properties were observed in the case of PAR7 (IC_{50} LS180 = 174 µg/ml) and PN7 (IC_{50} LS180 = 169 µg/ml). The results of

LDH assay revealed that all tested extracts were not cytotoxic against normal colon epithelial cells, whereas in the cancer cells, all compounds significantly damaged cell membranes, and the observed effect was dose-dependent. The highest cytotoxicity was observed in LS180 cells in response to PAR7, which, in concentrations ranging from 25 to 250 µg/ml, increased LDH release by 110%–1,062%, respectively. Performed studies have revealed that all *Potentilla* species may be useful sources for anti-colorectal cancer agents; however, additional research is required to prove this definitively.

KEYWORDS

***Potentilla*, Rosaceae, LC-PDA-HRMS, polyphenols, colorectal cancer, LS180 cells, cytotoxicity**

Introduction

The modern world struggles with the increasing problem of cancer, a significant cause of death worldwide. In 2020, the third most commonly diagnosed type of cancer after breast and lung cancers was colorectal cancer, estimated to represent 10.0% of total cancer cases and the second leading cause of cancer death (9.4% of total cancer deaths) (Sung et al., 2021). However, due to the Western lifestyle, which is closely associated with low physical activity, a high-fat diet, and high red meat consumption, the projected number of global new colorectal cancer cases will rise from 1.93 million in 2020 to 3.15 million cases in 2040 (Xi and Xu, 2021). Therefore, the economic burden of treatment and the high mortality rate of patients resulting from cancer recurrence after chemotherapy suggests a significant need for more efficient and safer drug candidates. However, access to the most effective and modern diagnostic methods and treatments is limited for a large proportion of people. Especially in rural areas, people predominantly still depend on phytotherapy (Edgar et al., 2007). Notably, *Potentilla* species, known as cinquefoils, are widely used, since they are well known in traditional medicine throughout the Asian and European continents as valuable phytomedicines in a remedy *inter alia* against diarrhoea, ulcers, fever, jaundice, oral inflammations, topical infections, and thyroid gland disorders (Tomczyk and Latte, 2009). Moreover, ancient Chinese medical works, in particular *Compendium of Chinese Materia Medica* and *Mingyi Bielu* mentioned that aerial parts of two *Potentilla* species, namely *P. indica* and *Duchesnea chrysantha* were used as anticancer agents in monotherapy or as a main ingredient of complex formulas against unspecified types of cancers (Peng et al., 2009). A number of studies have reported on the abundance of secondary metabolites in *Potentilla* species, which determine their anti-inflammatory, antimicrobial, and antioxidative properties (Augustynowicz et al., 2021b). Moreover, earlier studies on several *Potentilla* species have shown their anti-cancer potential against various cell lines, e.g., triterpenoids isolated from *P. chinensis* were cytotoxicity against MCF7 (human breast cancer), Hep G2 (human hepatocellular carcinoma) and T84 (human colonic adenocarcinoma), while extracts and fractions from aerial parts of *P. alba* decreased proliferation and viability of HT-29 (human colon adenocarcinoma) (Zhang et al., 2017; Kowalik et al., 2020).

We hypothesized that aerial parts of selected *Potentilla* species, similarly to other species from this genus, would exhibit broad pharmacological potential. Therefore, the primary aim of our study was to assess their cytotoxicity and antiproliferative activities against human colon adenocarcinoma cell line LS180 and human colon epithelial cell line CCD841 CoN. Moreover, we identified the marker metabolites present in extracts through LC-PDA-HRMS analysis to uncover correlations between the qualitative chemical composition of extracts and their possible mechanism of action.

Materials and methods

Reagents

The reference substances, including procyanidin B1, procyanidin B2 and procyanidin C1 were obtained from Cayman Chemical (Ann Arbor, MI, United States). Quercetin 3-*O*-glucuronide, kaempferol 3-*O*-glucuronide and isorhamnetin 3-*O*-glucoside were obtained from Extrasynthese (Genay, France) (+)-Catechin, (-)-epicatechin and gallic acid were the products of Carl Roth (Karlsruhe, Germany). Quercetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside, kaempferol 3-*O*-glucoside (purity >96%) were isolated from flowers of *Ficaria verna* L. Hud (Ranunculaceae) (Gudej and Tomczyk, 1999). Quercetin 3-*O*-galactoside (purity >96%) was isolated from aerial parts of *Rubus saxatilis* L. (Rosaceae) (Tomczyk and Gudej, 2005) and pedunculagin was isolated from leaves of *Rubus caesius* L. (Rosaceae) (Grochowski et al., 2020). Quercetin 3-*O*-arabinofuranoside, ellagic acid and tiliroside (purity >96%)

were isolated from aerial parts of *Drymocalis rupestris* (L.) Soják (Rosaceae) (Tomczyk, 2011). Agrimoniin and ellagic acid 3,3′-di-*O*-methyl ether 4-*O*-xyloside (purity >96%) were isolated from aerial parts of *P. recta* (Tomczyk, 2011; Bazylko et al., 2013). Apigenin and 3-*O*-caffeoylquinic acid (purity >96%) were isolated from leaves and inflorescences of *Arctium tomentosum* Mill. (Asteraceae) (Strawa et al., 2020). All other chemicals of analytical grade used in the study were purchased from Sigma-Aldrich (St. Louis, MO, United States). A POLWATER DL3-100 Labopol (Kraków, Poland) assembly was used to obtain ultra-pure water. Stock solutions of investigated extracts (100 mg/ml), as well as 5-fluorouracil (50 mM), were prepared by dissolving the compounds in dimethyl sulfoxide (DMSO) (POCH, Gliwice, Poland). Working solutions of investigated compounds were prepared by dissolving an appropriate stock solution in a culture medium. The final concentration of DMSO in all working solutions used in the studies was the same including control and it was 0.25%.

Plant materials and procedure of plant extracts preparation

Seeds of five species, namely *P. alba* (ind. sem. 354), *P. grandiflora* (ind. sem. 758), *P. norvegica* (ind. sem. 303), *P. recta* (ind. sem. 1549) and *P. rupestris* (ind. sem. 763) were kindly provided by the Botanical Garden of Vilnius University (Vilnius, Lithuania), Giardino Botanico Alpino (Cogne, Italy), Hortus Botanicus Universitatis Masarykianae (Brno, Czech Republic) and Hortus Botanicus University of Tartu (Tartu, Estonia). Plants were cultivated in common plots at the Medicinal Plant Garden at the Medical University of Białystok (Białystok, Poland), and aboveground materials were collected in June-August 2016–2019. Aerial parts of *P. argentea* were collected in June-July 2017–2019 from natural habitat, at Puszcza Knyszyńska (Poland, 53°15′6″N 23°27′58″E). The taxonomic identification of plant material was carefully authenticated by one of the authors (M.T.). Voucher specimens of *P. alba* (PAL-17039), *P. argentea* (PAR-02009), *P. grandiflora* (PGR-06020), *P. norvegica* (PNO-08024), *P. recta* (PRE-06019) and *P. rupestris* (PRU-06021) have been deposited at the Herbarium of the Department of Pharmacognosy, Medical University of Białystok (Poland). Collected dried materials were subsequently finely grounded with an electric grinder and stored in air-tight containers at ambient temperature. Powdered dry plant materials (2.0 g each time) were separately submitted to ultrasound-assisted extraction with 70% acetone (3 × 50 ml) using an ultrasonic bath (Sonic-5, Polsonic, Warszawa, Poland) at a controlled temperature (40 ± 2 °C) for 45 min in a 1:75 (*w:v*) solvent ratio. The obtained raw extracts after solvent evaporation were diluted with water (50 ml) and subsequently portioned with chloroform (10 × 20 ml). The acetone extracts were obtained using this method for *P. alba* (**PAL7**), *P. argentea* (**PAR7**), *P. grandiflora* (**PGR7**), *P. norvegica* (**PN7**), *P. recta* (**PRE7**) and *P. rupestris* (**PRU7**).

Determination of total phenolic content

The total phenolic content (TPC) was measured by the Folin-Ciocalteu assay with some modifications (Slinkard and Singleton, 1977). Briefly, 25 µl of tested solution (1 mg/ml) was mixed with 100 µl of diluted Folin-Ciocalteu reagent (1:9, *v/v*) and the mixture was allowed to react for 3 min. Thereafter, 75 µl of 1% Na_2CO_3 solution was added and the prepared mixture was incubated for 2 h at ambient temperature. The absorbance was measured at 760 nm using a microplate reader EPOCH2 BioTech (Winooski, VT, United States). The TPC determination was repeated at least three times for each sample solution. Obtained results were expressed as milligrams of gallic acid equivalents per Gram of dry extract (mg GAE/g extract).

Determination of total tannin content

The total tannin content (TTC) of each extract was measured by the employment of the protein-binding method and Folin-Ciocalteu assay described in the European Pharmacopoeia 10th ed (European Pharmacopoeia, 2019). with modifications. Briefly, each extract dissolved in water (1 mg/ml) was partitioned into two parts. For the first part of extracts total polyphenols were determined for each aliquot (25 µl) by mixing with 100 µl of diluted Folin-Ciocalteu reagent (1:9, *v/v*). After 3 min 75 µl of 1% Na_2CO_3 was added and the mixture was allowed to stand for 2 h at room temperature. Thereafter the absorbance of each sample (A_1) was recorded at 760 nm using a EPOCH2 microplate reader. Subsequently, the second part of aliquots of 0.5 ml each were mixed with 10 mg of hide powder. These preparations were shaken for 1 h without light and then centrifugated. A 25 µl of supernatants were assayed for total polyphenolics as described above and the absorbance of each sample (A_2) was recorded at 760 nm. Afterwards, the total tannin content was determined by subtraction of absorbances of total polyphenols (A_1) from total non-tannin polyphenols (A_2) and the obtained absorbance values were referred to a gallic acid calibration curve to obtain their values as milligrams of gallic acid equivalents per Gram of dry extract (mg GAE/g extract). The determination of TTC was repeated at least three times for each sample solution.

TABLE 1 Total phenolic (TPC), tannin (TTC), proanthocyanidin (TPrC), flavonoid (TFC) and phenolic acid contents (TPAC) of selected acetone extracts of *Potentilla* species.

Samples	TPC (mg GAE/g extract)	TTC (mg GAE/g extract)	TPrC (mg CE/g extract)	TFC (mg RE/g extract)	TPAC (mg CAE/g extract)
PAL7	159.87 ± 1.79	84.89 ± 1.40	21.28 ± 0.04	71.85 ± 1.40	124.18 ± 1.18
PAR7	339.72 ± 5.29	246.92 ± 4.64	6.95 ± 0.07	56.79 ± 0.98	78.95 ± 0.90
PGR7	228.36 ± 3.40	156.53 ± 3.71	3.80 ± 0.06	47.61 ± 0.35	58.61 ± 0.34
PN7	332.11 ± 1.40	252.30 ± 1.70	1.14 ± 0.02	38.06 ± 0.79	92.78 ± 1.03
PRE7	257.68 ± 2.95	170.45 ± 2.86	2.70 ± 0.08	43.37 ± 0.84	75.20 ± 1.23
PRU7	304.08 ± 2.51	209.43 ± 2.57	1.11 ± 0.02	47.74 ± 0.73	55.45 ± 0.59

*GAE, gallic acid equivalent; CE, catechin equivalent; RE, rutin equivalent; CAE, caffeic acid equivalent.

Determination of total proanthocyanidin content

The total proanthocyanidin content (TPrC) was analysed with the employment of a 4-dimethylamino-cinnamaldehyde (DMCA) reagent (Feliciano et al., 2012). The analysis was carried out in a microplate reader. A 50 µl of sample solution (1 mg/ml) dissolved in methanol was mixed with 250 µl of 0.1% DMCA in 6 M HCl in methanol. The mixture was incubated at ambient temperature for 15 min, and thereafter, the absorbance was recorded at 635 nm. The TPrC determination was repeated at least five times for each sample solution and was expressed as milligrams of catechin equivalents per Gram of dry extract (mg CE/g extract).

Determination of total flavonoid content

The total flavonoid content (TFC) of each extract was determined using the previously described aluminium chloride ($AlCl_3$) colorimetric method (Augustynowicz et al., 2021a) with slight modifications. In brief, 100 µl of tested solution or 100 µl of blank sample (methanol) was mixed with 100 µl of 2% (*w:v*) $AlCl_3$ solution. The mixture was kept at ambient temperature for 10 min. Then the absorbance of the mixture was recorded at 415 nm using a EPOCH2 microplate reader. The TFC determination was repeated at least three times for each sample solution. TFC was expressed as milligrams of rutin equivalents per Gram of dry extract (mg RE/g extract).

Determination of total phenolic acid content

The total phenolic acid content (TPAC) determination was carried out using the procedure with the use of Arnov's reagent (1 g of sodium molybdate and 1 g of sodium nitrate dissolved in 10 ml of distilled water) (Polumackanycz et al., 2019). A 30 µl of the tested solution, 180 µl of water, 30 µl of 0.5 M HCl, 30 µl of Arnov's reagent and 30 µ of 1 M NaOH were sequentially added to the microplate well. After incubation of mixture at room temperature for 20 min, the absorbance was measured at 490 nm. The TPAC determination was repeated at least three times for each sample solution and the obtained values were expressed as milligrams of caffeic acid equivalents per Gram of dry extract (mg CAE/g extract).

Estimation of qualitative composition with the employment of LC-PDA-HRMS

Evaluation of the secondary metabolite composition of each extract was conducted using an Agilent 1260 Infinity LC chromatography system coupled to a photo-diode array (PDA) detector and 6230 time-of-flight (TOF) mass spectrometer (Santa Clara, CA, California). The MS conditions were as follows: electrospray ionization (ESI) source in both negative and positive ionization mode, drying and sheath gas flow 11 L/min and temperature of 350°C, nebulizer pressure of 60 psi, capillary voltages of 2,500 and 4500 V for negative and positive ion modes, respectively and fragmentor experiments at 60, 180 and 320 V. The data were collected in the 120–3,000 m/*z* range. The separation was performed using a Kinetex XB-C18 column (150 × 2.1 mm, 1.7 µm, Phenomenex, Torrance, CA, United States). The mobile phases were ultra pure water (A) and acetonitrile (B) with 0.2% formic acid. The separation was achieved by a gradient of 0–3 min 65% B; 3–35 min 1% B, 35–80 min 12% B, 80–113 min 45% B, extended by 7 min of equilibrating time. The flow rate was 0.2 ml/min, and the column temperature was maintained at 35 ± 0.8°C. The UV-vis spectra were recorded in the range of 190–540 nm with selective wavelength monitoring at 280 and 360 nm. Data were processed with the employment of MassHunter Qualitative 10.0. Analysis software. Compounds were characterized based on UV–Vis and MS spectra and retention time of standards.

TABLE 2 MS and UV-Vis data of compounds detected in acetone extracts prepared from aerial parts of selected *Potentilla* species.

No.	Compounds	Rt [min]	UV spectra [λ max nm]	Observed[a]	Δ [ppm]	Formula	Fragmentation		Presence in extracts						Ref
							Negative	Positive	PAL7	PAR7	PGR7	PN7	PRE7	PRU7	
1	Gallic acid	5.70	270	169.01370	-3.44	$C_7H_6O_5$	**169**, 125			+	+	+	+	+	(s)
2	2-Pyrone-4,6-dicarboxylic acid	6.65	316	182.99276	-3.46	$C_7H_4O_6$	366, **182**, 139			+	+	+	+	+	Wilkes and Glasl. (2001)
3	Pedunculagin α or β	15.35	260sh	783.06883	0.38	$C_{34}H_{24}O_{22}$	**783**, 481, 301			+	+	+	+	+	Grochowski et al. (2020), (s)
4	Polyphenol derivative	15.70	280	337.11359	-1.03	$C_{13}H_{22}O_{10}$	**193**, 125		+						
5	5-*O*-Caffeoylquinic acid	20.07	295sh, 325	353.08747	-0.96	$C_{16}H_{18}O_9$	**353**, 191, 179	355, **163**	+		+	+			(s)
6	Galloyl-HHDP-glucose	22.26	250sh	633.07245	-0.24	$C_{27}H_{22}O_{18}$	**633**, 301							+	
7	Unknown	22.45	276	345.11788	-2.82	$C_{15}H_{22}O_9$	**345**, 299, 161							+	
8	Pedunculagin α or β	23.30	260sh	783.06929	0.85	$C_{34}H_{24}O_{22}$	**783**, 481, 301			+	+	+	+	+	Grochowski et al. (2020), (s)
9	Galloyl-HHDP-glucose	24.07	280sh	633.07359	0.36	$C_{27}H_{22}O_{18}$	**633**, 481, 301				+	+	+		
10	*p*-Coumaroylquinic acid isomer	24.41	308	337.09247	-0.92	$C_{16}H_{18}O_8$	**337**, 191, 163	339, **147**	+						
11	*p*-Coumaroylquinic acid isomer	25.27	312	337.09218	-1.59	$C_{16}H_{18}O_8$	**337**, 191, 163	339, **147**	+						
12	Procyanidin B1	26.20	280	577.13507	0.81	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 291, 139		+					(s)
13	Catechin	27.10	280	289.07136	-1.42	$C_{15}H_{14}O_6$	**289**, 245	**291**, 139		+			+		(s)
14	3-*O*-Caffeoylquinic acid	28.21	295sh, 325	353.08729	-1.45	$C_{16}H_{18}O_9$	**353**, 191	355, **163**	+		+	+	+		Strawa et al. (2020), (s)
15	Digalloyl-HHDP-glucose	28.56	275	785.08369	-0.63	$C_{34}H_{26}O_{22}$	785, **301**							+	
16	Feruloylquinic acid isomer	29.86	295sh, 325	367.10365	0.61	$C_{17}H_{20}O_9$	**367**, 193	369, **177**	+		+				
17	Caffeoylquinic acid isomer	30.91	295sh, 325	353.08779	-0.08	$C_{16}H_{18}O_9$	**353**, 191, 179	355, **163**	+						
18	Galloyl-HHDP-glucose	31.61	275	633.07366	-0.27	$C_{27}H_{22}O_{18}$	**633**, 463, 301			+	+	+	+	+	
19	Brevifolincarboxylic acid	32.11	278, 360	291.01385	-2.02	$C_{13}H_8O_8$	**291**, 247	293		+	+	+	+	+	Luo et al. (2020)
20	Procyanidin B2	33.73	278	577.13556	0.82	$C_{30}H_{26}O_{12}$	577, 289	**579**, 291, 139	+						(s)
21	Ellagic acid derivative	33.89	285sh	898.13313	-1.54	$C_{20}H_{30}O_{32}$	898, 633, **301**				+				
22	Ellagic acid derivative	34.13	325sh	632.06474	-1.85	$C_{27}H_{21}O_{18}$	632, 463, **301**				+				
23	Quercetin *O*-hexoso *O*-uronic acid derivative	34.20	255, 350	639.12125	1.20	$C_{27}H_{28}O_{18}$	**639**, 300	**641**, 479, 303			+				
24	Quercetin *O*-di-uronic acid derivative	34.40	255, 355	653.09958	-0.45	$C_{28}H_{26}O_{19}$	**653**, 477, 301	**655**, 479, 303		+	+		+		
25	*p*-Coumaroylquinic acid isomer	35.13	312	337.09285	0.02	$C_{16}H_{18}O_8$	**337**, 191	**339**, 147	+		+				

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TABLE 2 (*Continued*) MS and UV-Vis data of compounds detected in acetone extracts prepared from aerial parts of selected *Potentilla* species.

No.	Compounds	Rt [min]	UV spectra [λ max nm]	Observed[a]	Δ [ppm]	Formula	Fragmentation		Presence in extracts						Ref
							Negative	Positive	PAL7	PAR7	PGR7	PN7	PRE7	PRU7	
26	Quercetin *O*-hexoso *O*-uronic acid derivative	35.35	270, 350	639.12105	-0.61	$C_{27}H_{28}O_{18}$	**639**, 463, 301	**641**, 465, 303		+				+	
27	Epicatechin	35.74	280	289.07133	-1.43	$C_{15}H_{14}O_6$	**289**, 245	**291**, 139	+						(s)
28	2-Caffeoylisocitric acid	36.30	300sh, 328	353.05046	-2.69	$C_{15}H_{14}O_{10}$	**353**, 191, 155					+	+	+	Manyelo et al. (2020)
29	*p*-coumaroylquinic acid isomer	36.52	312	337.09314	-0.26	$C_{16}H_{18}O_8$	**337**, 163	**339**, 147	+						
30	Caffeoylmalic acid	38.51	295sh, 326	295.04541	-1.72	$C_{13}H_{12}O_8$	591, **295**, 179, 133	**135**	+		+	+	+		Szajwaj et al. (2011)
31	Kaempferol *O*-di-uronic acid derivative	38.97	265, 350	637.10470	-0.62	$C_{27}H_{26}O_{18}$	637, 461, 285	**639**, 463, 287		+			+		
32	Trigalloylglucose isomer	39.10	276	635.08918	0.20	$C_{27}H_{24}O_{18}$	**635**, 465, 313, 169							+	Luo et al. (2020)
33	Brevifolin	39.4	275, 350	247.02433	-1.83	$C_{12}H_8O_6$	**247**, 191	**249**		+	+	+	+	+	Luo et al. (2020)
34	Procyanidin A-type trimer	40.51	280	863.18353	1.19	$C_{45}H_{36}O_{18}$	**863**, 573, 289	**865**, 287	+						
35	Ellagic acid *O*-hexoside derivative	41.20	252, 365	463.05162	-0.79	$C_{20}H_{16}O_{13}$	**463**, 301					+		+	
36	Isorhamnetin *O*-di-uronic acid derivative	41.78	254, 352	667.11555	1.29	$C_{28}H_{28}O_{19}$	**667**, 315, 300	**669**, 493, 317		+	+		+		
37	Procyanidin C1	41.88	280	865.19924	0.68	$C_{45}H_{38}O_{18}$	**865**, 577, 289	**867**, 579, 291	+						(s)
38	Galloyl-bis-HHDP-glucose	43.98	255	935.07947	0.30	$C_{41}H_{28}O_{26}$	**935**, 633, 467, 301			+		+	+	+	
39	Laevigatin isomer	44.46	255	1,567.14331	-1.15	$C_{68}H_{48}O_{44}$	1,567, **783**, 301			+	+	+	+	+	Fecka et al. (2015)
40	Laevigatin isomer	45.94	255	1,567.14331	-1.15	$C_{68}H_{48}O_{44}$	1,567, **783**, 301				+		+		Fecka et al. (2015)
41	Quercetin *O*-hexoso *O*-deoxyhexoside isomer	46.98	255, 352	609.14615	-0.73	$C_{27}H_{30}O_{16}$	**609**, 446, 299	**611**, 499, 303				+			
42	Galloyl-bis-HHDP-glucose	47.33	276sh	935.07900	-0.79	$C_{41}H_{28}O_{26}$	935, 783, 633, 467, **301**			+		+	+	+	
43	Quercetin *O*-hexoso-deoxyhexo-pentoside isomer	47.90	255, 355	741.18912	-0.64	$C_{32}H_{38}O_{20}$	741, 447, **300**	743, 611, 465, **303**	+						
44	Quercetin *O*-deoxyhexoso-*O*-hexoso-deoxyhexoside isomer	48.66	256, 356	755.20326	-0.67	$C_{33}H_{40}O_{20}$	**755**, 609, 446, 299	**757**, 611, 449, 303				+			
45	Quercetin *O*-hexoso-pentoside isomer	49.55	255, 355	595.13019	-0.10	$C_{26}H_{28}O_{16}$	595, **300**, 271	597, 465, **303**	+				+		
46	Quercetin *O*-hexoso-pentoside isomer	50.48	255, 355	595.13046	-0.72	$C_{26}H_{28}O_{16}$	595, **300**, 271	597, 465, **303**	+					+	

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10.3389/fphar.2022.1027315

TABLE 2 (*Continued*) MS and UV-Vis data of compounds detected in acetone extracts prepared from aerial parts of selected *Potentilla* species.

No.	Compounds	Rt [min]	UV spectra [λ max nm]	Observed[a]	Δ [ppm]	Formula	Fragmentation		Presence in extracts						Ref
							Negative	Positive	PAL7	PAR7	PGR7	PN7	PRE7	PRU7	
47	Laevigatin isomer	51.09	255	1,567.14239	-1.74	$C_{68}H_{48}O_{44}$	1,567, **783**, 301			+		+	+	+	Fecka et al. (2015)
48	Quercetin *O*-pentoso-*O*-uronic acid derivative	52.30	255, 354	609.11075	-1.11	$C_{26}H_{26}O_{17}$	609, **301**	611, **479**, 303		+				+	
49	Ellagic acid 3′-*O*-methyl ether *O*-uronic acid derivative	54.10	254, 360	491.04709	0.29	$C_{21}H_{16}O_{14}$	**491**, 315, 301			+	+	+	+		
50	Ellagic acid *O*-pentoside	55.7	252, 360	433.04108	0.30	$C_{19}H_{14}O_{12}$	**433**, 301			+			+		
51	Ellagic acid	56.71	254, 370	300.99841	-1.60	$C_{14}H_6O_8$	**301**, 271	**303**		+	+	+	+	+	Tomczyk (2011), (s)
52	Quercetin 3-*O*-glucoside	59.30	255, 355	463.08816	0.13	$C_{21}H_{20}O_{12}$	**463**, 300, 271	**465**, 303	+		+	+	+		(s)
53	Unknown	59.80	290	435.09238	-2.77	$C_{20}H_{20}O_{11}$	871, **435**, 285, 151							+	
54	Ellagic acid 3′- *O*-methyl ether 4-*O*-pentoside	60.40	252, 362	447.05600	-1.85	$C_{20}H_{16}O_{12}$	**447**, 301							+	
55	Tetragalloylglucose isomer	62	278	787.09898	-1.57	$C_{34}H_{28}O_{22}$	**787**, 617, 465, 169							+	Luo et al. (2020)
56	Quercetin 3-*O*-rutoside	63.38	256, 354	609.14571	-0.32	$C_{27}H_{30}O_{16}$	**609**, 300, 271	**611**, 465, 303			+	+	+		Gudej and Tomczyk, (1999), (s)
57	Quercetin 3-*O*-galactoside	64.03	255, 355	463.08816	-0.70	$C_{21}H_{20}O_{12}$	**463**, 300, 271	**465**, 303	+	+	+		+	+	Tomczyk and Gudej, (2005), (s)
58	Quercetin *O*-glucuronide	64.83	255, 355	477.06649	-1.73	$C_{21}H_{20}O_{13}$	**477**, 300, 271	**479**, 303		+			+	+	(s)
59	Kaempferol *O*-hexoso-pentoside	64.85	265, 350	579.13594	1.19	$C_{26}H_{28}O_{15}$	**579**, 284	581, 449, **287**	+						
60	Quercetin *O*-uronic acid derivative	66.18	256, 354	477.06713	-0.49	$C_{21}H_{18}O_{13}$	**477**, 301	**479**, 303			+				
61	Kaempferol *O*-hexoso-pentoside	66.85	265, 350	579.13520	-0.41	$C_{26}H_{28}O_{15}$	577, **284**	581, **449**, 287	+						
62	Kaempferol *O*-hexoside	67.40	252, 350	447.09365	-0.44	$C_{21}H_{20}O_{11}$	**447**, 284	**449**, 287			+		+		
63	Galloyl-bis-HHDP-glucose	69	260sh	935.07978	-0.18	$C_{41}H_{28}O_{26}$	**935**, 467, 301			+	+	+	+		
64	Isorhamnetin *O*-hexoso-pentoside	73.99	255, 352	609.14611	0.20	$C_{27}H_{30}O_{16}$	**609**, 314, 271	611, 479, **317**	+				+		
65	Quercetin 3-*O*-arabinofuranoside	85.20	254sh, 350	433.07665	-2.12	$C_{20}H_{18}O_{11}$	**433**, 300	435, **303**						+	Tomczyk (2011), (s)
66	Kaempferol 3-*O*-glucoside	88.33	265, 350	447.09298	-1.70	$C_{21}H_{20}O_{11}$	**447**, 284	**449**, 287	+	+	+	+	+	+	Gudej and Tomczyk, (1999), (s)
67	Kaempferol 3-*O*-glucuronide	89.30	265, 346	461.07171	-0.92	$C_{21}H_{18}O_{12}$	**461**, 285	**463**, 287		+			+	+	(s)

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TABLE 2 (*Continued*) MS and UV-Vis data of compounds detected in acetone extracts prepared from aerial parts of selected *Potentilla* species.

No.	Compounds	Rt [min]	UV spectra [λ max nm]	Observed[a]	Δ [ppm]	Formula	Fragmentation		Presence in extracts						Ref
							Negative	Positive	PAL7	PAR7	PGR7	PN7	PRE7	PRU7	
68	Apigenin *O*-hexoside	90.04	266, 340	431.09754	-0.71	$C_{21}H_{20}O_{10}$	**431**, 268	**433**, 271			+				
69	Agrimoniin	90.30	250sh	1870.15689	-0.95	$C_{82}H_{54}O_{52}$	1870, 1,085, **934**, 783, 301			+	+	+	+	+	Bazylko et al. (2013), (s)
70	Ellagic acid 3′-*O*-methyl ether 4-*O*-pentoside	90.42	280sh, 365	447.05604	-0.72	$C_{20}H_{16}O_{12}$	**447**, 315, 301			+		+	+		Luo et al. (2020)
71	Isorhamnetin 3-*O*-glucoside	91.43	265, 355	477.10387	0.49	$C_{22}H_{22}O_{12}$	**477**, 314	**479**, 317	+						(s)
72	Pentagalloylglucose isomer	91.68	280	939.11105	0.49	$C_{41}H_{32}O_{26}$	**939**, 769, 469, 169							+	
73	Isorhamnetin *O*-deoxyhexoso-hexoso-*O*-pentoside isomer	91.92	254sh, 355	753.18766	-0.45	$C_{33}H_{38}O_{20}$	**753**, 314, 299	755, 623, **317**					+		
74	Isorhamnetin *O*-uronic acid derivative	92.81	255, 354	491.08356	0.77	$C_{22}H_{20}O_{13}$	**491**, 315, 300	**493**, 317		+			+		
75	Ellagic acid 3,3′-di-*O*-methyl ether 4-*O*-xyloside	94.14	245, 370	461.07148	-1.26	$C_{21}H_{18}O_{12}$	**461**, 328, 297	**463**, 331				+	+		Tomczyk (2011), (s)
76	Quercetin *O*-uronic acid derivative	94.80	270sh, 370	477.06758	0.10	$C_{21}H_{18}O_{13}$	**477**, 301	**479**, 303				+			
77	Kaempferol derivative	94.90	266sh, 348	533.09391	0.27	$C_{24}H_{22}O_{14}$	**533**, 489, 284	**535**, 287			+				
78	Apigenin	98.04	268, 338	269.04538	-2.04	$C_{15}H_{10}O_5$	**269**, 227	**271**			+				Strawa et al. (2020), (s)
79	*trans*-Tiliroside	101.48	268, 315	593.12979	-0.55	$C_{30}H_{26}O_{13}$	**593**, 284	**595**, 287	+	+	+	+	+	+	Tomczyk (2011), (s)
80	Kaempferol derivative	101.87	268, 330	623.14131	1.02	$C_{31}H_{28}O_{14}$	**623**, 284	**625**, 595, 287	+				+	+	
81	*cis*-Tiliroside	102.37	268, 315	593.12928	0.44	$C_{30}H_{26}O_{13}$	**593**, 284	**595**, 287		+	+	+	+	+	Luo et al. (2020)
82	Unknown	102.54	280	445.18621	-1.13	$C_{24}H_{30}O_8$	**445**, 385		+					+	
83	N^1, N^5, N^{10}-tricoumaroyl spermidine	104.47	295, 310sh	582.26072	-0.06	$C_{34}H_{37}N_3O_6$	**582**, 462, 342, 285	**584**, 438, 292, 147	+	+	+	+	+	+	Elejalde-Palmett et al. (2015)

[a]Exact mass of $[M-H]^-$ ion; sh–peak shoulder; bold–most aboundantion; (s)—reference substance; HHDP, hexahydroxydiphenoyl group.

TABLE 3 IC_{50} values (concentration causing viability/proliferation inhibition by 50% compared to control) of acetone extracts isolated from the aerial parts of *Potentilla* L and 5-fluorouracil (5-FU). IC_{50} values were calculated for human colon epithelial cell line CCD841 CoN and human colon adenocarcinoma cell line LS180 based on results of MTT and BrdU assays performed after 48 h of cells treatment with investigated compounds.

Sample	MTT assay						BrdU assay					
	LS180			CCD841 CoN			LS180			CCD841 CoN		
	IC_{50} (μg/ml)	Trust range (μg/ml)	R^2	IC_{50} (μg/ml)	Trust range (μg/ml)	R^2	IC_{50} (μg/ml)	Trust range (μg/ml)	R^2	IC_{50} (μg/ml)	Trust range (μg/ml)	R^2
PAL7	182	169–196	0.983	233	209–261	0.971	12,008	2096–68,805	0.752	4,164	1759–9,859	0.867
PAR7	38	32–44	0.974	1,134	575–2,235	0.902	174	165–183	0.982	217	203–231	0.977
PGR7	58	50–67	0.957	982	498–1938	0.890	372	338–409	0.968	570	488–666	0.965
PN7	32	28–37	0.981	757	459–1,248	0.903	169	159–179	0.974	217	202–233	0.958
PRE7	35	30–42	0.969	918	449–1879	0.882	237	223–251	0.966	268	248–289	0.943
PRU7	36	30–42	0.974	846	481–1,489	0.916	360	311–416	0.95	538	425–681	0.926
5-FU	31	28–33	0.977	113	81–157	0.884	15	13–16	0.956	94	80–111	0.933

Cell cultures

Human colonic epithelial cell line CCD841 CoN was purchased from the American Type Culture Collection (ATCC, Manassas, VA, United States). Human colon adenocarcinoma cell line LS180 was obtained from the European Collection of Cell Cultures (ECACC, Centre for Applied Microbiology and Research, Salisbury, United Kingdom). Cell cultures were conducted in accordance with the guidelines of the collections in which they were purchased.

Examination of the anticancer potential of extracts

Both colon epithelial, as well as colon adenocarcinoma cells, were seeded on 96-well microplates at a density of 5×10^4 cells/mL. The following day, the culture medium was exchanged for fresh medium supplemented with investigated extracts or 25 μM 5-fluorouracil (5-FU). After 48 h of cell treatment, the compounds' antiproliferative effect was determined using Cell Proliferation ELISA BrdU, following the manufacturer's instructions (Roche Diagnostics GmbH, Penzberg, Germany), while the compounds' cytotoxicity was examined by the *In Vitro* Toxicology Assay Kit Lactate Dehydrogenase Based according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, United States). Furthermore, cell viability in response to 48 h of exposure to investigated compounds was determined by MTT assays. A detailed description of the execution of the above-mentioned assays was presented by Langner and co-authors (Langner et al., 2019).

Statistical analysis

The analysed data were presented as the mean ± SEM. Statistical analyses were performed using One way-ANOVA with the Tukey *post-hoc* test and column statistics. Statistical significance was accepted at $p < 0.05$. The IC_{50} value (concentration leading the 50% inhibition of proliferation compared to the control) was calculated using GraphPad PRISM.

Results

In the first set of experiments, the studied extracts were examined for their TPC, TTC, TPrC, TFC, and TPAC using colorimetric methods. The obtained results are presented in Table 1. **PAR7** and **PN7**, followed by **PRU7**, were found to contain the highest TPC (339.72, 332.11, and 304.08 mg GAE/g extract, respectively) and TTC (246.92, 252.30, and 209.43 mg GAE/g extract, respectively). **PAL7** had the lowest TPC and TTC values (159.87 and 84.89 mg GAE/g). However, **PAL7** was found to contain the highest TPrC (21.28 mg CE/g extract), while the other extracts had low proanthocyanidin content. The TFC levels for all tested samples were found to be similar, with the highest values for **PAL7** and **PAR7** (71.85 and 56.79 mg RE/g extract, respectively). Moreover, the highest TPAC values were revealed for **PAL7** and **PN7** (124.18 and 78.95 mg CAE/g extract, respectively).

To unveil the secondary metabolite composition, acetone extracts of selected *Potentilla* species were analysed via LC-PDA-HRMS. The analysis demonstrated the presence of 83 compounds, predominately polyphenolic compounds, ascribed to hydrolysable and condensed tannins, flavonoids, and phenolic acids. Hydrolysable tannins were present in all

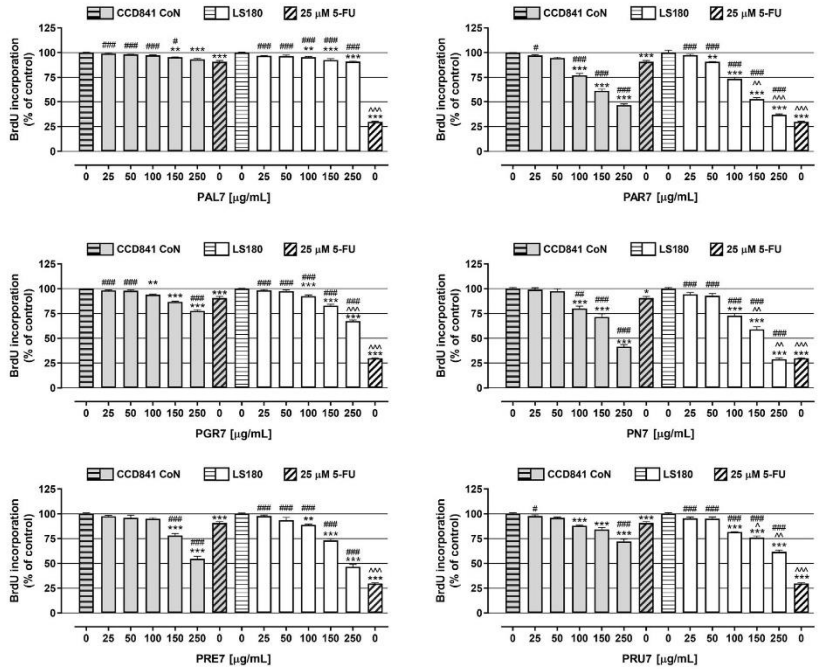

FIGURE 2
Antiproliferative effect of acetone extracts isolated from the aerial parts of *Potentilla* L. on human colon epithelial cell line CCD841 CoN and human colon adenocarcinoma cell line LS180. The cells were exposed for 48 h to the culture medium alone (control), or the extracts at concentrations of 25, 50, 100, 150, and 250 μg/ml, or 25 μM 5-fluorouracil (5-FU; positive control). The antiproliferative impact of the investigated compounds was measured by immunoassay based on BrdU incorporation into newly synthesized DNA. Results are presented as mean ± SEM of at least six measurements. $^{**}p < 0.01$; $^{***}p < 0.001$ vs. control, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ vs. positive control, $^{\wedge}p < 0.05$; $^{\wedge\wedge}p < 0.01$; $^{\wedge\wedge\wedge}p < 0.001$ colon cancer cells treated with extract/5-FU vs. colon epithelial cells exposed to the extract/5-FU at the corresponding concentration; one-way ANOVA test; post-test: Tukey.

highest tested concentration decreased LS180 cell proliferation by 87.3% (IC_{50} PN7 LS180 = 32 μg/ml), while the weakest effect was noted for **PAL7**, which at a concentration of 250 μg/ml inhibited cancer cells division by 57.5% (IC_{50} PAL7 LS180 = 182 μg/ml). In the case of colon epithelial cells, the strongest reduction (49.5%) of their metabolic activity was observed after exposure to 250 μg/ml **PAL7** (IC_{50} PAL7 CCD841 CoN = 233 μg/ml), while the weakest effect, as reflected by the IC_{50} value, was for **PAR7** (IC_{50} PAR7 CCD841 CoN = 1,134 μg/ml). Although all investigated extracts affected both normal and cancer colon cells, LS180 cells were more sensitive to the tested compounds. Comparing the metabolic activity in both analysed cell lines in response to extracts at the corresponding concentrations, greater sensitivity of cancer cells was observed in the entire range of analysed concentrations in the case of **PAR7**, **PRE7**, **PRU7**, and **PN7**, while **PGR7** showed statistically significant differences in concentrations from 50 μg/ml to 250 μg/ml. Even **PAL7** at the highest tested concentrations (150 and 250 μg/ml) strongly inhibited the viability of cancer cells than colon epithelial cells. As a positive control of the experiment, 5-fluorouracil (5-FU) at a concentration of 25 μM was used (Figure 1). The metabolic activity of CCD841 CoN and LS180 cells decreased in response to 5-FU by 22.2% and 46.2%, respectively. Comparing data obtained from the extracts with cell responses to 5-FU revealed that four of six investigated fractions at higher concentrations inhibited the metabolic activity of CCD841 CoN cells more strongly than 25 μM 5-FU: **PAL7** (100, 150, 250 μg/ml); **PRE7** (150, 250 μg/ml); and both **PAR7** and **PRU7** (250 μg/ml). In the case of colon cancer cells, **PGR7**, **PAR7**, **PRE7**, **PRU7**, and **PN7** at

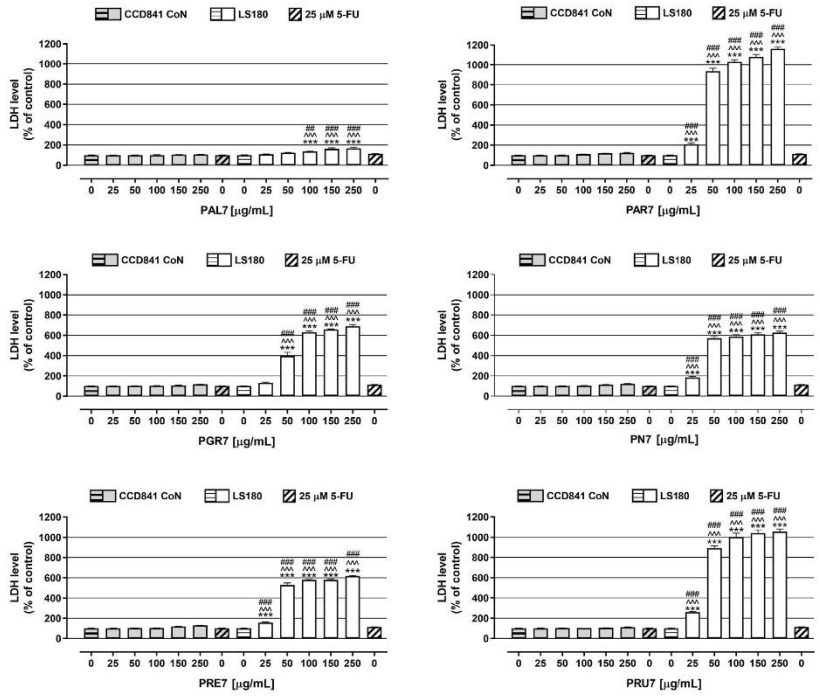

FIGURE 3
Influence of acetone extracts isolated from the aerial parts of *Potentilla* L. on cell membrane integrity of human colon epithelial CCD841 CoN cells and human colon adenocarcinoma LS180 cells. The cells were exposed for 48 h to the culture medium alone (control), or extracts at concentrations of 25, 50, 100, 150, and 250 μg/ml, or 25 μM 5-fluorouracil (5-FU; positive control). Compound cytotoxicity (level of LDH released into the cell culture medium from damaged cell membranes) was measured using LDH assay. Results are presented as mean ± SEM of at least six measurements. ***$p < 0.001$ vs. control, #$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$ vs. positive control, ^$p < 0.001$ colon cancer cells treated with extract/5-FU vs. colon epithelial cells exposed to the extract/5-FU at the corresponding concentration; one-way ANOVA test; post-test: Tukey.

concentrations from 50 to 250 μg/ml and **PAL7** at a concentration of 250 μg/ml showed a stronger anti-metabolic effect than the analysed cytostatic.

In the next step, the antiproliferative activity of *Potentilla* extracts was assessed in the abovementioned cell lines using BrdU assay (Figure 2; Table 3). A significant decrease of DNA synthesis in colon cancer cells was observed in response to all investigated extracts at concentrations ranging from 100 μg/ml to 250 μg/ml, and simultaneously in the case of **PAR7** a statistically significant antiproliferative effect was also noted at the concentration 50 μg/ml. Furthermore, **PAR7** and **PN7** showed the strongest inhibition of cancer cell proliferation, as reflected by the lowest IC_{50} values (IC_{50} PAR7 LS180 = 174 μg/ml and IC_{50} PN7 LS180 = 169 μg/ml) and the greatest decrease of DNA synthesis in LS180 cells in response to the extracts at a concentration of 250 μg/ml (cell proliferation was reduced by 63.1% (**PAR7**) and 71.1% (**PN7**)). Colon cancer cell division was least inhibited by **PAL7** (IC_{50} PAL7 LS180 = 12 mg/ml), which at the highest tested concentration decreased DNA synthesis by only 9.1%. The investigated extracts also affected the proliferation of colon epithelial cells and statistically significant inhibition of DNA synthesis was noted in response to all compounds at concentrations of 150 and 250 μg/ml, while in the case of **PN7**, **PAR7**, **PRU7**, and **PGR7** the antiproliferative effect was observed also at a concentration of 100 μg/ml. Similar to the colon cancer cells, epithelial cells were the most sensitive to **PN7** and **PAR7**, which at a concentration of 250 μg/ml reduced their proliferation by 58.4% and 53.4%, respectively (IC_{50} PN7 CCD841 CoN = 217 μg/ml and IC_{50} PAR7 CCD841 CoN = 217 μg/ml). The weakest

antiproliferative effect in CCD841 CoN cells was observed after exposure to **PAL7**, which at the highest tested concentration inhibited cell division by only 6.7%. Studies have revealed the antiproliferative abilities of *Potentilla* extracts in both normal and cancer colon cells, nevertheless **PGR7** at the highest tested concentration, as well as **PAR7**, **PRU7**, and **PN7** at concentrations 150 and 250 μg/ml, inhibited DNA synthesis significantly more strongly in LS180 cells than CCD841 CoN cells. As presented in Figure 2, 25 μM 5-fluorouracil (5-FU) decreased DNA synthesis in the investigated cell lines by 90.7% (CCD841 CoN) and 29.7% (LS180). The antiproliferative effect of 5-FU observed in colon cancer cells was significantly stronger than changes induced by examined extracts. On the contrary, data collected from colon epithelial cells revealed that five out of six investigated extracts in higher concentrations inhibited DNA synthesis more strongly than 25 μM 5-FU: both **PAR7** and **PN7** (100, 150, 250 μg/ml); **PRE7** (150, 250 μg/ml); and both **PGR7** and **PRU7** (250 μg/ml). The obtained data indicated a higher selectivity of the analysed cytostatic compared with examined extracts in the case of influence on DNA synthesis.

In the last step of the *in vitro* studies, extracts cytotoxicity was examined in CCD841 CoN cells and LS180 cells using LDH-based assay. As presented in Figure 3, the tested extracts were not cytotoxic against human colon epithelial cells, while they significantly damaged the cell membranes of colon cancer cells, and the observed effect was dose-dependent. The strongest release of LDH was noted in LS180 cells in response to **PAR7**, which in concentrations ranging from 25 to 250 μg/ml increased the LDH level by 110% and 1,062%, respectively. Very similar results were obtained after LS180 cell exposure to **PRU7**, which in the mentioned range of concentrations increased LDH release by 161% (25 μg/ml) and 956% (250 μg/ml). The weakest cytotoxic effect was noted in colon cancer cells treated with **PAL7**, which at the highest tested concentration caused an increase in the LDH level of 68%. Used as a positive control, 5-FU at a concentration of 25 μM was not cytotoxic against colon epithelial or colon cancer cells (Figure 3). The LDH levels of the cells were 100.7% (CCD841 CoN) and 113.4% (LS180). All investigated extracts damaged colon cancer cell membranes more effectively than 5-FU, and this difference was especially evident in the case of **PRU7**, **PAR7**, **PN7**, and **PRE7**, which even at the lowest tested concentration (25 μg/ml) increased the LDH level to 261, 210, 185, and 156%, respectively.

Discussion

Many studies have shown that *Potentilla* species are a source of a wide spectrum of secondary metabolites, mainly polyphenols, such as hydrolysable and condensed tannins, flavonoids and their glycosides, and phenolic acids, which show a variety of biological activities (Augustynowicz et al., 2021b). *Potentilla* species have a long history of use to treat intestinal problems, such as diarrhoea, inflammatory bowel disease (Tomczyk and Latte, 2009). Selected species for the present study are common in the Europe and were rarely selected as a subject of anticancer evaluation. Basing on the literature search we hypothesized that similarly to other species from this genus would exhibit anticancer potential. The quantitative identification of polyphenolic classes present in extracts using colorimetric methods offers information on their general contents. In our study, high TPC, TFC, and TTC values were observed in all tested samples. The TPC and TFC results were significantly higher compared to previous studies on aerial parts of *P. argentea, P. grandiflora, P. recta,* and *P. norvegica* (Tomczyk et al., 2010; Sut et al., 2019; Augustynowicz et al., 2021a). The differences in results can be partially explained by the type of solvent used in the extraction process. Aqueous acetone during the extraction process inhibits the interaction between tannins and proteins, and decreases the cleaving of depside bonds in hydrolysable tannins in comparison to aqueous alcohols. These mechanisms may lead to higher contents of high-molecular tannins in acetone extracts (Hagerman, 1988; Mueller-Harvey, 2001). Moreover, on several occasions, acetone has been reported as a good solvent for the extraction of flavonoids with higher contents, in comparison to water and alcoholic solvents (Dirar et al., 2019; Patel and Ghane, 2021). Furthermore, LC-PDA-HRMS analysis revealed a number of polyphenols, such as ellagitannins and products of their degradation, flavonoids, and phenolic acids, that were present in all extracts. Ellagitannins are plant secondary metabolites that tend to form relatively high molecular weight dimers and oligomers, ranging from 300 to 20,000 Da. Plants from the Rosaceae family accumulate a series of oligomeric, macrocyclic oligomeric, and *C*-glycosidic ellagitannins that can be used as chemophenetic markers (Grochowski et al., 2017; Gesek et al., 2021). On several occasions the presence of dimeric ellagitannin - agrimoniin in aerial parts of *Potentilla* species, in particular *P. anserina and P. kleiniana, P. recta*, have been reported (Okuda et al., 1982; Fecka, 2009; Bazylko et al., 2013). The chromatographic analysis reported herein indicates that agrimoniin is the most abundant ellagitannin in all extracts except **PAL7**. Moreover, several other phenolic compounds, such as pedunculagin, laevigatins, brevifolincarboxylic acid, and ellagic acid, an artifact, are released as a product of hydrolysis of ellagitannins. These compounds are widely present in the aerial parts of different species belonging to the genus *Potentilla*, including *P. indica, P. freyniana, Duchesnea chrysantha,* and *P. anserina*, and therefore could be considered significant in the chemophenetics of this genus (Okuda et al., 1992; Lee and Yang, 1994; Fecka, 2009; Luo et al., 2020). Furthermore, flavonol derivatives, such as quercetin 3-*O*-rutoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucuronide, quercetin 3-*O*-arabinoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-glucuronide, and tiliroside, were found in at least one of the

17 investigated *Potentilla* species (Tomczyk and Latte, 2009; Augustynowicz et al., 2021b). More interestingly, *N*-acylated biogenic amine derivative, N1, N5, N10-tricoumaroyl spermidine, was reported for the first time in the genus *Potentilla*. This compound accumulates exclusively in the pollen coat and has been detected in several other genera in the Rosaceae family (Elejalde-Palmett et al., 2015).

The anticancer potential of acetone extracts isolated from selected *Potentilla* species was examined in both colon cancer LS180 cells as well as normal colon epithelial CCD841 CoN cells by investigation compounds influence on cell viability (MTT assay), proliferation (BrdU assay), and cytotoxicity (LDH assay). All investigated extracts decreased viability of both normal and cancer colon cells in a dose-dependent manner; however, LS180 cells were more sensitive to the tested compounds. The results of MTT assay indicated that the tested extracts effectively decreased the mitochondrial metabolism of human colon cancer cells, which could be associated with the presence of hydrolysable tannins in all extracts except the **PAL7**, which revealed the weakest anticancer effect. Moreover, the highest impact in decrease of cancer cells viability by **PAR7**, **PN7** and **PRU7** correlate with their highest TPC and TTC values. Agrimoniin was shown to have prominent antioxidative, anti-inflammatory, and anticancer effects. Hoffman et al. (2016) found that agrimoniin-enriched fractions from rhizomes of *P. erecta* directly inhibit UVB-induced cyclooxygenase-2 (COX-2) expression and production of PGE2 in human keratinocytes (HaCaT), as well as in an *in vivo* model, and inhibit epidermal growth factor receptor (EGFR) phosphorylation. Shi et al. (2015) demonstrated that lyophilized strawberries (*Fragaria* x *ananasa*, Rosaceae) containing 16.2% agrimoniin downregulated the mRNA expression of COX-2, IL-1β, IL-6, TNF-α, and iNOS in AOM/DSS-induced colon cancer in mice. BrdU assay revealed a significant decrease of DNA synthesis in both colon cancer and non-cancer cells in response to all investigated extracts. The strongest antiproliferation effect in cancer cells was observed after treatment with **PAR7** and **PN7**. Those extracts revealed to posess the highest total polyphenol and tannin contents. Notably, the antiproliferative effect of 5-FU observed in colon cancer cells was significantly stronger than that of the examined extracts. Similarly, data collected from colon epithelial cells revealed that five out of six investigated extracts in higher concentrations inhibited DNA synthesis stronger than the positive control. The *in vivo* bioavailability of high weight ellagitannins is relatively low. Ellagitannins at neutral or alkaline pH are hydrolysed with the release of free ellagic acid, which exerts a number of biological activities (Ismail et al., 2016). Whitley et al. (2003) found that the human colorectal adenocarcinoma cell line Caco-2 strongly accumulated ellagic acid and, furthermore, 93% of it was irreversibly bounded to cellular DNA and proteins. Moreover, ellagic acid significantly decreased the expression of genes involved in the p53, PI3K-Akt, mitogen-activated protein kinase (MAPK), and TGF-β signaling pathways in human colorectal carcinoma cell line HCT 116 (Zhao et al., 2017). Ellagic acid also reduced the viability of human nasopharyngeal carcinoma cell line NPC-BM1 via activation of caspase-3 and inhibition of Bcl-2 and telomerase (Huang et al., 2009). Our results are in agreement with the studies by Kowalik and co-authors (2020), showing that selected extracts and fractions from aerial parts of *P. alba* significantly decreased proliferation of human colon cancer HT-29 cells. Additionally the authors found out that selected extracts and fractions from *P. alba* increased proliferation of human normal epithelial CCD 841 CoTr cells. Moreover, the tested samples damaged cell membranes and decreased their viability (Kowalik et al., 2020). Kaempferol 3-*O*-glucoside, present in all investigated extracts, exhibits anti-inflammatory, antioxidant, and anticancer effects. A recent study conducted on human colon cancer HCT 116 cells revealed that kaempferol 3-*O*-glucoside induces cell apoptosis by increasing expression of pro-apoptotic caspases (caspase 3, caspase 6, caspase 7, caspase 8, and caspase 9), protein p53, and Bax, and decreasing expression of anti-apoptotic proteins, cleaved caspase 3, and Bcl-2. Moreover, the investigated compound causes G0/G1 arrest, inhibits the expression of metalloproteinases MMP-2 and MMP-9, and decreases the activity of the NF-κB signalling pathway (Yang et al., 2021). Notably, tiliroside isolated from *P. argentea* exerted inhibitory activity against topoisomerase I and II and showed moderate cytotoxicity against human breast carcinoma cell line MCF-7 (Tomczyk et al., 2008). Finally, the LDH assay showed that the tested extracts even at the lowest concentration (25 μg/ml) significantly damaged the cell membranes of investigated colon cancer cells, releasing the high doses of LDH into the cell culture medium. The weakest effect was observed for PAL7, which may be due to the absence of hydrolysable tannins, which modify the permeability of cell membranes. However, strong observed effect of rest of tested extracts can be explained by high TTC. Moreover, the exerted the strongest cytotoxic effects of **PAR7** and **PRU7** among all extracts can be explained by their higher TPC and TTC values. At the same time, all tested samples were not cytotoxic against normal colon cells. In a recent paper, Borisowa and co-authors (2019) found that hydrolysable tannins selectively block calcium-activated chloride channels and form selective pores in the cell membrane (Borisova et al., 2019). Moreover, pedunculagin increased cytotoxicity of 5-FU against human liver cancer cells QGY-7703, probably through increased permeability of the cancer cell membrane, as observed by the authors through a microscope (Xiao et al., 2012). A recent study revealed that agrimoniin stimulates apoptosis via the mitochondria pathway, inducing activity of the mitochondrial permeability transition pore (MPTP), which leads to mitochondria swelling and a decrease in energy production. Moreover, the authors found that agrimoniin is cytotoxic against K562 and HeLa cell lines (Fedotcheva et al., 2021). The *in vivo* effects of tannin-rich acetone extracts from

selected *Potentilla* species may vary from obtained *in vitro* results. A recent study on aerial parts of *P. anserina* and rhizomes of *P. erecta* revealed that human intestinal microbiota convert ellagitannins to urolithins, which possess potent anti-inflammatory and anticancer activities (Piwowarski et al., 2014). Moreover, several studies suggest that the chronic application of tannin-rich extracts may lead to iron-deficiency anemia. Hydrolysable tannins posess antinutritional properties, due to their potential to complex iron ions and reduce their absorption (Petry et al., 2010). However, those effect may be offset by the development of formulations with modified release of extract or by the inclusion in diet of other bioactives, such as ascorbic acid, which prevents the inhibitory effect of polyphenols on iron absorption (Petroski and Minich, 2020). The acute complications of advanced stages of colorectal cancers includes a number of complications, such as bleeding, perforation and/or obstruction (Yang and Pan, 2014). Hydrolysable tannins are well known for their anti-bleeding properties. The tannin-rich extracts from *Potentilla* species may be used as potent, plant-based styptic agents as a complementary therapy in advanced stages of colorectal cancers.

Conclusion

In conclusion, this study reports, for the first time, analysis of the LC-PDA-HRMS profile of acetone extracts of selected *Potentilla* species. The analysis revealed the presence of several phenolic compounds, such as agrimoniin, pedunculagin, brevifolincarboxylic acid, ellagic acid, tiliroside, and tricoumaroyl spermidine. These secondary metabolites can be considered as chemophenetic markers for the genus *Potentilla*. Four of six investigated extracts (**PAR7**, **PRE7**, **PRU7**, **PN7**) showed great chemopreventive potential, manifested by the effective elimination of colon cancer cells, causing both damage to their cell membranes and inhibition of their proliferation and metabolic activity, with a simultaneous lack of a cytotoxic effect on normal colon epithelial cells and a significantly weaker effect on their metabolism and DNA synthesis compared to cancer cells. While it is impossible to specify the extract with the greatest therapeutic potential, these studies unequivocally showed that **PAL7** had the lowest anti-cancer potential in a cellular model of colon cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

DA and MT designed the research, DA performed experiments, analysed the data, and wrote the draft of the manuscript. MKL and JWS performed experiments, analysed the data, and revised the manuscript. AW and MT supervised the research and revised the manuscript. All authors approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1027315/full#supplementary-material

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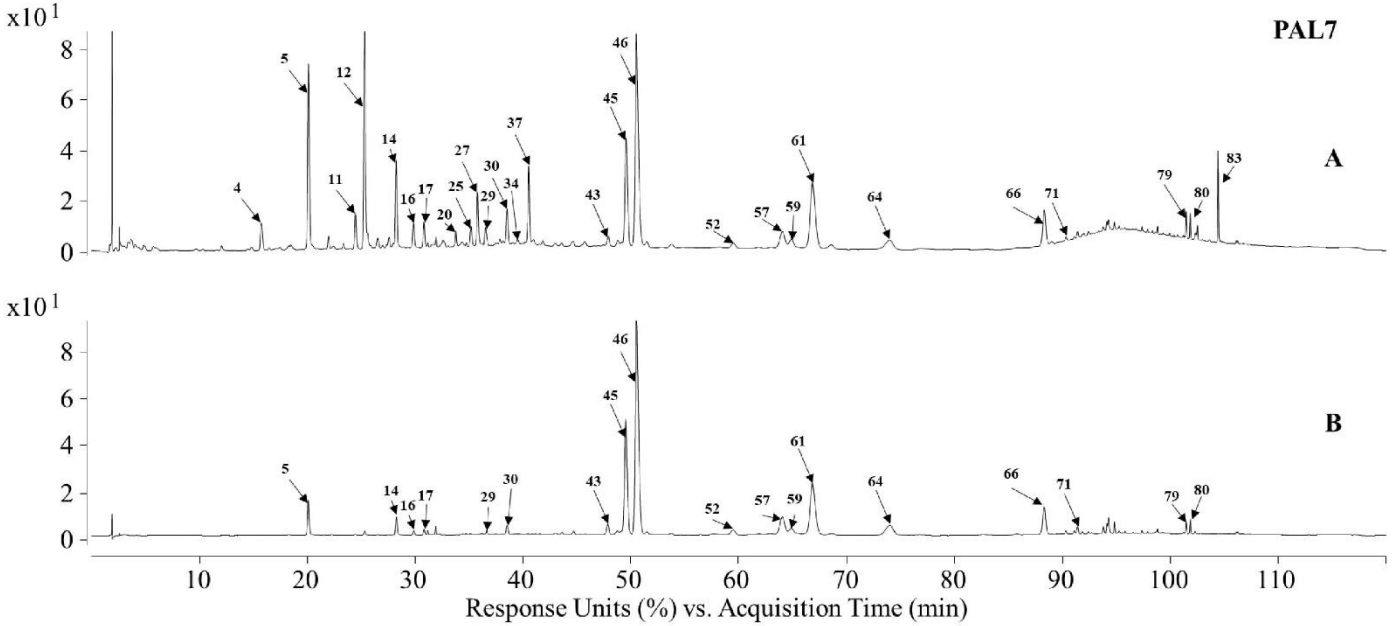
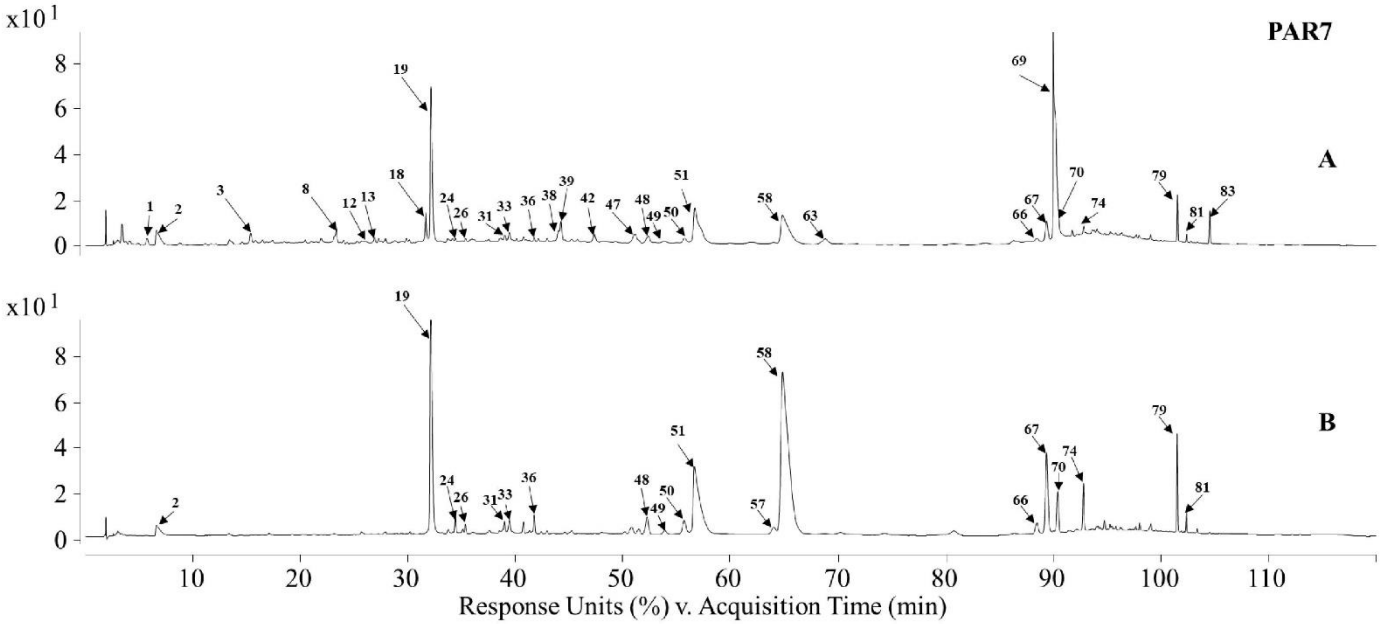
Suplement do publikacji 1

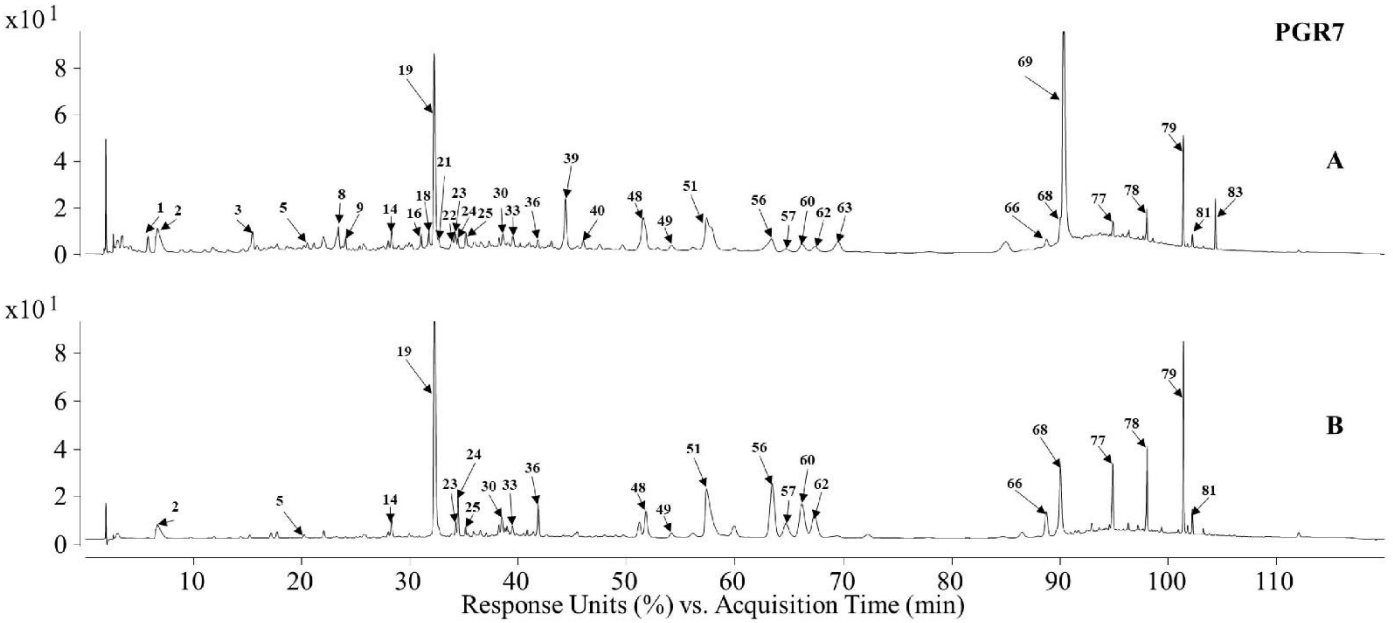
Supplementary Material



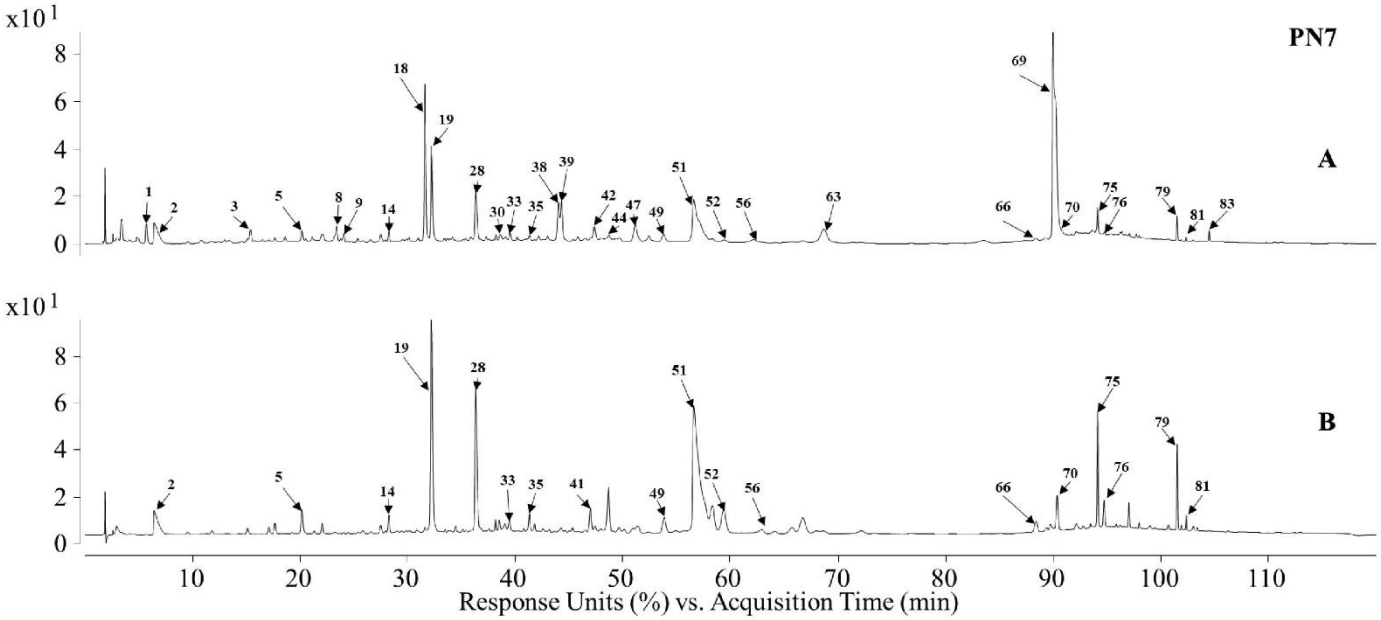
Supplementary Figure 1. The UV chromatograms with a designation of the main components of the analyzed PAL7 extract, recorded at lengths of 280 nm (A) and 360 nm (B).



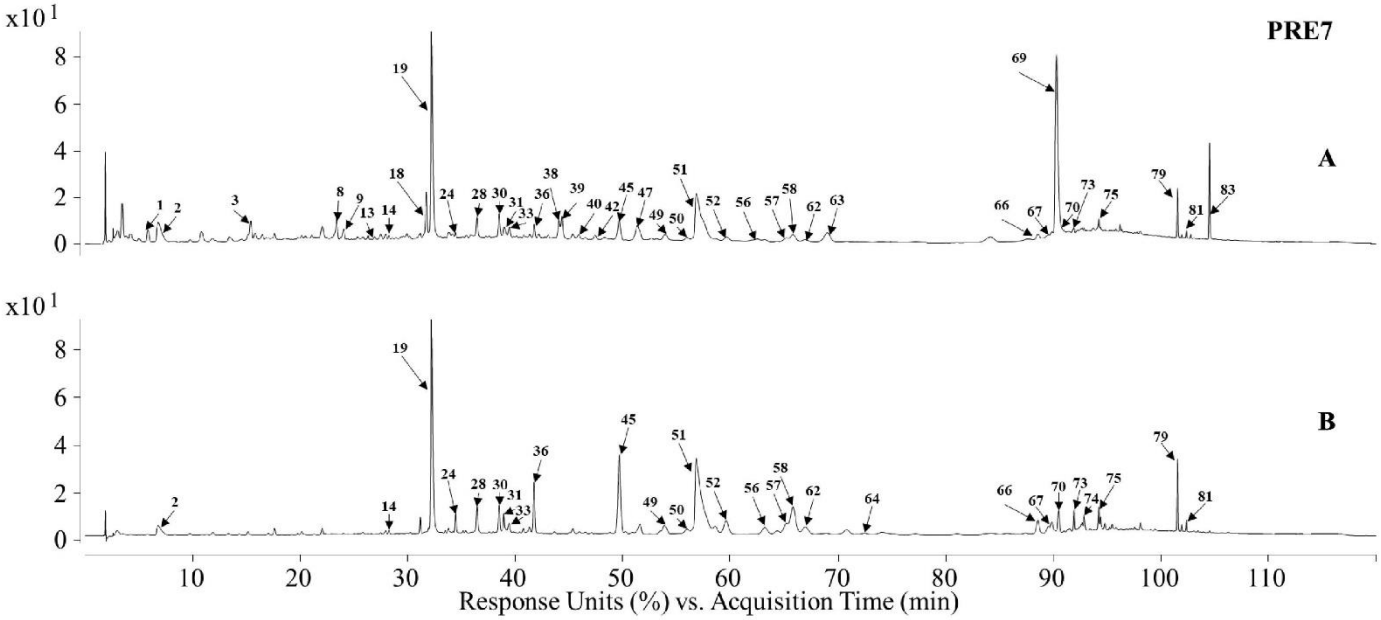
Supplementary Figure 2. The UV chromatograms with a designation of the main components of the analyzed PAR7 extract, recorded at lengths of 280 nm (A) and 360 nm (B).



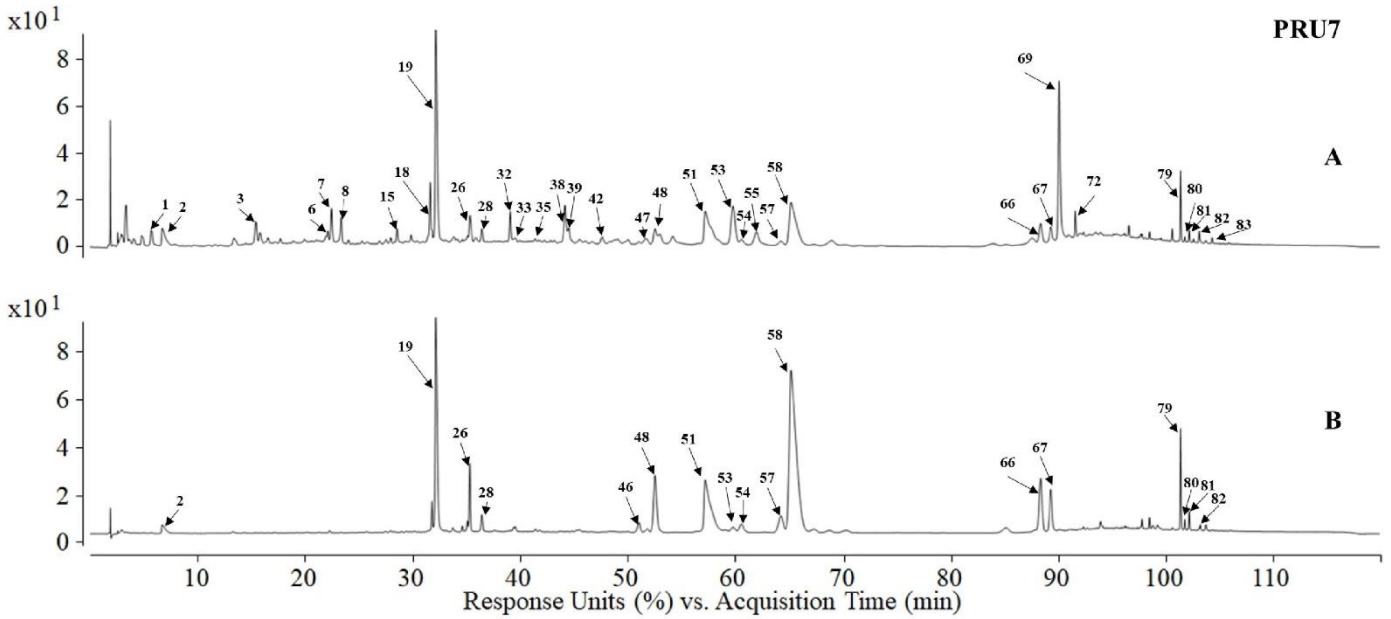
Supplementary Figure 3. The UV chromatograms with a designation of the main components of the analyzed PGR7 extract, recorded at lengths of 280 nm (A) and 360 nm (B).



Supplementary Figure 4. The UV chromatograms with a designation of the main components of the analyzed PN7 extract, recorded at lengths of 280 nm (A) and 360 nm (B).



Supplementary Figure 5. The UV chromatograms with a designation of the main components of the analyzed PRE7 extract, recorded at lengths of 280 nm (A) and 360 nm (B).



Supplementary Figure 6. The UV chromatograms with a designation of the main components of the analyzed PRU7 extract, recorded at lengths of 280 nm (A) and 360 nm (B).

Publikacja 2

Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Phytochemical profiling of extracts from rare *Potentilla* species and evaluation of their anticancer potential. *International Journal of Molecular Sciences*. 2023; 24, 4836. DOI: 10.3390/ijms24054836

International Journal of
Molecular Sciences

MDPI

Article

Phytochemical Profiling of Extracts from Rare *Potentilla* Species and Evaluation of Their Anticancer Potential

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Abstract: Despite the common use of *Potentilla* L. species (Rosaceae) as herbal medicines, a number of species still remain unexplored. Thus, the present study is a continuation of a study evaluating the phytochemical and biological profiles of aqueous acetone extracts from selected *Potentilla* species. Altogether, 10 aqueous acetone extracts were obtained from the aerial parts of *P. aurea* (**PAU7**), *P. erecta* (**PER7**), *P. hyparctica* (**PHY7**), *P. megalantha* (**PME7**), *P. nepalensis* (**PNE7**), *P. pensylvanica* (**PPE7**), *P. pulcherrima* (**PPU7**), *P. rigoi* (**PRI7**), and *P. thuringiaca* (**PTH7**), leaves of *P. fruticosa* (**PFR7**), as well as from the underground parts of *P. alba* (**PAL7r**) and *P. erecta* (**PER7r**). The phytochemical evaluation consisted of selected colourimetric methods, including total phenolic (TPC), tannin (TTC), proanthocyanidin (TPrC), phenolic acid (TPAC), and flavonoid (TFC) contents, as well as determination of the qualitative secondary metabolite composition by the employment of LC–HRMS (liquid chromatography–high-resolution mass spectrometry) analysis. The biological assessment included an evaluation of the cytotoxicity and antiproliferative properties of the extracts against human colon epithelial cell line CCD841 CoN and human colon adenocarcinoma cell line LS180. The highest TPC, TTC, and TPAC were found in **PER7r** (326.28 and 269.79 mg gallic acid equivalents (GAE)/g extract and 263.54 mg caffeic acid equivalents (CAE)/g extract, respectively). The highest TPrC was found in **PAL7r** (72.63 mg catechin equivalents (CE)/g extract), and the highest TFC was found in **PHY7** (113.29 mg rutin equivalents (RE)/g extract). The LC–HRMS analysis showed the presence of a total of 198 compounds, including agrimoniin, pedunculagin, astragalin, ellagic acid, and tiliroside. An examination of the anticancer properties revealed the highest decrease in colon cancer cell viability in response to **PAL7r** (IC_{50} = 82 µg/mL), while the strongest antiproliferative effect was observed in LS180 treated with **PFR7** (IC_{50} = 50 µg/mL) and **PAL7r** (IC_{50} = 52 µg/mL). An LDH (lactate dehydrogenase) assay revealed that most of the extracts were not cytotoxic against colon epithelial cells. At the same time, the tested extracts for the whole range of concentrations damaged the membranes of colon cancer cells. The highest cytotoxicity was observed for **PAL7r**, which in concentrations from 25 to 250 µg/mL increased LDH levels by 145.7% and 479.0%, respectively. The previously and currently obtained results indicated that some aqueous acetone extracts from *Potentilla* species have anticancer potential and thus encourage further studies in order to develop a new efficient and safe therapeutic strategy for people who have been threatened by or suffered from colon cancer.

Keywords: *Potentilla*; Rosaceae; polyphenols; LC–HRMS; colorectal cancer; LS180 cells; cytotoxicity; CCD841 CoN cells

Citation: Augustynowicz, D.; Lemieszek, M.K.; Strawa, J.W.; Wiater, A.; Tomczyk, M. Phytochemical Profiling of Extracts from Rare *Potentilla* Species and Evaluation of Their Anticancer Potential. *Int. J. Mol. Sci.* **2023**, *24*, 4836. https://doi.org/10.3390/ijms24054836

Academic Editor: Daniel Gabriel Pons

Received: 9 February 2023
Revised: 23 February 2023
Accepted: 28 February 2023
Published: 2 March 2023

1. Introduction

Cancer, a non-infectious disease, is one of the most dreadful diagnoses that severely impacts a patient's life quality. Unfortunately, cancer is a significant and increasing cause

of death worldwide. The European Cancer Information System (ECIS) estimated an increase in new cases of cancer in the European Union (EU-27) from 2.68 million in 2020 to 3.24 million in 2040, a 21% increase, while the cancer-related death toll is estimated to increase from 1.26 million to 1.66 million cases, a 31.8% increase. Colorectum cancer is the second-most-diagnosed cancer type in EU-27 countries, with over 0.34 million cases in 2020; however, in 2040, it will overtake breast cancer as the most commonly diagnosed cancer type with over 0.43 million cases [1]. The most frequently used method to treat early-stage colorectal cancer is surgical resection, which effectively relieves the patient's symptoms. However, approximately 25% to 30% of patients after successful surgery will develop metastases within 5 years [2]. Moreover, in the further stages, unresectable metastatic cancer systemic therapy includes chemotherapy, radiotherapy, immunotherapy, and biological therapy, such as antibodies to cellular growth factors, as well as their combinations [3]. Unfortunately, these treatment methods are inextricably linked with many side effects, such as pain, emotional stress, fatigue, a negative impact on fertility, and subsequent cancers [4]. Biologically active molecules in medicinal plants can be employed to reduce side effects and support the efficacy of the therapy. Notably, *Potentilla* species are widely used in traditional medicine for the treatment of dysentery, diarrhoea, diabetes mellitus, unspecified forms of cancer, and inflammation of the skin [5,6]. The pharmacological properties of *Potentilla* species stem from their secondary metabolite composition, which includes a predominant presence of polyphenols, such as hydrolysable and condensed tannins, flavonoids, and phenolic acid, as well as triterpenoids. These substances are associated with antioxidant, anti-inflammatory, and antimicrobial properties [5]. Numerous in vitro experiments on compounds obtained from *Potentilla* species have shown efficacy against various cancer cell lines, e.g., methanol extract from *P. discolor* inhibited the proliferation and induced the apoptosis of MC3 and YD-15 (human mucoepidermoid carcinoma) [7], ethyl acetate extracts from *P. recta* and *P. astracanica* decreased viability of HEp-2 (human cervix carcinoma) [8], and selected extracts and fractions from aboveground materials of *P. alba* significantly reduced the viability and proliferation of HT-29 (human colon adenocarcinoma) [9]. In a previous study, we demonstrated that aqueous acetone extracts from the aerial parts of selected *Potentilla* species showed great chemopreventive potential by decreasing the viability and proliferation of LS180 (human colon adenocarcinoma) cells, simultaneously causing substantial damage to their cell membranes while having a significantly weaker impact on normal colon epithelial cell line CCD841 CoN [10]. The present study is a continuation of that previous investigation conducted by the authors, concerning an assessment of the cytotoxicity and antiproliferative effect of aqueous acetone extracts from selected, rare *Potentilla* species against human colon cancer cell line LS180 and normal colon epithelial cell line CCD841 CoN. Additionally, identification of the marker metabolites present in extracts using LC–HRMS analysis was conducted to reveal and validate correlations between the qualitative chemical composition of the investigated samples and possible mechanisms of action.

2. Results and Discussion

2.1. Determination of Total Secondary Metabolites Content

Polyphenols are among the major secondary metabolites that are accountable for the pharmacological activities of plant-based preparations. The major group of polyphenols include flavonoids, phenolic acids, hydrolysable and condensed tannins, lignans, and stilbenes [11]. *Potentilla* species are well-known for their abundance of tannins and flavonoids, which contribute to certain traditional applications aimed at tackling diarrhoea, microbial infections, inflammations of the upper and lower gastrointestinal tract, diabetes mellitus, etc. [5,12]. In our study, extracts from the aerial and underground parts of common and rare *Potentilla* species were prepared using 70% acetone and were quantitative assessed for the general polyphenolic classes contents using colourimetric methods. The level of phenolic compounds in the extracts from selected *Potentilla* species are presented in Table 1. Extracts from the underground parts, namely, **PAL7r** and **PER7r**, were found to contain the

highest total phenolic (TPC) and total tannin (TTC) contents (268.63, 237.56, and 326.28, 269.79 mg gallic acid equivalent (GAE)/g extract, respectively). On the other hand, among extracts from the aerial parts, **PFR7** and **PPE7** had the highest TPC and TTC values (240.1, 178.65, and 218.85, 195.97 mg GAE/g extract, respectively), while **PAU7** and **PTH7** revealed the lowest TPC and TTC values (148.38, 129.2, and 149.77, 132.55 mg GAE/g extract, respectively). Moreover, **PFR7** was found to contain the highest total proanthocyanidin content (TPrC) (53.59 mg catechin equivalent (CE)/g extract), notably higher than that of other herb extracts. According to our previous study and the results herein, extracts from rhizomes, namely, **PAL7r** and **PER7r**, had remarkably higher proanthocyanidin contents than their above-ground counterparts (72.63 and 61.61 vs. 21.28 and 2.05 mg CE/g extract, respectively) [12]. Moreover, **PAL7r** and **PER7r** had the highest total phenolic acid content (TPAC), followed by **PFR7** (221.08, 263.54, and 197.83 mg caffeic acid equivalent (CAE)/g extract, respectively). On the contrary, **PAL7r** and **PER7r** had the lowest total flavonoid content (TFC) values, which were significantly lower than those of all other extracts. **PHY7** and **PPE7** revealed the highest TFC values (113.29 and 108.2 mg rutin equivalent (RE)/g extract, respectively). All the obtained results were significantly higher than the values available in the literature data reported for various extracts from the aerial parts of *P. erecta*, *P. fruticosa*, *P. nepalensis*, *P. pensylvanica*, and *P. thuringiaca* [13–15]. Notably, the selection of the solvent in the extraction process is a crucial factor in the explanation of those differences. An aqueous acetone solvent extracts much fewer non-phenol compounds, such as carbohydrates, than methanol and water, which results in higher TPC and TFC values [16]. Moreover, aqueous acetone was reported as an excellent solvent for extracting higher molecular weight flavonoids and proanthocyanidins [17]. The aforementioned solvent prevents the decomposition of hydrolysable tannins during the extraction process, leading to a higher tannin content in the obtained extracts [18].

Table 1. Total phenolic (TPC), tannin (TTC), proanthocyanidin (TPrC), phenolic acid (TPAC), and flavonoid contents (TFC) of aqueous acetone extracts.

Samples	TPC (mg GAE/g Extract) [1]	TTC (mg GAE/g Extract) [1]	TPrC (mg CE/g Extract) [2]	TPAC (mg CAE/g Extract) [3]	TFC (mg RE/g Extract) [4]
PAL7r	268.6 ± 6.9	237.6 ± 5.7	72.6 ± 2.5	221.1 ± 7	15 ± 0.3
PAU7	148.4 ± 2.3	129.2 ± 2	3.4 ± 0.1	44.2 ± 1.4	59.7 ± 1.3
PER7	201.2 ± 4.3	169.2 ± 7	2.1 ± 0.1	59.9 ± 1.3	54.9 ± 0.4
PER7r	326.3 ± 3.5	269.8 ± 2.4	61.6 ± 1.1	263.5 ± 7.5	11 ± 0.1
PFR7	240.1 ± 6.1	178.7 ± 5.5	53.6 ± 0.9	197.8 ± 6.2	94.6 ± 2.4
PHY7	199.2 ± 1.7	178.2 ± 3.9	1.6 ± 0.1	44 ± 1.1	113.3 ± 1.5
PME7	195.3 ± 4.4	168.5 ± 3.6	13.1 ± 0.4	80.8 ± 2	84.6 ± 0.1
PNE7	188.8 ± 2.5	163.5 ± 0.5	1.1 ± 0.1	33.4 ± 0.3	66.5 ± 2.5
PPE7	218.9 ± 1.8	196 ± 3.1	0.2 ± 0.1	50.5 ± 0.5	108.2 ± 0.5
PPU7	151.5 ± 2.4	135.9 ± 2.4	5.5 ± 0.1	50.2 ± 2.1	64.9 ± 0.6
PRI7	212.2 ± 5.5	170.5 ± 4.4	5.6 ± 0.6	58.1 ± 1.7	84.4 ± 0.7
PTH7	149.8 ± 2.3	132.6 ± 2.3	4.9 ± 0.1	58.8 ± 2.5	76.4 ± 1.6

[1] GAE—gallic acid equivalent; [2] CE—catechin equivalent; [3] CAE—caffeic acid equivalent; [4] RE—rutin equivalent. All values represent the mean ± standard deviation of three replicates for each sample (n = 3).

2.2. LC–HRMS Qualitative Analysis of Selected Extracts

The identification of the secondary metabolite composition of the aqueous acetone extracts of selected *Potentilla* species using LC–HRMS (liquid chromatography–high-resolution mass spectrometry) analysis demonstrated the presence of 198 compounds. Among them, three groups of phenolic compounds were dominant in the analysed extracts: tannins, flavonoids, and phenolic acids. Monomeric and dimeric ellagitannins, such as agrimoniin, sanguinis and pedunculagin, are important chemophenetic markers in the Rosaceae family, especially in the *Potentilla*, *Rubus*, and *Fragaria* genera [19]. The chromatographic analysis reported herein led to the identification of a series of hydrolysable tannins that are repre-

sented by ellagitannin derivatives, such as laevigatin isomers (**84**, **109**, **114**, **124**, and **128**), laevigatin E isomers (**37** and **40**), agrimoniin (**162**) and its structural isomer (**151**), agrimonic acid A or B (**102**), galloyl-HHDP-glucose (**16**, **21**, **43**, and **48**), digalloyl-HHDP-glucose (**33** and **60**) and trigalloyl-HHDP-glucose (**131** and **133**), galloyl-bis-HHDP-glucose (**108**, **118**, and **144**), ellagic acid (**135**) and its *O*-pentosides (**132** and **163**), *O*-hexosides (**73**, **97**, and **101**), and uronic acid (**82**, **95**, and **130**) derivatives. The analysis indicated that the one of the most abundant phytochemicals in all the extracts, except **PAL7r**, was agrimoniin. Agrimoniin has been frequently described as the major phenolic compound in several *Potentilla* species, such as *P. argentea*, *P. anserina*, *P. grandiflora P. kleiniana P. norvegica, P. recta*, and *P. rupestris* [10,20–22]. Other present ellagitannins, namely, leavigatins and agrimonic acid, are formed from the partial hydrolysis of agrimoniin (dehydrodigalloyl-di-(bis-HHDP-glucose)) [23]. Furthermore, few degradation products of hydrolysable tannins degradation, such as ellagic acid (**135**), brevifolincarboxylic acid (**46**) and its structural isomer (**50**), and brevifolin (**83**), were found. Gallotannins were present in a few extracts, which showed the presence of di-, tri-, tetra-, and pentagalloylglucose isomers (**35**, **36**, **80**, **86**, **103**, **137**, and **168**). However, the analysis revealed the absence of hydrolysable tannins in **PAL7r**. These findings are in agreement with the previous study, which demonstrated the absence of these metabolites in the aerial parts of *P. alba* [9]. Moreover, the analysis revealed the presence of condensed tannins, especially in **PAL7r**, such as catechin (**28**), epicatechin (**61**), and their glucosides (**11**, **22**, **23**, **41**, and **106**), as well as products of their polymerisation, such as A-type procyanidins (**24**, **54**, **71**, **90**, **96**, and **110**) and dimeric (**66**, **88**, and **107**), trimeric (**7**, **42**, **45**, and **64**) and tetrameric (**56** and **93**) B-procyanidins, including procyanidin B1 (**25**), procyanidin B2 (**47**), procyanidin B3 (**27**), procyanidin C1 (**94**), and procyanidin C2 (**34**).

Based on the chromatographic profiles, a number of flavonoids were detected and characterised, including apigenin (**92**, **119**, **161**, **166**, **184**, and **185**) as well as isorhamnetin (**87**, **91**, **98**, **100**, **125**, **150**, **158**, **167**, **169**, **171–173**, **179**, **182**, **187**, **188**, **191**, **194**, and **196**), naringenin (**180**), kaempferol (**62**, **67**, **72**, **78**, **81**, **104**, **113**, **117**, **140**, **142**, **143**, **147**, **148**, **153**, **155**, **157**, **159**, **160**, **164**, **165**, **170**, **175**, **176**, **178**, **190**, **192**, and **193**), quercetin (**39**, **44**, **51**, **53**, **55**, **58**, **59**, **63**, **74**, **79**, **85**, **99**, **105**, **111**, **116**, **120–122**, **126**, **127**, **129**, **134**, **136**, **138**, **139**, **141**, **145**, **149**, **152**, **154**, **156**, **177**, and **186**), acacetin (**183**), and tricin (**189** and **195**) derivatives. From a chemophenetic perspective, a few of them may be useful as chemical markers of the *Potentilla* genus, such as both isomers of tiliroside (**190**), astragalin (kaempferol 3-*O*-glucoside) (**155**), isorhamnetin 3-*O*-glucoside (**169**), kaempferol 3-*O*-glucuronide (**157**), avicularin (quercetin 3-*O*-arabinoside) (**149**), hyperoside (quercetin 3-*O*-galactoside) (**139**), isoquercitrin (quercetin 3-*O*-glucoside) (**136**), and rutin (quercetin 3-*O*-rutinoside) (**138**), which were previously reported to be present in at least one of the *Potentilla* species investigated to date [5,6,10]. The analysis also revealed the presence of phenolic acids, such as gallic acid (**1**), caffeic acid (**29**) and its derivatives (**5**, **9**, **10**, **15**, **22**, **65**, and **75**), coumaric acid (**12**, **17**, **57**, and **197**), dihydroxybenzoic acid (**13**), and syringic acid (**89**) derivatives. The detailed chromatographic data of the analysed samples are shown in Table 2 and in Supplementary Figures S1–S12. To summarize, the number of compounds shared by all the analysed *Potentilla* species may typify their chemical profile as homogeneous.

Table 2. LC–HRMS qualitative analysis of aqueous acetone extracts from aerial and underground parts of selected *Potentilla* species.

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
1	Gallic acid	5.72	270	169.01335	−3.02	$C_7H_6O_5$	**169**, 125			+	+	+	+	+	+	+	+	+	+	+	(s)
2	2-Pyrone-4,6-dicarboxylic acid	6.82	316	182.99292	−2.56	$C_7H_4O_6$	366, **183**, 139	**185**		+	+	+	+		+	+	+	+	+	+	[24]
3	Bis-HHDP-gluconic acid	11.2	255*sh*	799.06359	0.27	$C_{34}H_{24}O_{23}$	**799**, 497, 301								+				+		
4	Unknown	13.25	310	281.02976	−1.48	$C_{12}H_{10}O_8$	**281**, 237	**283**, 191, 163			+				+	+	+				
5	*O*-Caffeoylglucaric acid isomer	15.26	298, 326	371.06060	−1.98	$C_{15}H_{16}O_{11}$	**371**, 209, 191							+	+			+		+	[25]
6	Pedunculagin α or β	15.74	260*sh*	783.06839	−0.48	$C_{34}H_{24}O_{22}$	**783**, 481, 301			+	+	+	+	+	+	+	+	+	+	+	(s)
7	Procyanidin B-type trimer	15.9	278	865.19739	−1.98	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 291	+												
8	Bis-HHDP-glucose	16.58	260*sh*	783.06730	−1.29	$C_{34}H_{24}O_{22}$	**783**, 481, 301									+	+				
9	*O*-Caffeoylglucaric acid isomer	17.75	310*sh*, 326	371.06162	−1.12	$C_{15}H_{16}O_{11}$	**371**, 209, 191				+			+	+		+	+		+	[25]
10	5-*O*-caffeoylquinic acid	20.09	295*sh*, 325	353.08747	−0.96	$C_{16}H_{18}O_9$	**353**, 191, 179	355, **163**										+	+		(s)
11	Catechin or epicatechin *O*-hexoside isomer	20.35	278	451.12458	−1.55	$C_{21}H_{24}O_{11}$	**451**, 289, 245	**291**	+				+								
12	*O*-*p*-Coumaroylglucaric acid isomer	20.42	312	355.06630	−1.84	$C_{15}H_{16}O_{10}$	**355**, 209, 191, 147										+	+		+	[25]
13	Dihydroxybenzoic acid *O*-pentoside	21.9	280	285.06146	−0.55	$C_{12}H_{14}O_8$	**285**, 152											+		+	
14	Methylgallate *O*-glucoside	22.01	268	345.08239	−1.59	$C_{14}H_{18}O_{10}$	**345**, 183, 168	**185**											+	+	
15	*O*-Caffeoylglucaric acid isomer	22.05	300, 326	371.06085	−3.3	$C_{15}H_{16}O_{11}$	**371**, 209, 191							+	+		+	+			[25]
16	Galloyl-HHDP-glucose	22.26	250*sh*	633.07245	−0.24	$C_{27}H_{22}O_{18}$	**633**, 301							+		+					
17	*O*-*p*-Coumaroylglucaric acid isomer	23.12	312	355.06611	−2.36	$C_{15}H_{16}O_{10}$	**355**, 209, 191, 147										+	+		+	[25]

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
18	Pedunculagin α or β	23.3	260*sh*	783.06805	−0.82	$C_{34}H_{24}O_{22}$	**783**, 481, 301	**303**		+	+	+	+	+	+	+	+	+	+	+	(s)
19	Procyanidin B-type dimer *O*-hexoside	23.36	280	739.18619	−2.06	$C_{36}H_{36}O_{17}$	**739**, 451, 289	**741**, 579, 291	+												
20	Digalloyl-HHDP-gluconic acid	23.89	274	801.07970	1.57	$C_{34}H_{26}O_{23}$	**801**, 633, 301, 169			+									+		
21	Galloyl-HHDP-glucose	24.13	280*sh*	633.07357	0.4	$C_{27}H_{22}O_{18}$	**633**, 481, 301			+		+	+	+	+	+	+	+	+	+	
22	Catechin or epicatechin *O*-hexoside isomer	24.45	280	451.12352	−2.38	$C_{21}H_{24}O_{11}$	**451**, 289	453, **291**	+					+							
23	Catechin or epicatechin *C*-hexoside isomer	25.22	280	451.12257	−4.09	$C_{21}H_{24}O_{11}$	**451**, 289, 271	453, **291**	+												
24	Procyanidin A-type tetramer	25.6	280	1151.24372	−2.22	$C_{60}H_{48}O_{24}$	**1151**, 863, 575, 289	**1153**, 865, 577, 291	+												
25	Procyanidin B1	25.9	280	577.13480	−0.55	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 289, 257	+	+		+		+			+				(s)
26	*O*-Feruloylglucaric acid isomer	25.95	282, 326	385.07702	−2.26	$C_{16}H_{18}O_{11}$	**385**, 209, 191, 147							+			+	+			[25]
27	Procyanidin B3	26.25	280	577.13426	−1.28	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 289, 257				+	+	+				+	+	+	(s)
28	Catechin	27.05	280	289.07096	−2.33	$C_{15}H_{14}O_6$	**289**, 245	**291**, 139	+	+	+	+	+	+			+	+	+	+	(s)
29	Caffeic acid	27.55	292, 320*sh*	179.03455	−2.54	$C_9H_8O_4$	**179**, 135	**181**								+					(s)
30	*O*-Feruloylglucaric acid isomer	27.98	300*sh*, 318	385.07654	−2.63	$C_{16}H_{18}O_{11}$	**385**, 209, 191, 147							+			+	+		+	[25]
31	Digalloyl-pentose	28.02	278	453.06751	0.08	$C_{19}H_{18}O_{13}$	**453**, 301				+					+					
32	3-*O*-caffeoylquinic acid	28.35	295*sh*, 326	353.08691	−2.82	$C_{16}H_{18}O_9$	**353**, 191	355, **163**					+					+		+	(s)
33	Digalloyl-HHDP-glucose	28.56	275	785.08401	−0.87	$C_{34}H_{26}O_{22}$	**785**, 301, 275						+						+		
34	Procyanidin C2	28.87	280	865.19788	−0.51	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 289	+			+	+	+						+	[23]
35	Digalloylglucose isomer	29.32	276	483.07714	−2.2	$C_{20}H_{20}O_{14}$	**483**, 169, 125						+								
36	Digalloylglucose isomer	29.98	278	483.07745	−1.11	$C_{20}H_{20}O_{14}$	**483**, 169, 125			+			+	+		+	+				

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts													Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7		
37	Laevigatin E isomer	30.2	274	1265.13990	1.26	$C_{54}H_{42}O_{36}$	**1265**, 632, 301					+								+		
38	Methylgalloylmalic acid	30.77	278	299.04042	−1.91	$C_{12}H_{12}O_9$	**299**, 183, 168, 133												+			
39	Quercetin *O*-hexoso-*O*-deoxyhexoso-hexoside	30.9	254, 354	771.19840	−0.79	$C_{33}H_{40}O_{21}$	**771**, 609, 462, 299	**773**, 611, 465, 303		+												
40	Laevigatin E isomer	31.07	275*sh*	1265.13669	−1.27	$C_{54}H_{42}O_{36}$	**1265**, 632, 301							+								
41	Catechin or epicatechin *O*-hexoside isomer	31.43	280	451.12343	−2.47	$C_{21}H_{24}O_{11}$	**451**, 289	**453**, 291	+													
42	Procyanidin B-type trimer	31.54	280	865.19810	0.01	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 291	+				+	+								
43	Galloyl-HHDP-glucose	31.73	272	633.07313	−0.04	$C_{27}H_{22}O_{18}$	**633**, 481, 301			+	+		+	+	+	+	+	+	+	+		
44	Quercetin *O*-hexoso-*O*-hexoso-pentoside	31.82	256, 354	757.18267	−0.82	$C_{32}H_{38}O_{21}$	**757**, 462, 299	**759**, 627, 465, 303		+												
45	Procyanidin B-type trimer	32.01	280	865.19703	−1.1	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 291	+			+										
46	Brevifolincarboxylic acid	32.32	278, 360	291.01408	−1.94	$C_{13}H_8O_8$	**291**, 247	**293**		+	+		+	+	+	+	+	+	+	+		
47	Procyanidin B2	33.71	278	577.13502	−0.04	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 291, 139	+													(s)
48	Galloyl-HHDP-glucose	33.8	256, 342	633.07336	−0.48	$C_{27}H_{22}O_{18}$	**633**, 481, 301										+			+		
49	Ellagic acid derivative	33.84	280*sh*	898.13120	2.12	$C_{36}H_{35}O_{27}$	**898**, 783, 633, 301			+				+								
50	Brevifolincarboxylic acid isomer	34.01	284*sh*, 342	291.01448	−0.94	$C_{13}H_8O_8$	**291**, 247								+					+		
51	Quercetin *O*-hexoso-*O*-hexoso-pentoside	34.03	254, 342	757.18241	−0.85	$C_{32}H_{38}O_{21}$	**757**, 595, 462, 299	**759**, 597, 465, 303											+			
52	Laevigatin E isomer	34.19	275*sh*	1265.13618	−1.68	$C_{54}H_{42}O_{36}$	**1265**, 632, 463, 301							+								
53	Quercetin *O*-hexoso-*O*-uronic acid derivative	34.2	254, 346	639.11995	−0.44	$C_{27}H_{28}O_{18}$	**639**, 463, 300	**641**, 479, 303		+								+		+		
54	Procyanidin A-type tetramer	34.46	280	1151.24437	−1.65	$C_{60}H_{48}O_{24}$	**1151**, 863, 575, 289	**1153**, 865, 577, 287	+													

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
55	Quercetin *O*-diuronic acid derivative	34.5	256, 352	653.09909	−0.32	$C_{27}H_{26}O_{19}$	**653**, 447, 301	**655**, 479, 303			+		+	+				+			
56	Procyanidin B-type tetramer	34.54	280	1153.26132	−0.53	$C_{60}H_{50}O_{24}$	**1153**, 576, 289	**1155**, 867, 577, 289				+									
57	*p*-Coumaroylquinic acid isomer	35.14	312	337.09286	0.02	$C_{16}H_{18}O_8$	**337**, 191	**339**, 147										+			
58	Quercetin *O*-hexoso-*O*-hexoside	35.15	256, 352	625.14017	−1.24	$C_{27}H_{30}O_{17}$	**625**, 462, 299	**627**, 465, 303		+						+	+		+		
59	Quercetin *O*-hexoso-*O*-uronic acid derivative	35.4	254, 346	639.12105	−2.16	$C_{27}H_{28}O_{18}$	**639**, 463, 301	**641**, 465, 303		+				+		+	+			+	
60	Digalloyl-HHDP-glucose	35.47	276	785.08456	−0.06	$C_{34}H_{26}O_{22}$	**785**, 615, 301, 169						+						+		
61	Epicatechin	35.74	280	289.07181	0.6	$C_{15}H_{14}O_6$	**289**, 245	**291**, 139	+			+									(s)
62	Kaempferol *O*-hexoso-*O*-uronic acid derivative	36	264, 338	623.12581	0.57	$C_{27}H_{28}O_{17}$	**623**, 284	**625**, 463, 287			+										
63	Quercetin *O*-hexoso-*O*-hexoso-deoxyhexoside	36.03	256, 348	771.19893	−0.45	$C_{33}H_{40}O_{21}$	**771**, 462, 299	**773**, 627, 465, 303								+			+		
64	Procyanidin B-type trimer	36.07	280	865.19912	0.04	$C_{45}H_{38}O_{18}$	**865**, 575, 289	**867**, 579, 291				+	+								
65	Caffeoylisocitric acid	36.32	300*sh*, 328	353.05058	−2.24	$C_{15}H_{14}O_{10}$	**353**, 191, 179, 173, 155									+	+		+	+	[25]
66	Procyanidin B-type dimer	36.35	280	577.13479	−0.45	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 291				+	+								
67	Kaempferol *O*-hexoso-deoxyhexoso-*O*-uronic acid derivative	37.05	266, 346	769.18282	−0.4	$C_{33}H_{38}O_{21}$	**769**, 284	**771**, 625, 463, 287			+										
68	Dimeric hydrolysable tannin	37.55	270	1569.15737	−1.82	$C_{68}H_{50}O_{44}$	**1569**, 784, 469, 301													+	
69	Valoneic acid dilactone	37.6	255*sh*, 362	469.00441	0.32	$C_{21}H_{10}O_{13}$	**469**, 425, 301								+		+				[26]
70	Ellagic acid derivative	37.76	268, 342	741.18713	−0.79	$C_{32}H_{38}O_{20}$	**741**, 579, 446, 301												+		

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
71	Procyanidin A-type trimer	37.81	280	863.18110	−2.08	$C_{45}H_{36}O_{18}$	**863**, 711, 573, 411, 289	**865**, 575, 287	+												
72	Kaempferol *O*-hexoso-deoxyhexoso-deoxyhexoso-*O*-uronic acid derivative	37.86	266, 346	915.24077	−0.13	$C_{39}H_{48}O_{25}$	**915**, 285	**917**, 771, 625, 463, 287			+										
73	Galloyl-ellagic acid-*O*-hexoside	38.3	250, 374	615.06204	−1.43	$C_{27}H_{20}O_{17}$	**615**, 463, 301					+		+			+	+		+	
74	Quercetin *O*-uronic acid derivative	38.4	254, 350	725.11985	−0.75	$C_{30}H_{30}O_{21}$	**725**, 505, 300	**727**, 479, 303		+								+		+	
75	*O*-Caffeoylmalic acid	38.54	298, 326	295.04504	−2.45	$C_{13}H_{12}O_8$	591, **295**, 179, **133**	**295**, **135**									+	+		+	[27]
76	Methylgalloyl-galloyl-glucose	38.85	270	497.09317	−1.17	$C_{21}H_{22}O_{14}$	**497**, 345, 183, 169												+	+	
77	Sanguisorbic acid dilactone	38.88	255*sh*, 362	469.00439	0.38	$C_{21}H_{10}O_{13}$	**469**, 425, 301								+						[26]
78	Kaempferol *O*-diuronic acid derivative	38.92	265, 350	637.10483	0.37	$C_{27}H_{26}O_{18}$	**637**, 461, 285	**639**, 463, 287			+			+				+			
79	Quercetin *O*-(malonyl-hexoso)-*O*-hexoside	39.08	256, 354	711.14146	−0.09	$C_{30}H_{32}O_{20}$	**711**, 667, 462, 299	**713**, 551, 465, 303		+				+					+		
80	Trigalloylglucose isomer	39.1	276	635.08854	−0.04	$C_{27}H_{24}O_{18}$	635, 465, 313, **169**						+				+		+		[28]
81	Kaempferol *O*-hexoso-*O*-hexoside	39.31	262, 348	609.14565	−1.26	$C_{27}H_{30}O_{16}$	**609**, 446, 283	**611**, 449, 287									+			+	
82	Ellagic acid *O*-uronic acid derivative	39.4	252, 362	477.03029	−1.24	$C_{20}H_{14}O_{14}$	**477**, 301					+									[23]
83	Brevifolin	39.6	275, 350	247.02448	−1.92	$C_{12}H_8O_6$	**247**, 191	**249**			+		+		+	+	+				[28]
84	Laevigatin isomer	39.77	255	1567.14302	−0.99	$C_{68}H_{48}O_{44}$	1567, **783**, 633, 301												+	+	[23]
85	Quercetin *O*-deoxyhexoso-*O*-deoxyhexoso-hexoside	39.8	256, 354	755.20392	−0.22	$C_{33}H_{40}O_{20}$	**755**, 609, 446, 299	**757**, 611, 449, 303		+											

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts													Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7		
86	Trigalloylglucose isomer	40.05	276	635.08863	−0.5	$C_{27}H_{24}O_{18}$	635, 465, 313, **169**						+									[28]
87	Isorhamnetin *O*-hexoso-*O*-hexoso-pentoside	40.1	254, 352	771.19887	−0.64	$C_{33}H_{40}O_{21}$	**771**, 476, 315, 300	**773**, 641, 479, 317											+			
88	Procyanidin B-type dimer *O*-gallate	40.28	278	729.14551	−0.59	$C_{37}H_{30}O_{16}$	**729**, 577, 559, 289, 169	**731**, 289					+									
89	Syringic acid derivative	40.44	280	313.05569	−2.4	$C_{13}H_{14}O_9$	**313**, 197, 182												+			
90	Procyanidin A-type trimer	40.52	280	863.18180	−0.75	$C_{45}H_{36}O_{18}$	**863**, 573, 411, 289	**865**, 287	+													
91	Isorhamnetin *O*-hexoso-*O*-uronic acid derivative	40.67	266, 348	653.13595	0.25	$C_{28}H_{30}O_{18}$	**653**, 477, 314	**655**, 479, 317												+		
92	Apigenin *C*-dihexoside	40.75	270, 332	593.15106	−0.09	$C_{27}H_{30}O_{15}$	**593**, 473, 383, 353	**595**, 439, 355, 325		+								+		+		
93	Procyanidin B-type tetramer	40.8	280	1153.25158	−0.3	$C_{60}H_{50}O_{24}$	**1153**, 863, 576, 289	**1155**, 865, 577, 289				+										
94	Procyanidin C1	41.2	280	865.19784	−0.81	$C_{45}H_{38}O_{18}$	**865**, 577, 289	**867**, 579, 291	+													(s)
95	Ellagic acid *O*-uronic acid derivative	41.47	252, 360	477.03021	−0.63	$C_{20}H_{14}O_{14}$	**477**, 301						+									
96	Procyanidin A-type tetramer	41.51	280	1151.24448	−1.56	$C_{60}H_{48}O_{24}$	**1151**, 863, 575, 289	**1153**, 865, 577, 287	+													
97	Ellagic acid *O*-hexoside	41.64	252, 362	463.05127	−0.58	$C_{20}H_{16}O_{13}$	**463**, 301					+	+		+	+	+	+	+	+		[23]
98	Isorhamnetin *O*-diuronic acid derivative	41.8	254, 352	667.11526	0.2	$C_{28}H_{28}O_{19}$	1335, **667**, 491, 315	**669**, 493, 317			+			+				+		+		
99	Quercetin *O*-deoxyhexoso-*O*-hexoso-pentoside	42.35	254, 352	741.18832	−0.66	$C_{32}H_{38}O_{20}$	**741**, 446, 299	**743**, 611, 449, 303		+												
100	Isorhamnetin *O*-hexoso-*O*-uronic acid derivative	42.43	254, 352	653.13560	−0.3	$C_{28}H_{30}O_{18}$	**653**, 477, 315	**655**, 479, 317								+						

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
101	Ellagic acid *O*-hexoside	42.7	250, 370	463.05107	−1.61	$C_{20}H_{16}O_{13}$	**463**, 301					+		+			+		+		[23]
102	Agrimonic acid A or B	43.11	270*sh*	1103.08618	0.64	$C_{43}H_{32}O_{31}$	**1103**, 935, 783, 301, 169					+						+		+	[23]
103	Tetragalloylglucose isomer	43.13	278	787.10004	−0.98	$C_{34}H_{28}O_{22}$	**787**, 465, 169						+								[28]
104	Kaempferol *O*-deoxyhexoso-hexoso-*O*-deoxyhexoside	43.16	266, 346	739.20805	−0.74	$C_{33}H_{40}O_{19}$	**739**, 593, 430, 283	**741**, 595, 433, 287		+										+	
105	Quercetin *O*-hexoso-*O*-hexoside	43.17	264, 344	625.14038	−1.14	$C_{27}H_{30}O_{17}$	**625**, 463, 300	**627**, 465, 303									+				
106	Catechin or epicatechin *O*-hexoside isomer	43.3	278	451.12511	0.25	$C_{21}H_{24}O_{11}$	**451**, 289	**289**					+								
107	Procyanidin B-type dimer	43.6	280	577.13537	−0.24	$C_{30}H_{26}O_{12}$	**577**, 289	**579**, 287	+				+								
108	Galloyl-bis-HHDP-glucose	44.2	255	935.08057	−0.13	$C_{41}H_{28}O_{26}$	**935**, 633, 467, 301			+	+		+		+	+	+		+	+	[20]
109	Laevigatin isomer	44.6	255	1567.14331	−0.8	$C_{68}H_{48}O_{44}$	1567, **783**, 633, 301			+	+	+	+	+				+	+	+	[23]
110	Procyanidin A-type tetramer	45.03	280	1151.24657	0.26	$C_{60}H_{48}O_{24}$	**1151**, 863, 575, 289	**1153**, 865, 577, 289	+												
111	Quercetin *O*-hexoso-*O*-hexoside	45.39	254, 346	625.14019	−1.3	$C_{27}H_{30}O_{17}$	**625**, 463, 300	**627**, 465, 303									+				
112	HHDP-NHTP-glucose	45.56	254	933.06390	−0.43	$C_{41}H_{26}O_{26}$	**933**, 631, 466, 301				+				+	+					
113	Kaempferol *O*-diuronic acid derivative	45.86	266, 336	637.10484	−0.55	$C_{27}H_{26}O_{18}$	**637**, 461, 285	**639**, 463, 287		+											
114	Laevigatin isomer	45.99	255	1567.14487	0.19	$C_{68}H_{48}O_{44}$	1567, **783**, 301							+				+			[23]
115	Procyanidin B-type pentamer	46.13	278	1441.32708	1.23	$C_{75}H_{62}O_{30}$	**1441**, 1153, 863, 575, 289	**1443**, 1155, 865, 577, 289				+									
116	Quercetin *O*-hexoso-*O*-uronic acid derivative	46.5	264, 340*sh*	639.12041	0.17	$C_{27}H_{28}O_{18}$	**639**, 463, 301	**641**, 465, 303									+				

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
117	Kaempferol *O*-hexoso-*O*-hexoso-deoxyhexoside	46.63	264, 350*sh*	755.20300	−0.67	$C_{33}H_{40}O_{20}$	**755**, 593, 447, 285	**757**, 595, 449, 287								+		+		+	
118	Galloyl-bis-HHDP-glucose	47.7	275*sh*	935.07950	−0.36	$C_{41}H_{28}O_{26}$	**935**, 633, 467, 301								+	+	+			+	
119	Apigenin *C*-hexoso-*C*-pentoside	49.2	268, 340	563.13970	−1.45	$C_{26}H_{28}O_{14}$	**563**, 519, 473, 443, 383, 353	**565**, 379, 355, 325												+	
120	Quercetin *O*-hexoso-pentoside	50.08	255, 352	595.13009	0.61	$C_{26}H_{28}O_{16}$	**595**, 300	597, 465, **303**					+		+		+				
121	Quercetin *O*-hexoso-pentoside	50.95	256, 354	595.12982	−0.59	$C_{26}H_{28}O_{16}$	**595**, 300	**597**, 465, 303		+			+				+				
122	Quercetin *O*-deoxyhexoso-*O*-uronic acid derivative	51.17	254, 352	623.12438	−1.13	$C_{27}H_{28}O_{17}$	**623**, 301	**625**, 479, 303										+			
123	Feruloylisocitric acid	51.46	284, 326	367.06617	−2.08	$C_{16}H_{16}O_{10}$	**367**, 173									+	+			+	[25]
124	Laevigatin isomer	51.56	255	1567.14348	−0.69	$C_{68}H_{48}O_{44}$	1567, **783**, 301			+		+	+	+				+		+	[23]
125	Isorhamnetin *O*-hexoso-hexoside	51.65	270, 350	639.15591	−1.2	$C_{28}H_{32}O_{17}$	**639**, 314, 300	641, 479, **317**						+	+	+	+	+	+	+	
126	Quercetin *O*-deoxyhexoso-hexoside	52.15	256, 354	609.11075	−0.28	$C_{27}H_{30}O_{16}$	**609**, 300	**611**, 449, 303		+											
127	Quercetin *O*-galloyl-hexose	52.7	264, 352	615.09845	−1.15	$C_{28}H_{24}O_{16}$	**615**, 463, 300, 169	**617**, 303					+								
128	Laevigatin isomer	52.76	255	1567.14432	−0.15	$C_{68}H_{48}O_{44}$	1567, **783**, 301					+		+			+	+		+	[23]
129	Quercetin *O*-pentoso-hexoside	53.41	256, 354	595.12983	−1.16	$C_{26}H_{28}O_{16}$	**595**, 300	**597**, 435, 303											+		
130	Ellagic acid *O*-methyl ether *O*-uronic acid derivative	54.1	254, 360	491.04680	0.22	$C_{21}H_{16}O_{14}$	**491**, 315, 299.9					+			+		+	+		+	[23]
131	Trigalloyl-HHDP-glucose	54.3	270	937.09372	−1.14	$C_{41}H_{30}O_{26}$	**937**, 783, 468, 301, 169			+				+					+		
132	Ellagic acid *O*-pentoside	56.35	252, 360	433.04120	−0.54	$C_{19}H_{14}O_{12}$	**463**, 301					+			+	+					[23]

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
133	Trigalloyl-HHDP-glucose	56.57	278	937.09504	−0.77	$C_{41}H_{30}O_{26}$	**937**, 468, 301												+		
134	Quercetin *O*-galloyl-hexose	57.03	264, 354	615.09882	−1.5	$C_{28}H_{24}O_{16}$	**615**, 463, 300, 169	**617**, 303					+								
135	Ellagic acid	57.7	254, 370	300.99864	−0.98	$C_{14}H_6O_8$	**301**, 275	**303**		+	+	+	+	+	+	+	+	+	+	+	(s)
136	Isoquercitrin (Quercetin 3-*O*-glucoside)	59.8	254, 354	463.08790	−0.94	$C_{21}H_{20}O_{12}$	**463**, 300, 271	**465**, 303					+		+		+	+		+	(s)
137	Tetragalloylglucose isomer	62	278	787.09906	−1.22	$C_{34}H_{28}O_{22}$	**787**, 465, 169						+							+	[28]
138	Rutin (Quercetin 3-*O*-rutinoside)	63.7	256, 354	609.14556	−0.6	$C_{27}H_{30}O_{16}$	**609**, 300, 271	**611**, 465, 303					+			+		+	+		(s)
139	Hyperoside (Quercetin 3-*O*-galactoside)	64.13	255, 355	463.08829	−0.76	$C_{21}H_{20}O_{12}$	**463**, 300	**465**, 303		+			+	+		+	+	+	+	+	(s)
140	Kaempferol *O*-hexoso-pentoside	65.75	266, 348	579.13529	−0.02	$C_{26}H_{28}O_{15}$	**579**, 284	581, 449, **287**							+						
141	Quercetin *O*-uronic acid derivative	66.03	256, 354	477.06730	−0.23	$C_{21}H_{18}O_{13}$	**477**, 301	**479**, 303			+		+	+		+	+	+		+	
142	Kaempferol *O*-hexoside	67.4	252, 350	447.09290	−1.32	$C_{21}H_{20}O_{11}$	**447**, 284	**449**, 287		+								+			
143	Kaempferol *O*-uronic acid derivative	68.88	254, 348	461.07221	−1.04	$C_{21}H_{18}O_{12}$	**461**, 285	**463**, 287		+											
144	Galloyl-bis-HHDP-glucose	69.66	260*sh*	935.07940	−0.31	$C_{41}H_{28}O_{26}$	**935**, 467, 301			+		+	+	+	+	+	+	+	+	+	
145	Quercetin *O*-pentoso-*O*-pentoso-uronic acid derivative	71.48	254, 352	739.17255	−0.32	$C_{32}H_{36}O_{20}$	**739**, 300	**741**, 609, 433, 303							+						
146	Dimeric ellagitannin	72.47	270	1871.16610	−0.21	$C_{82}H_{56}O_{52}$	1871, 1265, **935**, 783, 301												+		
147	Kaempferol *O*-deoxyhexoso-hexoside	72.86	266, 345*sh*	579.13479	−1.17	$C_{26}H_{28}O_{15}$	**579**, 284	**581**, 449, 287											+		

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts													Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7		
148	Kaempferol *O*-deoxyhexoso-deoxyhexoso-*O*-hexoside	74.17	264, 346	739.20843	−0.33	$C_{33}H_{40}O_{19}$	**739**, 593, 284	**741**, 595, 449, 287			+											
149	Quercetin 3-*O*-arabinoside	85.55	254*sh*, 350	433.07623	−3.28	$C_{20}H_{18}O_{11}$	**433**, 300	435, **303**					+									(s)
150	Isorhamnetin *O*-pentoso-hexoside	86.15	254, 354	609.14566	−1.28	$C_{27}H_{30}O_{16}$	**609**, 314, 300	**611**, 479, 317											+			
151	Dimeric ellagitannin	87.1	250*sh*	1869.14746	−1.81	$C_{82}H_{54}O_{52}$	1869, **934**, 783, 301												+	+		
152	Quercetin *O*-pentoso-deoxyhexoside	87.15	256, 352	579.13374	−3.01	$C_{26}H_{28}O_{15}$	**579**, 300	**581**, 435, 303					+									
153	Kaempferol *O*-deoxyhexoso-*O*-hexoside	88.6	264, 346	593.15030	−1.35	$C_{27}H_{30}O_{15}$	**593**, 447, 284	**595**, 449, 287								+		+	+			
154	Quercetin derivative	88.75	256, 350	607.12966	−1.56	$C_{27}H_{28}O_{16}$	**607**, 300	**609**, 303												+		
155	Astragalin (Kaempferol 3-*O*-glucoside)	88.8	264, 350	447.09299	−0.34	$C_{21}H_{20}O_{11}$	**447**, 284	**449**, 287		+	+		+	+	+	+	+	+	+	+		(s)
156	Quercetin *O*-deoxyhexoso-hexoside	89.1	256, 348	609.14510	−1.89	$C_{27}H_{30}O_{16}$	**609**, 300	**611**, 448, 303					+									
157	Kaempferol 3-*O*-glucuronide	89.25	265, 350	461.07183	−1.38	$C_{21}H_{18}O_{12}$	**461**, 285	**463**, 287						+		+	+					(s)
158	Isorhamnetin *O*-deoxyhexoso-deoxyhexoso-*O*-hexoside	89.33	254, 352	769.21962	−0.11	$C_{34}H_{42}O_{20}$	**769**, 315	**771**, 625, 479, 317			+											
159	Kaempferol *O*-uronic acid derivative	89.6	268, 342*sh*	461.07133	−2.38	$C_{21}H_{18}O_{12}$	**461**, 285	**463**, 287					+				+					
160	Kaempferol *O*-deoxyhexoso-*O*-hexoso-deoxyhexoside	89.7	266, 348	737.19349	0.28	$C_{33}H_{38}O_{19}$	**737**, 593, 284	**739**, 593, 433, 287												+		
161	Apigenin *O*-hexoside	90.1	266, 336	431.09795	−1.23	$C_{21}H_{20}O_{10}$	**431**, 268	**433**, 271										+				
162	Agrimoniin	90.3	250*sh*	1869.14917	−0.89	$C_{82}H_{54}O_{52}$	1870, 1085, **934**, 783, 301			+	+	+	+	+	+	+	+	+	+	+		(s)

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
163	Ellagic acid *O*-methyl ether *O*-pentoside	90.45	280*sh*, 365	447.05603	−1.61	$C_{20}H_{16}O_{12}$	**447**, 315, 301								+						[28]
164	Kaempferol derivative	90.61	264, 348	723.17755	0.1	$C_{32}H_{36}O_{19}$	**723**, 621, 579, 284	**725**, 593, 287							+						
165	Kaempferol derivative	91.08	266, 348	635.16048	−2.02	$C_{29}H_{32}O_{16}$	**635**, 284	**637**, 287												+	
166	Apigenin *O*-uronic acid derivative	91.35	266, 336	445.07769	0.26	$C_{21}H_{18}O_{11}$	891, **445**, 269	**447**, 271		+											
167	Isorhamnetin *O*-deoxyhexoso-hexoside	91.43	254, 352	623.16185	0.37	$C_{28}H_{32}O_{16}$	**623**, 314	**625**, 479, 317								+			+		
168	Pentagalloylglucose isomer	91.7	280	939.11106	−0.58	$C_{41}H_{32}O_{26}$	**939**, 769, 469, 169						+						+		
169	Isorhamnetin 3-*O*-glucoside	91.72	254, 350	477.10350	−0.23	$C_{22}H_{22}O_{12}$	**477**, 314, 300	**479**, 317								+					(s)
170	Kaempferol derivative	91.94	266, 350	737.19229	−1.6	$C_{33}H_{38}O_{19}$	**737**, 284	**739**, 593, 287												+	
171	Isorhamnetin *O*-deoxyhexoso-hexoso *O*-pentoside	92.02	254*sh*, 355	753.18765	−0.65	$C_{33}H_{38}O_{20}$	**753**, 314	755, 623, **317**							+						
172	Isorhamnetin *O*-uronic acid derivative	92.79	254, 354	491.08194	−1.69	$C_{22}H_{20}O_{13}$	**491**, 315, 300	**493**, 317						+		+		+			
173	Isorhamnetin *O*-pentoso-O-deoxyhexoso-*O*-uronic acid derivative	92.93	254, 354	767.20286	−1.49	$C_{34}H_{40}O_{20}$	**767**, 621, 314	**769**, 623, 493, 317												+	
174	Chrysoeriol *O*-uronic acid derivative	93.7	266*sh*, 346	475.08764	−0.91	$C_{22}H_{20}O_{12}$	951, **475**, 299	**477**, 301		+											
175	Kaempferol *O*-acetylhexoside	94.62	264, 346	489.10342	−1.01	$C_{23}H_{22}O_{12}$	**489**, 284	**491**, 287									+	+	+	+	
176	Kaempferol derivative	94.8	266, 348	591.13497	−0.5	$C_{27}H_{28}O_{15}$	**591**, 284	**593**, 287												+	

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts												Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7	
177	Quercetin *O*-uronic acid derivative	94.87	266*sh*, 360	477.06702	−0.36	$C_{21}H_{18}O_{13}$	**477**, 301	**479**, 303		+	+			+				+			
178	Kaempferol *O*-malonylhexoside	94.97	266*sh*, 348	533.09266	−1.62	$C_{24}H_{22}O_{14}$	**533**, 284	**535**, 287										+			
179	Isorhamnetin *O*-galloyldeoxyhexoside	95.2	270, 348	629.11322	−2.41	$C_{29}H_{26}O_{16}$	**629**, 314, 299, 169	**631**, 317					+								
180	Naringenin O-hexoside	95.52	276*sh*, 362	433.11345	−1.15	$C_{21}H_{22}O_{10}$	**433**, 271	435, **273**									+				
181	Isorhamentin derivative	95.83	254, 352	621.14502	−1.78	$C_{28}H_{30}O_{16}$	**621**, 314, 300	**623**, 317												+	
182	Isorhamnetin *O*-acetylhexoside	95.96	254, 352	519.11432	−0.23	$C_{24}H_{22}O_{13}$	**519**, 314, 299	**521**, 317								+			+	+	
183	Acacetin	96.26	254	283.06188	0.6	$C_{16}H_{12}O_5$	**283**, 268	**285**, 242							+						[29]
184	Apigenin *O*-acetylhexoside	97.3	266, 326	473.10900	−0.77	$C_{23}H_{22}O_{11}$	**473**, 269											+			
185	Apigenin	98.1	268, 338	269.04538	−1.93	$C_{15}H_{10}O_5$	**269**	**271**										+			(s)
186	Quercetin *O*-deoxyhexoso-deoxyhexoso-*O*-hexoside	98.43	266, 346	753.22397	−0.86	$C_{34}H_{42}O_{19}$	**753**, 299	**755**, 609, 463, 301			+										
187	Isorhamnetin *O*-hexoside	98.9	256, 356	477.10244	−3.05	$C_{22}H_{22}O_{12}$	**477**, 314, 299	**479**, 317					+								
188	Isorhamnetin *O*-hexoside	99.21	256, 354	477.10227	−2.92	$C_{22}H_{22}O_{12}$	**477**, 314, 271	**479**, 317					+								
189	Tricin *O*-deoxyhexoso-deoxyhexoso-*O*-hexoside	99.5	254, 354	783.23498	−0.36	$C_{35}H_{44}O_{20}$	**783**, 329	**785**, 639, 493, 331			+										
190	*trans*-Tiliroside	101.41	268, 315	593.13011	0.27	$C_{30}H_{26}O_{13}$	**593**, 284	**595**, 287		+	+		+	+	+	+	+	+	+	+	(s)
191	Isorhamnetin *O*-pentoside	101.5	258, 354	447.09386	−1.61	$C_{21}H_{20}O_{11}$	**447**, 315, 271	**449**, 317					+								
192	Kaempferol derivative	101.87	268, 330	623.13981	−0.98	$C_{31}H_{28}O_{14}$	**623**, 284	**625**, 287		+	+			+	+		+	+	+	+	
193	*cis*-Tiliroside	102.37	268, 315	593.12995	−0.3	$C_{30}H_{26}O_{13}$	**593**, 284	**595**, 287		+	+		+	+	+	+	+	+	+	+	

Table 2. *Cont.*

No.	Compounds	Rt (min)	UV Spectra (λ Max nm)	Observed [1]	Δ (ppm)	Formula	Fragmentation		Presence in Extracts													Ref.
							Negative	Positive	PAL7r	PAU7	PER7	PER7r	PFR7	PHY7	PME7	PNE7	PPE7	PPU7	PRI7	PTH7		
194	Isorhamnetin *O*-deoxyhexoside	102.65	256, 350	461.10762	−2.95	$C_{22}H_{22}O_{11}$	**461**, 314, 271	**463**, 317, 274					+									
195	Tricin *O*-uronic acid derivative	103.04	254*sh*, 352	505.09843	−0.58	$C_{23}H_{22}O_{13}$	**505**, 329	**507**, 331, 316			+											
196	Isorhamnetin derivative	103.4	256, 350	593.14977	2.29	$C_{27}H_{30}O_{15}$	**593**, 314, 299	**595**, 317					+									
197	N^1, N^5, N^{10}-Tricoumaroyl spermidine	104.47	295, 310*sh*	582.26028	−0.48	$C_{34}H_{37}N_3O_6$	**582**, 462, 342, 285	**584**, 438, 292, 147		+	+			+	+	+	+	+	+	+	[30]
198	Ellagic acid derivative	111.73	350*sh*, 362	422.99970	0.41	$C_{20}H_8O_{11}$	**423**, 343, 269							+								

[1] Exact mass of $[M-H]^-$ ion; *sh*—peak shoulder; bold—most aboundantion; (s)—reference substance; HHDP—hexahydroxydiphenoyl group; NHTP—nonahydroxytriphenoyl group.

2.3. Examination of the Anticancer Potential of Extracts

In the first step, the extract's influence on both human colon epithelial cell line CCD841 CoN as well as human colon adenocarcinoma cell line LS180 was examined using an MTT assay. Studies were conducted after 48 h of the cells being exposed to either a culture medium (control) or extracts (25–250 μg/mL). As presented in Figure 1 and Table 3, all the investigated extracts inhibited the metabolic activity of both normal and cancer cells, and the observed effect was dose-dependent. The most significant anticancer effect was presented by extracts **PAL7r** and **PFR7**, which, at the highest tested concentration, deceased LS180 cells' proliferation by 91.3% ($IC_{50\ PAL7r\ LS180}$ = 82 μg/mL) and 94.8% ($IC_{50\ PFR7\ LS180}$ = 89 μg/mL), respectively. On the contrary, the weakest influence on the metabolic activity of colon cancer cells was noted after treatment with **PTH7** and **PRI7**, which, at a concentration of 250 μg/mL, inhibited cell viability by 58.7% ($IC_{50\ PTH7\ LS180}$ = 225 μg/mL) and 57.9% ($IC_{50\ PRI7\ LS180}$ = 213 μg/mL), respectively. The strongest reduction (by 36.7%) of the viability of colon epithelial cells was caused by both **PME7** and **PHY7** ($IC_{50\ PME7\ CCD841\ CoN}$ = 380 μg/mL; $IC_{50\ PHY7\ CCD841}$ CoN = 489 μg/mL), while the weakest effect, as reflected by the IC_{50} value, was shown by PRI7 ($IC_{50\ PRI7\ CCD841\ CoN}$ = 2402 μg/mL).

Used as a positive control for the experiment, 5-fluorouracil (5-FU) at a concentration of 25 μM decreased the metabolic activity of CCD841 CoN and LS180 by 22.2% and 46.2%, respectively. All the investigated extracts at the highest tested concentrations revealed a stronger anticancer effect than 5-FU. Seven of twelve extracts inhibited LS180 cells' viability better than 5-FU, when used at lower concentrations; the beneficial effect of **PER7r**, **PHY7**, **PME7**, and **PPE7** was observed at concentrations of 150 and 250 μg/mL, while the beneficial effect of **PAL7r**, **PFR7**, and **PNE7** was observed at concentrations from 100 to 250 μg/mL. In the case of CCD841 CoN cells, only 3 out of 12 of the investigated extracts inhibited the metabolic activity of colon epithelial cells stronger than 25 μM 5-FU: **PAL7r** (250 μg/mL); **PHY7** (250 μg/mL); **PME7** (150 and 250 μg/mL).

The obtained results for the MTT assay may be strongly associated with high TPrC, especially in the **PAL7r**, **PER7r**, and **FFR7** extracts. On several occasions, proanthocyanidins were reported to have a strong influence on colon cancer cell viability. Especially oligomeric proanthocyanidins from grape seeds (*Vitis vinifera* L., Vitaceae), which induce the apoptotic cell death of Caco-2 (human colorectal adenocarcinoma) cells manifested by nuclear condensation, caspase-3 and PARP cleavage, and formation of apoptotic bodies [31]. Additionally, proanthocyanidins from hops (*Humulus lupulus* L., Cannabaceae) increased the intracellular formation of reactive oxidative species, which was manifested by the augmented accumulation of protein carbonyls and induced cytoskeletal disorganisation of human colon cancer cell line HT-29 [32]. However, in a comparison with a previous study, all extracts exerted a weaker effect on cancer cell viability than extracts obtained from five out of six tested aqueous acetone extracts, namely, *P. argentea*, *P. grandiflora*, *P. norvegica*, *P. recta*, and *P. rupestris* [10]. This difference may be associated with the lower TPC and TTC obtained herein. Ellagitannins display great chemopreventive and chemotherapeutic activities. Among them, agrimoniin, the main ellagitannin present in all extracts, except **PAL7r**, was shown to have prominent anticancer, antioxidant, and anti-inflammatory activities [33]. It is widely recognised that there is a strict correlation between chronic inflammation and colorectal cancerogenesis [34]. Preclinical and clinical studies showed that non-steroidal and anti-inflammatory drugs are effective in preventing the formation of colorectal tumours; however, there are limitations due to severe and fatal side effects, such as gastric bleeding, ulcers, and renal toxicity [35]. Phytochemicals have fewer side effects compared with synthetic drugs, which is advantageous. A study conducted by Shi and co-authors revealed that the use of lyophilised strawberries (*Fragaria x ananasa* L., Rosaceae) containing agrimoniin as the second-most-abundant phytochemical, in an inflammation-induced colorectal carcinogenesis model, led to downregulating the mRNA expression of the proinflammatory mediators, such as COX-2, iNOS, IL-1β, IL-6, and TNF-α [36]. Moreover, in two consecutive studies, an agrimoniin-enriched fraction from the underground parts of *P. erecta* showed the dose-dependent inhibition of UVB-induced or TNF-α

stimulated IL-6 and PGE_2 production as well as reduced NFκB activation in HaCaT cells (human keratinocytes). Further, a UV erythema study in healthy volunteers revealed that an agrimoniin-enriched fraction significantly inhibited the UVB-induced inflammation process [37,38].

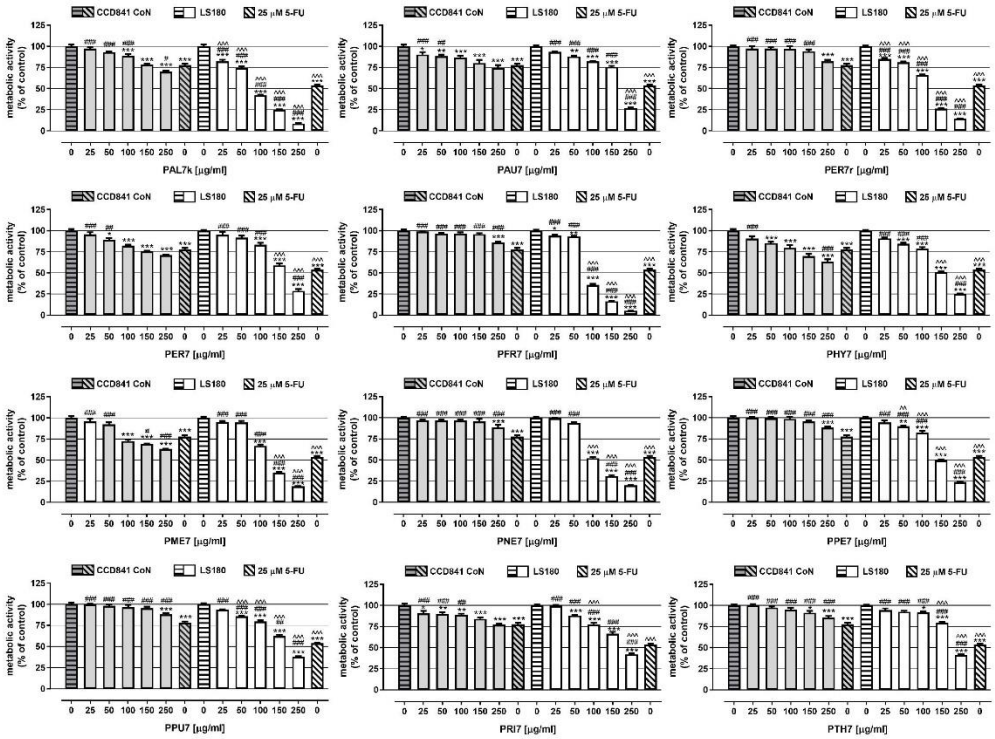

Figure 1. Influence of obtained extracts on the viability of CCD841 CoN and LS180 cell lines. The cells were exposed for 48 h to the culture medium alone (control). extract at concentrations of 25–250 μg/mL. or 25 μM 5-fluorouracil (5-FU; positive control). Metabolic activity of investigated cell lines in response to tested compounds was examined photometrically by MTT assay. Results are presented as mean ± SEM of at least 5 measurements. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. control. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ vs. positive control. ^^ $p < 0.01$; ^^^ $p < 0.001$ colon cancer cells treated with extract/5-FU vs. colon epithelial cells exposed to the extract/5-FU at the corresponded concentration; one-way ANOVA test; post-test: Tukey's.

Table 3. IC_{50} values (concentration causing viability/proliferation inhibition by 50% compared to control) of aqueous acetone extracts isolated from selected *Potentilla* L. species. IC_{50} values were calculated for human colon epithelial cell line CCD841 CoN and human colon adenocarcinoma cell line LS180 based on results of MTT as well as BrdU assays performed after 48 h of cells' treatment with investigated compounds.

Samples	MTT Assay						BrdU Assay					
	LS180			CCD841 CoN			LS180			CCD841 CoN		
	IC_{50} (μg/mL)	Trust Range (μg/mL)	R^2	IC_{50} (μg/mL)	Trust Range (μg/mL)	R^2	IC_{50} (μg/mL)	Trust Range (μg/mL)	R^2	IC_{50} (μg/mL)	Trust Range (μg/mL)	R^2
PAL7r	82	77–87	0.980	496	396–623	0.908	52	41–64	0.917	412	351–483	0.841
PAU7	192	180–206	0.920	1575	536–4632	0.672	1495	1311–1704	0.871	2058	1626–2604	0.542
PER7	176	166–186	0.957	672	474–952	0.891	1001	847–1183	0.845	3705	2368–5796	0.336
PER7r	110	101–120	0.943	523	326–839	0.595	54	44–66	0.925	337	281–405	0.856
PFR7	89	85–92	0.989	707	450–1113	0.737	50	40–62	0.916	282	244–327	0.809
PHY7	156	146–167	0.952	489	334–717	0.838	425	350–516	0.843	631	495–804	0.765
PME7	128	122–133	0.983	380	291–495	0.870	417	325–536	0.774	837	661–1061	0.740
PNE7	112	106–118	0.977	1795	329–9800	0.365	343	298–395	0.915	586	451–763	0.748
PPE7	158	150–167	0.966	620	367–1047	0.663	343	283–414	0.850	911	728–1140	0.768
PPU7	197	185–210	0.967	865	359–2081	0.531	881	761–1019	0.836	937	803–1093	0.846
PRI7	213	200–228	0.968	2402	788–7326	0.717	542	452–649	0.848	1230	837–1806	0.553
PTH7	225	215–236	0.956	969	443–2119	0.643	606	521–704	0.876	1039	791–1364	0.693
5-FU	31	28–33	0.977	113	81–157	0.884	15	13–16	0.956	94	80–111	0.933

In the next step, the antiproliferative activity of *Potentilla* L. extracts was examined in both the normal and cancer cell lines using a BrdU assay (Figure 2 and Table 1). All the investigated extracts decreased DNA synthesis in the colon cancer cells in a dose-dependent manner. Nevertheless, a significant decrease in LS180 cells' proliferation in response to the extract, for the whole range of tested concentrations, was only observed for **PAL7r**, **PFR7**, and **PER7r**, which, at concentrations of 100, 150, and 250 μg/mL, reduced DNA synthesis by around 80%. Furthermore, the aforementioned extracts were characterised by the lowest IC_{50} values ($IC_{50\ PAL7r\ LS180}$ = 52 μg/mL; $IC_{50\ PFR7\ LS180}$ = 50 μg/mL; $IC_{50\ PER7r\ LS180}$ = 54 μg/mL). On the contrary, the lowest antiproliferative abilities were revealed by PAU7, which, even at the highest tested concentration, decreased DNA synthesis in LS180 cells by only 14.9% ($IC_{50\ PAU7\ LS180}$ = 1495 μg/mL). The antiproliferative effect of the examined extracts was also observed in colon epithelial cells; however, the observed effect was weaker than in cancer cells. The only extract that did not affect divisions of CCD841 CoN was **PER7**, which was characterised by the highest IC_{50} value of 3705 μg/mL. On the contrary, the most significant changes in normal cells were observed in response to **PAL7r**, **PFR7**, and **PER7r**, which, at the highest tested concentration, reduced the proliferation of epithelial cells by 36.1%, 38.3%, and 43.9%, respectively ($IC_{50\ PAL7r\ CCD841\ CoN}$ = 412 μg/mL; $IC_{50\ PER7\ CCD841\ CoN}$ = 282μg/mL; $IC_{50\ PER7r\ CCD841\ CoN}$ = 337 μg/mL). As presented in Figure 2, 25 μM 5-fluorouracil (5-FU) decreased DNA synthesis in the investigated cell lines to 90.7% (CCD841 CoN) and 29.7% (LS180). The antiproliferative effect of 5-FU recorded in colon cancer cells was significantly stronger than the changes induced by most of the examined extracts (9 of 12); however, **PAL7r**, **PFR7**, and **PER7r**, in concentrations from 100 to 250 μg/mL, decreased DNA synthesis more than 5-FU. On the contrary, data collected from colon epithelial cells revealed that most of the investigated extracts (**PAL7r**, **PER7r**, **PFR7**, **PHY7**, **PME7**, **PNE7**, **PPE7**, **PPU7**, **PRI7**, and **PTH7**) at higher concentrations inhibited DNA synthesis stronger than 25 μM 5-FU, while the antiproliferative effect of **PFR7** for the whole range of tested concentrations was higher than the changes induced by analysed cytostatic. However, the presented results correspond with data from our previous study, showing that tested *Potentilla* species possess similar anticancer potential; moreover, for the **PAL7r**, **PER7r**, and **PFR7** extracts, the results from a BrdU assay were significantly higher than those for all other tested samples [10]. The observed effect may be attributed to the high TPrC values. Kresty and co-authors found that a cranberry (*Vaccinium macrocarpon* Aiton, Ericaceae) proanthocyanidin-rich fraction significantly inhibited the viability and proliferation of human oesophageal adenocarcinoma SEG-1 cells. The mechanism involved cell cycle arrest in the G1 phase as well as a significant apoptosis induction [39]. Notably, the antiproliferative effect of other extracts may be connected with the presence of ellagitannins and the main product of their decomposition, namely, ellagic acid. The anticancer mechanism of ellagic acid is multidirectional. A study conducted on human colorectal carcinoma HCT-116 and the Caco-2 cell line revealed that ellagic acid induced cell cycle arrest in the G phase, reduced proliferating cell nuclear antigen (PCNA) expression and mitotic activity, and induced apoptosis via increasing the expression of caspase-8 and Bax [40]. Additionally, a further study conducted on HCT-116 cells revealed the involvement of ellagic acid in the decreased gene expression of signalling pathways' proteins such as mitogen-activated protein (MAPK), p53, PI3K-Akt, and TGF-β [41]. Recently, Han and co-authors found that tiliroside acted as an inhibitor of carbonic anhydrase XII (CAXII), a membrane enzyme that produces a favourable intracellular pH and sustains optimum P-glycoprotein (Pgp) efflux activity in cancer cells. Moreover, tiliroside downregulated E2F1 and E2F3 expression and promoted caspase-3 activity [39]. In addition, the meta-analysis revealed that a high intake of flavonoids, such as quercetin and kaempferol derivatives, in the diet may decrease the risk of colon cancer [42].

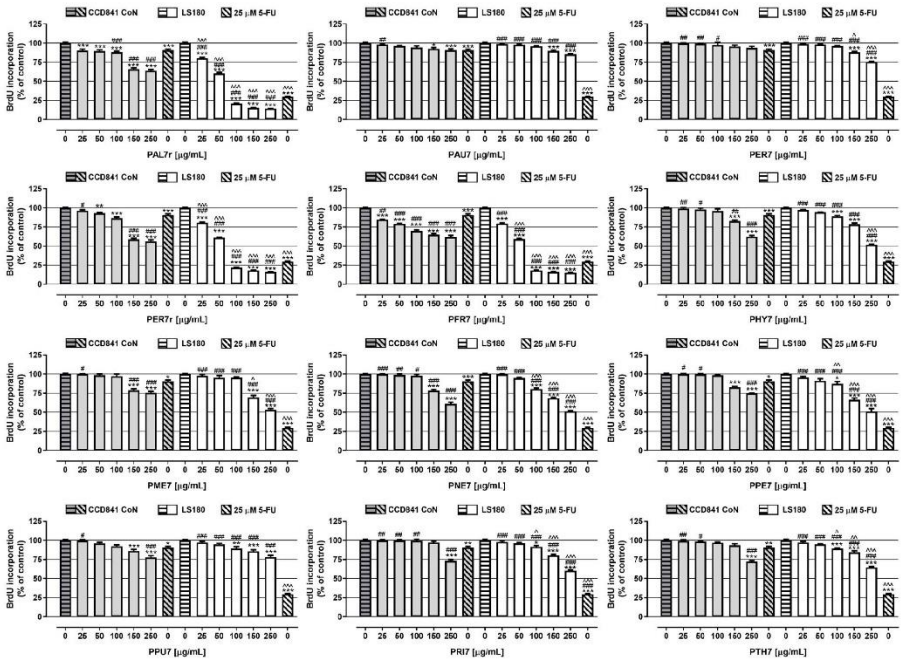

Figure 2. Antiproliferative effect of extracts on CCD841 CoN and LS180 cell lines. The cells were exposed for 48 h to the culture medium alone (control). extract at concentrations of 25–250 μg/mL. or 25 μM 5-fluorouracil (5-FU; positive control). The antiproliferative impact of investigated compounds was measured using BrdU assay (incorporation of BrdU to newly synthesised DNA). Results are presented as mean ± SEM of at least 4 measurements. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. control. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ vs. positive control. ^ $p < 0.05$; ^^ $p < 0.01$; ^^^ $p < 0.001$ colon cancer cells treated with extract/5-FU vs. colon epithelial cells exposed to the extract/5-FU at the corresponded concentration; one-way ANOVA test; post-test: Tukey's.

Extract cytotoxicity was also examined in both normal and cancer colon cell lines using an LDH (lactate dehydrogenase)-based assay (Figure 3). Most of the examined extracts were not cytotoxic against human colon epithelial cells; however, **PME7** was, for the whole range of tested concentrations, while **PAL7r**, **PER7**, and **PHY7**, in concentrations from 100 to 250 μg/mL, damaged the membranes of epithelial cells. The indicated extracts at the highest tested concentration increased the LDH level by an average of 11%. Studies conducted on colon cancer cells showed the cytotoxic effect of all the examined extracts for the whole range of tested concentrations. The strongest damage of cancer cell membranes was caused by **PAL7r**, which in concentrations from 25 to 250 μg/mL increased the LDH level by 145.7% and 479.0% respectively. The weakest cytotoxic effect was noted in colon cancer cells treated with **PRI7**, **PPU7**, and **PTH7**, which, at the highest tested concentration, caused an increase in the LDH level by 245.1%, 254.7%, and 256.0%, respectively. An LDH assay showed that 5-FU in a concentration of 25 μM was not cytotoxic against colon epithelial, while LDH releases were increased in colon cancer cells by 13.4%. All the investigated extracts damaged the colon cancer cell membranes significantly more than 5-FU. For CCD841 CoN cells, significant differences in the LDH concentration between 25 μM 5-FU and the examined extracts were observed in the case of four extracts (**PME7**, **PAL7r**, **PER7**, and **PHY7**), for which the cytotoxic impact on colon epithelial cells was reported

above. The results of the LDH assay are presumably directly associated with the TTC in the investigated samples. Tannins, due to their specific chemical structure, are known to affect the physical properties of membranes, initiate membrane protein aggregation, increase bilayer adhesion, and regulate cell metabolism [43,44]. The most abundant hydrolysable tannin present in most extracts, agrimoniin, induces the intrinsic pathway of apoptosis, directly influencing the permeability of the mitochondrial membrane via the activation of the mitochondrial permeability transition pore (MPTP) [45]. However, further in vivo studies are required to evaluate the exact mechanism of action. The bioavailability of large ellagitannins is generally low. Therefore, the method of application is limited to topical application. The gut microbiota metabolise ellagitannins and ellagic acid to produce a series of bioavailable metabolites, known as urolithins. Urolithins possess a series of biological activities, such as anti-inflammatory, antioxidant, anticancer, and immunomodulatory activities. The chemopreventive effects of urolithins were extensively studied in several models, including prostate and colorectal cancer models. Urolithins were shown to inhibit colon cancer cell growth in a dose-dependent manner, alter the expression of the genes and proteins modulating the cell cycle, and induce apoptosis [46]. Notably, a clinical study on the aerial parts of *P. anserina* and the rhizomes of *P. erecta* confirmed the formation of urolithins in ex vivo conditions [47].

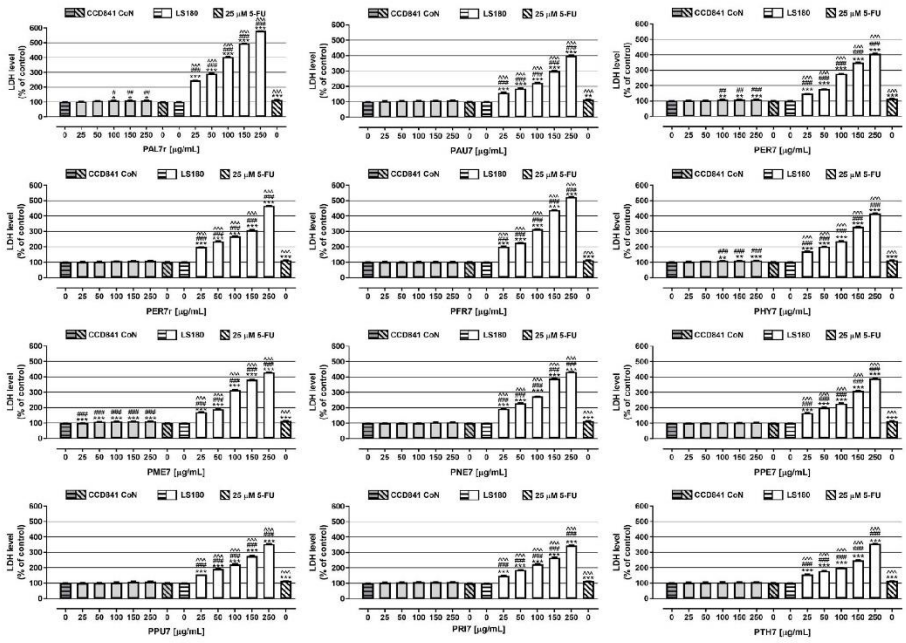

Figure 3. The influence of extracts on cell membrane integrity of CCD841 CoN and LS180 cell lines. The cells were exposed for 48 h to the culture medium alone (control). extract at concentrations of 25–250 µg/mL. or 25 µM 5-fluorouracil (5-FU; positive control). Extracts' cytotoxicity (level of LDH released into the cell culture medium from damaged cell membranes) was measured using an LDH assay. Results are presented as mean ± SEM of at least 3 measurements. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. control. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ vs. positive control. ^^^ $p < 0.001$ colon cancer cells treated with extract/5-FU vs. colon epithelial cells exposed to the extract/5-FU at the corresponded concentration; one-way ANOVA test; post-test: Tukey's.

3. Materials and Methods

3.1. Chemicals

The reference phytochemicals, including isorhamnetin 3-*O*-glucoside, kaempferol 3-*O*-glucuronide, and quercetin 3-*O*-glucuronide were obtained from Extrasynthese (Genay, France). Gallic acid, catechin, and epicatechin were obtained from Carl Roth (Karlsruhe, Germany). Procyanidin B1, procyanidin B2, procyanidin B3, and procyanidin C1 were purchased from Cayman Chemical (Ann Arbor, MI, USA), while agrimoniin, apigenin, 3-*O*-caffeoylquinic acid, ellagic acid, astragalin (kaempferol 3-*O*-glucoside), pedunculagin, avicularin (quercetin 3-*O*-arabinoside), hyperoside (quercetin 3-*O*-galactoside), isoquercitrin (quercetin 3-*O*-glucoside), and tiliroside (purity > 96%) were previously isolated in the Department of Pharmacognosy of Medical University of Białystok (Białystok, Poland) [22,48–51]. All other analytical grade chemicals used in the study were obtained from Sigma-Aldrich (St. Louis, MO, USA). To obtain ultra-pure water, a POLWATER DL3-100 Labopol (Kraków, Poland) system was used. Investigated extracts (100 mg/mL) and 5-fluorouracil (50 mM) were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions. Working solutions were prepared by dissolving stock solutions in a culture medium. The final concentration of DMSO in all working solutions used in the studies was 0.25%.

3.2. Plant Materials and Extraction Procedure

Plants used to obtain material for investigations come from the Medicinal Plant Garden at the Medical University of Białystok (Białystok, Poland) and were collected in June-August 2017–2020. Plants were carefully identified by one of the authors (M.T.), and individual voucher specimens were deposed at the Herbarium of the Department of Pharmacognosy, Medical University of Białystok (Białystok, Poland). Plant material was dried at room temperature in the shade and air temperature and subsequently finely ground with an electric grinder. Accurately weighed 2 g of each powdered dry plant material were separately extracted using an ultrasonic bath (Sonic-5, Polsonic, Warszawa, Poland) with 70% acetone at a controlled temperature (40 ± 2 °C) for 45 min in a 1:75 (*w:v*) solvent ratio to obtain raw extracts. Subsequently. extracts were evaporated to dryness, diluted with water (50 mL). and successively portioned between chloroform (10 × 20 mL). Afterwards. purified extracts were freeze-dried. The list of obtained aqueous acetone extracts from selected *Potentilla* species detailing plant species, voucher specimen, the parts used and extraction yields are presented in Table 4.

Table 4. List of plants from the *Potentilla* genus that were screened in the study and extraction yields.

Sample Name	Lant Species	Voucher Specimen No.	Parts Used [1]	Extraction Yield (%) [2]
PAL7r	*Potentilla alba* L.	PAL-17039	R	11.2%
PAU7	*Potentilla aurea* L.	PAU-20045	A	32.8%
PER7	*Potentilla erecta* (L.) Raeusch	PER-06016	A	17.8%
PER7r			R	15.7%
PFR7	*Potentilla fruticosa* L. (syn. *Dasiphora fruticosa* (L.) Rydb.)	PFR-06018	L	36.6%
PHY7	*Potentilla hyparctica* Malte	PHY-20046	A	26.5%
PME7	*Potentilla megalantha* Takeda	PME-18043	A	34.1%
PNE7	*Potentilla nepalensis* Hook.	PNE-06023	A	33.4%
PPE7	*Potentilla pensylvanica* L.	PPS-08025	A	22.4%
PPU7	*Potentilla pulcherrima* Lehm.	PPU-18044	A	28%
PRI7	*Potentilla rigoi* Th. Wolf	PRI-20047	A	30.6%
PTH7	*Potentilla thuringiaca* Bernh.	PTH-06022	A	22.8%

[1] A, aerial parts; L, leaves; R, rhizomes; [2] Extraction yield of purified fraction.

3.3. Phytochemical Profile

3.3.1. Determination of Total Phenolic (TPC) and Total Tannin Content (TTC)

The content of total phenolic compounds was measured using standard the Folin-Ciocalteu colourimetric method, with slight modification according to [29]. The content total tannin determination was carried out using the hide powder-binding method and Folin–Ciocalteu assay reported in the corresponding monograph in the European Pharmacopoeia 10th ed. [52]. The absorbance was measured at 760 nm using a EPOCH2 BioTech (Winooski, VT, USA) microplate reader. The obtained results for were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). The determination was repeated at least in triplicate for each sample solution.

3.3.2. Determination of Total Proanthocyanidin Content (TPrC)

Total proanthocyanidin content was analysed using the procedure based on the previously published protocol [53]. The analysis was carried out by mixing 50 µL of the sample solution (1 mg/mL) dissolved in methanol and 250 µL of 0.1% methanolic solution of 4-dimethylamino-cinnamaldehyde (DMCA) reagent in 6M HCl. After incubation of the mixture at room temperature for 15 min, the absorbance was measured at 635 nm, and results were expressed as milligrams of catechin equivalents per gram of extract (mg CE/g extract). The determination was repeated at least five times for each sample solution.

3.3.3. Determination of Total Phenolic Acid Content (TPAC)

Total phenolic acid content was estimated with the procedure using Arnov's reagent (1 g of sodium molybdate and 1 g of sodium nitrate dissolved in 10 mL of distilled water) [54]. Each time the tested solution (30 µL) was mixed with 180 µL of water, 30 µL of 0.5 M HCl, 30 µL of Arnov's reagent, and 30 µL of 1 M NaOH were sequentially added to the microplate well, and then it was incubated for 10 min at ambient temperature. Afterwards, the absorbance was measured at 490 nm, and results were expressed as milligrams of caffeic acid equivalents per gram of extract (mg CAE/g extract). The determination was repeated at least three times for each sample solution.

3.3.4. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was estimated by the previously described colourimetric method [29]. Each aliquot (100 µL) was mixed with aluminum chloride ($AlCl_3$) solution (100 µL, 2% *w:v*). After incubation of the mixture at room temperature for 10 min, the absorbance was measured at 415 nm, and results were expressed as milligrams of rutin equivalents per gram of extract (mg RE/g extract). The determination was repeated at least three times for each sample solution.

3.3.5. LC–HRMS Profiling of Extracts

The separation and qualitative evaluation of each extract were conducted using a Kinetex XB-C18 column (150 × 2.1 mm, 1.7 µm, Phenomenex, Torrance, CA, USA) and Agilent 1260 Infinity LC chromatography system coupled to a photo-diode array (PDA) detector and 6230 time-of-flight (TOF) mass spectrometer (Santa Clara, CA, USA). A detailed description of the execution of the above-mentioned assay was presented in the previous study [10].

3.4. Cell Cultures

For the cell culture study, human colon adenocarcinoma cell line LS180 and human colonic epithelial cell line CCD841 CoN were purchased from the European Collection of Cell Cultures (ECACC, Centre for Applied Microbiology and Research, Salisbury, UK) and American Type Culture Collection (ATCC, Menassas, VA, USA), respectively. LS180 cells and CCD841 CoN cells were maintained in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham and Dulbecco's Modified Eagle's Medium (DMEM), respectively. Then, 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 g/mL) were

added to the cell culture media. Cells were incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

3.5. Evaluation of the Anticancer Potential of Extracts

Examination of the anticancer potential of extracts was conducted simultaneously on both cancer (LS180) and normal (CCD841 CoN) colon cells. Cells at a density of 5 × 104 cells/mL were plated on 96-well plates. The next day, the cell growth medium was exchanged for fresh medium supplemented with investigated extracts or 25 µM 5-fluorouracil (5-FU). After 48 h of treatment, compound impacts on cell membrane integrity, metabolic activity, and DNA syntexis were examined using LDH, MTT, and BrdU assays, respectively. The description of the execution of indicated tests was previously presented [10].

3.6. Statistical Analysis

The data were presented as the mean ± standard error of mean (SEM). Statistical analyses were carried out using one-way ANOVA with Tukey's post hoc test and column statistics. Significance was accepted at $p < 0.05$. The IC_{50} value (concentration causing proliferation inhibition by 50% compared to control) was calculated according to the method of Litchfield and Wilcoxon [55] using GraphPad Prism 5.

4. Conclusions

In conclusion, the presented study reports, for the first time, an analysis of the LC–HRMS profile of aqueous acetone extracts from rare *Potentilla* species. The analysis revealed a series of marker metabolites such as agrimoniin, pedunculagin, dimeric and trimeric B-type procyanidins, tiliroside, astragalin (kaempferol 3-*O*-glucoside), hyperoside (quercetin 3-*O*-galactoside, ellagic acid, and tri-coumaroyl spermidine. The performed studies revealed that all of the investigated acetone extracts obtained from rare *Potentilla* species decreased the viability and proliferation of human colon adenocarcinoma LS180 cells. Nevertheless, most of the investigated extracts also decreased metabolic activity and DNA synthesis in human colon epithelial CCD841 CoN cells, and 4 out of 12 of the tested extracts (**PAL7r**, **PER7**, **PHY7**, and **PME7**) showed cytotoxic effects against normal epithelial cells. Despite the fact that the investigated extracts affected both normal and cancer colon cells, the LS180 cells were more sensitive to tested extracts. Considering the data obtained from all the performed studies, the 2 of the 12 investigated extracts (**PFR7** and **PER7r**) revealed the greatest chemopreventive potential, as manifested by the effective elimination of colon cancer cells, which caused both damage to their cell membranes and inhibition of their proliferation and metabolic activity, with a simultaneous lack of any cytotoxic effect on normal colon epithelial cells and a significantly weaker effect on their metabolism and DNA synthesis compared to cancer cells. The previous [10] and currently obtained results indicated that some acetone extracts from *Potentilla* species have anticancer potential, however, additional animal and clinical studies, especially including the influence of intestinal flora are required to verify discovered beneficial properties of investigated extracts. Nevertheless, discovered selectivity of the anticancer effects of tested extracts encourages further studies to develop a new efficient and safe therapeutic strategy for people who have been threatened by or suffered from colon cancer.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ijms24054836/s1.

Author Contributions: Conceptualisation, D.A. and M.T.; methodology, D.A., M.K.L., J.W.S. and M.T.; formal analysis, D.A., M.K.L., A.W. and M.T.; investigation, D.A., M.K.L. and J.W.S.; resources, M.K.L. and M.T.; writing—original draft preparation, D.A., M.K.L. and J.W.S.; writing—review and editing, A.W. and M.T.; visualization, D.A.; supervision, A.W. and M.T.; project administration, A.W. and M.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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Suplement do publikacji 2

International Journal of
Molecular Sciences

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Supplementary Material

Phytochemical Profiling of Extracts from Rare *Potentilla* Species and Evaluation of Their Anticancer Potential

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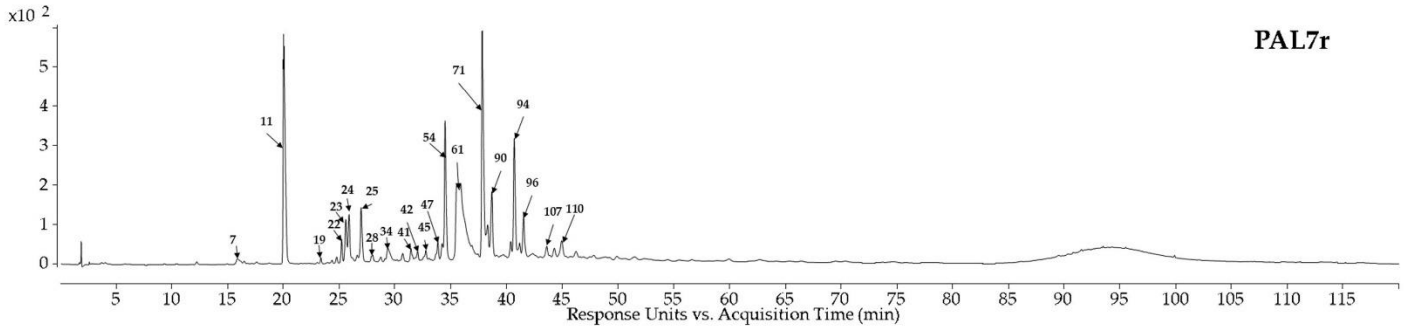
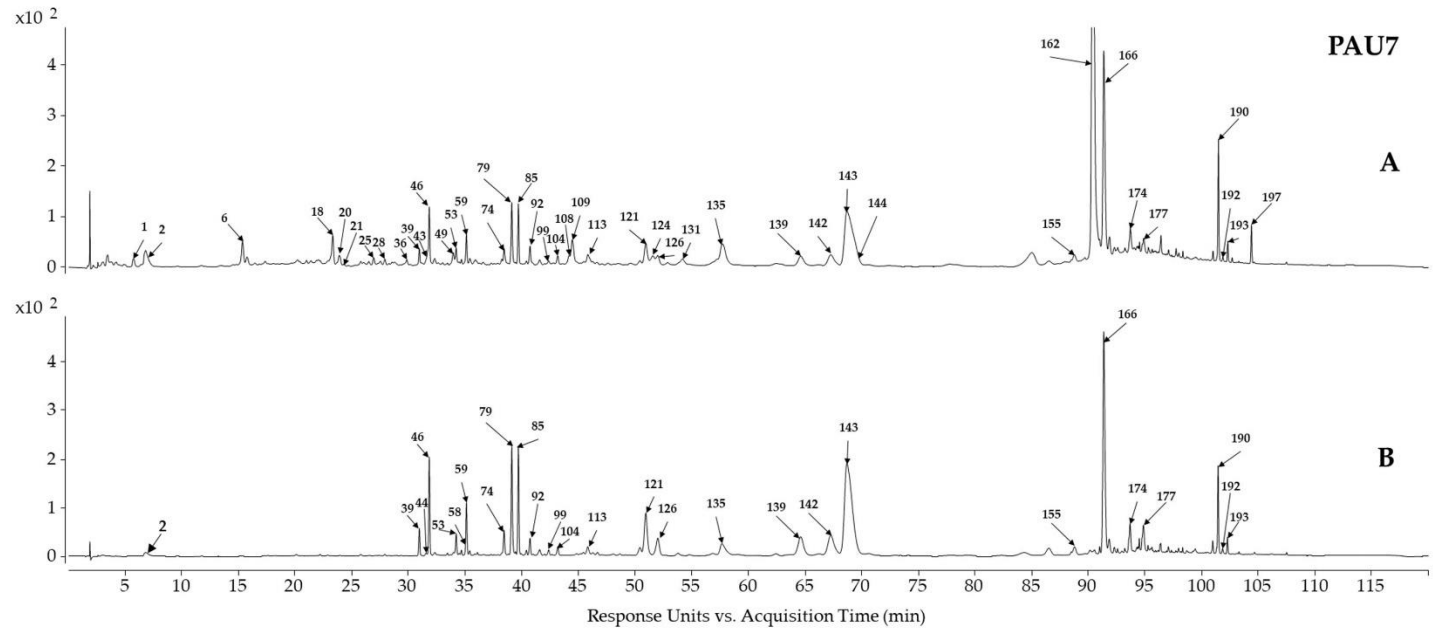
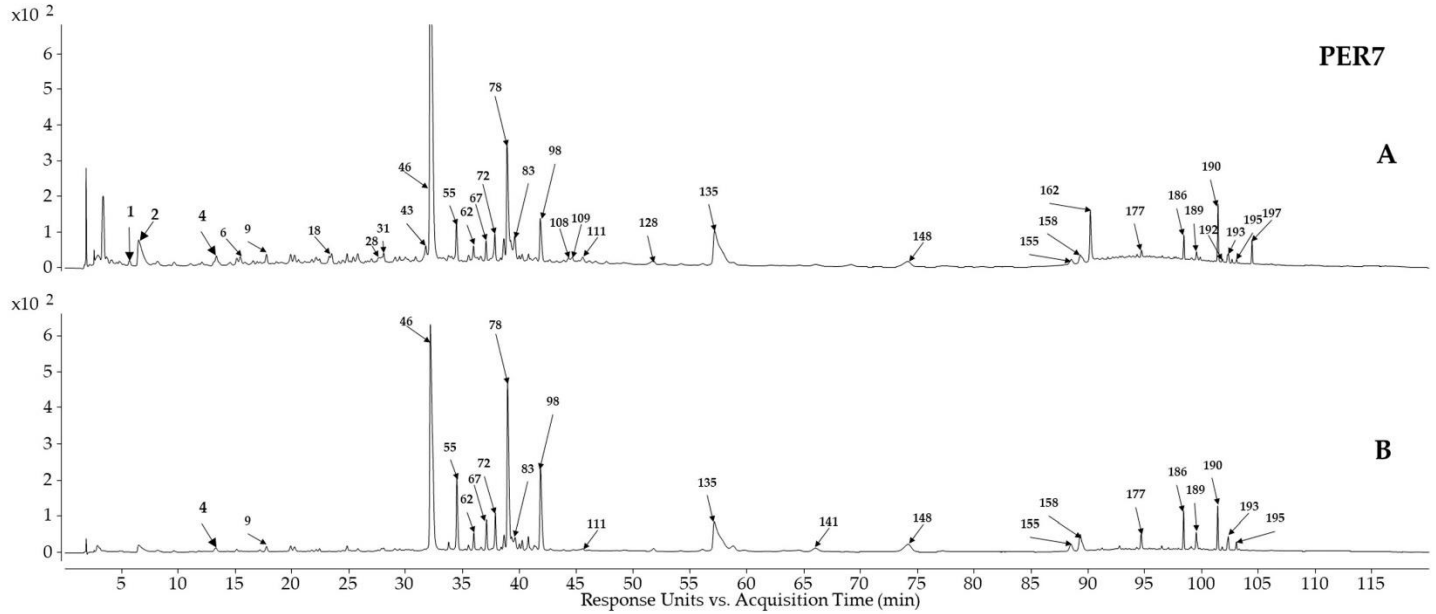


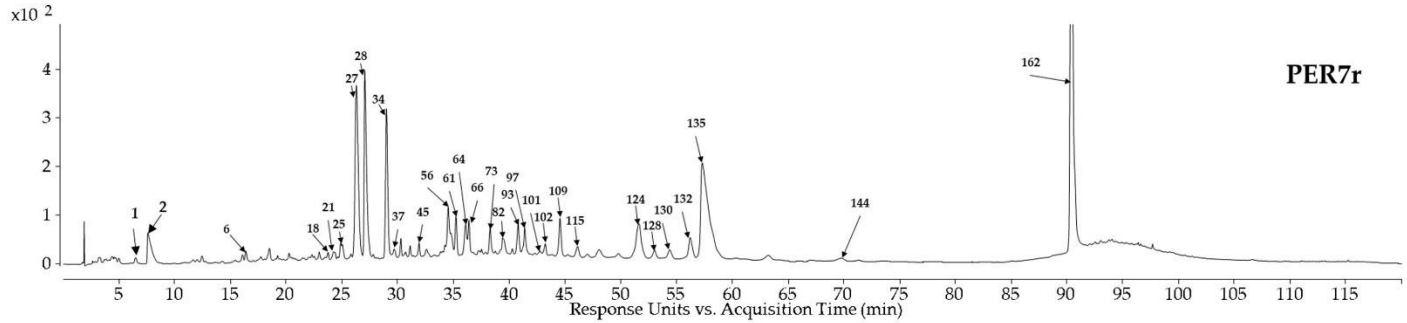
Supplementary Figure S1. The UV chromatogram with a designation of the main components of the analyzed **PAL7r** extract, recorded at length of 280 nm.



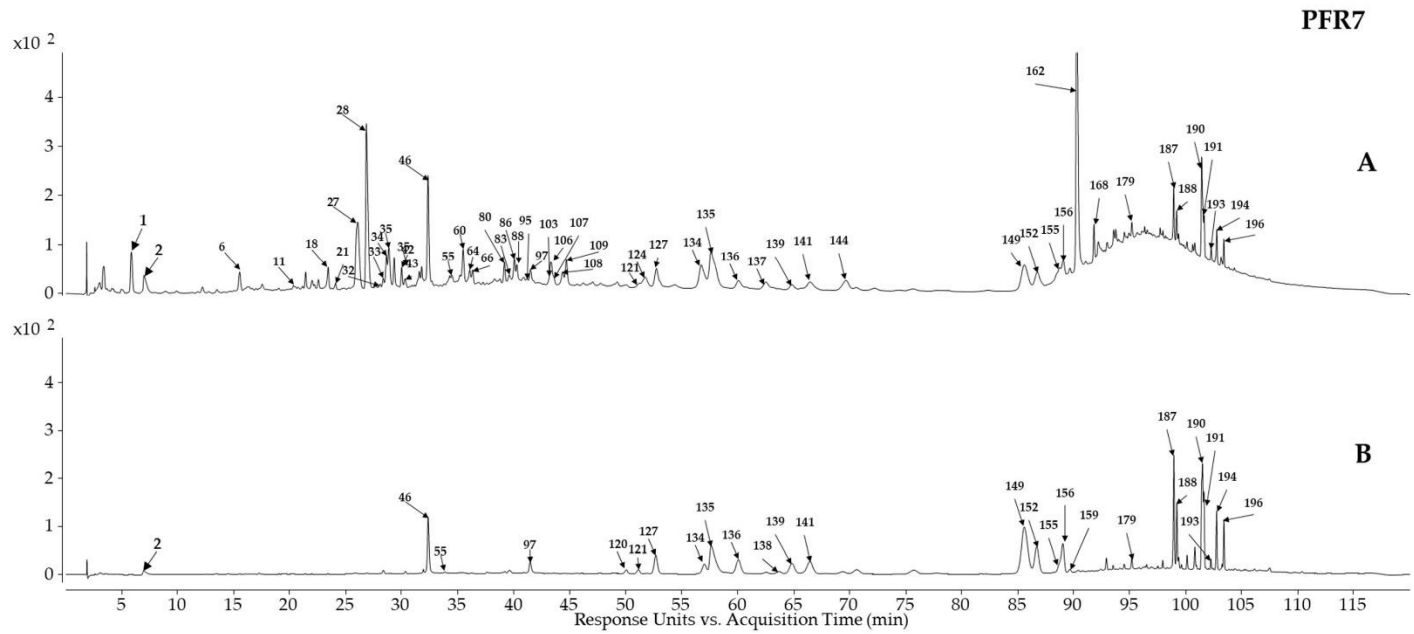
Supplementary Figure S2. The UV chromatograms with a designation of the main components of the analyzed **PAU7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



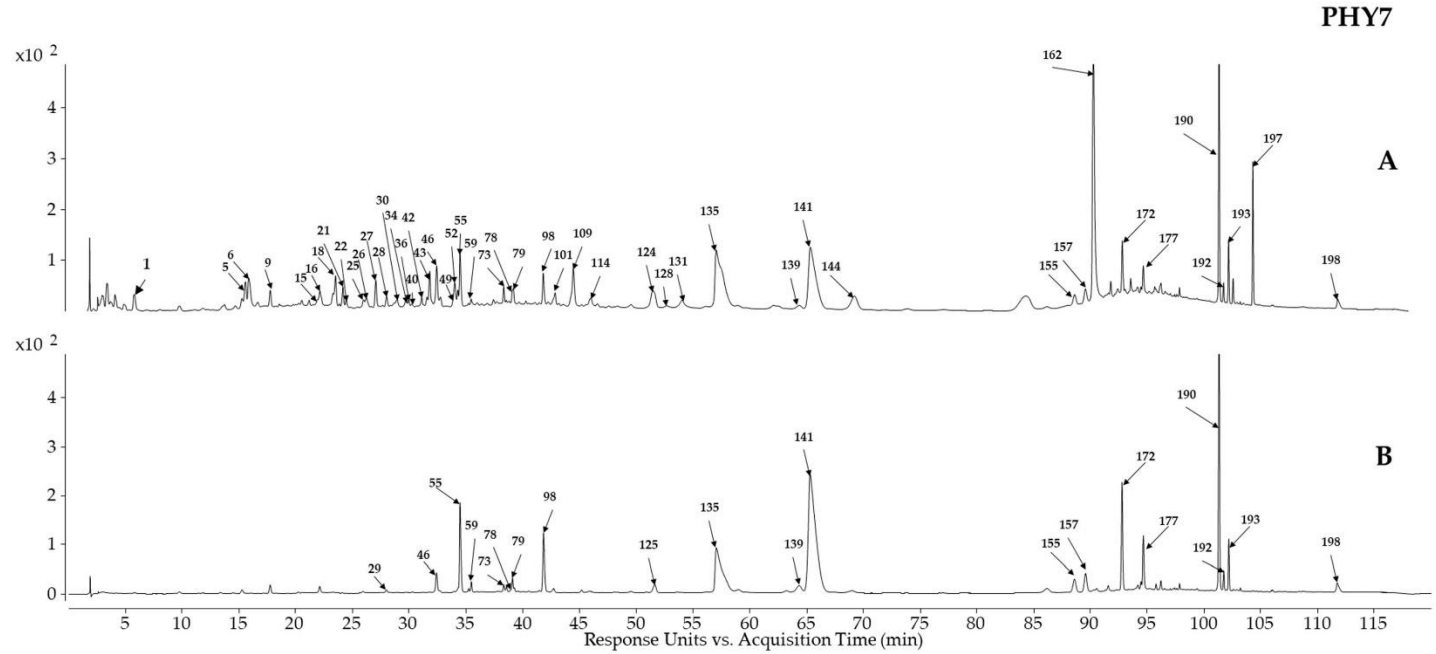
Supplementary Figure S3. The UV chromatograms with a designation of the main components of the analyzed **PER7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



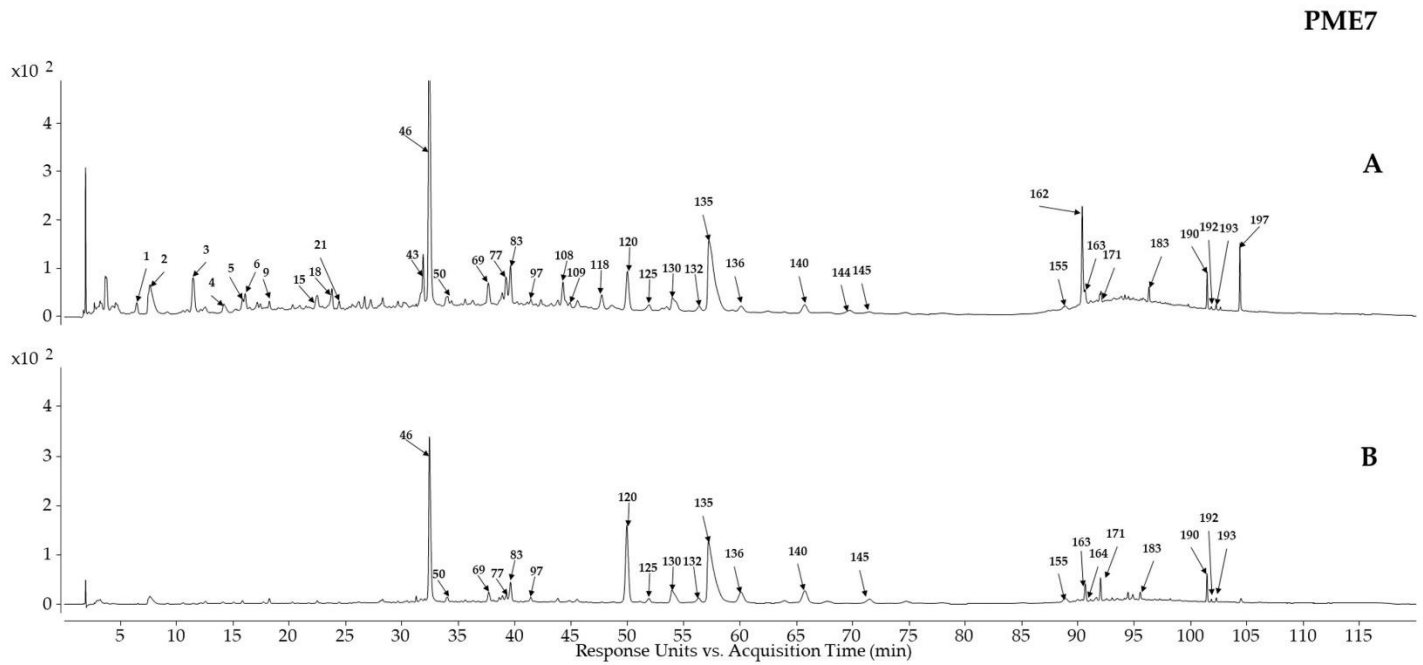
Supplementary Figure S4. The UV chromatogram with a designation of the main components of the analyzed **PER7r** extract, recorded at length of 280 nm.



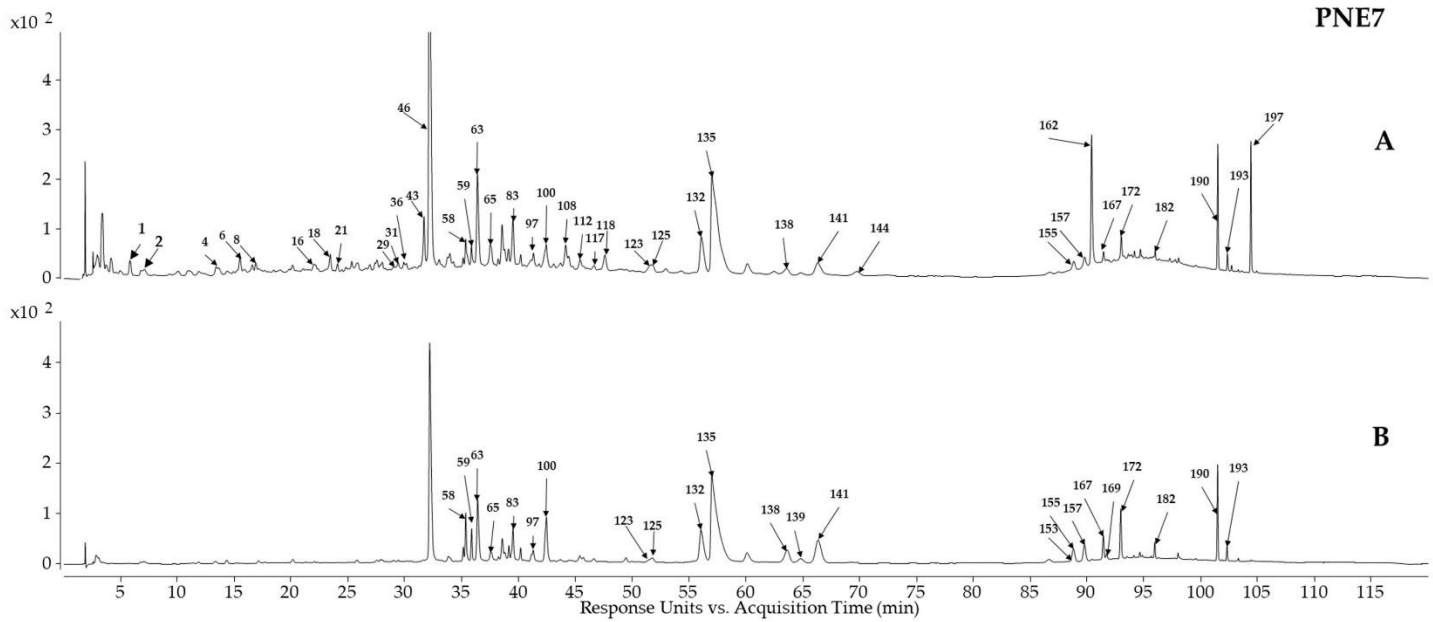
Supplementary Figure S5. The UV chromatograms with a designation of the main components of the analyzed **PFR7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



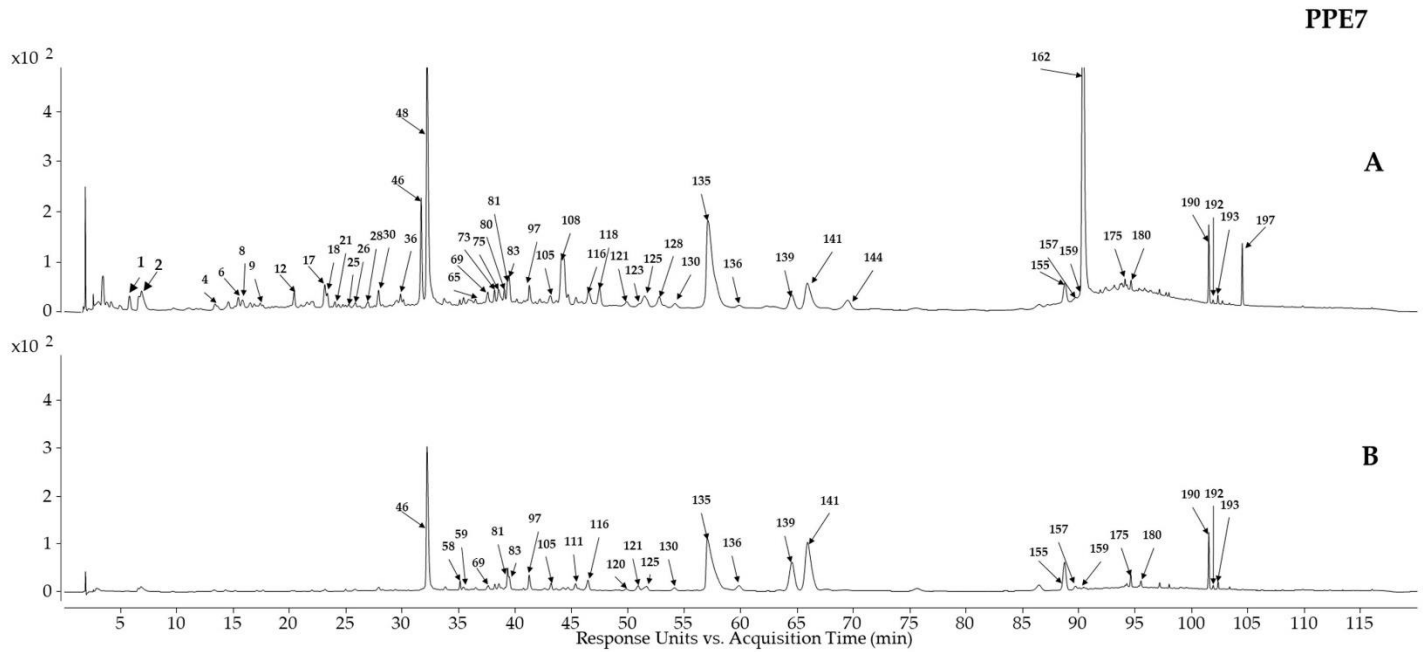
Supplementary Figure S6. The UV chromatograms with a designation of the main components of the analyzed **PHY7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



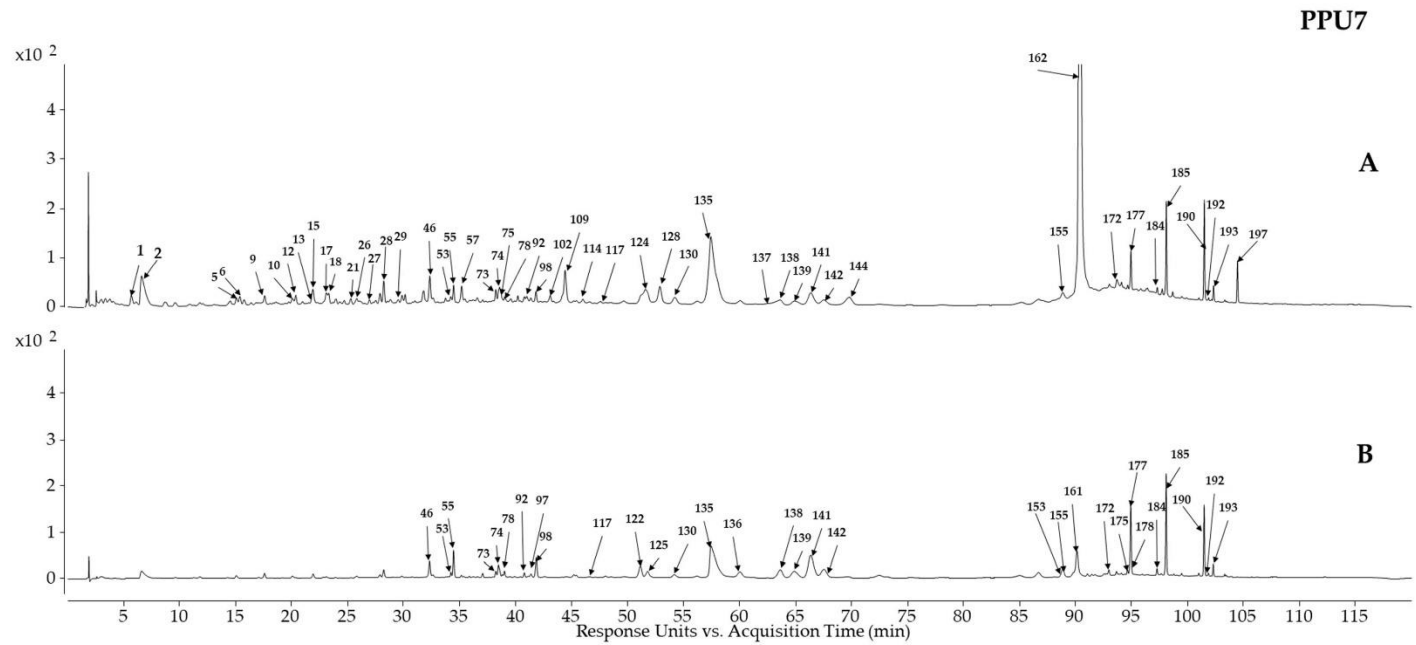
Supplementary Figure S7. The UV chromatograms with a designation of the main components of the analyzed **PME7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



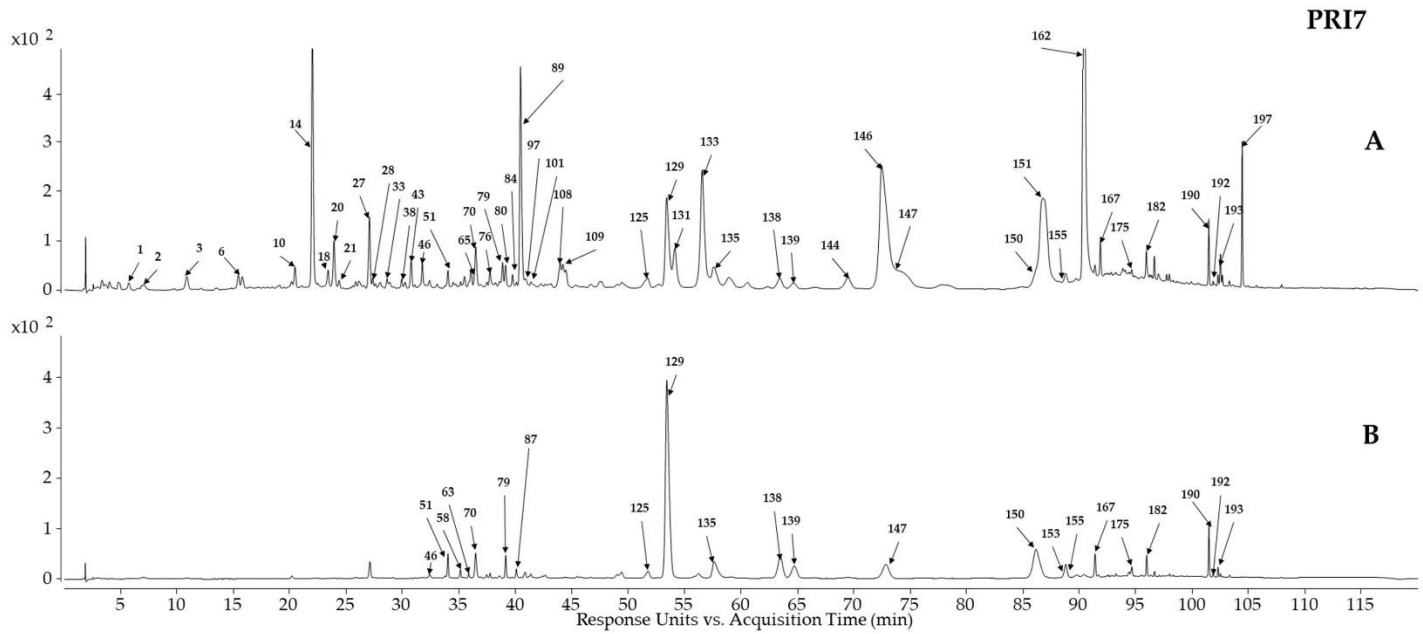
Supplementary Figure S8. The UV chromatograms with a designation of the main components of the analyzed **PNE7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



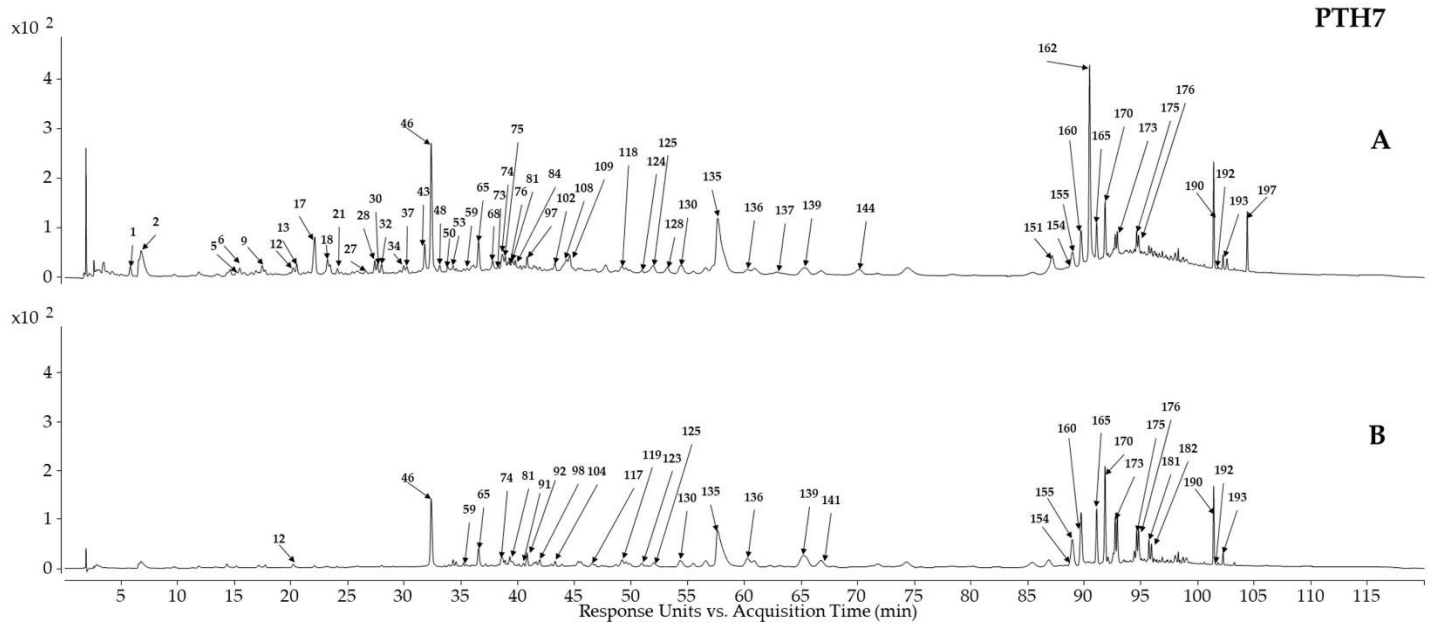
Supplementary Figure S9. The UV chromatograms with a designation of the main components of the analyzed **PPE7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



Supplementary Figure S10. The UV chromatograms with a designation of the main components of the analyzed **PPU7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



Supplementary Figure S11. The UV chromatograms with a designation of the main components of the analyzed **PRI7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).



Supplementary Figure S12. The UV chromatograms with a designation of the main components of the analyzed **PTH7** extract, recorded at lengths of 280 nm (A) and 360 nm (B).

Publikacja 3

Augustynowicz D., Latte, KP., Tomczyk, M. Recent phytochemical and pharmacological advances in the genus *Potentilla* L. *sensu lato*—An update covering the period from 2009 to 2020. *Journal of Ethnopharmacology*. 2021; 266, 113412. DOI: 10.1016/j.jep.2020.113412

Journal of Ethnopharmacology 266 (2021) 113412

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Review

Recent phytochemical and pharmacological advances in the genus *Potentilla* L. *sensu lato* – An update covering the period from 2009 to 2020

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ARTICLE INFO

Keywords:
Potentilla
Rosaceae
Phytochemistry
Pharmacology
Review
Update

ABSTRACT

Ethnopharmacological relevance: *Potentilla* plants are still common herbal medicines used in folk medicine. This review provides an update of research undertaken on *Potentilla* from 2009 until 2020.
Aim of the study: This comprehensive review considers biological updates, recent advances in phytochemical and pharmacological research, and toxicological reports on *Potentilla sensu lato* based on available data since 2009.
Methods: A literature search was conducted using available databases including ScienceDirect, PubMed, Scopus, Web of Science, China National Knowledge Infrastructure and Google Scholar.
Results: Until now, more than 210 new and known compounds, including flavonoids, tannins, triterpenes and phenolic compounds, have been confirmed and elucidated for numerous *Potentilla* species, i.e., in the underground and aerial parts of this genus. Modern pharmacology studies have revealed that those structures are responsible for a broad spectrum of pharmacological activities, such as anti-neoplastic, antihyperglycemic, anti-inflammatory, antioxidant, hepatoprotective, neuroprotective, antibacterial and anti-yeast effects.
Conclusions: However, *in vitro* studies must be re-considered due to the discovery of urolithins and their origins, including microbiota, which can lead to different results when applying *Potentilla* species and their extracts to *in vivo* conditions. Thus, future research should focus more on *in vivo* and particularly clinical studies to confirm the validity and safety of traditional uses. Particularly, the use of *Potentilla alba* extracts in the treatment of thyroid gland disorders should be further explored to confirm the underlying mechanism of their action, efficacy and safety. In addition, more clinical studies should focus on *Potentilla erecta* rhizome extracts for application as herbal remedies against dysentery, diarrhoea and inflammation of the skin.

1. Introduction

This review is an upgraded version of a previous study by the same authors (Tomczyk and Latté, 2009) on the genus *Potentilla* L. and was conducted with the help of a systematic literature search based on available records since 2009. Research has increasingly focused on medicinal plants in recent years, as human awareness of health increases every year. Moreover, approximately, 90% of the earth's total human population is located in the northern hemisphere, where a high percentage of people in most developing countries must rely on herbal-derived medicines due to their economic status. Thus, there is a growing interest in developing new plant-based medicaments, botanical supplements and plant-based cosmeceutical products. Consequently, researchers are interested in verifying the vast number of reports on the traditional use of plant species, among other *Potentilla* species, which have been traditionally used for centuries in folk medicine in eastern and western Asia, in the Indian subcontinent and in Europe to uncover pharmacological and plant-drug safety profiles (Melzig and Böttger, 2020; Ullah et al., 2013; Uysal et al., 2019). The purpose of this review is to list and compare the updated phytochemical and pharmacological profiles of the *Potentilla* genus, which have been identified in the literature to reveal the relationship between compounds and their effects. Previously reviewed phytochemical structures and pharmacological mechanisms of *Potentilla* are further elucidated, and new experiments are performed to determine the impact of other activities, such as anti-anthelmintic effects, on the cardiovascular system. In recent years, there has clearly been a growing interest in exploring new, not yet examined species. Those works have provided interesting data, confirming that most plants and constituents isolated from this genus can also exert potent antioxidative, anti-neoplastic and anti-inflammatory

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https://doi.org/10.1016/j.jep.2020.113412
Received 5 July 2020; Received in revised form 12 September 2020; Accepted 20 September 2020
Available online 25 September 2020

activities. However, in light of new reports concerning the mechanism of action involving the human microbiota and its metabolism leading to a conversion of the ellagitannins that are widely present in the *Potentilla* genus into urolithins (Piwowarski et al., 2014), some results achieved *in vitro* may not have a correlation when applied to *in vivo* and human studies.

2. Traditional use of *Potentilla* species

Potentilla species and their medicinal use have been known since antiquity. The oldest evidence for the use of *Potentilla* species is presumably the mention in the Papyrus Ebers which belongs to a group of papyri that contains a compendium of miscellaneous medical recipes. This papyrus from Egyptian ancient times is considered as the most comprehensive medical papyrus, dating to circa 1550 BC. According to a translation of chapter 89, 12–13, a paste consisting of mandrake (*Mandragora* species) and a species of *Potentilla* was described for "making healthy" the teeth. The two drugs were chewed and then spat out. The vehicle was sweet ale (Greeff, 2013).

In Greek language *Potentilla* species were called "Heptaphyllon" or "Pentaphyllon" and in Latin language "Septifolium" or "Quinquefolium" (Fuchs, 1543, Tabernaemontanus, 1664). Pliny the Elder (AD 23–79), a naturalist and natural philosopher of the Roman Empire, mentioned in his work "Naturalis historia" that the Greek word "Pentaphyllon" is derived from the number of leaflets of each leaf of this plant. Moreover, he recommended "Quinquefolium" as a remedy against lichen, a skin disorder (Yavuz et al., 2013). The Greek physician Pedanios Dioscorides (AD 459–370) recommended in his comprehensive work "De Materia Medica" the root of "Pentaphyllon" as a medicine against tooth ache, as a gargle against stomatitis and throat problems and also for the treatment of skin disorders. Internally he used "Pentaphyllon" against dysentery and diarrhoea, also against gout and sciatic problems. The leaves of "Pentaphyllon" were recommended as a remedy against epilepsia and jaundice. Berendes in his translation of "*De materia medica*" into German attributed "Pentaphyllon" to *P. reptans* (Berendes, 1902).

Avicenna, also known as Abu-Ali al-Hossein ibn Abdullah ibn Sin or ibn Sina (980–1037), was a famous Persian physician and scientist of the so called "Islamic Golden Age" in which translations of Greco-Roman, Persian and Indian texts were studied extensively. He is also known as the father of early modern medicine. In his book "Al-Qānūm fi Tibb" (The Canon of Medicine) consisting of five volumes he gathered results of herbal medicine experiments on humans and animals. He recommended the roots of a plant which is nowadays recognized as *P. reptans* for the treatment of jaundice and liver complaints, for the relief of tooth ache and pain in the throat, as a litholytic agent in order to expel kidney stones and also for the treatment of diabetes (Ahmed et al., 2016; Faridi et al., 2015; Pour et al., 2019; Sharofova et al., 2017).

In the medieval times, *Potentilla* species and their medicinal use were mentioned in several herbal books, e.g. in the work by the German Benedictine abbess Hildegard von Bingen (1098–1179). In the Renaissance time and with the upcoming art of printing invented by Johannes Gutenberg in the mid of the 15th century an enormous dissemination of herbal wisdom and herbal books written in (old) German language started. Leonhard Fuchs (1501–1566), Hieronymus Bock (1498–1554) and Otto Brunfels (1488–1534) often called the "ancestors of German botany" described the plants and the medicinal use and also depicted the respective plants in their herbal books (Latté, 2020).

In their herbal books L. Fuchs and also Jacobus Theodorus, called Tabernaemontanus, a German physician and early botanist (1525–1590), assigned the Pentaphyllon and Quinquefolium, respectively, to the "Fünffingerkraut" (translated "five finger herb").

L. Fuchs described in his herbal book dating from 1543 in chapter 239 "Fünffingerkraut" that the use of its underground parts as a remedy against tooth ache, as a gargarism for complaints in the mouth and throat, and internally against dysentery, ulcers and liver complaints including jaundice. The leaves were recommended against fever and jaundice and externally for wound-healing and the treatment of fistula. Interestingly, he depicted four "Fünffingerkraut" plants which are nowadays known as *P. neumanniana*, *P. reptans*, *P. anserina* and *P. alba* (Fuchs, 1543).

Tabernaemontanus known for the rich illustration of his herbal book dating from 1588 also described in chapter 22 ("Von dem Fünffingerkraut und seinen Geschlechten", translated "five finger herb and its species") the peroral application of the roots as a remedy against epilepsia, rheuma, paralysis, dysentery and fever and also ischuria. The herbal part was applied against tuberculosis, dysentery, liver complaints including jaundice and spleen disorders. Externally the use of the roots as a remedy against a sore throat, tooth ache, ulcera in the oral cavity and stomatitis and the use of the herbal parts against angina, nose bleeding, eye complaints, stomatitis, ulcera in the mouth, skin disorders and many more disorders were mentioned, also the application in wound-healing. Altogether 15 *Potentilla* species were described and 11 *Potentilla* species were depicted, including *P. alba*, *P. neumanniana*, *P. reptans*, and *P. anserina* (Tabernaemontanus, 1664).

Surprisingly, in the herbal books of Fuchs and Tabernaemontanus *P. erecta* (tormentil) were described in separate chapters. i.e. separated from the aforementioned *Potentilla* species. Both explained that this plant is equivalent with the Greek "Heptaphyllon" and Latin "Septifolium". Fuchs depicted *Potentilla erecta* In chapter 98 ("Von Tormentill") of his herbal book and mentioned the peroral and external application of the roots of *P. erecta* for the treatment of wounds and also the application against dysentery. The aerial parts were used externally for the treatment of fistula and the roots and herbal part together as a remedy against ulcer and for bleeding control. Either the roots or the aerial parts were applied against ischuria and ulcer in the oral cavity. Fuchs concluded that tormentil has the same effect as "Fünffingerkraut" (Fuchs, 1543).

According to Tabernaemontanus in chapter 23 ("Von dem Tormentill") of his herbal book the underground parts of *P. erecta* were used apart from wound-healing for the treatment of dysentery, intestinal disorders, diarrhoea, syphilis, lung disorders, jaundice and vertigo as well as helminthiasis. The herbal parts were applied against intestinal complaints, haemorrhoids, malaria. The external application of the roots was mentioned by Tabernaemontanus against stomatitis, vomiting, rheuma, wounds, ulcera, haemorrhoids and nose bleeding. The herbal part was externally used in the treatment of stomatitis, haemorrhoids and wounds (Tabernaemontanus, 1664).

In 1811 C.H. Pfaff performed the first analytical study on a *Potentilla* species and described the astringent effect of the rhizomes of *P. erecta*. In the mid of the 19th century the high value of *P. erecta* rhizomes was rediscovered due to the high costs of other drugs which were imported like Ratanhiae radix (*Krameria triandra*, syn. *K. lappacea*) (Latté, 2006). In 1985 the German Commission E described in a monograph the use of *P. erecta* rhizomes (Tormentillae rhizoma) for the treatment of unspecific, acute diarrhoea and also mild mucous membrane inflammations of the mouth and the pharynx (Commission E of the German Bundesgesundheitsamt, 1988). In the last decade the traditional use of *P. erecta* rhizomes was acknowledged by the Committee on Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA) and also the European Scientific Cooperative on Phytotherapy (ESCOP) in their respective monographs (Committee on Herbal Medicinal Products, 2010a, 2010b, ESCOP, 2013). In addition there are two monographs in the European Pharmacopoeia, i.e. the tormentil rhizomes and a tincture prepared thereof covering the quality aspects of the drug and its preparation.

Regarding *P. anserina*, the German Commission E was the only official committee so far which acknowledged the use of the aerial parts (Potententillae anserinae herba) for the treatment of mild dysmenorrhoea, as a supportive therapy for mild, unspecific and acute diarrhoea, and also as a remedy against mild inflammations in the mouth and throat (Commission E of the German Bundesgesundheitsamt, 1985). In respect to the quality of the drug a monograph on Anserinae herba is

included in the German Drug Codex, a supplement to the pharmacopoeia in Germany, which is published by the Federal Union of German Associations of Pharmacists.

Potentilla species have also been used as a medicine in the Traditional Chinese Medicine (TCM), e.g. for the treatment of diarrhoea, hepatitis, rheuma and scabies and as a remedy for detoxification (Tomczyk et al., 2009). Regarding the quality aspects the Chinese Pharmacoepoeia currently includes two monographs on the aerial parts of *Potentilla* species, namely *P. discolor* (fān bái cǎo) and *P. chinensis* (wěi líng cài).

In other regions of the world, i.e. within the natural habitat of *Potentilla* species in Europe, Asia and America, the local growing *Potentilla* species have also been applied in traditional medicine for a longer time (Tomczyk et al., 2009). In Table 1 a list of all *Potentilla* species covered by this review and their traditional uses are given. In Table 2 the traditional uses mentioned for at least one of the *Potentilla* species are summarized divided into the peroral and external application of respective preparations.

It is worth mentioning that till 2009 only two clinical studies on *P. erecta* rhizome extracts had been published, i.e. the study by Huber and other (Huber et al., 2007), on the efficacy in active colitis ulcerosa and the study by Subbotina and co-authors (Subbotina et al., 2003), on the efficacy in rotavirus-induced diarrhoea in children. At that time several *in vitro* and *in vivo* studies had been performed confirming the traditional use of various *Potentilla* species (Tomczyk et al., 2009).

3. Botanical description and taxonomical update

Potentilla is a genus belonging to the *Potentillinae* subtribe, *Potentilleae* tribe, one of 10 tribes of the Rosaceae family. Extensive apomixis, hybridization and/or polyploidization processes occurring in several genera, including *Argentina* and *Potentilla*, have generated problems in the valid taxonomic classification, including at some point in history genera such as *Dasiphora*, *Sibbaldia* and *Fragaria* directly in *Potentilla* (Mabberley, 2002; Paule et al., 2011, 2012; Persson et al., 2020). Recently, based on molecular phylogenetic work, researchers suggested the exclusion of only two genera from the *Potentillinae* subtribe. The first genus, *Potentilla sensu stricto*, also directly encompasses in the *Potentilla* molecular phylogenetic tree two species from *Duchesnea* - *Duchesnea indica* (Andrews) Focke and *Duchesnea chrysantha* (Zoll. & Moritzi) Miq. (Heo et al., 2019; Park et al., 2019). However, those data exclude species from *Potentilla*, such as *Dasiphora fruticosa* (L.) Rydb (syn. *Potentilla fruticosa* L.). and *Drymocalis rupestris* (L.) Soják (syn. *Potentilla ruprestris* L.), which are more closely related to *Fragaria* L. and *Potentilla palustris* (L.) Scop. Considered as *Comarum* L. species. Moreover, species native to North America from *Comarella*, *Horkelia* Cham. & Schlecht, *Horkeliella* Rydb. and *Ivesia* Torr. & Gray genera are part of a monophyletic group nested within this genus (Chen et al., 2020; Töpel et al., 2011). The second genus, *Argentina* Hill, with the most representative *Potentilla anserina*, consists mostly of species distributed in continental Asia and on islands of the Malay Archipelago. Morphological separation of both genera is characterized by the *Potentilla* subterminal styles position and lateral stipular auricles, while *Argentina* styles are in a lateral position and stipular auricles are ventral. However, due to the close genetic relationship, *Argentina* may be treated not as a distinct genus, but as a part of the *Potentilla* (Dobeš and Paule, 2010; Eriksson et al., 2015; Feng et al., 2017; Soják, 2010). Based on these genetic updates and available data, we also included in this review findings on species belonging to the genera *Duchesnea* and *Ivesia*, even if the scientific articles were published before 2009.

4. Chemical constituents of *Potentilla* species

As reported previously (Tomczyk and Latté, 2009), phytochemical studies of plants of the *Potentilla* genus historically started determining the constituents of the tannin fraction. *Potentilla* species, also known as cinquefoils, have been found to be rich in polyphenols and triterpenes, which show an array of biological activities and are present in both aerial and underground parts. Many species such as *P. anatolica, P. evestita, P. parvifolia, P. visianii*, which have not yet been studied for their phytochemical composition, have entered the limelight. However, further investigations carried out on previously studied species have led to the isolation of an even greater number of compounds. Since 2009, the largest number of new constituents has been described for *P. anserina* (93 new structures for the underground parts and 18 for the aerial parts).

4.1. Constituents of the roots and rhizomes

Altogether, 173 compounds were isolated from at least 1 of the 21 investigated *Potentilla* species (Table 3). Among them, more than 150 of the isolated chemical structures were reported for the *Potentilla* underground parts for the first time, with flavonoids being the group of natural compounds with the largest number of altogether 45 structures. The largest number of new constituents has been identified for *P. anserina*, with a total number of 93 structures comprising, amongst others, 18 triterpenes, 19 flavonoid *O*-glycosides and *O*-glucuronides and 5 flavonoid C-glycosides. However, researchers have also focused on the rich polysaccharide fraction of *P. anserina*. In addition, 33 structures have been reported for *P. discolor* and 27 for *P. fulgens*. Interestingly, only one new monomeric hydrolysable tannin – potentillin (Fig. 1) – was isolated during the phytochemical analysis of the roots and rhizomes of *Potentilla* species, i.e., from *P. erecta*. A rich fraction of degradation products of ellagitannins was elucidated in three species – *P. anserina*, *P. chinensis* and *P. fulgens*, while in *P. erecta*, the authors found three isomers of galloylquinic acid that under the conditions of the isolation process transformed to 5-*O*-galloylquinic acid (Fecka et al., 2015). Six new dimeric condensed tannins were isolated from *P. fulgens*, while several precursors of condensed tannins were confirmed to be present in three species.

A predominant investigated group from the underground parts was the triterpenoids. Altogether, 34 compounds based on the olean or ursan skeleton were isolated from more than 16 species, including *P. anserina*, *P. fulgens* and *P. freyniana*. Some typical and newly isolated terpenoid structures are depicted in Fig. 2. On several occasions, a few sterols, including β- and γ-sitosterol, ergosterol and 7-oxo-cholesterol, were reported for *P. discolor*. Currently, in comparison to the previous article, a number of flavonoid *O*-glycosides and C-glycosides with a large structural variety of aglycone were isolated for the first time from several underground parts of cinquefoils. A large number of them were also present in the aerial parts. Furthermore, several other compounds were reported for *Potentilla* underground parts, comprising organic and phenolic carboxylic acids (23 structures), two anthraquinone structures for *P. speciosa*, five isoflavone glycosides for *P. anserina*, adenine, adenosine, L-tryptophan, a pyran-2-acetic acid derivative and two carotenoid metabolites, loliolide and isololiolide for *P. anserina* and an essential oil for *P. discolor*.

4.2. Constituents of the aerial parts

Most of the phytochemical studies have been performed with the aerial parts of *Potentilla* species. At least 226 compounds and four fractions (amino acids, essential oil, fatty acids and polysaccharides) were identified in at least 41 species, and 160 structures were isolated for the first time (Table 4).

The largest number of constituents was elucidated for *P. discolor* (75 compounds), followed by *Duchesnea indica* (42 compounds), *P. parvifolia* (32 compounds) and *P. recta* (32 compounds). The dominating group of compounds was the flavonoids, identified in at least 26 species. Among them, 17 were identified as flavonoid aglycones, 44 flavonoid *O*-glycosides and *O*-glucuronides, and 7 as C-glycosides. Again, isoflavones were isolated from the aerial parts from three species for the first time in this genus. More interestingly, a diflavonol ester of μ-truxinic acid, named potentilin A, was isolated from *P. parvifolia*. Hydrolysable tannins were

Table 1
Traditional use of prominent *Potentilla* s.l. species (limited to species currently under investigation in respect to phytochemistry and/or pharmacology).

Species	Region	Plant part used	Traditional use	References
Duchesnea chrysantha	Asia, e.g. China, Korea, Japan, India	whole plant, aerial parts	asthma, certain forms of cancer, congenital fever, tooth ache, astringent, inflammations, impairment of immune system	Kim et al. (2008); Lee et al. (2012); Yang et al., (2008)
Duchesnea indica	Asia, e.g. China, Korea, Japan, India	whole plant, aerial parts	cough, inflammation of throat, skin inflammations, certain forms of cancer, astringent, viral infections, leprosy	Qiao et al. (2009); Xiang et al. (2019); Zhu et al. (2015)
Ivesia gordonii (syn.: *P. gordonii)*	North America	roots	general body tonic	Ahmed et al. (2014)
Potentilla alba	Europe, e.g. central Europe, Russia, Ukraine	aerial parts, roots	hyperplastic and autoimmune diseases of the thyroid gland	Neilla et al. (2015); Pankiv et al. (2020)
Potentilla anserina	Asia, e.g. China, Korea, Japan, India; Europe, e.g. central Europe, Sweden, Serbia and Montenegro, Russia, Bulgaria, Turkey	aerial parts, roots	acute, nonspecific diarrhoea with mild discomforts, mild inflammations of the oral and pharyngeal mucosa; tooth ache	Morikawa et al. (2014); Tomczyk et al. (2009)
Potentilla arguta	America, e.g. Canada	roots	viral infections	Tomczyk et al. (2009)
Potentilla atrosanguinea	Asia, e.g. China, Korea, Japan, India	roots	wound-healing	Tomczyk et al. (2009)
Potentilla aurea	Europe, e.g. central Europe, Sweden, Serbia and Montenegro, Russia, Bulgaria, Turkey	aerial parts	diabetes mellitus	Buchholz and Melzig (2016)
Potentilla chinensis	Asia, e.g. China, Korea, Japan, India	aerial parts, roots	certain forms of cancer	Jung et al. (2016); Tomczyk et al. (2009)
Potentilla discolor	Asia, e.g. China, Korea, Japan, India	aerial parts, roots	diarrhoea, diabetes mellitus, hemorrhage	Tomczyk et al. (2009)
Potentilla erecta	Europe, e.g. central Europe, Sweden, Serbia and Montenegro, Russia, Bulgaria, Turkey	roots	inflammations, treatment of wounds, bleeding, dysentery, diarrhoea, inflammatory bowel disease, bacterial, fungal and viral infections, certain forms of cancer, antiseptic for the mouth and throat	Tomczyk et al. (2009)
Potentilla evestita	Asia, e.g. India, Pakistan	aerial parts	analgesic, inflammations, diarrhoea, diabetes mellitus, certain forms of cancer, impairment of immune system	Rauf et al. (2014), 2016
Potentilla freyniana	Asia, e.g. China, Korea, Japan, India	roots	viral infections	Tomczyk et al. (2009)
Potentilla fruticosa	Europe, e.g. central Europe, Sweden, Serbia and Montenegro, Russia, Bulgaria, Turkey	aerial parts	viral infections, impairment of immune system	Tomczyk et al. (2009)
Potentilla fulgens	Asia, e.g. China, Korea, Japan, India	roots	stomach disorders, certain forms of cancer, diabetes mellitus, helminthiasis	Roy et al. (2010); Tomczyk et al. (2009)
Potentilla kleiniana	Asia, e.g. China, Korea, Japan, India	aerial parts	diarrhoea, bleeding, influenza, cough, parotitis, lymphadenitis, hepatitis, scare, numbness of the limbs, dysmenorrhea, ulcer	Tomczyk et al. (2009)
Potentilla mooniana	Asia, e.g. China, India, Pakistan	whole plant, roots	colic, spasmodic pain, gastric disorders, mouth ulcer, bacterial and fungal infections	Laloo et al. (2014)
Potentilla multicaulis	Asia, e.g. China, Korea, Japan, India	roots	certain forms of cancer	Tomczyk et al. (2009)
Potentilla multifida	Asia, e.g. China, Korea, Japan, India	aerial parts, roots	hepatitis, enterobiasis, functional uterine hemorrhage, type 2 diabetes	Tomczyk et al. (2009)
Potentilla parvifolia	Asia, e.g. China, Mongolia	aerial parts	skin diseases, mastitis, oedema, certain forms of cancer	Yuan et al. (2017)
Potentilla peduncularis	Asia, e.g. China, Korea, Japan, India	leaves, buds	fever, influenza, cough	Tomczyk et al. (2009)
Potentilla polyphylla	India	taproots	wounds, burns, chewing for dental problems	Gogoi et al. (2014); Nakhuru et al. (2016)
Potentilla recta	Europe, e.g. central Europe, Sweden, Serbia and Montenegro, Russia, Bulgaria, Turkey	aerial parts, roots	diarrhoea, microbial infections, external and internal inflammations, fever, tonic agent, bleeding, ulcer	Bazylko et al., (2013); Ökdem et al. (2018); Tomczyk et al. (2009)
Potentilla reptans	Europe, e.g. central Europe, Sweden, Serbia and Montenegro, Russia, Bulgaria, Turkey	leaves, roots	diarrhoea, tooth ache, dental problems, ulcers, inflammation of throat, mastitis, hemoroids	Mincheva et al. (2019); Tomczyk et al. (2009); Tomovic et al. (2015)
Potentilla rugulosa	Korea	aerial parts	fever, bleeding	Choi et al. (2020)
Potentilla simplex	America, e.g. Canada	stems, leaves	fungal infections	Tomczyk et al. (2009)
Potentilla speciosa			inflammations, anti-ulcer activity	

(*continued on next page*)

Table 1 (*continued*)

Species	Region	Plant part used	Traditional use	References
	Europe, e.g. central Europe, Sweden, Serbia and Montenegro, Russia, Bulgaria, Turkey	aerial parts, roots		Tomczyk et al. (2009)
Potentilla supina	Asia, e.g. China, Korea, Japan, India; North Africa and North America	aerial parts	arthritism, asthma. bloody discharge, bleeding, certain forms of cancer, dysentery, helminthiasis, fever	Lee et al. (2017); Nam et al. (2017)

Table 2
Summary of the traditional uses of *Potentilla* s. l. (i.e. indications known for at least one of the species).

Peroral application	External application
acute, nonspecific diarrhoea with mild discomforts	wound-healing
inflammatory bowel disease	bleeding
bacterial and fungal infections	burns
viral infections, including influenza	inflammations of the skin
hepatitis	inflammation of throat
impairment of immune system	mild inflammations of the oral and pharyngeal mucosa
helminthiasis	antiseptic for the mouth and throat
enterobiasis	tooth ache
stomach disorders	dental problems
diabetes mellitus (type 2)	
hyperplastic and autoimmune diseases of the thyroid gland	
parotitis	
hemorrhage	
functional uterine hemorrhage	
ulcers	
certain forms of cancer	
lymphadenitis	
dysmenorrhea	
fever	
cough	
scare	
numbness of the limbs	

isolated from at least 15 *Potentilla* species. Altogether, 10 monomers, the dimer agrimoniin and several degradation products of hydrolysable tannins and their congeners, such as ellagic acid and its 7 derivatives, brevifolin, brevifolincarboxylic acid, methyl and ethyl brevifolincarboxylate, were found in the aerial parts of *Potentilla* species. Moreover, several dimeric condensed tannins were present in *P. fulgens, P. fruticosa* and *P. parvifolia,* accompanied by the precursors of condensed tannins such as (+)-catechin, (−)-epicatechin, (+)-afzelechin and (−)-epiafzelechin described for several *Potentilla* species. Numerous triterpenoid compounds (43 structures), mostly aglycons and their glucosides based mainly on an olean or an ursan skeleton but also on a hopan and a lupan skeleton, were isolated from at least 9 cinquefoil species. The largest number of new triterpenoid structures were described for *P. discolor* (25 compounds). A significant number of other compounds were reported, including organic acids and phenolcarboxylic acids (37 compounds from several species), lignans and their precursors from *P. discolor, P. multicaulis* and *P. recta*, coumarins, chromones, sterols, amino acids, fatty acids and polysaccharide fractions (various *Potentilla* species), essential oils (from 2 cinquefoil species), raffinose and sucrose from *P. discolor*. Moreover, a novel constituent, the dimeric isobutyrylphloroglucinol derivative named ivesinol, was isolated from *Ivesia gordonii*. The structure of this compound is presented in Fig. 3.

5. Pharmacological profile (*in vitro*, *in vivo*, clinical studies)

The modern world struggles with the increasing problem of chronic diseases, e.g., diabetes mellitus, chronic inflammatory diseases, and tumours. In the past few years, scientists have focused on increasing knowledge about natural drugs from the *Potentilla* L. genus based on vast ethnopharmacological reports. Scientific research has confirmed effects known from traditional use and furthermore discovered new pharmacological profiles of *Potentilla* extracts based on *in vitro* and *in vivo* experiments. However, literature data concerning clinical studies is limited. An overview of the current status of pharmacological evaluations and their comparison to main ethnopharmacological uses of *Potentilla* species are outlined in Table 5. It should be emphasized that Table 5 contains data published between 2009 and 2020. For data before 2009, results were published by Tomczyk and Latté (2009).

5.1. Antihyperglycemic and antihyperlipidemic activity

Diseases associated with dysfunction of carbohydrates and lipid management are the major public problem. Various estimates imply that the number of people that have type 2 diabetes mellitus will rise in 2040 to over 640 million patients in the world. However, the majority of affected patients live in countries with low and middle-income (Mirzaei et al., 2020; Olokoba et al., 2012). Thus, the demand for new, cheap and effective drugs in those countries is high. Studies to date justify the use of several *Potentilla* species against diabetes mellitus, nevertheless, despite promising results obtained in *in vitro* and *in vivo* studies, there are no conducted clinical trials so far to assess the exact potential of herbal drugs in humans.

5.1.1. In vitro experiments

P. fulgens roots are traditionally used in certain regions of Asia as medicine for the treatment of diabetes mellitus (Kumar et al., 2013). During the phytochemical study, hyptadienic acid and four more triterpenes were obtained from a methanolic extract from the roots of *P. fulgens* and examined in an α-glucosidase inhibition assay. Inhibition of this enzyme lowers post-prandial glycemia via preventing the degradation of oligosaccharides into absorbable carbohydrates. Among these triterpenes, hyptadienic acid demonstrated high potency of inhibition of α-glucosidase with an IC_{50} of 39.67 ± 1.88 μM (Kumar et al., 2013). Also, *P. kleiniana* has been praised as traditional herbal medicine in Asia in treatment of several diseases e.g. diabetes mellitus. Basing on these reports, a study on the constituents of *P. kleiniana* led to the identification of five α-glucosidase inhibitors. Four out of five of these compounds revealed a higher inhibitory potency than acarbose (Liu et al., 2019). Moreover, a study by Şöhretoğlu and co-authors demonstrated that two new compounds isolated from *P. astracanica*, prunetin 5-*O*-β-glucopyranoside and genistein 5-*O*-β-glucopyranoside, together with their aglycons possessed strong inhibitory properties against α-glucosidase, comparable to positive control, acarbose, with genistein being the most potent compound with an IC_{50} value of 1.47 ± 0.11 μg/mL (Şöhretoğlu et al., 2017). The same study was performed for *P. inclinata*. Methanolic and butanolic extracts demonstrated very strong inhibition at IC_{50} values of 1.06 ± 0.02 and 0.93 ± 0.01 μg/mL, respectively. The isolation from the *n*-BuOH fraction provided nine compounds, of which rutin was the most active (Şöhretoğlu et al., 2018). Uysal and Aktumsek analysed various extracts obtained from *P. anatolica* and determined their enzyme inhibition potential. The ethyl acetate, methanol and water extracts exerted strong inhibitory potential against α-amylase, α-glucosidase, and other enzymes, such as acetylcholinesterase and tyrosinase, while butyrylcholinesterase was not affected (Uysal and Aktumsek, 2015). A further investigation carried out on the herbal and root parts of selected *Potentilla* species (*P. anatolica, P.*

Table 3
Constituents isolated from roots and rhizomes of *Potentilla* species between 2009 and 2020. For compounds isolated from *Potentilla* species before 2009 see Tomczyk and Latté (2009).

Compounds	*Potentilla* species	References
Hydrolysable tannins and related compounds		
Monomers		
Potentillin	*P. erecta*	Fecka et al. (2015)
Related compounds		
Ellagic acid	*P. anserina, P. discolor, P. fulgens*	Anal et al. (2014); Cheng et al. (2020); Morikawa et al. (2014)
Ellagic acid 4-*O*-α-L-arabinofuranoside	*P. anserina*	Morikawa et al. (2014)
Ellagic acid 3′-*O*-methyl ether-4-*O*-α-L-arabinofuranoside (Ducheside B)	*P. anserina*	Morikawa et al. (2014)
Ellagic acid 2,3,8-tri-*O*-methyl ether	*P. chinensis*	Jung et al. (2016)
Ellagic acid 3-*O*-methyl ether-4-*O*-α-L-arabinofuranoside (Potentillanoside G)	*P. anserina*	Morikawa et al. (2018)
Ellagic acid 3-*O*-α-L-rhamnopyranosyl-4′-*O*-α-L-arabinofuranoside (Potentillanoside H)	*P. anserina*	Morikawa et al. (2018)
Ellagic acid 3-*O*-α-L-rhamnopyranoside	*P. anserina*	Morikawa et al. (2018)
Ellagic acid 3,3′-*O*-dimethyl ether-sulfate	*P. discolor*	Cheng et al. (2020)
3-Galloylquinic acid	*P. erecta*	Fecka et al. (2015)
4-Galloylquinic acid	*P. erecta*	Fecka et al. (2015)
5-Galloylquinic acid	*P. erecta*	Fecka et al. (2015)
Condensed tannins (proanthocyanidins), their precursors and related compounds		
(+)-Catechin	*P. fulgens; P. reptans*	Choudhary et al. (2013); Tomovic et al. (2015)
(+)-Catechin 7-*O*-β-glucopyranoside	*P. anserina*	Morikawa et al. (2014)
(+)-8-carboxymethylcatechin methyl ester	*P. anserina*	Yang et al. (2020)
(−)-Epicatechin	*P. anserina, P. fulgens*	Jaitak et al. (2010b); Yang et al. (2020)
(−)-Epicatechin 7-*O*-β-glucoside	*P. anserina*	Wang et al., (2020b)
(−)-Epigallocatechin-7-*O*-β-D-glucoside	*P. anserina*	Wang et al., (2020b)
(+)-Afzelechin	*P. fulgens*	Choudhary et al. (2015)
(−)-Epiafzelechin	*P. fulgens, P. reptans*	Choudhary et al. (2015); Tomovic et al. (2015)
(2R, 3S)-3′,5′-dimethoxy-gallocatechin	*P. anserina*	Yang et al. (2020)
(2R, 3S)-8-carboxyl-gallocatechin methyl ester (potenserin A)	*P. anserina*	Yang et al. (2020)
6,8′-Methylenebiscatechin	*P. anserina*	Yang et al. (2020)
8,8′-Methylenebiscatechin	*P. anserina*	Yang et al. (2020)
Dimers		
(−)-Epiafzelchin-(6 → 8)-epiafzelchin (Potifulgene)	*P. fulgens*	Jaitak et al. (2010b)
(+)-Afzelechin-(4α→8)-(+)-catechin	*P. fulgens*	Choudhary et al. (2015)
(+)-Afzelechin-(4β→8)-(−)-epicatechin	*P. fulgens*	Choudhary et al. (2015)
(+)-Afzelechin-(4α→8)-(−)-epiafzelechin	*P. fulgens*	Choudhary et al. (2015)
(+)-Catechin-(4α→8)-(−)-epicatechin	*P. fulgens*	Choudhary et al. (2015)
(+)-Catechin-(4α→8)-(+)-catechin (procyanidin B3)	*P. anserina*	Yang et al. (2020)
(+)-Catechin-(4α→8)-(−)-epicatechin	*P. anserina*	Yang et al. (2020)
	P. fulgens	

Table 3 (*continued*)

Compounds	*Potentilla* species	References
(−)-Epiafzelechin-(4β→8)-(−)-epicatechin		Choudhary et al. (2015)
(2S,3R,2″S,3″S)-gallocatechin-(7 → 7″)-epigallocatechin (potenserin C)	*P. anserina*	Yang et al. (2020)
(+)-Gallocatechin-(4′→O→7)-(−)-epigallocatechin	*P. anserina*	Yang et al. (2020)
Gallocatechin-(4α→8)-catechin (Prodelphinidin B3)	*P. anserina*	Wang et al., (2020b)
Trimers		
Procyanidin C1	*P. discolor*	Cheng et al. (2020)
Prodelphinidin C	*P. anserina*	Wang et al., (2020b)
Triterpenes		
Alphitolic acid	*P. anserina, P. freyniana*	Morikawa et al. (2014); Wu et al. (2009)
3-Epialphitolic acid	*P. freyniana*	Wu et al. (2009)
Arjunic acid	*P. anserina*	Liu et al. (2014)
19α-Hydroxyasiatic acid	*P. freyniana*	Wu et al. (2009)
Betulinic acid	*P. freyniana*	Wu et al. (2009)
Cecropiacic acid	*P. anserina*	Morikawa et al. (2014)
Coleonolic acid	*P. freyniana*	Wu et al. (2009)
Euscapic acid	*P. bifurca, P. collina, P. freyniana, P. fulgens, P. recta, P. reptans* and more than other 8 species	Choudhary et al. (2013); Józwiak et al. (2014); Wu et al. (2009)
Hyptadienic acid	*P. fulgens*	Kumar et al. (2013)
Maslinic acid	*P. anserina*	Morikawa et al. (2014)
Myrianthic acid	*P. freyniana*	Wu et al. (2009)
Pomolic acid	*P. erecta, P. montenegrina, P. neumanniana, P. reptans, P. recta* and more than other 8 species	Józwiak et al. (2014)
Pomolic acid 28-*O*-β-D-glucoside	*P. anserina*	Morikawa et al. (2014)
2-oxopomolic acid	*P. anserina*	Morikawa et al. (2014)
3-epi-2-oxopomolic acid	*P. anserina*	Liu et al. (2014)
Rosamultic acid	*P. freyniana; P. fulgens*	Kumar et al. (2013); Wu et al. (2009)
Tormentic acid	*P. bifurca, P. collina, P. freyniana, P. fulgens, P. recta, P. reptans* and more than other 8 species	Kumar et al. (2013); Józwiak et al. (2014); Wu et al. (2009)
Ursolic acid	*P. erecta, P. fulgens, P. montenegrina, P. neumanniana, P. recta, P. reptans* and more than other 8 species	Choudhary et al. (2013); Józwiak et al. (2014)
2α-hydroxyursolic acid	*P. anserina*	Morikawa et al. (2014)
Euscaphic acid 28-*O*-β-d-glucoside (kajiichigoside F1)	*P. fulgens*	Kumar et al. (2013)
2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid	*P. anserina*	Morikawa et al. (2014)
2α,19α-dihydroxy-3-oxo-urs-12-en-28-oic acid 28-*O*-β-D-glucoside (potentillanoside A)	*P. anserina, P. fulgens*	Morikawa et al. (2014)
3α, 19α-dihydroxy-2-oxo-urs-12-en-28-oic acid 28-*O*-β-D-glucoside (potentillanoside B)	*P. anserina*	Kumar et al. (2013); Morikawa et al. (2014)
6′-*O*-acetylrosamultin (potentillanoside C)	*P. anserina*	Morikawa et al. (2014)
Cecropiacic acid 28-*O*-β-glucoside (potentillanoside D)	*P. anserina*	Morikawa et al. (2014)
Cecropiacic acid 3-methyl-28-*O*-β-glucopyranosyl ester (potentillanoside E)	*P. anserina*	Morikawa et al. (2014)

(*continued on next page*)

Table 3 (*continued*)

Compounds	*Potentilla* species	References
2α,3β,30-trihydroxyolean-13 (18)-en-28-oic acid 28-*O*-β-D-glucoside (potentillanoside F)	*P. anserina*	Morikawa et al. (2014)
2,19α-dihydroxy-2-oxo-urs-1,12-dien-28-oic acid (fupenjic acid)	*P. discolor*	Park et al. (2007)
2,19α-dihydroxy-3-oxours-1,12-dien-28-oic acid 28-*O*-β-D-glucoside	*P. anserina*	Liu et al. (2014)
3β-acetoxy-19α-hydroxyursa-12-en-28-oic acid	*P. anserina*	Liu et al. (2014)
Arjunglucoside I	*P. anserina*	Morikawa et al. (2014)
Corosolic acid	*P. anserina*	Morikawa et al. (2014)
Fulgic acid A	*P. fulgens*	Choudhary et al. (2013)
Fulgic acid B	*P. fulgens*	Choudhary et al. (2013)
Sterols		
7-Oxo-cholesterol	*P. discolor*	Zhang et al. (2018)
Ergosterol	*P. discolor*	Zhang et al. (2018)
Lanost-8-en-3-ol	*P. discolor*	Zhang et al. (2018)
β-Sitosterol	*P. discolor*	Park et al. (2007)
β-Sitosterol β-D-glucopyranoside	*P. discolor*	Park et al. (2007)
γ-Sitosterol	*P. discolor*	Zhang et al. (2018)
Organic acids and phenol carboxylic acids and their derivatives		
4-Hydroxybenzoic acid	*P. anatolica, P. argentea, P. recta, P. reptans*	Uysal et al. (2019)
3,4-Dihydroxybenzoic acid	*P. anserina*	Yang et al. (2020)
3,4-Dihydroxy-5-(2′,3′,6′-trihydroxy-5′-methoxycarbonylphenoxy)-benzoic acid	*P. anserina*	Yang et al. (2020)
3,4-Dihydroxy-5-(2,3,6-trihydroxy-5-methoxycarbonylphenoxy)-benzoic acid methyl ester	*P. anserina*	Yang et al. (2020)
Caffeic acid	*P. discolor*	Cheng et al. (2020)
Chlorogenic acid	*P. anatolica, P. argentea, P. erecta, P. recta*	Dróżdż et al. (2019); Uysal et al. (2019)
Cinnamic acid	*P. anatolica, P. argentea, P. recta, P. reptans*	Uysal et al. (2019)
Dicaffeoylquinic acid	*P. discolor*	Cheng et al. (2020)
Ferulic acid	*P. anatolica, P. argentea, P. erecta, P. recta*	Dróżdż et al. (2019); Uysal et al. (2019)
Fumaric acid	*P. anatolica, P. argentea, P. recta*	Uysal et al. (2019)
p-Coumaric acid	*P. anatolica, P. argentea, P. atrosanguinea, P. recta, P. reptans*	Walia et al. (2018); Uysal et al. (2019)
p-Coumaroylsucrose	*P. anserina*	Morikawa et al. (2014)
6-*O*-feruloylsucrose	*P. anserina*	Morikawa et al. (2014)
Gallic acid	*P. anatolica, P. anserina, P. argentea, P. discolor, P. fulgens, P. recta, P. reptans*	Cheng et al. (2020); Choudhary et al. (2013); Morikawa et al. (2014); Tomovic et al. (2015); Uysal et al. (2019)
Gallic acid methyl ester	*P. anserina*	Morikawa et al. (2014)
Gallic acid ethyl ester	*P. discolor*	Cheng et al. (2020)
Malic acid	*P. anatolica, P. argentea, P. recta, P. reptans*	Uysal et al. (2019)
Protocatechuic acid	*P. anatolica, P. argentea, P. erecta, P. recta, P. reptans*	Dróżdż et al. (2019); Tomovic et al. (2015); Uysal et al. (2019)
Quinic acid	*P. anatolica, P. argentea, P. recta, P. reptans*	Tomovic et al. (2015); Uysal et al. (2019)
Salicylic acid	*P. discolor*	Cheng et al. (2020)

Table 3 (*continued*)

Compounds	*Potentilla* species	References
Sinapic acid	*P. argentea*	Uysal et al. (2019)
Syringic acid	*P. anatolica, P. recta, P. reptans, P. speciosa*	Uysal et al. (2019); Zengin et al. (2016)
Vanillic acid	*P. anatolica, P. argentea, P. recta, P. reptans, P. speciosa*	Uysal et al. (2019); Zengin et al. (2016)
Flavonoids		
Flavonoid aglycones		
Isorhamnetin	*P. discolor*	Cheng et al. (2020)
Kaempferol	*P. fulgens*	Anal et al. (2014)
Luteolin	*P. discolor*	Cheng et al. (2020)
Naringenin	*P. anatolica, P. argentea, P. atrosanguinea, P. discolor, P. recta, P. reptans*	Cheng et al. (2020); Walia et al. (2018); Uysal et al. (2019)
Quercetin	*P. anserina, P. discolor, P. fulgens, P. reptans*	Anal et al. (2014); Cheng et al. (2020); Tomovic et al. (2015); Yang et al. (2020)
Tricetin 3′,5′-dimethyl ether (tricin)	*P. discolor*	Cheng et al. (2020)
Flavonoid *O*-glycosides and *O*-glucuronides		
Apigenin 7-*O*-β-D-glucoside (cosmosiin)	*P. anatolica, P. argentea, P. recta*	Uysal et al. (2019)
Apigenin 7-*O*-neohesperidoside (rhoifolin)	*P. anatolica, P. reptans*	Uysal et al. (2019)
Hesperitin 7-*O*-rutinoside (hesperidin)	*P. anatolica, P. argentea, P. recta, P. reptans*	Uysal et al. (2019)
Isoscutellarein 8-*O*-β-D-glucopyranoside	*P. anserina*	Xu et al. (2010)
Isorhamnetin 3-*O*-galactoside-7-*O*-rhamnoside	*P. discolor*	Cheng et al. (2020)
Kaempferol 3-*O*-β-D-glucoside (astragalin)	*P. anserina*	Xu et al. (2010)
Kaempferol 3-*O*-β-D-(2-*O*-trans-p-coumaroyl)-glucoside	*P. anserina*	Xu et al. (2010)
Kaempferol 3-*O*-β-D-(6″-*O*-trans-p-coumaroyl)-glucoside (tiliroside)	*P. anserina*	Xu et al. (2010)
Kaempferol 3-*O*-β-D-(6″-*O*-malonyl)-glucoside	*P. discolor*	Cheng et al. (2020)
Kaempferol 3-*O*-rutinoside (nicotiflorin)	*P. anatolica, P. recta, P. reptans*	Uysal et al. (2019)
Luteolin 3′-*O*-β-D-glucoside	*P. atrosanguinea*	Walia et al. (2018)
Luteolin 7-*O*-β-D-glucoside (cynaroside)	*P. anserina, P. reptans*	Morikawa et al. (2014); Tomovic et al. (2015)
Luteolin 7-*O*-β-D-glucuronide	*P. anserina*	Xu et al. (2010)
Myricetin 3-*O*-α-L-(3″-*O*-methoxyl)-rhamnoside (potenserin D)	*P. anserina*	Yang et al. (2020)
Naringenin 7-*O*-β-D-glycoside	*P. anserina*	Yang et al. (2020)
Pinocembrin 7-*O*-β-D-glycoside	*P. anserina*	Yang et al. (2020)
Quercetin 3-*O*-galactoside (hyperoside)	*P. discolor*	Cheng et al. (2020)
Quercetin 3-*O*-β-D-sambubioside	*P. anserina*	Morikawa et al. (2014)
Quercetin 3-*O*-β-D-xyloside (reynoutrin)	*P. anserina*	Xu et al. (2010)
Quercetin 3-*O*-β-D-glucoside (isoquercitrin)	*P. anserina, P. anatolica, P. argentea, P. recta, P. reptans*	Uysal et al. (2019); Xu et al. (2010)
Quercetin 3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranoside-3′-*O*-β-D-glucopyranoside	*P. anserina*	Morikawa et al. (2014)
Quercetin 3-*O*-rutinoside (rutin)	*P. anatolica, P. argentea, P. atrosanguinea, P. discolor, P. recta, P. reptans*	Cheng et al. (2020); Walia et al. (2018); Uysal et al. (2019)
Quercetin 3-*O*-β-D-glucuronide (miquelianin)	*P. anserina*	Xu et al. (2010)
Quercetin 3-*O*-α-L-rhamnopyranoside	*P. anserina*	Xu et al. (2010)
Quercetin 3,7-*O*-β-D-diglucoside	*P. anserina*	Xu et al. (2010)
Quercetin 7-*O*-β-D-glucoside	*P. anserina*	Xu et al. (2010)

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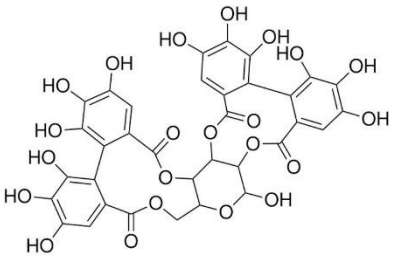
Table 3 (*continued*)

Compounds	*Potentilla* species	References
Quercetin 3-*O*-α-L-arabinopyranosyl-(1 → 6)-β-glucoside (quercetin 3-*O*-vicianoside, peltatoside)	*P. discolor*	Cheng et al. (2020)
Quercetin 3-*O*-β-D-(6″-*O*-acetyl)-glucoside	*P. discolor*	Cheng et al. (2020)
Quercetin 3-*O*-β-D-(6″-*O*-malonyl)-glucoside	*P. discolor*	Cheng et al. (2020)
Quercetin 3-*O*-α-L-(2″-gallate)-rhamnopyranoside	*P. anserina*	Yang et al. (2020)
5,7,3′,5′-tetrahydroxyflavanone 7-*O*-β-D-glucopyranoside	*P. anserina*	Yang et al. (2020)
Flavonoid C-glycosides		
Apigenin 6-C-β-D-glucoside (isovitexin)	*P. anserina*	Xu et al. (2010)
Apigenin 6-C-(2″-*O*-α-L-rhamnopyranosyl)-β-D-glucoside	*P. anserina*	Xu et al. (2010)
Apigenin 6,8-C-β-D-diglucoside	*P. anserina*	Xu et al. (2010)
Apigenin 6-*C*-β-D-glucoside-8-C-β-D-xyloside	*P. anserina*	Xu et al. (2010)
Apigenin 6-*C*-β-D-glucoside-8-C-α-D-arabinoside (schaftoside)	*P. anserina*	Xu et al. (2010)
Apigenin 6-C-α-D-arabinoside-8-C-β-D-glucoside (isoschaftoside)	*P. discolor*	Cheng et al. (2020)
Apigenin 8-C-β-D-glucoside (orientin)	*P. discolor*	Cheng et al. (2020)
Diflavonol ester of μ-truxinic acid		
Potentilin A	*P. anserina*	Xu et al. (2010)
Isoflavones *C*- and *O*-glycosides		
Daidzein 7-*O*-β-D-glucoside (daidzin)	*P. anserina*	Liu et al. (2011)
Daidzein 8-*C*-glucoside (puerarin)	*P. anserina*	Liu et al. (2011)
3′-Methoxydaidzein 8-*C*-glucoside	*P. anserina*	Liu et al. (2011)
Daidzein 8-*C*-apiosyl-(1 → 6)-glucoside	*P. anserina*	Liu et al. (2011)
Puerarin 3′-methyl ether	*P. anserina*	Zhang et al. (2019)
Antraquinones		
Chrysophanol	*P. speciosa*	Zengin et al. (2016)
Physcione	*P. speciosa*	Zengin et al. (2016)
Coumarins		
Coumarin	*P. anatolica, P. argentea, P. recta, P. reptans*	Uysal et al. (2019)
Fraxetin	*P. discolor*	Cheng et al. (2020)
Lignans		
(−)-olivil	*P. anserina*	Yang et al. (2020)
(+)-cycloolivil	*P. anserina*	Yang et al. (2020)
Others		
Adenine	*P. anserina*	Liu et al. (2014)
Adenosine	*P. anserina*	Liu et al. (2011)
L-tryptophan	*P. anserina*	Morikawa et al. (2014)
Loliolide	*P. anserina*	Xu et al. (2010)
Isololiolide	*P. anserina*	Xu et al. (2010)
2,6-Dimethyl-2,3-dihydro-4-oxo-4H-pyran-2-acetic acid	*P. anserina*	Xu et al. (2010)
Pentadecylbutyrate	*P. atrosanguinea*	Gupta et al. (2016)
Methyl pentatetraconta-30,32,34,36,38,40,42-heptaenoate	*P. atrosanguinea*	Gupta et al. (2016)
Essential oil	*P. discolor*	Zhang et al. (2018)
Fatty acids	*P. alba*	Neilla et al. (2015)
p-Hydroxy-benzaldehyde	*P. fulgens*	Choudhary et al. (2013)
Solasodine	*P. discolor*	Zhang et al. (2018)
Phloridzin	*P. anserina*	Yang et al. (2020)
3-Hydroxy-phloridzin	*P. anserina*	Yang et al. (2020)
(2R,3S)-3,5-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H,8H-pyrano [2,3-f]chromen-8-one (potenserin B)	*P. anserina*	Yang et al. (2020)
Resveratrol	*P. anserina, P. discolor*	Cheng et al. (2020); Yang et al. (2020)
Resveratrol 3-*O*-β-D-(6-*O*-galloyl)-glucopyranoside	*P. anserina*	Yang et al. (2020)
(Z)-Resveratrol 3,5-*O*-β-diglucoside	*P. anserina*	Yang et al. (2020)

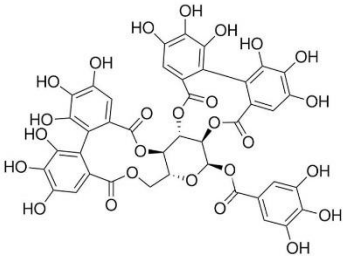
argentea, P. recta, P. reptans) confirmed the high α-amylase and α-glucosidase inhibitory effects for all the tested samples in terms of acarbose equivalents (Sut et al., 2019; Uysal et al., 2019). Similar properties possessed fractions extracted from *P. thuringiaca*. Significant inhibitory activities were reported for all obtained extracts against α-amylase as well as for acetylcholinesterase, tyrosinase and lipase; however, an anti-α-glucosidase activity was confirmed only for a water extract (Grochowski et al., 2017). In another study, German authors (Buchholz and Melzig, 2016) basing on traditional medicine reports described in the literature, found that methanol extracts from *P. aurea* exhibited strong inhibitory activity against pancreatic lipase and α-amylase, while the aqueous extract had moderate activity. *P. anserina* rhizome has a long history of use in China, serving as one of the ingredients in preparing of congee, but as well serving as a drug in traditional Chinese medicine. Therefore, during a phytochemical study on *P. anserina* underground parts, Yang and co-authors isolated and identified 5 new and 25 known compounds that were assessed for their enzyme inhibitory properties. During the study, it has been found that quercetin-3-*O*-α-L-(2″-gallate)-rhamnopyranoside and biflavanols compounds exerted significant α-glucosidase inhibitory activity (Yang et al., 2020). This study may provide a basis for the development of new, plant-derived drugs directed against diabetes mellitus.

Aldose reductase (AR) is an enzyme present mainly in the liver, kidneys, lens, retina, and Schwann's cells, allowing the formation of sorbitol. In the state of chronic hyperglycaemia, polyols accumulate in cells and cause osmotic damage leading to diabetic complications (Tarr et al., 2013). Majaw and Syiem (2016) observed strong AR inhibition value for the terpenoid/phenolic fraction *from P. fulgens*. A further study revealed that the aqueous-methanol (1:4) extract of *P. fulgens* inhibited the enzymes amylase, α-glucosidase, β-glucosidase and lipase, with the highest inhibition properties observed against α-glucosidase (94.57% ± 0.16 at 1 mg/mL) (Majaw et al., 2018). Ellagic acid and its derivatives isolated from *Duchesnea chrysantha* exerted moderate inhibitory properties against rat AR. However, ellagic acid, isovitexin, kaempferol 3-*O*-β-D-glucuronide methyl ester and quercetin 3-*O*-α-L-arabinopyranosyl-(1 → 6)-β-D-galactopyranoside were potent inhibitors of advanced glycation end product formation (Kim, J.M. et al., 2008).

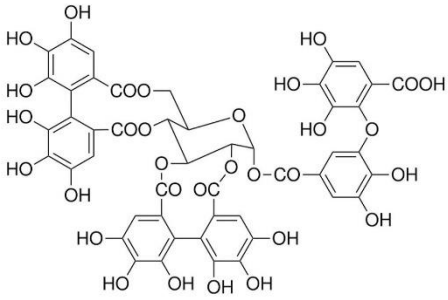
Protein tyrosine phosphatase 1B (PTP1B) is responsible for the negative regulation of signalling pathways of insulin and leptin. Thus, PTP1B inhibitors are promising drug candidates for diabetes mellitus treatment. Due to the ethnopharmacological relevance indicating *P. discolor* as a traditional drug against e.g. diabetes mellitus, Tuo and co-authors isolated from *P. discolor* roots seven oleanane triterpenes, that demonstrated strong PTP1B inhibitory activity comparable to RK682, used as a positive control, with IC_{50} values from 7.5 ± 0.5 to 22.7 ± 0.5 μmol/L. Four of seven compounds were assessed as selective PTP1B inhibitors (Tuo et al., 2016). Furthermore, it has been found, that purified phenolic extract from roots of *P. discolor* exerted higher inhibitory than acarbose on α-amylase. However, the inhibition of α-glucosidase by the aforementioned extract was weaker than acarbose. (Cheng et al., 2020). Wang et al. (2019) employed network pharmacology-based analysis of *P. discolor* secondary metabolites. Isolation in connection with gas chromatography-mass spectrometry, STRING and Cytoscape analysis led to the identification of 21 anti-diabetic compounds. One of them, tricetin, showed low cytotoxicity in the *in vitro* hyperglycaemic HepG2 cell line model, while glucose absorption and uptake processes were promoted. Recently, Chinese authors (Xu et al., 2017) evaluated the protective effects of *P. discolor* extracts in the streptozotocin induced β-cell injury model using the RIN-m5f cell line. Tested extracts increased



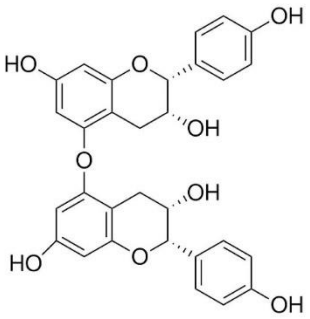
pedunculagin



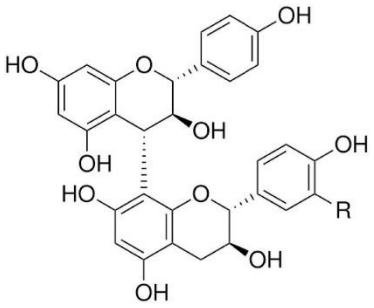
potentillin



agrimonic acid A



(-)-epiafzelechin-(6→8)-epiafzelechin (potifulgene)



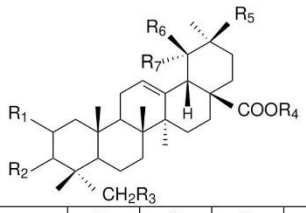
	R
afzelechin-(4α→8)-afzelechin	H
afzelechin-(4α→8)-catechin	OH

Fig. 1. Structures of selected hydrolysable and condensed tannins from *Potentilla* species.

cell viability and insulin secretion in a dose-dependent manner.

5.1.2. In vivo experiments

In a series of experiments, Indian authors evaluated the biological profile of various extracts from *P. fulgens* root on aldose reductase (AR) and sorbitol dehydrogenase (SDH) activity. The extracts exhibited inhibitory activities in a dose-dependent manner against SDH localized in the liver, kidney and eye tissues of both normal and alloxan-induced diabetic mice. It is also worth mentioning that a preparation administered to diabetic mice reduced the total cholesterol and triglyceride profiles and increased the HDL level comparable to metformin, glibenclamide and insulin. Moreover, an extract administered via the



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
ursolic acid	H	β-OH	H	H	H	CH_3	H
2α-hydroxyursolic acid	α-OH	β-OH	H	H	H	CH_3	H
pomolic acid	H	β-OH	H	H	H	CH_3	OH
pomolic acid 28-O-β-D-glucoside	H	β-OH	H	β-D-glc	H	CH_3	OH
2-oxopomolic acid	O	β-OH	H	H	H	CH_3	OH
maslinic acid	α-OH	β-OH	H	H	CH_3	H	H
arjunglucoside I	α-OH	β-OH	OH	β-D-glc	CH_3	H	OH
euscaphic acid	α-OH	α-OH	H	H	H	CH_3	OH
kaji-ichigoside F1	α-OH	α-OH	H	β-D-glc	H	CH_3	OH
tormentic acid	α-OH	β-OH	H	β-D-glc	H	CH_3	OH
tormentoside (rosamultin)	α-OH	β-OH	H	β-D-glc	H	CH_3	OH
potentillanoside A	α-OH	O	H	β-D-glc	H	CH_3	OH
potentillanoside B	O	α-OH	H	β-D-glc	H	CH_3	OH
potentillanoside C	α-OH	β-OH	H	β-D-6-O-ac-glc	H	CH_3	OH

Fig. 2. Structures of selected terpenoids from *Potentilla* species.

intraperitoneal route significantly increased liver hexokinase activity comparable to insulin (Syiem et al., 2009; Syiem and Majaw, 2010). A further study revealed that the terpenoid and phenolic fractions simultaneously exerted the highest inhibitory effect on AR and SDH activity in all tested tissues in both normoglycemic and diabetic mice (Syiem and Majaw, 2011). Recent ultrastructural studies carried out on diabetic mice tissues (eye lens, liver, kidney) revealed reduced structural tissue alterations after a treatment of 4 weeks with a *P. fulgens* root extract (Majaw et al., 2018).

Tiliroside (kaempferol 3-*O*-β-D-(6″-*O*-trans-*p*-coumaroyl)-glucoside) isolated from *P. chinensis* was evaluated for its antihyperglycemic, antihyperlipidemic and antioxidant activities. Those effects of tiliroside were evaluated on normal, alloxan-induced diabetic mice and streptozotocin-induced diabetic rats. Daily administration of tiliroside at doses from 0.4 to 1.6 mg/kg body weight (b.w.) significantly decreased in the same manner as metformin, the glucose, triglycerides and total cholesterol serum levels in both alloxan-induced diabetic mice and streptozotocin-induced diabetic rats. Furthermore, there was a decrease in the malondialdehyde concentrations and an increase in superoxide dismutase activity in the diabetic rats, which prevented pancreatic diabetes mellitus damage (Qiao et al., 2011). A previous study (Lu et al., 2008) also revealed that the crude flavonoid and alkaloid fractions increased serum adiponectine and reduced serum glucose and resistin levels.

On several occasions, the antidiabetic characteristics of *P. discolor* have been reported. This plant is traditionally used in many regions of Asia as a treatment of diabetes. A whole plant ethanol extract exhibited significant hypoglycaemic activity in alloxan-induced diabetic mice, while in normoglycemic animals the glucose levels were unaffected. Moreover, glucose disposal was increased in an oral glucose tolerance test in mice treated with this herbal preparation from *P. discolor* (Yang et al., 2010). In another study, the flavonoid and triterpenoid fractions obtained from *P. discolor* were administered for 15 days to high fat diet-fed and streptozotocin-induced diabetic rats. The authors reported similar improvements in both treated groups as in a positive control group administered with reference drug (glibenclamide) regarding fasting blood glucose, glycosylated serum protein, serum total cholesterol, triglycerides and LDL-c levels, with a simultaneous increase in HDL-c level. Furthermore, both extracts exerted a protective effect on the pancreatic β-cells, improved the glutathione and superoxide dismutase levels and lowered malondialdehyde and nitric oxide serum concentrations in diabetic rats (Zhang et al., 2010b). A 28-day treatment with a *P. discolor* water extract attenuated all measured parameters in Ob-db mice, including blood glucose and cholesterol levels (Song et al., 2012). A metabonomic analysis conducted in male C57BL/6 mice fed a high-fat diet revealed that the extract had an influence on 26 metabolite biomarkers of diabetes mellitus. A *P. discolor* ethanol extract and the ursolic-type triterpene corosolic acid reversed the pathological changes in diabetic mellitus by regulating amino acid, lipid and purine metabolism (Li et al., 2014). Zhang and co-authors found out that the mixture of *P. discolor* and *Astragalus membranaceus* lowered both fasting serum insulin and plasma glucose levels compared with a control group, with a simultaneous increased insulin sensitivity index. The authors suggested that the increased liver mRNA expression of PGC1 and PI3-K was a possible mechanism of action (Zhang et al., 2012).

5.2. Antimicrobial and antiviral activities

Overuse of antibiotics in hospitals, communities, as well in agriculture and in other important branches of industry led to the origin of antibiotic-resistant bacteria. Resistance against antibiotics can occur via several mechanisms, e.g. reduction of intracellular drug concentration, target site modification or inactivation of antibiotics. This problem may lead to difficulties in the treatment of bacterial infections and it is likely possible that in the near future it will cause a global health crisis. This problem led to focus on the interest of science on much more extensive plant screening. As a result, a number of works concerning promising antimicrobial properties of vast *Potentilla* species have been published.

Table 4
Constituents isolated from aerial parts of *Potentilla* species between 2009 and 2020. For compounds isolated from *Potentilla* species before 2009 see Tomczyk and Latté (2009).

Compounds	*Potentilla* species	References
Flavonoids		
Flavonoid aglycones		
Apigenin	*D. indica, P. discolor, P. fruticosa*[a], *P. grandiflora, P. rupestris, P. supina*	Bazylko et al. (2011); Miao et al. (2008); Syrpas et al. (2020); Zhang et al. (2011); Zheng and Piao (2012)
Ayanin	*P. parvifolia*	Yuan et al. (2017)
Chrysin	*P. evestita*[b]	Rauf (2013)
Isorhamnetin	*P. alba*	Kowalik et al. (2020)
Kaempferol	*D. indica, P. alba, P. glabra, P. multicaulis*[b]	Han et al. (2016); Jia et al. (2013); Kowalik et al. (2020); Miao et al. (2008)
Luteolin	*P. discolor, P. anserina*[a,e], *P. fruticosa, P. grandiflora, P. recta, P. rupestris, P. thuringiaca*	Bazylko et al. (2011); Zhang et al. (2011)
Myricetin	*P. nepalensis*[c], *P. rupestris*	Bazylko et al. (2011)
Dihydromyricetin (ampelopsin)	*P. fruticosa*[a]	Syrpas et al. (2020)
Naringenin	*P. glabra, P. reptans, P. speciosa*	Kim et al. (2020); Uysal et al. (2017)
Quercetin	*P. alba, P. glabra, P. multicaulis*[b], *P. parvifolia, P. visianii*[b]	Han et al. (2016); He et al. (2009); Kowalik et al. (2020); Radojević et al. (2018); Yuan et al. (2017)
Tricetin	*P. discolor*	Wang et al. (2019)
Kaempferol 4′,5,7-trimethyl ether	*P. chinensis*	Liu et al. (2009)
Luteolin 7,3′,4′-trimethyl ether	*P. fruticosa*[a]	Syrpas et al. (2020)
Quercetin 7-methyl esther (Rhamnetin)	*P. glabra*	Han et al. (2016)
Apigenin 4′-methyl ether (acacetin)	*P. evestita*[b]	Rauf (2013)
5,2′-Dihydroxy-6,7,8-trimethoxyflavone (tenaxin I)	*P. discolor*	Song et al. (2012)
Tricetin 3′,5′-dimethyl ether (tricin)	*P. parvifolia*	Yuan et al. (2017)
Flavonoid *O*-glycosides and *O*-glucuronides		
Apigenin 7-*O*-β-D-glucoside (cosmosiin)	*P. discolor*[b], *P. grandiflora, P. recta, P. reptans*	Tomczyk (2011); Tomovic et al. (2015); Yang et al. (2010)
Apigenin 7-*O*-β-D-glucuronide	*P. discolor, P. fulgens*	Chen et al. (2013); Song et al. (2012)
Ayanin 3′-*O*-β-D-glucopyranoside	*P. parvifolia*	Murata et al. (2013)
Acacetin 7-*O*-rutinoside	*D. indica, P. anserina*[b]	Mari et al. (2013); Xu et al. (2007b)
Kaempferol 3-*O*-β-D-glucoside (astragalin)	*D. indica, P. alba, P. anserina, P. discolor*[b], *P. fulgens, P. grandiflora, P. inclinata*[a], *P. parvifolia, P. reptans, P. supina, P. thuringiaca*	Bazylko et al. (2011); Chen et al. (2013); Grochowski et al. (2017); Kowalik et al. (2020); Mari et al. (2013); Miao et al. (2008); Murata et al. (2013); Tomovic et al. (2015); Şöhretoğlu et al. (2018); Yang et al. (2010); Zheng and Piao (2012)

Table 4 (*continued*)

Compounds	*Potentilla* species	References
Kaempferol 3-*O*-β-D-glucuronide	*P. discolor*[b], *P. grandiflora, P. thuringiaca*	Bazylko et al. (2011); Grochowski et al. (2017); Yang et al. (2010)
Kaempferol 6″-*O*-methyl-3-*O*-β-D-glucuronide	*D. chrysantha*[b], *P. inclinata*[a]	Kim et al. (2008); Şöhretoğlu et al. (2018)
Kaempferol 3-*O*-α-L-arabinofuranoside	*P. chinensis*	Liu et al. (2009)
Kaempferol 3-*O*-β-D-galactopyranoside	*D. indica, P. alba*	Kowalik et al. (2020); Xu et al. (2007b)
Kaempferol 3-*O*-rutinoside	*P. anserina*[b], *P. astracanica, P. inclinata*[a], *P. recta*	Mari et al. (2013); Şöhretoğlu and Kirmzbekmez (2011); Şöhretoğlu and Sterner (2011); Şöhretoğlu et al. (2018)
Kaempferol 3-*O*-β-D-(6″-*O*-trans-p-coumaroyl)-glucoside (tiliroside, potengriffioside A)	*D. indica, P. alba, P. chinensis, P. erecta, P. fulgens, P. grandiflora, P. nepalensis*[a,c], *P. parvifolia, P. recta, P. rupestris, P. thuringiaca*	Bazylko et al. (2011); Chen et al. (2013); Kowalik et al. (2020); Liu et al. (2009); Miao et al. (2008); Murata et al. (2013); Tomczyk (2011); Tomczyk et al. (2010a, 2010b)
Kaempferol 3-*O*-β-D-(6″-*O*-cis-p-coumaroyl)-glucoside (cis-tiliroside)	*P. chinensis, P. parvifolia*	Liu et al. (2009); Murata et al. (2013)
Kaempferol 3-*O*-α-arabinopyranosyl-(1 → 6)-β-glucoside (kaempferol 3-*O*-vicianoside)	*P. recta*	Şöhretoğlu and Kirmzbekmez (2011)
Kaempferol 3-*O*-α-L-rhamnoside	*P. anserina*	Olennikov et al. (2015)
Kaempferol 7-*O*-α-L-rhamnoside	*P. discolor*[b]	Zhang et al. (2010a)
Kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside (Kaempferol 3-robinobioside)	*D. indica*	Xu et al. (2007a)
Kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1 → 3)α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside	*D. indica*	Xu et al. (2007a)
Luteolin 7-*O*-β-D-glucoside (cynaroside)	*P. discolor*[b], *P. fulgens, P. grandiflora, P. recta, P. reptans*	Bazylko et al. (2011); Chen et al. (2013); Tomczyk (2011); Tomovic et al. (2015); Yang et al. (2010)
Myricetin 3-*O*-β-D-glucuronide	*P. arenaria, P. aurea*	Hriboová et al. (2014)
Quercetin 3-*O*-α-arabinoside (avicularin)	*P. chinensis, P. recta*	Liu et al. (2009); Tomczyk (2011)
Quercetin 3-*O*galactoside (hyperoside)	*D. indica, P. anserina*[a,e], *P. fulgens, P. glabra*[a], *P. recta, P. thuringiaca*	Bazylko et al. (2011); Chen et al. (2013); Şöhretoğlu and Kirmzbekmez (2011); Wang et al. (2013); Xu et al. (2007b)
Quercetin 3-*O*-β-D-glucoside (isoquercitrin)	*D. indica, P. alba, P. argentea, P. erecta, P. glabra, P. grandiflora, P. nepalensis*[a,c], *P. inclinata*[a], *P. parvifolia*[a], *P. recta, P. reptans, P.*	Bazylko et al. (2011); Han et al. (2016); Kowalik et al. (2020); Şöhretoğlu et al. (2018); Tomovic et al. (2015); Xu et al. (2007b)

(*continued on next page*)

Table 4 (*continued*)

Compounds	*Potentilla* species	References
	rupestris, P. thuringiaca	
Quercetin 3-*O*-β-D-glucuronide (miquelianin)	*P. argentea, P. astracanica, P. erecta, P. fruticosa, P. glabra, P. grandiflora, P. nepalensis*[b,e], *P. parvifolia, P. recta, P. rupestris, P. thuringiaca*	Bazylko et al. (2011); Han et al. (2016); Murata et al. (2013); Şöhretoğ;lu and Kirmzbekmez (2011); Şöhretoğlu and Sterner (2011); Tomczyk et al. (2012)
Quercetin 3-*O*-α-L-rhamnoside (quercitrin)	*P. argentea, P. chinensis, P.erecta, P. glabra, P. grandiflora, P. multicaulis*[b], *P. nepalensis*[b,e], *P. recta, P. rupestris, P. thuringiaca*	Bazylko et al. (2011); Han et al. (2016); Jia et al. (2013); Liu et al. (2009); Şöhretoğ;lu and Kirmzbekmez (2011); Yuan et al. (2017)
Quercetin 3-*O*-rutinoside (rutin)	*D. indica, P. alba, P. anserina, P. astracanica, P. fulgens, P. glabra*[a], *P. grandiflora, P. inclinata*[a], *P. nepalensis*[b,e], *P. parvifolia*[a], *P. recta, P. reptans, P. speciosa, P. thuringiaca, P. visianii*[b],	Bazylko et al. (2011); Han et al. (2016); Jaitak et al. (2010a); Kowalik et al. (2020); Mari et al. (2013); Radojević et al. (2018); Şöhretoğ;lu and Kirmzbekmez (2011); Şöhretoğlu and Sterner (2011); Şöhretoğlu et al. (2018); Tomovic et al. (2015); Uysal et al. (2017); Wang et al. (2013); Xu et al. (2007b)
Quercetin 3-*O*-β-D-xyloside (reynoutrin)	*P. glabra*	Han et al. (2016)
Quercetin 3-*O*-α-L-arabinopyranosyl-(1 → 6)-β-galactopyranoside	*D. chrysantha*[b]	Kim et al. (2008)
Quercetin 3-*O*-α-L-arabinopyranosyl-(1 → 6)-β-glucoside (quercetin 3-O-vicianoside, peltatoside)	*P. recta*	Şöhretoğ;lu and Kirmzbekmez (2011)
Quercetin 3-*O*-β-D-(6″-*O*-galloyl) glucoside	*P. parvifolia*	Murata et al. (2013)
Quercetin 3-*O*-β-D-(6″-*O*-galloyl) galactoside	*P. anserina, P. arenaria, P. argentea, P. erecta, P. parvifolia,*	Hríbová et al. (2014); Murata et al. (2013)
Quercetin 4′-*O*-β-D-glucoside (spiraeoside)	*P. bifurica*	Piao et al. (2009)
Quercetin 7-*O*-α-L-rhamnoside (vincetoxicoside B)	*P. discolor*[b]	Zhang et al. (2010a)
Quercetin 7-*O*-β-D-glucuronide	*P. fruticosa*[a,f]	Zeng et al. (2020)
Rhamnetin 3-*O*-glucoside	*P. glabra*	Han et al. (2016)
Rhamnetin 3-*O*-rhamnoside	*P. glabra*	Han et al. (2016)
Rhamnetin 3-*O*-rutinoside	*P. glabra*	Han et al. (2016)
Isorhamnetin 3-*O*-β-D-glucoside	*P. anserina, P. multicaulis*[b], *P. thuringiaca*	Grochowski et al. (2017); Jia et al. (2013); Olennikov et al. (2015)
Isorhamnetin 3-*O*-β-D-glucuronide	*P.fruticosa*[a], *P. thuringiaca*	Grochowski et al. (2017); Syrpas et al. (2020)
Naringenin 7-*O*-β-neohesperidoside (naringin)	*P. reptans, P. speciosa*	Uysal et al. (2017)
Herbacetin 8-methyl ether-3-*O*-β-sophoroside	*P. astracanica, P. multicaulis*[b]	Jia et al. (2013); Şöhretoğlu and Sterner (2011)
Scutellarein 7-*O*-β-D-glucuronide (scutellarin)	*P. fulgens*	Chen et al. (2013)

Table 4 (*continued*)

Compounds	*Potentilla* species	References
Cyanidin 3-*O*-rutinoside	*D. indica*[c]	Qin et al. (2009)
Peonidin 3-*O*-rutinoside	*D. indica*[c]	Qin et al. (2009)
Petunidin 3-*O*-rutinoside	*D. indica*[c]	Qin et al. (2009)
Flavonoid C-glycosides		
Apigenin 6-C-β-D-glucoside (isovitexin)	*D. chrysantha*[b], *D. indica, P. discolor*	Chen et al. (2013); Kim et al. (2008); Xu et al. (2007b)
Apigenin 8-C-β-D-glucoside (orientin)	*P. astracanica*	Şöhretoğlu and Sterner (2011)
Apigenin 8-C-β-D-glucoside-6-C-β-D-xyloside (vicenin 1)	*P. discolor*	Song et al. (2012)
Apigenin 6,8-di-C-β-D-glucoside (vicenin 2)	*P. discolor*	Song et al. (2012)
Apigenin 6-C-β-D-glucoside-8-C-β-D-xyloside (vicenin 3)	*P. discolor*	Song et al. (2012)
Apigenin 6-C-β-D-glucoside-8-C-α-D-arabinoside (schaftoside)	*P. discolor, P. multicaulis*[b]	Jia et al. (2013); Song et al. (2012);
Apigenin 6-C-α-D-arabinoside-8-C-β-D-glucoside (isoschaftoside)	*D. indica, P. discolor, P. multicaulis*[b]	Jia et al. (2013); Song et al. (2012); Zhu et al. (2015)
Diflavonol ester		
Potentilin A	*P. parvifolia*	Murata et al. (2013)
Isoflavones aglycons, C- and O-glycosides		
Genistein	*P. anserina*[b]	Mari et al. (2013)
Genistein 5-*O*-β-D-glucoside	*P. astracanica*	Şöhretoğlu and Sterner (2011)
Prunetin 5-*O*-β-D-glucoside	*P. astracanica, Potentilla inclinata*[a]	Şöhretoğlu and Sterner (2011); Şöhretoğlu et al. (2018)
Hydrolysable tannins and related compounds		
Monomers		
Agrimonic acid A	*P. anserina*	Olennikov et al. (2015)
Agrimonic acid B	*P. anserina*	Olennikov et al. (2015)
Digalloylglucose	*P. fruticosa*[a,f]	Zeng et al. (2020)
Trigalloylglucose	*P. fruticosa*[a,f]	Zeng et al. (2020)
Tetragalloylglucose	*P. fruticosa*[a,f], *P. parvifolia*	Murata et al. (2013); Zeng et al. (2020)
Pentagalloylglucose	*P. fruticosa*[a,f], *P. parvifolia*	Murata et al. (2013); Zeng et al. (2020)
Pedunculagin	*P. anserina*	Fecka (2009)
Potentillin	*D. chrysantha, P. anserina, P. recta*	Lee and Yang (1994); Olennikov et al. (2015); Ö kdem et al. (2018)
2,3-Hexahydroxydiphenoic acid α-D-glucoside	*P. inclinata*[a]	Şöhretoğlu et al. (2018)
2,3-Hexahydroxydiphenoic acid β-D-glucosidec	*P. inclinata*[a]	Şöhretoğlu et al. (2018)
Dimers		
Agrimoniin	*P. anserina, P. recta*	Bazylko et al. (2013); Fecka (2009)
Related compounds		
Brevifolin	*D. indica, P. discolor*[b]	Xu et al. (2007a); Zhang et al. (2010a)
Brevifolincarboxylic acid	*D. chrysantha, D. indica, P. kleiniana*[b]	Lee and Yang (1994); Liu et al. (2019); Xu et al. (2007a)
Methyl brevifolincarboxylate	*D. indica, P. anserina*[b,e], *P. erecta, P. nepalensis*[b,e], *P. recta, P. rupestris, P. thuringiaca*	Bazylko et al. (2011); Grochowski et al. (2017); Tomczyk et al. (2010a, 2010b); Tomczyk (2011); Xu et al. (2007a)
Ethyl brevifolincarboxylate	*P. kleiniana*[b]	Liu et al. (2019)
Ellagic acid		

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Table 4 (*continued*)

Compounds	*Potentilla* species	References
	D. chrysantha[b], *D. indica*, *P. discolor*[b], *P. glabra*[a], *P. grandiflora*, *P. parvifolia*[a],	Bazylko et al. (2011); Kim et al. (2008); Wang et al. (2013); Wang et al. (2020a); Wang et al. (2020b); Zhu et al. (2015)
Ellagic acid 3′-*O*-methyl ether-4-*O*-β-D-xyloside (ducheside A)	*D. indica*	Ye and Yang (1996)
Ellagic acid 3′-*O*-methyl ether-4-*O*-α-L-arabinoside (ducheside B)	*D. indica*	Ye and Yang (1996)
Ellagic acid 3-*O*-methyl ether	*P. discolor*	Zhang et al. (2011)
Ellagic acid 3,3′-di-*O*-methyl ether	*D. chrysantha*[b]; *P. kleiniana*[b]	Kim et al. (2008); Liu et al. (2019)
Ellagic acid 3,3′-di-*O*-methyl ether-4′-*O*-β-D-glucoside	*P. kleiniana*[b]	Liu et al. (2019)
Ellagic acid 3,3′-di-*O*-methyl ether-4-*O*-β-D-xyloside	*P. recta*, *P. rupestris*	Bazylko et al. (2011)
Ellagic acid 3,3′,4-tri-*O*-methyl ether	*D. chrysantha*[b]	Kim et al. (2008)
Condensed tannins and their precursors		
(+)-Catechin	*P. alba*, *P. astracanica*, *P. glabra*[a], *P. parvifolia*[a], *P. reptans*, *P. speciosa*, *P. visianii*[b]	Kowalik et al. (2020); Radojević et al. (2018); Şöhretoğlu and Sterner (2011); Uysal et al. (2017); Wang et al. (2013)
(+)-Catechin 3-*O*-β-D-glucoside	*P. astracanica*	Şöhretoğlu and Sterner (2011)
(−)-Epicatechin	*P.alba*, *P. reptans*, *P. speciosa*, *P.visianii*[b]	Kowalik et al. (2020); Radojević et al. (2018); Uysal et al. (2017)
(−)-Epiafzelechin	*P. fulgens*	Jaitak et al. (2010a)
Epigallocatechin 3-*O*-p-coumarate	*P. fruticosa*[a]	Syrpas et al. (2020)
Epigallocatechin-dimethylgallate	*P. fruticosa*[a]	Syrpas et al. (2020)
Catechin-(7,8-*bc*)-4*b*-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranone	*P. parvifolia*	Murata et al. (2013)
Dimers		
(+)-Afzelechin-(4α→8″)-(+)-catechin	*P. fulgens*	Jaitak et al. (2010a)
(−)-Afzelechin-(4α→8)-(−)-afzelechin	*P. fruticosa*[a,f]	Zeng et al. (2020)
(−)-Epi-afzelechin-(4β→8,2β→*O*→7)-(+)-afzelechin (Geranin A)	*P. parvifolia*	Murata et al. (2013)
(+)-Catechin-(4α→8)-(−)-catechin (procyanidin B3)	*P. parvifolia*	Yuan et al. (2016)
(+)-Catechin-(4α→6)-(−)-catechin (procyanidin B6)	*P. parvifolia*	Yuan et al. (2016)
(−)-Epicatechin-(4β→6)-(+)-catechin (procyanidin B7)	*P. parvifolia*	Yuan et al. (2016)
Related compounds		
6,8′-Methylenebiscatechin	*P. fruticosa*[a,f]	Zeng et al. (2020)
8,8′-Methylenebiscatechin	*P. fruticosa*[a,f]	Zeng et al. (2020)
6,8′-Methylene (7-*O*-glucosyl)-biscatechin	*P. fruticosa*[a,f]	Zeng et al. (2020)
Organic acids and phenol carboxylic acids		
p-Anisic acid	*P. recta*[a]	Sytar et al. (2018)
Benzoic acid	*P. reptans*, *P. speciosa*	Uysal et al. (2017)
3-Hydroxybenzoic acid	*P. reptans*, *P. speciosa*, *P. visianii*[b]	Radojević et al. (2018); Uysal et al. (2017)
4-Hydroxybenzoic acid	*P. recta*[a], *P. reptans*, *P. speciosa*	Sytar et al. (2018); Uysal et al. (2017)
3,5-Dihydroxybenzoic acid	*P. visianii*[b]	Radojević et al. (2018)
2,3-Dimethoxybenzoic acid	*P. reptans*, *P. speciosa*	Uysal et al. (2017)
	P. discolor[b]	Wang et al. (2020a)

Table 4 (*continued*)

Compounds	*Potentilla* species	References
Benzyl 1-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyloxy)-3-hydroxybenzoate		
Caffeic acid	*P. glabra*[a], *P. parvifolia*[a]	Wang et al. (2013)
Caffeic acid methyl ester	*D. chrysantha*	Lee and Yang (1994)
3-*O*-Caffeoylquinic acid (chlorogenic acid)	*P. alba*, *P. anserina*[b], *P. discolor*, *P. fruticosa*[a], *P. recta*[a], *P. reptans*, *P. speciosa*, *P. visianii*[b]	Kowalik et al. (2020); Mari et al. (2013); Radojević et al. (2018); Song et al. (2012); Syrpas et al. (2020); Sytar et al. (2018); Uysal et al. (2017)
4-*O*-Caffeoylquinic acid (cryptochlorogenic acid)	*P. alba*, *P. discolor*	Kowalik et al. (2020); Song et al. (2012)
5-*O*-Caffeoylquinic acid (neochlorogenic acid)	*P. alba*, *P. discolor*	Kowalik et al. (2020); Song et al. (2012)
Citric acid	*P. discolor*, *P. fruticosa*[a]	Syrpas et al. (2020); Wang et al. (2019)
o-Coumaric acid	*P. reptans*, *P. speciosa*	Uysal et al. (2017)
p-Coumaric acid	*P. discolor*, *P. fruticosa*[a,f], *P. visianii*[b]	Radojević et al. (2018); Song et al. (2012); Zeng et al. (2020)
Cinnamic acid	*P. recta*[a]	Sytar et al. (2018)
Methoxy-cinnamic acid	*P. recta*[a]	Sytar et al. (2018)
p-Hydroxy-cinnamic acid	*D. indica*	Miao et al. (2008)
Ferulic acid	*D. indica*, *P. glabra*[a], *P. parvifolia*[a], *P. visianii*[b]	Radojević et al. (2018); Wang et al. (2013); Zhu et al. (2015)
Gallic acid	*D. chrysantha*, *P. discolor*, *P. fruticosa*[a,f], *P. inclinata*[a], *P. multicaulis*[b], *P. reptans*, *P. speciosa*	He et al. (2009); Lee and Yang (1994); Şöhretoğlu et al. (2018); Uysal et al. (2017); Wang et al. (2019); Zeng et al. (2020)
Gallic acid methyl ester	*P. fruticosa*[a,f]	Zeng et al. (2020)
Malic acid	*P. fruticosa*[a]	Syrpas et al. (2020)
Maleic acid	*P. discolor*	Wang et al. (2019)
Xspolyphenol B (Orsellinic acid D-mannitol ester)	*P. discolor*[b]	Wang et al. (2020)
Oxalic acid	*P. discolor*	Wang et al. (2019)
Quinic acid	*P. fruticosa*[a], *P. reptans*	Tomovic et al. (2015); Syrpas et al. (2020)
3-*O*-p-Coumaroylquinic acid	*P. alba*	Kowalik et al. (2020)
5-*O*-p-Coumaroylquinic acid	*P. alba*	Kowalik et al. (2020)
3-*O*-Feruloylquinic acid	*P. alba*	Kowalik et al. (2020)
Protocatechuic acid	*D. chrysantha*, *P. discolor*, *P. fruticosa*[a,f], *P. reptans*	Lee and Yang (1994); Tomovic et al. (2015); Wang et al. (2019); Zeng et al. (2020)
Pyruvic acid	*P. discolor*	Wang et al. (2019)
Salicylic acid	*P. discolor*; *P. recta*[a]	Sytar et al. (2018); Wang et al. (2019)
5-Hydroxysalicylic acid	*P. fruticosa*[a,f]	Zeng et al. (2020)
Succinic acid	*P. discolor*	Wang et al. (2019)
Syringic acid	*P. recta*[a], *P. reptans*, *P. visianii*[b]	Radojević et al. (2018); Sytar et al. (2018); Uysal et al. (2017)

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Table 4 (*continued*)

Compounds	*Potentilla* species	References
Tartaric acid	*P. discolor*	Wang et al. (2019)
Vanillic acid	*P. recta*[a], *P. reptans, P. speciosa, P. visianii*[b]	Radojević et al. (2018); Sytar et al. (2018); Uysal et al. (2017)
Coumarins		
Aesculetin	*P. reptans, P. supina*	Tomovic et al. (2015); Zheng and Piao (2012)
Bergapten	*P. fruticosa*[a]	Syrpas et al. (2020)
Umbelliferone	*P. evestita*[b]	Rauf (2013)
Chromones		
5-Hydroxy-7-*O*-(6-*O*-*p*-cis-coumaroyl-β-D-glucopyranosyl)-chromone	*P. parvifolia*	Murata et al. (2013)
5-Hydroxy-7-*O*-(6-*O*-*p*-trans-coumaroyl-β-D-glucopyranosyl)-chromone	*P. parvifolia*	Murata et al. (2013)
Sterols		
Daucosterol	*P. discolor*[b], *P. multicaulis*[b], *P. supina,*	He et al. (2009); Zhang et al. (2010a); Zheng and Piao (2012)
β-Sitosterol	*D. indica, P. chinensis, P. discolor*[b], *P. multicaulis*[b], *P. supina,*	He et al. (2009); Liu et al. (2009); Xu et al. (2007a); Zhang et al. (2010a); Zheng and Piao (2012)
2,6*β*,7*β*-trihydroxy-4-methyl-19-norpregna-1,3,5 (10)-trien-17-one	*P. evestita*[b]	Khan et al. (2011)
11*α*,17*α*,21-trihydroxypregna-4,16 (22)-diene-3,20-dione	*P. evestita*[b]	Khan et al. (2011)
(24R)-6β-hydroxyl-24-ethyl-cholest-4-en-3-one	*D. indica*	Xu et al. (2007a)
Triterpenoids		
Aceriphyllic acid A	*P. discolor*[b]	Tuo et al. (2016)
Aceriphyllic acid A methyl ester	*P. discolor*[b]	Tuo et al. (2016)
α-Amyrin	*P. discolor*[b]	Yang et al. (2010)
β-Amyrin	*P. discolor*[b]	Yang et al. (2010)
Asiatic acid	*P. discolor*[b]	Yang et al. (2010)
Arjunic acid	*D. indica*	Miao et al. (2008)
Arjunolic acid	*P. discolor*	Yang et al. (2011)
Betulinic acid	*D. indica, P. discolor*[b], *P. fruticosa, P. parvifolia*	Kang et al. (2013); Miao et al. (2008); Yang et al. (2010); Yuan et al. (2018)
2α-Hydroxy-betulinic acid	*P. discolor*[b]	Yang et al. (2010)
Corosolic acid	*P. argentea, P. parvifolia, P. recta*	Sut et al. (2019); Yuan et al. (2018)
Euscaphic acid	*D. chrysantha*[b], *D. indica, P. argentea, P. parvifolia, P. recta, P. supina,*	Kim et al. (2008); Miao et al. (2008); Sut et al. (2019); Yuan et al. (2018); Zheng and Piao (2012)
Euscaphic acid 28-*O*-β-D-glucoside (Kajiichigoside F1)	*D. chrysantha*[b], *P. discolor*[b]	Kim et al. (2008); Yang et al. (2010)
Gypsogenic acid	*P. discolor*[b]	Tuo et al. (2016)
30-Methyl-17α-hopan-12-ene-3-one (Potentene A)	*P. fulgens*	Jaitak et al. (2010a)
Hopan-12-eno-11-oxo-28-oic acid 3-*O*-β-D-glucosyl-(1,2)-β-D-glucoside (potentene B)	*P. fulgens*	Jaitak et al. (2010a)
3α-Acetyl-19α- hydrogen-29-aldehyde-27-lupanoic acid	*P. discolor*[b]	Zhang et al. (2017)
3α-Hydroxy-19α-hydrogen-29-aldehyde-27-lupanoic acid	*P. discolor*[b]	Zhang et al. (2017)
3α-Hydroxy-19β-hydrogen-29-aldehyde-27-lupanoic acid	*P. discolor*[b]	Zhang et al. (2017)
Maslinic acid	*D. chrysantha*[b], *P. discolor*[b]	Kim et al. (2008); Yang et al. (2010)
Myrianthic acid	*P. discolor*[b]	Yang et al. (2011)
Oleanolic acid	*D. indica, P. argentea, P. discolor*[b], *P.*	He et al. (2009); Kang et al. (2013); Sut et al. (2019);

Table 4 (*continued*)

Compounds	*Potentilla* species	References
	fruticosa, P. multicaulis[b], *P. parvifolia, P. recta, P. supina*	Xu et al. (2007a); Yang et al. (2010); Yuan et al. (2018); Zheng and Piao (2012)
2α-hydroxyoleanolic acid	*D. indica*	Miao et al. (2008)
3α-hydroxyolean-12-en-27-oic acid	*P. discolor*[b]	Tuo et al. (2016)
3β-hydroxyolean-12-en-27-oic acid	*P. discolor*[b]	Tuo et al. (2016)
3-oxoolean-12-en-27-oic acid	*P. discolor*[b]	Tuo et al. (2016)
Platanic acid	*P. parvifolia*	Yuan et al. (2018)
Pomolic acid	*D. chrysantha*[b], *P. argentea, P. parvifolia, P. recta*	Kim et al. (2008); Sut et al. (2019); Yuan et al. (2018)
Tormentic acid	*D. chrysantha*[b], *D. indica, P. argentea, P. recta, P. supina*	Kim et al. (2008); Miao et al. (2008); Sut et al. (2019); Zheng and Piao (2012)
Tormentoside (rosamultin)	*D. indica, P. supina*	Miao et al. (2008); Zheng and Piao (2012)
Ursolic acid	*D. chrysantha*[b], *D. indica, P. argentea, P. discolor*[b], *P. recta, P. supina*	Kim et al. (2008); Sut et al. (2019); Xu et al. (2007a); Zheng and Piao (2012); Zhang et al. (2017)
2α-hydroxyursolic acid	*D. indica*	Miao et al. (2008)
2α,3β-dihydroxy-urs-12-en-18,19-epoxy-28-oic acid	*D. indica*	Qiao et al. (2009)
18,19-seco,2a,3a-dihydroxyl-19- oxo-urs-11,13 (18)-dien-28-oic acid	*D. indica*	Qiao et al. (2009)
2α, 3α, 19α, 23-tetrahydroxy-urs-12-en-28-oic acid	*P. supina*	Zheng and Piao (2012)
3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-xylopyranosyl-19α-hydroxyurs-12-en-28-oic acid	*P. discolor*	Li et al. (2013)
2α,3β,19α-trihydroxy-urs-12-en-28-oic acid	*D. indica, P. discolor*	Li et al. (2013); Ye and Yang (1996)
2α,3β,19α-trihydroxy-urs-12-en-24,28-dioic acid	*P. discolor*	Li et al. (2013)
2α,3β-dihydroxy-olean-12-en-28-oic acid	*P. discolor*	Li et al. (2013)
2α,3α,19α-trihydroxy-urs-12-en-28-oic acid	*P. discolor*	Li et al. (2013)
3β-hydroxyurs-12-en-28-oic acid	*D. indica*	Ye and Yang (1996)
2α,3α,19α-trihydroxy-urs-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester	*D. indica*	Ye and Yang (1996)
2α,3β,19α-trihydroxy-urs-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester	*D. indica*	Ye and Yang (1996)
2α,3β,19α,23,30-pentahydroxyurs-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester	*P. multicaulis*[b]	Jia et al. (2013)
Lignans		
(7S,8R)-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucoside	*P. multicaulis*[b]	Jia et al. (2013)
(+)-1-hydroxy-2-epipinoresinol-1-β-D-glucoside	*P. multicaulis*[b]	Jia et al. (2013)
(+)-1-hydroxy-pinoresinol-1-β-D-glucoside	*P. multicaulis*[b]	Jia et al. (2013)
(+)-1-hydroxy-pinoresinol-4-β-D-glucoside	*P. multicaulis*[b]	Jia et al. (2013)
(−)-4-*O*-Methyldihydrodehydrodiconiferyl alcohol 9′-O-β-glucoside	*P. recta*	Şöhretoğ;lu and Kirmzbekmez (2011)
Chaenomiside F	*P. discolor*[b]	Wang et al. (2020a)
Potentillalignan A	*P. discolor*[b]	Wang et al. (2020a)
Potentillalignan B	*P. discolor*[b]	Wang et al. (2020a)
	P. discolor[b]	Wang et al. (2020a)

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Table 4 (continued)

	Potentilla species	References
(7R,8S)-4,7,9,3′,9′-pentahydroxy-3-methoxy-8,4′-oxyneolignan-3′-*O*-β-D-glucoside		
(7S,8R)-4,7,9,3′,9′-pentahydroxy-3-methoxy-8,4′-oxyneolignan-3′-*O*-β-D-glucoside	*P. discolor*[b]	Wang et al. (2020a)
(7R,8R)-4,7,9,3′,9′-pentahydroxy-3-methoxy-8,4′-oxyneolignan-3′-*O*-β-D-glucoside	*P. discolor*[b]	Wang et al. (2020a)
(7S,8S)-4,7,9,3′,9′-pentahydroxy-3-methoxy-8,4′-oxyneolignan-3′-*O*-β-D-glucoside	*P. discolor*[b]	Wang et al. (2020a)
(+)-seco-5′-methoxyisolariciresinol-9′-O-α-L-rhamnoside	*P. discolor*[b]	Wang et al. (2020a)
Others		
Amino acids	*P. discolor*	Wang et al. (2019)
Fatty acids	*P. alba*, *P. alchemilloides*[d], *P. ambigua*[d], *P. anatolica*, *P. anserina*, *P. argyrophylla var. leucochroa*[d], *P. atrosanguinea*[d], *P. aurea*[d], *P. discolor*, *P. fruticosa*[a], *P. grandulosa*[d], *P. grammopetala*[d], *P. hippiana*[d], *P. pyrenaica*[d], *P. speciosa*[d], *P. visianii*[d], *P. uniflora*[d]	Liu et al. (2016); Matthaus and Özcan (2014); Neilla et al. (2015); Syrpas et al. (2020); Uysal and Aktumsek (2015); Xia et al. (2010)
Raffinose	*P. discolor*	Wang et al. (2019)
Sucrose	*P. discolor*	Wang et al. (2019)
Vemifoliol (Blumenol A)	*P. chinensis*	Liu et al. (2009)
(6S,9R)-Vomifoliol-9-*O*-β-xylsyl-(1′′→6′)-*O*-β-glucoside	*P. discolor*[b]	Wang et al. (2020a)
2-Pyrrolidone	*P. multicaulis*[b]	Jia et al. (2013)
Essential oil	*D. indica*, *P. grandiflora*	Falchero et al. (2009); Umesh and Thoppil (2006)
Acidic heteropolysaccharides	*D. indica*	Jiang et al. (2013)
N-hydroxyl-hexahydroazepin-2,4-diones	*P. multicaulis*[b]	Jia et al. (2013)
4-Acetylphenyl-β-D-glucoside (picein)	*P. multicaulis*[b]	Jia et al. (2013)
3,3′-diisobutyryl-2,6′-dimethoxy-4,6,2′,4′-tetrahydroxy-5,5′dimethyldiphenyl methane (ivesinol)	*I. gordonii (P. gordonii)*	Ahmed et al. (2014)
1,5-dihydroxy-2-(2′-methylpropionyl)-3-methoxy-6-methylbenzene	*I. gordonii (P. gordonii)*	Ahmed et al. (2014)
3-Hydroxy-4-Methoxy-benzaldehyde	*P. reptans*, *P. speciosa*	Uysal et al. (2017)
Benzyl-(6-*O*-α-l-rhamnosyl)-*O*-β-D-glucoside	*P. discolor*[b]	Wang et al. (2020a)
(1R,2S,4S,5R)-angelicoidenol-2-*O*-β-D-glucoside	*P. discolor*[b]	Wang et al. (2020a)

[a] Leaves.
[b] Whole plant.
[c] Fruits.
[d] Seeds.
[e] Flowers.
[f] Branches.

However, further studies should be carried out to determine compounds with the highest antimicrobial activities.

5.2.1. *In vitro experiments*

Mongolian flora consists of many species used in folk medicine. The antimicrobial screening revealed a moderate activity of crude ethanolic extracts from the aerial parts of *P. fruticosa* and *P. viscosa* against *Staphylococcus aureus* and *Micrococcus luteus*. Researchers identified that the *P. fruticosa* ethyl acetate fraction was responsible for its antimicrobial activity (Gonchig et al., 2008). These results correlate with a short report presented by Tomczyk et al. (2008), who tested the aqueous extracts from a total of nine species: *P. anserina*, *P. argentea*, *P. erecta*, *P. fruticosa*, *P. grandiflora*, *P. nepalensis* var. 'Miss Willmott', *P. recta*, *P. rupestris* (syn.: *Drymocallis rupestris*) and *P. thuringiaca*. Selected samples exhibited potent activity against *Helicobacter pylori*, while the effect on Gram-positive bacteria (*Staphylococcus aureus, Micrococcus luteus* and *Bacillus subtilis*) and fungi (*Candida albicans)* was moderate. Chinese authors (Wang et al., 2013) evaluated *P. fruticosa*, *P. glabra* and *P. parvifolia* leaf extracts for their phytochemical profiles as well as their antioxidant and antimicrobial activities. In comparison to the abovementioned report, the results revealed a wider antibacterial spectrum, including an inhibition of *Pseudomonas aeruginosa* (MIC from 6.25 to 25 mg/mL). Of all the species, *P. parvifolia* showed the most potent activity against all tested microorganisms and plant pathogenic fungi. Several other studies reported similar results for *P. fruticosa* aerial part extracts, reaffirming its antimicrobial spectrum against *Staphylococcus aureus, Candida albicans* and 4 phytopathogenic fungi: *Rhizoctonia cerealis*, *Botrytis cinerea*, *Alternaria alternata* and *Bipolaris sorokiniana* (Jurkštiene et al., 2011; Kang et al., 2013; Ma, 2014). Interestingly, an aqueous ethanol extract from the underground parts of *Duchesnea indica* exhibited potent anti-MRSA (methicillin-resistant *Staphylococcus aureus*) activity with an MIC of 2.71 ± 0.54 mg/mL (Zuo et al., 2008). Moreover, another study confirmed the antimicrobial effects against further bacterial and fungi strains – *Shigella flexneri, Microsporum canis* and *Aspergillus flavus*. However, all tested fractions were weaker than standard drugs (imipenem, miconazole and amphotericin B). Nevertheless, those results confirm the validity of ethnopharmacological use of *D. indica* in the treatment of skin inflammations (Khuda et al., 2012). *Staphylococcus aureus* is one of the main etiological factors in the development of mastitis, an inflammatory reaction that commonly occurs during lactation in women, as well in animals used in the production of dairy products. Bulgarian authors (Mincheva et al., 2019), based on an ethnopharmacological report, evaluated various *P. reptans* extracts against three strains of *Staphylococcus aureus* – ATCC 6538 P, FDA 209 and methicillin-resistant *Staphylococcus aureus* 1337. From all examined extracts and their fractions, the n-hexane fraction from the 70% ethanol extract of this *Potentilla* species demonstrated the most active potential against all tested strains, with an MIC value of 0.313 mg/mL against *Staphylococcus aureus* ATCC 6538 P. Water extracts from the underground parts of *P. erecta* and *P. alba* in a 1:20 dilution showed a moderate antimicrobial effect on *Staphylococcus aureus*, while the ethanol and acetone extracts were effective against *Escherichia coli* (Grujić-Vasić et al., 2006). Egyptian authors (Ahmed et al., 2014) noted that ivesinol, a new dimeric acylphloroglucinol isolated from *Ivesia gordonii* (syn. *P. gordonii*), showed potent activity against the antibiotic-resistant bacteria *inter alia* methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*, with MIC values of 0.5 and 1.25 μg/mL, respectively. However, antifungal activity in a moderate manner was confirmed only for the monomeric form of ivesinol against *Candida albicans*, *C. krusei* and *Cryptococcus neoformans*. It is worth mentioning that the screening of Mississippi river basin plants revealed that a prairie cinquefoil (*P. arguta*) seed extract possessed anti-yeast activity against *Candida albicans* in a manner similar to reference (Borchardt et al., 2009).

Rhizome and herb water extracts of *P. reptans* at the concentration ranging from 10 to 150 mg/mL exhibited potent activities against *Escherichia coli* and *Staphylococcus aureus*, which were similar to ceftriaxone. The effect was stronger for the rhizome samples than for the extracts prepared from the aerial parts (Tomovic et al., 2018). In northeast India, local tribes use *P. polyphylla* taproots in the treatment of wounds, burns and chew them for dental problems. An experiment designed to evaluate the antimicrobial effect against potential pathogens causing the abovementioned ailments was carried out. An aqueous

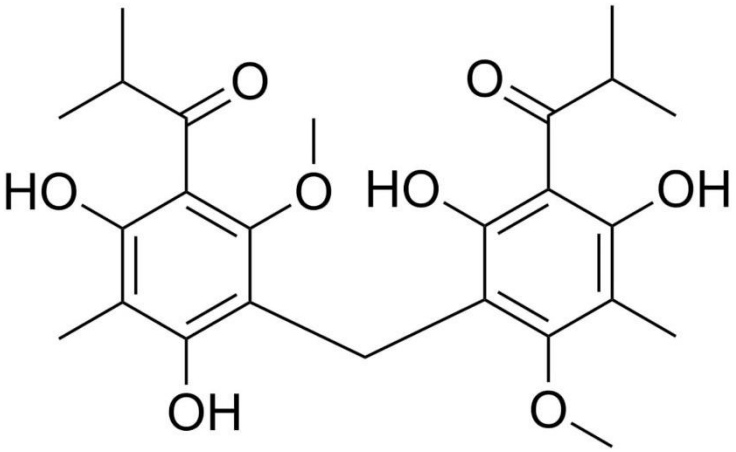

Fig. 3. Structure of ivesinol.

methanolic taproot extract showed zone inhibitions in a range from 17 to 30 mm and MIC values of 0.01, 0.4, 5 and 10 mg/mL against *Streptococcus mutans, Candida albicans, Staphylococcus aureus* and *Escherichia coli*, respectively. Obtained results indicated that tested extract exerted similar activity as reference drugs (Nakhuru et al., 2016). The authors of a recent patent submitted in the Republic of Korea showed that cosmetics containing a *P. kleiniana* leaf extract inhibited the growth of *Cutibacterium acnes*, a primary bacterial pathogen of acne vulgaris (Park et al., 2015). Further investigation by Xuan et al. (2019) revealed that the ethyl acetate fraction exerted the most potent inhibitory effect on *Staphylococcus aureus* with an MIC of 2.5 mg/mL, an MBC of 10 mg/mL and a zone diameter of 12 mm, similar to results obtained for methyl paraben. Substantial antimicrobial activities were also obtained against *Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*. Biological studies on *D. indica* undertaken by Korean authors showed that a water extract, apart from anti-inflammatory and anti-oxidant properties, also exerted excellent antibacterial activities against *Cutibacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis* with MIC values ranging from 78.1 μg/mL to 1.25 mg/mL. Therefore, this extract may be considered a candidate for cosmeceutical applications (Kang et al., 2016).

Gastric juice alkalization, as a result of urease activity, promotes *Helicobacter pylori* growth. In a study of 42 plants growing in the Czech Republic, antiurease activity was examined. *P. anserina, P. argentea, P. erecta, P. fruticosa* water extracts yielded an antiurease activity (72.7–73.7%) comparable to acetohydroxamic acid, used as a standard, while two other species, *P. arenaria* and *P. aurea*, moderately inhibited the enzyme (44.4% and 56.9%, respectively) (Hřibová et al., 2014).

TLC-Direct Bioautography is a useful analysis method enabling search for antibiotics in the complex plant matrix. Dichloromethane crude extracts of *P. collina, P. crantzii, P. erecta* and *P. megalantha* were chromatographed on silica layer, with individually optimized nonaqueous mobile phases. Direct bioautography revealed that selected fractions from every tested species inhibited *Bacillus subtilis* growth (Jóźwiak et al., 2016).

Glucosyltransferases are bacterial virulence factors produced by the cariogenic bacteria *Streptococcocus mutans* and *S. sobrinus*. Tomczyk and co-authors examined the antibacterial activity, inhibition of glucan synthesis and biofilm formation for ten *Potentilla* aerial part water extracts (*P. anserina, P. argentea, P. crantzii, P. erecta, P. fruticosa, P. grandiflora, P. nepalensis, P. norvegica, P. pensylvanica, P. thuringiaca*). All tested extracts, especially *P. fruticosa*, effectively inhibited glucan synthesis and biofilm formation of *S. mutans* (Tomczyk et al., 2010b). Similarly, in the following study, the ethyl acetate fraction exhibited the strongest anti-biofilm activity against *S. sobrinus* GCM 20381 among all tested *P. recta* extracts. The minimum biofilm inhibition concentrations for *S. mutans* were 6.25 and 25 μg/mL, respectively (Tomczyk et al., 2011). Interestingly, a hydrogel preparation of a *P. erecta* dry extract at a concentration of 2 mg/mL, i.e., lower than the minimum inhibitory concentration (6.4 mg/mL), strongly inhibited streptococci biofilm formation in a dose-dependent manner (Tomczyk et al., 2017). More substantial antibacterial and antibiofilm effects were obtained for *P. visianii* preparations. During biofilm formation, the most sensitive species was *Staphylococcus aureus* ATCC 25923, with a minimum biofilm inhibition concentration (BIC50) for the ethyl acetate extract of 1.5 μg/mL, while in a preformed biofilm, the most potent influence was observed for a methanol extract on *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 25922 strains (BIC50 were 1.1 and 3.8 μg/mL, respectively) (Radojević et al., 2018).

Neuraminidase, an influenza virus surface glycoprotein, is the most important target for new drug development directed against influenza viruses. *D. indica* is usually used in traditional medicine as a treatment for viral infections. Basing on this relevance, Tian et al. (2011) found that a *D. indica* herbal extract exhibited strong neuraminidase inhibition. Moreover, samples tested in concentrations of 25–250 μg/mL decreased the virus yield and increased the viability of infected MDCK cells. Simultaneously, other authors observed a suppressive effect of *Duchesnea indica* ethanol extract on herpes simplex virus-induced neuron injury through increased microglial apoptosis (Li et al., 2011). In another study, two novel non-sulphated acidic heteropolysaccharides, DIP_{30} and DIP_{60}, isolated from the aerial parts of *D. indica* exerted potent anti-varicella-zoster virus activity with half maximal effective concentration (EC_{50}) values of 265.2 ± 35.4 μg/mL and 325.6 ± 42 μg/mL, respectively (Jiang et al., 2013). In addition, a *D. indica* herbal preparation could express potential immunomodulatory activity with a dose- and a time-dependent increase in the viability and proliferation of mouse splenocytes and thymocytes, increasing proliferation by 18 and 35%, respectively (Ang et al., 2014).

5.2.2. In vivo experiments

Interestingly, one-day-old chicks experimentally infected with *Salmonella pullorum* were treated with a water extract from *P. kleiniana*.

Table 5
An overview on the current status of pharmacological evaluations and comparison to main ethnopharmacological uses of *Potentilla* species based on studies published between 2009 and 2020. For results obtained before 2009 see Tomczyk and Latté (2009).

Ethnopharmacological use	Effects *in vitro*	Effects *in vivo*	Clinical trials	References
Diabetes mellitus	Inhibition of α-amylase (*P. anatolica, P. argentea, P.aurea, P. discolor. P. fulgens, P. recta, P. reptans, P. thuringiaca*); inhibition of α-glucosidase (*P. anatolica, P. anserina, P. astracanica, P. argentea, P. discolor, P. inclinata, P. kleiniana, P. recta, P. reptans, P. thuringiaca*); inhibition of aldose reductase (*D. chrysantha, P. fulgens*); inhibition of advanced glycation end product formation (*D. chrysantha*); Protein tyrosine phosphatase 1B inhibition; promotion of glucose absorption and uptake (*P. discolor*)	Normal and alloxan-induced diabetic mice: inhibition of Aldose reductase and sorbitol dehydrogenase activity in liver, kidney and eye lens, increase of hexokinase activity (*P. fulgens*); Ob.-db and alloxan-induced diabetic mice: decrease of glucose serum levels; C57BL/6 mice: regulation of aminoacid, lipid and purine metabolism; streptozotocin-induced diabetes rats: decrease of fasting blood glucose levels, protective pancreas activity (*P. discolor*); Normal and alloxan-induced diabetic mice and streptozotocin-induced diabetic rat: decrease of glucose serum level, decrease of malondialdehyde concentrations, protective against diabetes-induced pancreas damages (*P. chinensis*)		Buchholz and Melzig (2016); Cheng et al. (2020); Grochowski et al. (2017); Kim et al. (2008); Kumar et al. (2013); Li et al. (2014); Liu et al. (2019); Lu et al. (2008); Majaw and Syiem (2016); Majaw et al. (2018); Qiao et al. (2011); Song et al. (2012); Şöhretoğlu et al. (2017, 2018); Sut et al. (2019); Syiem and Majaw (2010, 2011) Syiem et al. (2009); Tarr et al. (2013); Uysal and Aktumsek (2015); Uysal et al. (2019); Wang et al. (2019); Xu et al. (2017); Yang et al. (2010); Yang et al. (2020); Zhang et al. (2010b, 2012)
Bacterial infections	Moderate or potent activity against *Staphylococcus aureus*, MR *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium, Micrococcus luteus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Cutibacterium acnes, Helicobacter pylori*; suppresion of bacterial urease activity; inhibition of glucan synthesis, *Streptococcus mutans* and *Streptococcus sorbinus* biofilm formation (various *Potentilla* species)	One-day-old chicks: potent antibacterial effect against *Salmonella pullorum* and *Escherichia coli* (*P. kleiniana*)		Gonchig et al. (2008); Grujić-Vasić et al. (2006); Hríbová et al. (2014); Józwiak et al. (2016); Jurkštiene et al. (2011); Khuda et al. (2012); Ma (2014); Mincheva et al. (2019); Park et al. (2015); Radojević et al. (2018); Tomczyk et al. (2008, 2010b, 2011, 2017); Tomovic et al. (2018); Wang et al. (2013); Xuan et al. (2019); Zuo et al. (2008)
Fungi infections	Moderate or potent activity against *Aspergillus flavus, Candida albicans, C. krusei, C. neoformans, Microsporum canis* (various *Potentilla* species)			Ahmed et al. (2014); Borchardt et al. (2009); Jurkštiene et al. (2011); Kang et al. (2016); Nakhuru et al. (2016); Tomczyk et al. (2008); Wang et al. (2013)
Viral infections	Inhibition of neuroamidase activity and decrease of influenza virus yield; decrease herpes virus-induced injuries; potent anti-varicella-zoster virus activity (*Duchesnea indica*)			Jiang et al. (2013); Li et al. (2011); Tian et al. (2011)
Parasitosis	Paralysis and following death of cestode *Raillietina echinobothrida* and trematode *Gastrothylax crumenifer*, decrease of parasites enzymes activity (*P. fulgens*); moderate activity against *Plasmodium falciparum*, inhibition of protozoan xanthine oxidase (*P. evestita*)			Giri et al. (2013); Rauf et al. (2016); Roy et al. (2010)
Immunomodulatory activity	Immunomodulating activity in mice splenocytes and thymocytes (*D. indica*); increased pinocytic activity (*P. anserina*)	BALB/c mice: increase of IL-10 and IFN-γ serum levels; improvement of spleen and thymus indicies (*P. anserina*)		Ang et al. (2014); Chen et al. (2010)
Cancer	Anti-neoplastic activity against various cell lines (various *Potentilla* species)	Activity against tumours in mice (*D. indica*); mitigation of gastric tumour-initiating processes in mice (*P. fulgens*)		Chen et al. (2017); Ganguly et al. (2019); Hu et al. (2018); Jin et al. (2011); Kim et al. (2007, 2008); Kowalik et al. (2020); Li et al. (2009); Lei et al. (2016); Meng et al. (2020) Peng et al. (2007, 2008, 2009); Qiao et al. (2009); Radovanovic et al. (2013); Radhika et al. (2012); Rauf et al. (2015a); Shoemaker et al. (2005); Şöhretoğlu et al. (2015); Tripathy et al. (2015); Wan et al. (2016); Yang et al. (2019); Yu et al. (2018); Zhang et al. (2017,2018); Zhang et al., (2020)
Inflammations				

(*continued on next page*)

Table 5 (*continued*)

Ethnopharmacological use	Effects *in vitro*	Effects *in vivo*	Clinical trials	References
	Reduction of pro-inflammatory cytokines formation (various *Potentilla* species); inhibition of COX-2 and iNOS (*P. chinensis*, *P. discolor*, *P. erecta*, *P. fragarioides* var. *Major*, *P. glabra* var. *Mandshurica*, *P. supina*)	Anti-inflammatory activity in atopic dermatitis and asthma (*D. chrysantha*); mice with LPS-induced endotoxemia: reduction of iNOS, COX-2 activity and pro-inflammatory cytokines formation (*P. supina*); anti-inflammatory activity in ear/paw oedema (*D. indica*, *P. chinensis*, *P. evestita*, *P. reptans*); mice and rats: blocade of painful sensations (*P. evestita* fractions and isolated compounds)	Prevention of UV-induced skin inflammations, skin blanching effect (*P. erecta*, external application)	Bazylko et al. (2013); Choi et al. (2014); Chon et al. (2016) Hoffmann et al. (2016); Han et al. (2019); Khuda et al. (2014); Kim et al. (2020); Lee et al. (2012); Lee et al. (2017); Lim et al. (2009); Nam et al. (2017); Ökdem et al. (2018); Paduch et al. (2015); Rauf et al. (2014a, 2014b, 2015b); Tomczyk et al. (2013); Tomovic et al. (2015); Wölfle et al. (2017); Yang et al. (2008); Yoon and Lee (2014); Yun et al. (2010); Zhao et al. (2008)
Antioxidants	Antioxidative activity against $ABTS^{+}$, DPPH, FRAP assays (various *Potentilla* species extracts, fractions and isolated compounds); reduction of bisphenol-induced reactive oxygen species formation and following adipocytes cellular damages (*P. rugulosa*)			Choi et al. (2020); Damien Dorman et al. (2011); Gogoi et al. (2014); Gupta et al. (2016); Han et al. (2016); Hu et al. (2009, 2010); Jaitak et al. (2010b); Lincheva et al. (2017); Liu et al. (2016a, 2016b, 2018) ; Liu et al., (2018); Liu et al., (2016c); Liu et al., (2016a, 2016b); Murata et al. (2013); Savel'yeva et al. (2020); Seleshe et al. (2017); (Hu et al., 2010; Shuai et al. (2009); Sut et al. (2019); Wan et al. (2016); Wang et al. (2015); Zhao et al. (2013, 2020)
Protective against neuronal injuries	Neuroprotective properties in Alzheimmer disease SH-SY5Y cell model (*P. parvifolia*); protective against hypoxia in rat hippocampal and cortical neurons (*P. anserina*)	Prevention of cerebellum, spinal cord and retinal damage in rat following traumatic brain injury (*P. fulgens*)		Baloglu et al. (2019); Hüseyin and Engin (2017); Hüseyin et al. (2017, 2018); X.J. Qin et al. (2012); Sun et al. (2017); Yuan et al. (2017, 2018)
Wound healing		Enhance of wound healing in rats (*P. fulgens*)		Kundu et al. (2016)
Cardioprotective	Cardioprotective effect against acute hypoxia	Protective activity against acute myocardial ischemia in rats (*P. anserina*); ischemia/reperfusion injury in mice and rats (*P. anserina*, *P. reptans*)		Enayati et al. (2018, 2019); Li et al. (2009); Mironova et al. (2008, 2010); Murzaeva et al. (2013); Qi et al. (2011), Qin et al., (2012a); Zhang et al. (2019)
Hepatitis impairments	Inhibition of D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes (*P. anserina*); Decrease lipogenesis process, cellular damage and proliferation of HepG2 cell line (*P. chinensis*)	Hepatoprotective activity against LPS/D-galactosamine, ethanol induced injury and non-alcoholic fatty liver disease (*P. anserina*, *P. chinensis*)		Lin et al. (2014); Min et al. (2016); Morikawa et al. (2014, 2018); Pang et al. (2020); Wang et al. (2018); Wei et al. (2013)
Thyroid impairments			Efficacy in treatment of hypothyroic and toxic goiter, hypo- and hyperthyroidism, non-toxic goiter, nodular goiter and autoimmune thyroiditis (*P. alba*)	Hotsko et al. (2020); Kiselëva et al. (2012); Kvacheniuk and Kvacheniuk (2012); Pankiv (2012); Pankiv et al. (2017); Turchaninova (2014);
Respiratory diseases		Antitussive and expectorant activities in mice and guinea pigs (*P. anserina*)		Guo et al. (2016)

Herbal preparations exhibited strong antimicrobial activity against *S. pullorum* and *Escherichia coli* in chicken treated with a dose of 0.75 mL once a day for 3 consecutive days (Tao et al., 2012). Chen et al. (2010) found that the polysaccharide from *P. anserina* displayed potent immunomodulatory activity. An *in vitro* assay revealed that the polysaccharide stimulated pinocytic activity in treated macrophages. Moreover, immunosuppressed BALB/c mice treated with the polysaccharide had increased spleen and thymus indices, as well as acid phosphatase and lactate dehydrogenase activity and IL-10 and IFN-γ serum levels.

5.3. Anti-neoplastic activity

Neoplastic diseases are a significant cause of the worldwide death toll. This situation is mainly due to late-stage presentation of a tumour, problems with access to diagnosis and treatment or cancer drug resistance. Those problems are reported more frequently in countries with low-income. Also, the serious problem is the occurrence of severe side-effects during chemotherapy, leading to a decrease in patient compliance. Therefore there is a significant need for more efficient and safer drug candidates. Numerous experiments on *Potentilla* species has focused primarily on pharmacological effects *in vitro* against various cancer cell lines achieving promising results. However, future

phytochemical studies should focus deeply on isolation compounds responsible mainly for the anti-neoplastic activity of tested extracts.

5.3.1. In vitro experiments

Anal et al. (2014) observed significant activity of a *P. fulgens* dichloromethane-methanol extract at a dose of 100 μg/mL against the A-549 cancer cell line. Moreover, the methanol extract at the highest tested concentration (100 μg/mL) demonstrated a potent cytotoxic effect on Hep-2 and a weaker effect on OVCAR-5, A-549, PC-3 and SF-295 cancer cell lines. Methanolic fraction samples from *P. fulgens* root extract inhibited the growth of the MCF-7 cell line in the clonogenic assay in a dose-dependent manner. Moreover, the authors intraperitoneally transplanted Ehrlich ascites carcinoma cells into mice. Treatment with the extract of *P. fulgens* roots increased the survival of the animals due to the accumulation of protein p53 in cancer cells, leading to their increased apoptosis through Bax enzyme (Radhika et al., 2012). Further studies on the methanolic root extract fractionated into hexane, ethyl-acetate and n-butanol fractions showed a significantly higher cytotoxic action against MCF-7 and U87 cancer cell lines than normal cells. The ethyl-acetate fraction was the most potent fraction, with ursolic acid being the most active component (Tripathy et al., 2015).

Shoemaker et al. (2005) found that a crude herbal preparation from the aerial parts of *Duchesnea indica* demonstrated growth inhibition of LLC (murine Lewis lung carcinoma) and Panc 02 (murine pancreatic carcinoma) cell lines. In two consecutive studies, the *D. indica* phenolic fraction was attributed to exert significant cytotoxic effects against SKOV-3 ovarian, HeLa, C33A human and U14 mouse cervical cancer cell lines (Peng et al., 2008, 2009). Additionally, two triterpenes isolated from the herbal parts of *D. indica* demonstrated moderate cytotoxic activities against the carcinoma HeLa and L929 cell lines by the MTT assay (Qiao et al., 2009). In several other experiments, a *D. indica* aqueous ethanol extract attenuated cell invasion, motility, adhesion of human lung adenocarcinoma as well as human oral squamous carcinoma cell lines by the down-regulating activity of metalloproteinase-2 and urokinase-type plasminogen activator (Chen et al., 2017; Yang et al., 2019). More interestingly, a neutral polysaccharide isolated from this species revealed high antioxidant and anti-neoplastic activities against Hep-G2 and SKOV-3 cell lines, with half maximal inhibitory concentrations of 1.42 and 1.23 mg/mL, respectively (Xiang et al., 2019). It has been observed that the combination of the fungi *Ganoderma lucidum* and *D. chrysantha* together with ionizing radiation significantly increased the apoptosis process of the human leukaemia HL-60 cell line due to G1-phase arrest (Kim, K.C. et al., 2007, 2008). Chinese medicinal herbs, such as the abovementioned *D. chrysantha* and *D. indica,* are often combined in complex formulas. Anticancer activities were observed against various cell lines, such as Bel-7042 human hepatocellular carcinoma and Ishikawa human endometrial adenocarcinoma (Hu et al., 2018; Li, Z.L. et al., 2009). Brevifolincarboxylic acid isolated from *D. chrysantha* was tested for its effect on PC14 and MKN45 human carcinoma cell lines. The compound showed strong cytotoxic activity against both cell lines (Lee and Yang, 1994).

A study conducted using the HepG-2 carcinoma cell line proved that a *P. discolor* ethanol extract decreased cell viability in a dose-dependent manner. The authors showed an increase in apoptosis activity associated with increased caspase-3 activity (Jin et al., 2011). Additionally, ethyl acetate extracts obtained from two Turkish species, *P. recta* and *P. astracanica* reduced the viability of the Hep-2 cell line at a concentration of 100 μg/mL due to the high phenolic content (Şöhretoğlu et al., 2015). Chinese authors isolated three lupane-triterpenoid derivatives from a *P. discolor* whole plant ethanolic extract that showed cytotoxicity against MCF-7, T-84 and HepG-2 cell lines based on the Cell Counting Kit-8 assay. Furthermore, 3α-hydroxy-19α-hydrogen-29-aldehyde-27-lupanoic acid (in the further studies named as PDB-1) augmented HepG-2 cellular apoptosis in a dose-dependent manner, probably in association with increased ROS production, an induction of caspase-3 and caspase-9 and a lowering of Bcl-2 activity (Zhang et al., 2017). Further study indicated that in the A549 cell line the inhibition of PI3K/Akt/mTOR pathway had been responsible for the antitumor activity of PDB-1 (Zhang et al., 2020). In addition to this report, the triterpenoid mentioned before inhibited the proliferation of lung cancer A-549 and H460 cell lines with decreased proliferating cell nuclear antigen (PCNA) expression, and also downregulated the MMP-2 and MMP-9 pathway, which attenuated cancer cell migration and invasion (Meng et al., 2020).

The Fanbaicao oil (*Herba Potentillae Discoloris*), composed of 45 compounds, was evaluated for its cytotoxicity against the human hepatoma cell line HepG2. Investigated samples reduced neoplasm cell growth with an IC_{50} of 2.03 mg/mL. The authors also observed, in a dose-dependent manner, lower CDK4 expression and an upregulation of p21 and phosphorylated p21, increasing the apoptosis process in the analysed cell line (Lei et al., 2016). Another study screened the essential oil obtained from the underground parts of *P. discolor* against several cancer lines for its cytotoxic activity. The results demonstrated that the preparation in the investigated cell lines induced apoptosis by a mitochondrial intrinsic pathway, and the lowest IC_{50} value against T24 cell line was observed at a concentration of 19.02 μg/mL (Zhang et al., 2018a). The other apoptosis study conducted using salivary glands human mucoepidermoid carcinoma cell lines (MC3 and YD15) revealed that a *P. discolor* methanol extract induced p53 upregulated modulator of apoptosis (PUMA) expression with simultaneous phosphorylation reduction of STAT3 leading to cell growth inhibition and apoptosis (Yu et al., 2018). The MTT assay was performed using the 4T1 mouse breast cell line to evaluate the cytotoxic effect of aqueous extracts of *P. reptans* rhizome and aerial parts. Despite weak cytotoxicity of both samples (IC_{50} at 280.51 μg/mL and 310.79 μg/mL for rhizome and aerial parts extracts, respectively), the rhizome extract showed stronger antitumor activity than the herb infusion (Radovanovic et al., 2013). Uysal et al. (2017) found a highly time-dependent cytotoxicity of *P. reptans* extracts toward A549 and MCF-7 cell lines, while the *P. speciosa* effects were much weaker. Recently, Kowalik and co-authors (2020) found that selected extracts and fractions of *P. alba* significantly decreased proliferation of human colon cancer cell line HT-29, while the proliferation of human non-cancerous colon epithelial cells line was significantly promoted. Moreover, the extracts caused damage to the cell membranes in both cell lines, mildly decreasing their viability (Kowalik et al., 2020).

The human osteosarcoma MG63 cell line was treated with *P. chinensis* ethyl acetate root extract to evaluate its effect on apoptosis induction. The samples revealed a reduction of cell migration and higher dose-dependent cytotoxicity for the MG63 cell line than the fR-2 normal epithelial cell line. Moreover, phase contrast microscopy showed cell shape changes, such as volume reduction and membrane blebbing, and an increased number of total apoptotic cells in the Annexin V binding assay from a control value of 5.6%–24.2, 38.8 and 55.7% for concentrations of 40, 80 and 150 μg/mL, respectively. The authors suggested a G0/G1-cell cycle arrest as a responsible mechanism for the observed effects (Wan et al., 2016). Chrysin and umbelliferone isolated from *P. evesitita* exhibited a significant antitumor promoting effect in an Epstein-Barr virus early antigen activation assay (IC_{50} at 462 and 308 mol ratio/32 pmol TPA, respectively) (Rauf et al., 2015a).

5.3.2. In vivo experiments

The incidence of oral and gastric cancers in north-east India due to raw areca-nut and lime consumption is lower in people who consume *P. fulgens* root. An *in vivo* study with 160 mice demonstrated that the presence of a *P. fulgens* root preparation or a mixture of epicatechin, catechin, gallic acid and ursolic acid mitigated gastric diet induced tumour-initiating processes (Ganguly et al., 2019). Teng-Long-Bu-Zhong-Tang, a Chinese herbal formula, contains a *Duchesnea indica* water root extract. This extract combination has shown potent anticancer effects against a CT26 colon carcinoma cell line established in BALB/c mice and enhanced the anticancer effect of 5-fluorouracil (Deng et al., 2013). Moreover, the phenolic fraction of *D. indica* inhibited the growth of a U14 cell line transplanted into mice, and

induced antineoplastic activity through an increased cell-mediated and humoral immune response in mice (Peng et al., 2007).

5.4. Anti-inflammatory activity

A number of studies assessed the anti-inflammatory potential of *Potentilla* species, as well as the possible mechanism of action. Majority of studies revealed promising results, that may serve in future in the elaboration of new herbal-derived drugs, with fewer side effects than classic drugs, e.g. non-steroidal anti-inflammatory drugs. In the light of the discovery of urolithins, metabolites of ellagitannins, *in vitro* studies may not correlate with results obtained *in vivo*. However, the answer requires further research aimed at indicating compounds responsible for anti-inflammatory action and determining the correlation between observed effect *in vitro* and *in vivo* and the possible influence of microbiota on secondary metabolites present in *Potentilla* extracts.

5.4.1. In vitro experiments

An ethanolic extract from the whole plant of *Duchesenea indica* in the RAW264.7 cell line induced by LPS significantly reduced the production of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 and the secretion of IL-10, while the release of the anti-inflammatory mediator HO-1 was also slightly promoted (Zhao et al., 2008). Another species from this genus, *D. chrysantha*, inhibited the production and release of pro-inflammatory cytokines such as IL-6, IL-8 and monocyte chemotactic protein-1 in a human monocytic THP-1 cell line and an eosinophilic leukaemia EOL-1 cell line, and it might contribute to the attenuation of atopic dermatitis-like lesions (Lee et al., 2012).

A series of experiments were carried out using various aqueous extracts of *Potentilla* species (*P. anserina, P. argentea, P. crantzii, P. erecta, P. fruticosa, P. grandiflora, P. nepalensis, P. norvegica, P. pensylvanica, P. rupestris, P. thuringiaca)* to evaluate their biological activities in normal human colon cells. All the tested samples apart from showing potent free radical scavenging effects in a dose-dependent manner, especially at a lower concentration, decreased myofibroblast IL-6 and IL-10 formation. In contrast, in epithelial cells, this effect was achieved at higher concentrations. Moreover, the tested preparations had an impact on the cellular cytoskeleton organization (Tomczyk et al., 2013; Paduch et al., 2015).

Pre-treatment with fupenjic acid isolated from *P. discolor* strongly inhibited the LPS-induced inflammatory response while lowering the activity of cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS), resulting in reduced prostaglandin E_2 (PGE_2) and nitric oxide production (NO) as well as TNF-α and IL-6 levels (Yun et al., 2010). In similar experiments with LPS-induced RAW264.7 macrophages, a *P. supina* ethanolic extract as well as an ethyl acetate fraction significantly suppressed iNOS activity and NO and PGE_2 formation. An ethanolic preparation reduced the transcriptional activity and DNA-binding activity of NF-κB. This effect was achieved through a decrease in IKK-α/β, p38 kinase phosphorylation and a suppression of c-Jun and c-Fos expression in the nucleus (Lee et al., 2017; Nam et al., 2017). Recently, Kim and co-authors carried out an experiment focused on the potential anti-inflammatory mechanism of a *P. glabra* var. *Mandshurica* ethanol extract in both *in vitro* and *in vivo* assays. The herbal preparation applied to LPS-induced RAW264.7 cells inhibited Src kinase and the translocation of p50 and p65 into the nucleus and therefore decreased NFκB phosphorylation and thus mRNA expression levels of proinflammatory cytokines (TNF-α, IL-1β, IL-6) and iNOS. Moreover, an *in vivo* study in a mouse HCl/Ethanol generated acute gastritis model revealed that the ethanol extract significantly decreased inflammatory lesions at a dose of 150 mg/kg b. w., comparable to ranitidine (Kim et al., 2020). The schematic of the mechanism of action both *P. supina* and *P. glabra* is shown in Fig. 4.

Decreased NO production has also been noticed in a study on *P. fragarioides* var. *Major* extracts. The preparations exerted a potent and dose-dependent decrease in NO production, and as well an inhibition of the translocation of NF-κB to the nucleus (Choi et al., 2014). *P. recta* is traditionally used in the treatment of skin disorders. The study of the herbal preparation revealed strong antioxidant properties and significant hyaluronidase and lipoxidase inhibition activities of the ethyl acetate fraction. Agrimoniin isolated from the ethyl acetate fraction inhibited both enzymes to an even greater extent, partially explaining the beneficial effects of this plant (Bazylko et al., 2013). Moreover, Ökdem and co-authors found that a *P. recta* methanol herbal preparation decreased TNF-α and pro-inflammatory interleukin (IL-1β, IL-6) levels and resulted in significantly higher concentrations of pro-inflammatory IL-10 in the LPS-stimulated RAW 264.7 cell assay (Ökdem et al., 2018).

The *P. erecta* rhizome, which contains a rich tannin fraction with agrimoniin as the main compound, is well-known in traditional medicine as a drug with excellent anti-inflammatory properties. In a study involving fractions obtained from *P. erecta*, underground parts were enriched with agrimoniin and studied in HaCaT keratinocytes. The extract inhibited UV-B radiation-induced COX-2 expression and reduced the PGE_2 concentration (Hoffmann et al., 2016). Similarly, German authors (Wölfle et al., 2017) measured the levels of IL-6 and PGE_2 in irradiated or TNF-α-stimulated HaCaT cell lines after application of Tormentillae rhizoma (*P. erecta*) extract. The preparations strongly inhibited pro-inflammatory formation and NF-κB activation.

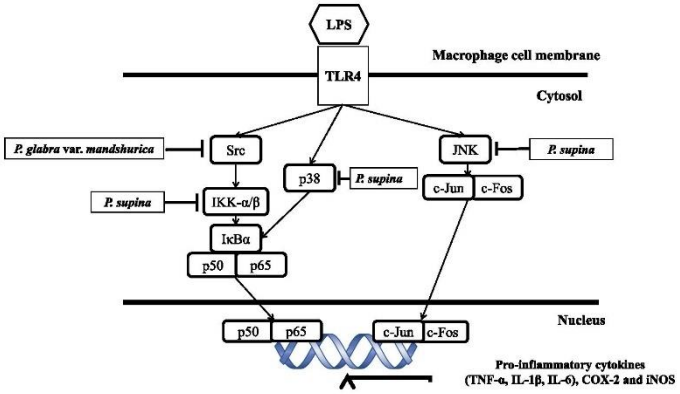

Fig. 4. Schematic representation of the anti-inflammatory mechanisms of action of *P. supina* and *P. glabra* var. *Mandshurica* ethanolic extracts on RAW 264.7 cell line.

The cosmeceutical industry is one of the fastest-growing sectors acquiring benefits for both health and aesthetics. A root extract from *P. chinensis* was examined as a promising cosmeceutical material. The tested sample decreased COX-2 and metalloproteinase-1 expression, melanin synthesis and stimulated elastin and aquaporin-3 synthesis (Chon et al., 2016).

5.4.2. In vivo experiments

Atopic dermatitis is a chronic inflammatory skin disease, and phosphodiesterase 4 (PDE4) plays an important role in its development. Therefore, PDE4 inhibitors are promising therapeutic agents. Yoon and Lee (2014) selected 51 plant species and studied their ethanol extracts using phosphodiesterase 4 and 7 inhibitory assays. Among them, *Duchesnea chrysantha* had potent activity against the enzymes tested. A further *in vivo* experiment showed a decrease in histamine release and IgE serum levels. An 80% ethanol extract obtained from *D. chrysantha* has been studied for anti-inflammatory activities in BALB/c mice in an induced asthma model. The results showed that the preparation inhibited the expression of eotaxin, IL-5, mucus production and the migration of inflammatory cells, especially eosinophils, which play a key role in the pathogenesis of asthma. Moreover, ovalbumin-specific IgE levels were decreased in both serum and bronchoalveolar lavage (Yang et al., 2008). The previously mentioned *P. supina* ethanol extract, administered to mice with LPS-induced endotoxemia, reduced the level of pro-inflammatory interleukins, iNOS, COX-2 and increased their survival (Lee et al., 2017).

In 2009, Lim et al. (2009) evaluated the impact of a *P. chinensis* water extract on paw oedema volume. At oral doses from 50 to 200 mg/kg b. w., the extract showed significant anti-inflammatory activities by decreasing the paw volume and vascular permeability. Furthermore, a dose of 100 mg/kg b. w. Decreased the activity of PAR2 agonist-induced myeloperoxidase. Similarly, a *Duchesnea indica* crude extract also exerted anti-inflammatory activity against carrageenan-induced hind paw oedema, and an ethyl acetate fraction *in vitro* significantly inhibited lipooxidase activity (Khuda et al., 2014). In another experiment, the contents of polyphenols and flavonoids in aqueous extracts from rhizomes of *P. reptans* exhibited strong antioxidant activity and suppression of the inflammatory process at a dose of 10 mg/ear in mouse phenol-in-acetone induced ear oedema (Tomovic et al., 2015). A recent study conducted using mice with streptozotocin and high-fat diet-induced diabetes treated with a *P. discolor* water extract, demonstrated a significant decrease in LPS faecal and serum levels and of IL-1β, IL-6 and TNF-α serum levels. This activity resulted from modulatory activity of the gut microbiota, which improved the function of the mucosal barrier, increased short-chain fatty acid production and G-protein-coupled receptor 41 and 43 expression and alleviated the intestinal inflammation process mediated via the down-regulation of TLR4, MyD88 and NFκB (Han et al., 2019). In three consecutive studies, Pakistani authors found several activities of selected extracts, fractions and isolated compounds – acacetin, chrysin and umbelliferone – from *P. evestita* in various rodent models. Briefly, researchers using the formalin test and acetic acid-induced writhing test demonstrated a potent blockade of painful sensation at doses of 5 and 10 mg/kg b. w. For all the above mentioned compounds. Moreover, the chloroform fraction and one isolate of this extract, acacetin, exhibited strong antipyretic effects in the yeast-induced fever assay at doses of 50 and 100 mg/kg b. w. and of 5 and 10 mg/kg b. w., respectively. The anti-inflammatory potential was measured for chrysin and umbelliferone with the carrageenan-induced paw oedema method. Both compounds at doses of 5 and 10 mg/kg b. w. Displayed an amelioration of the paw oedema volume after 4 h and 3 h of administration, respectively. Furthermore, an *in silico* docking study revealed that the possible anti-inflammatory effect was associated with a strong interaction of chrysin with COX-2 (Rauf et al., 2014a, 2014b; 2015b).

5.4.3. Studies in humans

Recently, Hoffmann et al. (2016) studied the agrimoniin-enriched fraction obtained from *P. erecta* in a placebo-controlled UV-erythema test after external application. The anti-inflammatory effect of the richest fraction was determined in 8 healthy volunteers. It was demonstrated that the tested samples inhibited the ultraviolet-induced inflammation process. A further study evaluated the vasoconstrictive properties obtained from Tormentillae rhizoma. The occlusive patch test was carried out in 40 healthy volunteers in comparison to 1% hydrocortisone. The extract exerted a blanching effect comparable to hydrocortisone. The vasoconstrictive effect was partly attributable to the inhibitory effects on endothelial nitric oxide synthase (eNOS) and the scavenging abilities to neutralize NO without any impact on the nuclear translocation of the glucocorticoid receptor (Wölfle et al., 2017).

Ellagitannin-rich plant materials are traditionally used to treat the inflammatory process in the gastrointestinal tract. Piwowarski et al. (2014) demonstrated that after oral application, human intestinal microbiota metabolize ellagitannins from both *P. anserina* and *P. erecta* to urolithins, present in investigated human faecal samples, which exert a potent anti-inflammatory effect.

5.5. Antioxidative, protective and wound healing activity

Reactive oxygen species are one of the main factors in the pathogenesis of various diseases, e.g. atherosclerosis, cancer, diabetes mellitus, inflammatory diseases. Polyphenolic compounds widely present in *Potentilla* genus carry potent antioxidant capacity and thus protective effects by preventing ROS-generated lipid and protein peroxidations and therefore the death of the cell. Interestingly, prominent antioxidative and protective effects were also exerted by polysaccharides isolated from *P. anserina*. However, despite obtaining promising effects in both *in vitro* and *in vivo* assays, the experiments concerning the impact of antioxidants derived from *Potentilla* species and their possible protective effect on human health are still missing.

5.5.1. In vitro experiments

On several occasions, the antioxidative characteristics of *P. fruticosa* have been reported. Chinese authors using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^+$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) assays, isobolographic analysis and a microbial test system reported that a *P. fruticosa* leaf extract combined with *Ginkgo biloba* and green tea polyphenols displayed strong synergistic antioxidant activity. These results correspond to the high contents of tannins and flavonoids, especially to the high contents of (+)-catechin, ellagic acid, rutin and hyperoside. A few reports have revealed the impact of environmental factors on the active component concentration and antioxidative activity (Liu et al., 2016a, 2016b; 2018; Liu et al., 2016a, 2016b; 2018; Luo et al., 2016; Wan et al., 2016; Wang et al., 2015). Yu et al. (2016) found that the total polyphenol content in *P. fruticosa* organs correlates with their ability to sweep free radicals, with the highest activity determined for the aerial parts. Indian authors selected three medical plants traditionally used in Indian folk medicine and observed that the radical scavenging properties of a *P. polyphylla* aqueous methanolic extract on ABTS and DPPH radicals were stronger than ascorbic acid (Gogoi et al., 2014). In another study, the *P. fulgens* butanol fraction together with catechin and a newly isolated biflavonoid - potifulgene - exerted potent scavenging activity in terms of TEAC (Trolox equivalent antioxidant capacity) using $ABTS^+$, DPPH and FRAP assays (Jaitak et al., 2010b). Similar activity has also been observed for *D. chrysantha*, *D. indica*, *P. alba*, *P. approximata*, *P. argentea*, *P. atrosanguinea*, *P. canescens*, *P. paradoxa*, *P. parvifolia*, *P. recta*, *P. reptans* and *P. tergemina* (Damien Dorman et al., 2011; Gupta et al., 2016; Hu, W. et al., 2009; Lincheva et al., 2017; Murata et al., 2013; Savel'yeva et al., 2020; Seleshe et al., 2017; Sut et al., 2019). Food containers and water bottles are commonly produced from polycarbonate polymers. The monomers of this polymer are

bisphenols. Bisphenols A, F and S induced the adipogenesis process through increased lipid and reactive oxygen species production. A *P. rugulosa* leaf extract was investigated for its antioxidant and protective activities in the 3T3-L1 cell line against bisphenol-induced cellular damage. The authors reported that the herbal preparation exhibited potent antioxidant properties due to a high content of total phenolics and flavonoids and a restrained bisphenol-induced lipid accumulation, which led to reduced formation of reactive oxygen species. The inhibition of the adipogenesis process was also achieved through decreased expression of the transcription factors PPAR-γ, C/EBP-α, and aP2 (Choi et al., 2020). Antioxidant and radical scavenging properties have been verified individually for the flavonoids occurring in the highest concentrations in *P. glabra*, which are commonly also present in other *Potentilla* species: kaempferol, quercetin, rutin and isoquercitrin using FRAP and DPPH assays. The observed effect for all flavonoids in both tests was stronger than for ascorbic acid (Han et al., 2016). Selenium polysaccharides are excellent polymers with high activity and low toxicity. Zhao et al. (2013) modified *P. anserina* polysaccharide by the nitric and selenious acid method with the assistance of microwaves. Data obtained in this study showed an enhancement of antioxidant activity in comparison to the non-modified polysaccharide. Recent work has also reported that a sulphation process of the crude polysaccharide fraction augmented its antioxidant activity, as measured with the help of the DPPH, FRAP, ferrous ion chelating (FIC), hydroxyl radical (HORAC) and superoxide radicals assays (Zhao et al., 2020). Furthermore, a study conducted on a non-modified polysaccharide extracted from silver cinquefoil roots (*P. anserina*) at concentrations of 50–400 μg/mL decreased murine splenic lymphocytes apoptotic cell formation in a dose-dependent manner by reducing oxidative stress damage induced by hydrogen peroxide (Hu et al., 2010; Shuai et al., 2009). Moreover, polysaccharides from underground parts of *P. anserina* augmented antioxidant activity and thus increased cell viability of RAW264.7 cell line after exposure to H_2O_2 (Huber et al., 2007). Additionally, tormentoside, a triterpenoid present *inter alia* in *P. anserina* strongly attenuated apoptosis induced by hydrogen peroxide oxidative stress in H9c2 cardiomyocytes by reducing the expression of caspase cascade pathway proteins, increasing the Bcl-2 level and pCryABS59 activity followed by a decrease in oxygen radical production (Zhang et al., 2018b). Recently, in two consecutive studies, Chinese authors confirmed that kaji-ichigoside F1 and rosamultin acid, as well as their aglycones euscaphic acid and tormentic acid isolated from silver cinquefoil roots, exerted anti-hypoxic activity on the EA. hy926 cell line through activation of the ERK1/2 pathway, which upregulated the NFκB and downregulated the PI3K/AKT signalling pathway (Shi, C. et al., 2020a, 2020b) In addition to these reports, a *P. anserina* n-butanol fraction was also investigated for its effect in hypoxia injuries. The results demonstrated that the tested extract markedly increased human umbilical vein endothelial cell (EAhy926) and primary cultured rat hippocampal neuron viability by amelioration of cell metabolism with inhibition of the caspase cascade pathway (Liu et al., 2011; Qin et al., 2012a). Furthermore, in 2017, Chinese authors tested a *P. anserina n*-BuOH fraction and demonstrated a neuroprotective effect against neuronal cell death induced by oxygen deprivation. They explained this effect by a participation of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α (Sun et al., 2017). A methanol extract from *D. indica* exerted a cytoprotective effect on human skin fibroblasts (CCD-986Sk) in H_2O_2-induced injury. Further investigation led to the conclusion that the herbal sample enhanced protection against oxidative stress and inhibited cell apoptosis by suppression of Bax gene (Hu et al., 2011).

A series of altogether 6 triterpenic acids and 7 flavonoids isolated from the aerial parts of *P. parvifolia* was tested for neuroprotective effects. The human neuroblastoma SH-SY5Y cell line was treated with all trans-retinoic acid and okadaic acid to establish an Alzheimer disease cell model. Western blot and morphology analyses suggested that corosolic acid, euscaphic acid, platanic acid and pomolic acid, as well as quercetin and tiliroside, had the most potent neuroprotective effects (Yuan et al., 2017, 2018).

5.5.2. In vivo experiments

A number of authors have recognized a protective effect of *P. fulgens* against selected ischemia-reperfusion injuries. It has been found that a herbal preparation administered intraperitoneally for 5 days before injury at a dose of 400 mg/kg b. w. Decreased cellular apoptotic development in the rat intestine, testicles, tuba uterine and ovary (Acar et al., 2016; Karabulut et al., 2016; Toğrul et al., 2015; Tunç et al., 2015). Traumatic head injury leads to a considerable number of patients with permanent disability, or even to death. Turkish authors evaluated the neuroprotective effect of *P. fulgens* against cerebellum and retinal damage induced by traumatic head injury in various rat models. Biochemical, histopathological and immunohistopathological analyses revealed that catalase, glutathione peroxidase and superoxide dismutase activities were increased in tissues after treatment with the herbal preparation (Hüseyin and Engin, 2017; Hüseyin et al., 2017, 2018). Those results correlate with another study, which reported a remarkable augmentation of the development of bipolar and multipolar nerve cells and vascular structures in the injured rat spinal cord after a 7-day administration of a *P. fulgens* preparation (Baloglu et al., 2019).

In 2012, Saio et al. (2012) found that a methanol extract of *P. fulgens* ameliorated the diabetic-induced increase in oxidative stress markers in mice with alloxan-induced diabetes. Similarly, in another study, the dichloromethane-methanol fraction alleviated oxidative stress in aging male mice by protection against oxidative stress and an increase in antioxidant defence (Saio et al., 2016).

Interestingly, *P. fulgens* preparations, in a rat tibial defect model, exerted a protective effect on new bone formation and supported osteocyte development and osteoblast matrix secretion after 14 and 28 days of treatment (Koparal et al., 2016). A previous study by the aforementioned Korean authors conducted on skin of hairless mice demonstrated a decrease in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced H_2O_2 production by *Duschesnea indica* methanol extract (Hu et al., 2011).

Numerous *in vivo* studies have investigated the antioxidant and protective effect of *P. anserina* polysaccharide. In 2009, Hu and co-authors found that a 7-day administration of this polysaccharide at doses from 50 to 200 mg/kg b.w. reversed dexamethasone-induced oxidative stress in mice by increasing the endogenous antioxidant capacity level and the thymus and spleen indices (Hu et al., 2009Hu, T.J. et al., 2009). In another study, pre-treatment with the polysaccharide fraction in the presence of cadmium significantly improved redox homeostasis in *in vitro* and *in vivo* assays, and also protected against apoptosis. Moreover, cadmium-induced mitochondrial dysfunction, kidney degeneration and fibrosis effects were also reversed by the polysaccharide fraction (Shen et al., 2017). Recently, Chinese authors explored the potential mechanism of the polysaccharide in high altitude cerebral oedema treatment. The results demonstrated that the tested samples in both rat and mouse high altitude cerebral oedema models exerted a protective effect by a decrease in brain water content and of tissue injuries, reduction of oxidative stress and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) levels and blockade of HIF-1α and NF-κB signalling pathway activation (Shi et al., 2020Shi, J. et al., 2020).

P. fulgens roots are traditionally used in northern India as a remedy to treat animal-generated wounds. Based on these ethnopharmacological reports, Kundu et al. (2016) demonstrated that the topical administration of a crude root extract and an ethyl acetate fraction enhanced the wound healing process in experimental rats. The effect was demonstrated by a significant increase in the content of hydroxyproline, hexosamine, total protein and total DNA, as well as morphologically through re-epithelization of the wounded area.

5.6. Effect on the cardiovascular system

5.6.1. In vitro experiments

In a series of experiments, Russian authors found that the *P. fruticosa* flavonoid fraction at doses from 0.05 to 3 μg/mL increased ATP-dependent K^+ transport in a study on isolated rat heart and liver mitochondria. The authors suggested that this extract exerted its protective effect against hypoxia through activation of the mitochondrial ATP-dependent K^+ channel and decreased formation of reactive oxygen species. Further, an *in vivo* assay in a rat model revealed the protective effect of this extract at a dose of 20 mg/kg b. w. under acute hypoxic conditions (Mironova et al., 2008, 2010; Murzaeva et al., 2013). Polysaccharides isolated from *P. anserina* roots were investigated for an antiaggregatory effect. This fraction exhibited weak to moderate inhibitory activity in a concentration-dependent manner on ADP-induced platelet aggregation at concentrations from 6.25 to 50 mg/kg (Guo et al., 2014).

5.6.2. In vivo experiments

The cardioprotective effect of selected *P. anserina* extracts has been reported. First, Qi et al. (2011) investigated the impact of an ethanolic extract on myocardial function after acute myocardial ischemia in rats. Pre-treatment with this herbal preparation decreased the ST-segment elevation, lactase dehydrogenase (LDH) and creatine kinase (CK) serum activities and significantly improved cardiomyocyte function. Then, in 2012, other authors validated that treatment with an ethanol extract at doses of 1.8 and 3.6 g/kg b.w. exerted a significant decrease in caspase-3 and caspase-9 protein and mRNA expression (Qin et al., 2012b). In another study, Chinese authors determined a significant protective effect of the *P. anserina* n-butanol fraction in ischemia/reperfusion injury in mice. A further experiment led to the isolation of 14 compounds that showed a significant increase in rat cardiomyocyte survivability through decreased cell apoptosis. The possible underlying mechanism involves an increase in catalase, superoxide dismutase, and glutathione activities and decreased malondialdehyde formation. Furthermore, expression of Cyt C, cleaved caspase-3 and alpha B-crystallin (CryAB) were decreased, while phosphorylated alpha B-crystallin (pCryAB Ser 59) levels were increased (Li et al., 2009; Zhang et al., 2019).

Recently, Iranian authors investigated the potential cardioprotective and antiapoptotic activities of a polyphenolic fraction of *P. reptans* roots in an isolated rat heart ischemia/reperfusion model. Isolated rat hearts were subjected to 30 min of ischemia and 100 min of reperfusion. The administered preparation before the main ischemia significantly decreased arrhythmia scores, infarct size and the ventricular fibrillation incidence, while hemodynamic parameters were augmented. The cardioprotective effect was mediated through augmentation of endogenous oxidant scavenging abilities, suppression of nuclear factor erythroid 2-related factor 2 (Nrf-2) and reduction of apoptotic indices (Enayati et al., 2018, 2019). A polyphenol-rich extract from the underground parts of *P. erecta* had an effect on the arachidonic acid cascade, which also affected the thrombotic process. It has been found that a 14-day administration of a Tormentil extract (400 mg/kg b. w.) significantly reduced thrombus formation and prolonged the time to carotid artery occlusion without changes in blood pressure. An *ex vivo* experiment revealed reduced thromboxane synthesis and decreased tissue plasminogen activator activity (t-PA), without changes in the total t-PA concentration (Marcinczyk et al., 2017).

5.7. Hepatoprotective effect

5.7.1. In vitro experiments

A hepatoprotective effect was observed for a methanolic extract of *P. anserina* roots. A study led to the isolation of 32 known compounds and 6 new triterpenes (potentillanosides A-F). Four compounds (potentillanoside A, rosamultin, kaji-ichigoside F1, 28-*O*-β-D-glucopyranosyl pomolic acid) exhibited *in vitro* cytoprotective effects on mouse hepatocytes against D-galactosamine-induced cytotoxicity. Moreover, application of the *in vivo* LPS/D-galactosamine liver injury assay confirmed the hepatoprotective properties for the first three compounds at oral doses ranging from 50 to 100 mg/kg b. w. (Morikawa et al., 2014). A further study on this extract led to the isolation of two new ellagic acid glycosides, named potentillanosides G and H, respectively, of which the second molecule showed hepatoprotective activity in the *in vitro* assay (Morikawa et al., 2018).

In an *in vitro* study conducted on an oleic acid-induced steatosis HepG2 cell line, asiatic acid isolated from *P. chinensis* decreased the process of lipidosis and cellular damage and demonstrated a potent inhibition of proliferation. Furthermore, the process of lipogenesis was reduced by the down-regulation of genes encoding carboxylase, fatty acid synthase, liver X Receptor Rα and sterol regulatory element-binding protein 1c and up-regulation of that encoding AMP-activated protein kinase (Wang et al., 2018).

5.7.2. In vivo experiments

Studies performed in rats treated with ethanol and asiatic acid derived from *P. chinensis* demonstrated a hepatoprotective effect against ethanol-induced injury. The studies showed that this effect was achieved through various mechanisms, such as decreased malondialdehyde and myeloperoxidase formation and enhanced antioxidant capacities, by recruiting essential antioxidant enzymes. Furthermore, asiatic acid decreased liver TNF-α and IL-1β levels and iNOS and COX-2 expression and inhibited Kupffer cell activation by down-regulating TLR4, CD14 and MyD88 expression and decreasing the endotoxin plasma level (Wei et al., 2013). Then, in 2018, Wang et al. (2018) carried out a further study on asiatic acid, which showed a further protective effect against non-alcoholic fatty liver disease by reducing endoplasmic reticulum stress, lipid deposition and hepatocyte apoptosis. Furthermore, it has been found that asiatic acid through reduction of endoplasmic reticulum stress and autophagy stimulation significantly mitigated LPS/D-galactosamine induced acute liver injury in mice (Pang et al., 2020). Lin et al. (2014) reported that tormentic acid isolated from *P. chinensis* significantly averted LPS/D-galactosamine induced fulminant hepatic failure through various mechanisms, such as a decrease in bilirubin, serum alanine aminotransferase and aspartate aminotransferase levels, suppression of lipid peroxidation, reduction of the proinflammatory cytokines TNF-α and IL-6 with increasing IL-10 levels, increase in HO-1 expression, the inhibition of NFκB, resulting in lower iNOS expression and finally increasing Bcl-2 with simultaneous cytochrome c and caspase-3,8,9 activity reduction.

More interestingly, the polysaccharide fraction from *P. anserina* exerted a potent hepatoprotective effect against acute injury induced by D-galactosamine after intraperitoneal administration. The authors found that 7 days of administration of the studied fraction at concentrations ranging from 50 to 200 mg/kg b. w. led to increased activity of hepatic antioxidant defence by increasing the activity of superoxide dismutase, glutathione peroxidase, and the glutathione level, while the malondialdehyde concentrations were decreased (Min et al., 2016).

5.8. Effect on the thyroid gland

In Eastern Europe, in the traditional medicine aerial and underground parts of *P. alba* (white cinquefoil) are commonly used alone or as a part of comprehensive therapy in the treatment of hyperplastic and autoimmune diseases of the thyroid gland (Pankiv, 2020). Several clinical studies have evaluated the influence of herbals on the thyroid gland. Ukrainian authors evaluated the clinical efficacy of a *P. alba* root extract in 46 patients with toxic goitre. Addition of the herbal preparation to the basic treatment according to international guidelines resulted in a marked improvement in the structure of the thyroid gland, a significant increase in thyroid-stimulating hormone (TSH) levels and a reduction of the concentration of antibodies against TSH receptor (AB-r

TSH) after 3 months (Pankiv, 2012). In another study, 55 patients with hyperthyroidism received the abovementioned extract for 6 consecutive months. The results showed a reduction of the thyroid gland volume, an improvement of its function and a decrease in AB-r TSH levels (Kiselëva et al., 2012). Moreover, Kvacheniuk and Kvacheniuk (2012) reported a possible positive effect of this phytotherapy in the treatment of toxic and hypothyroid goitres. A further study also revealed that children treated orally with a *P. alba* root extract displayed normalized thyroid gland function and a reduction of the size of the gland (Turchaninova, 2014). A large clinical study conducted in different regions of the Ukraine involving a total of 1107 patients determined the dynamics of the thyroid gland volumes in patients with diffuse non-toxic goitre, nodular goitre and autoimmune thyroiditis after 6 months of monotherapy with a *P. alba* root extract. The authors found that in all tested groups of patients, the probability of achieving effects on gland volume reduction after treatment with the *P. alba* extract increased with a larger initial thyroid volume (Pankiv et al., 2017). Recently, Hotsko et al. (2020) evaluated the efficiency of a 3-month treatment of a herbal medicine containing a *P. alba* extract against autoimmune thyroiditis. Sixty patients divided into control and treatment groups were enrolled. A subgroup with subcompensated hypothyroidism showed a significant decrease in TSH and increase in free thyroxine levels after the treatment. Moreover, the administration of this herbal drug was safe and well tolerated. Those prominent results greatly support the traditional use of white cinquefoil. However, further clinical studies are needed to confirm its mechanism of action and the beneficial effects on the thyroid gland, as well as to evaluate its safety. Furthermore, a phytochemical study should be assessed to determine the major components responsible for the observed effect.

5.9. Anti-anthelmintic, antimalarial effects

Based on ethnopharmacological reports, Indian scientists examined the anti-anthelmintic properties of *P. fulgens*. Roy et al. (2010) tested a crude ethanolic peel root extract against the cestode *Raillietina echinobothrida* and the trematode *Gastrothylax crumenifer*. At the highest concentration of 100 mg/mL, paralysis of the worms was observed after 2.00 ± 0.05 h and 1.21 ± 0.06 h, respectively. The death of the parasites was observed after 2.8 ± 0.06 h and 2.18 ± 0.04 h, respectively. Those results indicated mild to moderate anti-anthelmintic effect dependent on used concentrations in comparison to reference drug praziquantel. Moreover, the total enzyme activities of acid phosphatase, alkaline phosphatase and adenosine phosphatase were reduced significantly in both worm species compared with the positive controls. Three years later, a *P. fulgens* methanolic extract was assessed in *Raillietina echinobothrida* at various concentrations up to 10 mg/mL, leading to a paralysis of the cestode after 6.3 ± 0.04 h of exposition, followed by the death of the worm after 8 ± 0.06 h (Giri et al., 2013). Those research confirmed validity of traditional use *P. fulgens* against helminthiasis, however, this effect is milder, when we compare it to standard anti-anthelmintic drugs. Acacetin isolated from the chloroform fraction of *P. evestita* exhibited weaker antimalarial activity against *Plasmodium falciparum* (IC_{50} 55.14 ± 0.19 μM) compared with the control chloroquine diphosphate (IC_{50} 0.11 ± 0.07 μM). Moreover, the anti-inflammatory effect in malaria was enhanced by the inhibition of xanthine oxidase activity by acacetin, chrysin and umbelliferone, with IC_{50} values of 11.92 ± 0.01 μM, 73.74 ± 0.02 μM, and 97.12 ± 0.01 μM, respectively, compared with allopurinol as a control with IC_{50} values of 0.59 ± 0.01 μM (Rauf et al., 2016).

5.10. Other effects

Russian authors (Shikov et al., 2011) obtained interesting results when testing the influence of a *P. alba* rhizome extract on mice. The tested sample significantly increased the mouse swimming endurance time and exerted anxiolytic-like action with a predominant locomotor component. Ethanol and aqueous extracts and polysaccharides from *P. anserina* exhibited antitussive and expectorant activities in tested mice and guinea pigs. The authors suggested that the polysaccharides represented the main active fraction responsible for the observed effects (Guo et al., 2016).

6. Toxicity

Due to many ethnopharmacological reports on the internal and external use of the genus *Potentilla,* there is a need to investigate the safety of the corresponding preparations. Few studies have determined the acute toxicity with the help of animal models. A *P. fulgens* ethanolic extract using single doses up to 4000 mg per kg b. w. resulted in no signs or symptoms of acute toxicity in rats. Furthermore, intraperitoneal injection of a dose of 450 mg per kg b. w. of a methanolic root extract caused no mortality in albino mice (Kundu et al., 2016; Laloo et al., 2013; Syiem and Majaw, 2010). Similar research including a *P. mooniana* ethanol extract at a dose of 5000 mg/kg b. w. had no impact on toxicity or mortality in the tested rats (Laloo et al., 2014). However, a *P. fulgens* root extract led to the death of one mouse within 72 h at a dose of 3200 mg/kg b. w. (Tangpu et al., 2014). Administration of a water extract of *P. erecta* rhizomes led to the conclusion that single doses of the extract of 2.5 and 6.8 g/kg b. w. in rats and mice, respectively, are non-toxic and safe in a 2-week observation period (Shushunov et al., 2009). Additionally, an extract from *P. reptans* showed no toxic effects after an acute oral toxicity study and a repeated dose 28-day oral toxicity study in rodents (Mincheva et al., 2018). Recently, Chinese authors determined the safety of a *P. anserina* preparation. At a maximum oral dose of 345.6 g per kilogram b. w. administered within 12 h to Kunming mice, neither external toxic symptoms nor anatomical or histopathological changes in organs were observed after 2 weeks (Dram et al., 2020).

A large number of studies focused mainly on *P. alba* species. White cinquefoil aerial parts did not cause toxicity symptoms in any of the tested animals during the 3-month chronic administration trial. Oral acute toxicity studies demonstrated that these extracts were non-toxic (Bortnikova et al., 2019; Khishova et al., 2016). However, *P. alba* oral application had a negative impact on embryogenesis in antenatal and postnatal stages of development in the rat pregnancy model (Savinova et al., 2018). Immunotoxicity studies assessed that a white cinquefoil dry extract at a dose of 3 mg/kg b. w. stimulated a primary humoral immune response and did not affect the cell-mediated response (Bortnikova et al., 2018). From a cosmeceutical industry perspective, the application of a *Duchesnea indica* water extract did not induce any adverse reactions at a concentration of 100 μg/mL in a human skin primary irritation test performed in 31 healthy female Korean subjects (Kang et al., 2016).

Results of current toxicological studies indicated that *Potentilla* species are safe both in short and long-term usage. This state of knowledge is compatible with previous reports and as well as with traditional medicine that no toxic effects of administration of *Potentilla* species are known to occur.

Both n-butanol and water fractions of *P. fulgens* exerted potent antimutagenic activity in two strains of *Salmonella typhimurium* TA98 and TA100 in a study conducted using the Ames histidine reversion assay (Monga et al., 2014). In the other study, *D. indica* water extract demonstrated moderate antimutagenic activity against benzo-γ-pyrene-induced mutation (Lee and Lin, 1988).

7. Discussion regarding the updated profile of the genus *Potentilla*

Compared with the time prior to 2009, a vast amount of new data on the genus *Potentilla* was gathered all over the world. In the last decade, chemical analysis of *Potentilla* species led to the elucidation of more than 120 compounds for the underground parts – either new or new for the

distinct species – belonging to the flavonoids, hydrolysable and condensed tannins, triterpenes, organic acids and some other compounds with various structures. In 2009, only 66 structures were described for the underground parts of *Potentilla* species (Tomczyk and Latté, 2009). These recent findings extend the knowledge on the chemical constituents. Furthermore, the occurrence of isoflavones, coumarins and even anthraquinones in the roots and rhizomes, respectively, of the genus *Potentilla* could be shown in recent studies. It worth mentioning that the compounds published in the time before 2009 (i.e., 1950s till 2009) were isolated from approximately 11 *Potentilla* species, mainly *P. erecta*, *P. anserina* and *P. viscosa*. From 2009 to 2020, the new constituents were elucidated from 21 *Potentilla* species, especially *P. anserina* and *P. fulgens* and from *P. anatolica*, *P. argentea* and *P. recta*. Interestingly, no compounds were known for these *Potentilla* species apart from *P. anserina*. These findings imply that the focus on the chemical analysis of *Potentilla* species shifted within the last decade to further *Potentilla* species that had not been the subject of any analysis before 2009.

Regarding the phytochemical analysis of the herbal parts, a comparable shift to other *Potentilla* species for which no data on the constituents were available in 2009 is apparent. Whereas approximately 134 compounds from approximately 30 *Potentilla* species were known in 2009, with *P. erecta*, *P. anserina* and *P. fruticosa* as the dominating *P.* species, the research in the last decade has focused on approximately 40 *Potentilla* species, especially *P. discolor*, *P. recta* and *P. parvifolia*. It is worth mentioning that *Duchesnea indica* and *D. chrysantha,* as well as *Ivesia gordonii,* which are considered herein as *Potentilla s. l.*, have also been subjects for chemical analysis, especially the two *Duchesnea* species. Altogether, approximately 210 compounds – either new or new for distinct species – were elucidated in the last 10 years.

Regarding the pharmacological evaluation of extracts prepared from *Potentilla* species, a vast amount of new *in vitro* and *in vivo* studies (animal studies) was performed to confirm the traditional uses of the *Potentilla* species. Compared with 2009, the main focus in pharmacological research is still on testing for anti-neoplastic, antiviral and antimicrobial, antihyperglycemic, anti-inflammatory, hepatoprotective and antioxidative activities. Researchers worldwide have also addressed new activities, e.g., anthelminthic and antimalarial, wound-healing, and cardiovascular system effects, as well as the influence on the thyroid gland. However, anti-ulcerogenic and anti-diarrhoeic activities, especially described for *P. erecta*, were not subjects of pharmacological studies in the period from 2009 to 2020. In our review dating from 2009 (Tomczyk and Latté, 2009), pharmacological results were known for approximately 28 *Potentilla* species, mainly *P. erecta*, *P. anserina* and *P. fruticosa*. Currently, however, pharmacological data are published for approximately 42 *Potentilla* species, with *P. fulgens*, *P. anserina.*, *P. discolor* and *P. erecta* as the dominating *Potentilla* species. Additionally, several studies are available for *D. indica*. Thus *P. erecta* and *P. anserina* are still in the focus of pharmacological research, but a tendency toward an even broader testing of other *Potentilla* species can be observed.

In 2009 clinical studies were only known for extracts of *P. erecta* rhizomes with respect to effects on patients suffering from colitis ulcerosa and diarrhoea, respectively. Within the last decade, studies on healthy volunteers were performed with *P. erecta* rhizome extracts applied externally as an ailment for the cure of skin inflammation. Considering *P. erecta,* the mentioned studies confirmed the traditional use of the rhizomes of this *Potentilla* species in the treatment of inflammation, dysentery, diarrhoea and inflammatory bowel disease. However, more and larger studies are urgently needed to establish *P. erecta* rhizomes as a herbal remedy in light of the rational phytotherapy. *P. erecta* rhizomes have been evaluated by the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA). In 2010, a community herbal monograph was published by the HMPC for the rhizomes of *P. erecta* and preparations thereof together with an assessment report. Based on the available data and long-standing use, this expert group acknowledged the regulatory status of "traditional use" for *P. erecta* rhizomes and preparations thereof within the European Union for the symptomatic treatment of mild diarrhoea and minor inflammation of the oral mucosa (Committee on Herbal Medicinal Products, 2010a). In the assessment report (Committee on Herbal Medicinal Products, 2010b), the HMPC came to the conclusion that the published clinical studies provided only preliminary data for the mentioned indications and that the level of evidence did not support the regulatory status of "well-established use". Only a short time later, in 2013, the European Scientific Cooperative of Phytotherapy published a monograph on *P. erecta* rhizomes, and after considering the existing data, concluded that the efficacy in these indications (non-specific, acute diarrhoea, adjuvant treatment of acute and subacute enteritis and colitis, as well as slight irritations of the mucosa of mouth and throat) was plausible on the basis of human experience and long-standing use (ESCOP, 2013).

Within the last decade, the underground parts of *P. alba* have been extensively investigated with respect to effects on the thyroid gland. In traditional medicine of Eastern Europe, the aerial and underground parts of this *Potentilla* species are highly valued in folk medicine in the treatment of hyperplastic and autoimmune diseases of the thyroid gland. Several clinical studies, mainly conducted in the Ukraine, were performed with patients. Up to 6 months, promising results were obtained for patients suffering from different forms of thyroid gland disorders, i.e., toxic goitre, hyperthyroidism, hypothyroidism and auto-immune-thyroiditis. No clinical studies were performed on *P. alba* before 2009. Therefore, the underground parts of *P. alba* might become a valuable new treatment option derived from a *Potentilla* species. More clinical studies are needed to confirm the beneficial effects of *P. alba* on the thyroid gland.

Although *Potentilla* species have a long tradition in folk medicine, toxicological data are still rare. In 2009, very limited data on the toxicology of *Potentilla* species were available, and only for *P. erecta* and *P. anserina*. In the last decade, only few toxicological studies were performed, i.e., on *P. fulgens*, *P. mooniana*, *P. reptans*, *P. erecta*, *P. alba*, *P. anserina* and *D. indica*. It must be emphasized that most of these studies cover a single application of the corresponding extracts in animals, but studies on mutagenic effects, teratogenicity and repeated dose application schemes for periods up to 28 days are missing, among others. Therefore, more data on toxicological issues are necessary for a proper safety assessment of *Potentilla* species, although the longstanding use of these species provides a first hint that there are no toxicological risks.

It should be kept in mind that the underground parts of *P. erecta* were the first subjects of chemical analysis in the genus *Potentilla,* with the structural elucidation in the 1950s and first systematic pharmacological screening in the 1960s (Tomczyk and Latté, 2009). Thus, research on *Potentilla* species has proceeded extensively since these first studies, especially within the last decade, with a broad array of *Potentilla* species being investigated worldwide. All efforts demonstrate that *Potentilla* species are a rich source of a wide spectrum of natural compounds and that the extracts have activity toward many different pharmacological targets. Therefore, *Potentilla* species are an excellent example of the advancement of research with respect to phytochemical and pharmacological issues, and how knowledge on the genus *Potentilla s. l.* has improved within only a short time due to worldwide efforts.

8. Conclusions

In the modern world, despite the great advances in medicine and the pharmaceutical industry, a large proportion of humankind still has no access to novel therapeutic methods, mainly due to economic reasons. Therefore, many people, especially in rural areas, must still rely on the traditional use of plants characteristic of the local flora. It is also necessary to develop new safer treatment perspectives for a still growing group of patients with chronic diseases, such as diabetes mellitus, cardiovascular diseases and chronic inflammatory diseases.

Phytomedicines often have the advantage of fewer side effects compared with synthetic drugs. In addition, plant extracts contain a large number of compounds and therefore generally work on different targets concurrently. A steadily increasing number of structures isolated and identified from the genus *Potentilla* have provided essential information about the main constituents responsible for the plant-drug mechanism of action, such as the antihyperglycemic, antimicrobial, anti-inflammatory, and hepatoprotective activities. Pharmacological studies performed *in vitro* and *in vivo* have confirmed the effects reported in traditional medicine, as well as their low toxicity. However, these results may change in the future with recent findings concerning interactions among *Potentilla* ellagitannins, human metabolism and the human gut microbiota since comprehensive investigations *in vivo* concerning those relationships are missing.

Despite the great medical potential of *Potentilla* preparations, clinical studies are very limited. Results obtained from clinical studies to date during treatment with preparations from only two species, such as *P. erecta* and *P. alba*, indicate alternative therapies for gastrointestinal and thyroid gland disorders, respectively, as a complementary element of a comprehensive therapy. Moving forward, further work is needed in the fields of phytochemistry and pharmacology, as well as to address toxicological issues. These efforts will provide essential data on the clinical use of *Potentilla* preparations and will help to develop new, cheap and safe plant-based drugs, which are urgently needed, especially in the least developed countries. In addition, new plant-based medicines will also be greatly acknowledged by all patients who prefer alternative medicines.

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Rozdział 11. Oświadczenie autora rozprawy doktorskiej

Białystok, 17.03.2023 r.

mgr farm. Daniel Augustynowicz

Zakład Farmakognozji
Uniwersytet Medyczny w Białymstoku

Oświadczenie autora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Anticancer potential of acetone extracts from selected Potentilla species against human colorectal cancer cells. Frontiers in Pharmacology. 2022; 13, 1027315. DOI: 10.3389/fphar.2022.1027315*

wchodzącej w skład mojej rozprawy doktorskiej polegał na współtworzeniu założeń metodycznych i merytorycznych pracy, pozyskaniu i obróbce materiału roślinnego, wykonaniu ekstraktów do badań spektrofotometrycznych, analizie ilościowej metabolitów w ekstraktach metodami spektrofotometrycznymi, optymalizacji procesu rozdziału związków techniką LC-HRMS, analizie jakościowej techniką LC-HRMS w badanych ekstraktach, przygotowaniu ekstraktów do badań biologicznych, opracowaniu ostatecznej wersji manuskryptu, co określam jako **60%** udziału w przygotowaniu wyżej wymienionej publikacji.

2. *Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Phytochemical profiling of extracts from rare Potentilla species and evaluation of their anticancer potential. International Journal of Molecular Sciences. 2023; 24, 4836. DOI: 10.3390/ijms24054836*

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3. *Augustynowicz D., Latté, KP., Tomczyk, M. Recent phytochemical and pharmacological advances in the genus Potentilla L. sensu lato—An update covering the period from 2009 to 2020. Journal of Ethnopharmacology 2021; 266, 113412, DOI: 10.1016/j.jep.2020.113412.*

wchodzącej w skład mojej rozprawy doktorskiej polegał na polegał na stworzeniu koncepcji pracy, zgromadzeniu i zestawieniu dostępnej literatury, stworzeniu ostatecznej wersji manuskryptu, co określam jako **70%** udziału w przygotowaniu wyżej wymienionej publikacji.

Daniel Augustynowicz
.....
Podpis autora rozprawy doktorskiej(czytelny)

.....
Podpis promotora rozprawy doktorskiej(czytelny)

Rozdział 12. Oświadczenia współautorów rozprawy doktorskiej

Białystok, 17.03.2023 r.

dr hab. n. farm. Michał Tomczyk

Zakład Farmakognozji
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Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Anticancer potential of acetone extracts from selected *Potentilla* species against human colorectal cancer cells. *Frontiers in Pharmacology* 2022; 13, 1027315.
   DOI: 10.3389/fphar.2022.1027315

   wchodzącej w skład rozprawy doktorskiej Pana mgr farm. Daniela Augustynowicza polegał na współtworzeniu koncepcji pracy, analizie merytorycznej uzyskanych wyników, edycji finalnej wersji manuskryptu oraz pełnieniu funkcji autora korespondencyjnego.

2. Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Phytochemical profiling of extracts from rare *Potentilla* species and evaluation of their anticancer potential. *International Journal of Molecular Sciences* 2023; 24, 4836.
   DOI: 10.3390/ijms24054836

   wchodzącej w skład rozprawy doktorskiej Pana mgr farm. Daniela Augustynowicza polegał na współtworzeniu koncepcji pracy, współtworzeniu metodologii, analizie merytorycznej uzyskanych wyników, edycji finalnej wersji manuskryptu oraz pełnieniu funkcji autora korespondencyjnego.

3. Augustynowicz D., Latté KP., Tomczyk, M. Recent phytochemical and pharmacological advances in the genus *Potentilla* L. sensu lato—An update covering the period from 2009 to 2020. *Journal of Ethnopharmacology* 2021; 266, 113412.
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Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej prac przez Pana mgr farm. Daniela Augustynowicza jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

.....

Podpis (czytelny)

Lublin, 10.03.2023 r.

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Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Anticancer potential of acetone extracts from selected Potentilla species against human colorectal cancer cells. Frontiers in Pharmacology 2022; 13, 1027315. DOI: 10.3389/fphar.2022.1027315*

wchodzącej w skład rozprawy doktorskiej Pana mgr farm. Daniela Augustynowicza polegał na współtworzeniu metodologii badań oraz analizie przeżywalności, analizie wpływu na syntezę DNA oraz analizę cytotoksyczności wobec ludzkich komórek raka nabłonkowego okrężnicy (LS180) i ludzkich prawidłowych komórek nabłonkowych okrężnicy (CCD841 CoTr) traktowanych ekstraktami pozyskiwanymi z badanych gatunków, jak i również korekcie merytorycznej oraz edycji ostatecznej wersji manuskryptu.

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Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy/prac przez Pana mgr farm. Daniela Augustynowicza jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Marta Lemieszek

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mgr farm. Jakub Władysław Strawa

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Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Anticancer potential of acetone extracts from selected Potentilla species against human colorectal cancer cells. Frontiers in Pharmacology. 2022; 13, 1027315. DOI: 10.3389/fphar.2022.1027315*

wchodzącej w skład rozprawy doktorskiej Pana mgr Daniela Augustynowicza polegał na współtworzeniu metodyki analizy jakościowej techniką LC-HRMS, nadzorze merytorycznym wykonywanych analiz chromatograficznych oraz edycji finalnej wersji manuskryptu.

2. *Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Phytochemical profiling of extracts from rare Potentilla species and evaluation of their anticancer potential. International Journal of Molecular Sciences. 2023; 24, 4836. DOI: 10.3390/ijms24054836*

wchodzącej w skład rozprawy doktorskiej Pana mgr farm. Daniela Augustynowicza polegał na współtworzeniu metodyki analizy jakościowej techniką LC-HRMS, nadzorze merytorycznym wykonywanych analiz chromatograficznych oraz edycji finalnej wersji manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy/prac przez Pana mgr farm. Daniela Augustynowicza jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Strawa Jakub

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dr hab. Adrian Wiater, prof. UMCS
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Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Anticancer potential of acetone extracts from selected Potentilla species against human colorectal cancer cells. Frontiers in Pharmacology. 2022; 13, 1027315. DOI: 10.3389/fphar.2022.1027315*

wchodzącej w skład rozprawy doktorskiej Pana mgr Daniela Augustynowicza polegał na nadzorowaniu badań, korekcie merytorycznej oraz edycji ostatecznej wersji manuskryptu.

2. *Augustynowicz D., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Phytochemical profiling of extracts from rare Potentilla species and evaluation of their anticancer potential. International Journal of Molecular Sciences. 2023; 24, 4836. DOI: 10.3390/ijms24054836*

wchodzącej w skład rozprawy doktorskiej Pana mgr Daniela Augustynowicza polegał na współudziale w opracowaniu wyników, nadzorze projektu oraz edycji ostatecznej wersji manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy/prac przez Pana mgr Daniela Augustynowicza jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Wiater Adrian

Podpis (czytelny)

Berlin, March 6th, 2023

Dr. Klaus Peter Latté

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Co-author's name (first, middle, last)

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.....

Employment/affiliation

Co-author's declaration

I hereby declare that my participation in the preparation of the publication:

Augustynowicz D., Latté, KP., Tomczyk, M. Recent phytochemical and pharmacological advances in the genus *Potentilla* L. *sensu lato*—An update covering the period from 2009 to 2020. Journal of Ethnopharmacology. 2021; 266, 113412, DOI: 10.1016/j.jep.2020.113412.

Comprising a part of the doctoral dissertation of Mr Daniel Augustynowicz, MSc, consisted in * analysis and interpretation of literature data and critical revision of the manuscript.

I also agree that Mr Daniel Augustynowicz, MSc, presents the aforementioned work as a part of his doctoral dissertation comprising of a series of single-subject articles published peer-reviewed scientific journal.

Klaus Peter Latté

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Signature (legible)

for co-authored works included in the publication series, it is recommended that **the co-author declares their material (NOT percentage) work [e.g. formulation of hypothesis, initiation of the study, specific study tasks (e.g. specific experiments, data collection and processing, statistical summaries etc.), analysis of the results, preparation of the manuscript, etc.]. Identification of co-author's contribution should be sufficiently precise for an accurate assessment of their participation and role in each of the listed studies.*

Rozdział 13. Dorobek naukowy

Wykształcenie

01.10.2013 r. – 31.03.2019 r.	mgr farmacji, jednolite, magisterskie studia na kierunku Farmacja. Uniwersytet Medyczny w Białymstoku, Wydział Farmaceutyczny z Oddziałem Medycyny Laboratoryjnej. Praca magisterska „Przeciwpłytkowy efekt karwedilolu. Badania *in vitro*."

Doświadczenie zawodowe

01.10.2019 r. - obecnie	doktorant, Zakład Farmakognozji, Uniwersytet Medyczny w Białymstoku
29.04.2019 r. – 21.09.2019 r.	Apteka Cefarm Białystok S.A., Suwałki, magister farmacji
01.10.2018 r. – 31.03.2019 r.	Apteka Cefarm Białystok S.A., Suwałki – staż zawodowy

Wykaz publikacji stanowiących rozprawę doktorską

1. **Augustynowicz D.**, Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Anticancer potential of acetone extracts from selected *Potentilla* species against human colorectal cancer cells. *Frontiers in Pharmacology*. 2022; 13, 1027315. DOI: 10.3389/fphar.2022.1027315.
2. **Augustynowicz D.**, Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Phytochemical profiling of extracts from rare *Potentilla* species and evaluation of their anticancer potential. *International Journal of Molecular Sciences*. 2023; 24, 4836. DOI: 10.3390/ijms24054836.
3. **Augustynowicz D.,** Latté, KP., Tomczyk, M. Recent phytochemical and pharmacological advances in the genus *Potentilla* L. *sensu lato*—An update covering the period from 2009 to 2020. *Journal of Ethnopharmacology*. 2021; 266, 113412. DOI: 10.1016/j.jep.2020.113412.

Wykaz pozostałych publikacji naukowych

1. **Augustynowicz D.**, Podolak M., Latté KP., Tomczyk M., New perspectives for the use of *Potentilla alba* rhizomes to treat thyroid gland impairments. *Planta Medica*. 2023; 89, 19-29. DOI: 10.1055/a-1663-6461.
2. **Augustynowicz D.**, Jakimiuk K., Uysal S., Strawa JW., Juszczak AM., Zengin G., Tomczyk M. LC-ESI-MS profiling of *Potentilla norvegica* and evaluation of its biological activities. *South African Journal of Botany*. 2021; 142, 259-265. DOI: 10.1016/j.sajb.2021.06.042.

Łączny dorobek naukowy

Sumaryczny wskaźnik oddziaływania Impact Factor (IF) = 23,509
Sumaryczna liczba punktów MEiN = 580 pkt
***h*-index (Web of Science/Scopus) = 2/2**

Wykaz doniesień zjazdowych

1. **Augustynowicz D**., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. The anticancer effect of rare *Potentilla* species - Preliminary study in human colon cancer cell line LS180. 3rd International Conference on Future of Preventive Medicine and Public Health, Barcelona, Hiszpania, 30-31.03.2023 r.
2. Strawa JW., Brożyna M., Junka A., **Augustynowicz D**., Wiater A., Tomczyk M. *In Vitro* efficacy of bacterial cellulose dressings chemisorbed with selected *Potentilla* extracts against biofilm formed by pathogens isolated from chronic wounds. 3rd International Conference on Future of Preventive Medicine and Public Health, Barcelona, Hiszpania, 30-31.03.2023 r.

3. **Augustynowicz D**., Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Potential anticancer activity of acetone extracts from selected *Potentilla* species against human colorectal cancer cells. International Scientific Conference on Plant Biodiversity and Sustainability, online, Włochy, Chieti 13-14.10.2022 r.
4. Szadkowska D., Posłuszny M., Chłopecka M., Strawa JW., Jakimiuk K., **Augustynowicz D.**, Tomczyk M., Mendel M. Effects of selected *Cirsium palustre* extracts on intestinal motility - an *ex vivo* study. *Planta Medica*. 2022; 88, 1483. DOI: 10.1055/s-0042-1759119. 70th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research, Grecja, Saloniki, 28-31.08. 2022 r.
5. **Augustynowicz D.**, Lemieszek MK., Wiater A., Snarska J., Tomczyk M. Evaluation of secondary metabolites contents and anticancer properties of acetone extracts from selected *Potentilla* species. Trends in Natural Products Research: a PSE Young Scientists' Meeting, Grecja, Kolymbari, 23-26.05.2022 r.
6. **Augustynowicz D.**, Lemieszek MK., Strawa JW., Wiater A., Tomczyk M. Preliminary study of anticancer properties of acetone extracts from selected *Potentilla* species against colon cancer cell line LS180. 1st Joint Meeting on Natural Products Pharmacology SIF - SIPHAR - IMGNPP, Neapol, Włochy 24-26.02.2022 r.
7. **Augustynowicz D.**, Jakimiuk K., Strawa JW., Tomczyk. Determination of anti-tyrosinase activity of acetone extracts from selected *Potentilla* L. species. *Planta Medica*. 87(15): 1289. DOI: 10.1055/s-0041-1736907. 69th GA Annual Meeting 2021,Virtual Conference, Niemcy, 5-8.09.2021 r.
8. **Augustynowicz D.**, Uysal S., Zengin G., Jakimiuk K., Strawa JW., Juszczak AM., Tomczyk M. LC-MS determination of marker metabolites and biological activities of *Potentilla norvegica*. PSE e-Congress "Plant Derived Natural Products as Pharmacological and Nutraceutical Tools", September 15-18-22-25.09.2020 r.

Realizacja projektów badawczych (projekty subwencyjne)

Kierownik projektów

1. Badania aktywności biologicznej wybranych gatunków z rodzaju *Potentilla* L. (Rosaceae). B.SUB.23.130
2. Ocena aktywności cytotoksycznej ekstraktów z wybranych gatunków z rodzaju *Potentilla* L. SUB/2/DN/22/005/2212.
3. Ocena wpływu ekstraktów, frakcji oraz związków z części nadziemnych wybranych gatunków z rodzaju *Potentilla* L. na aktywność enzymów w modelu *in vitro*. SUB/2/DN/21/002/2212
4. Ocena aktywności cytotoksycznej wybranych ekstraktów z *Potentilla alba*. SUB/2/DN/20/002/2212.