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ROZPRAWA DOKTORSKA

Analiza fitochemiczna oraz badanie aktywności biologicznej nasion *Scorzonera hispanica* L. w komórkach raka piersi

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Wykaz skrótów

Akt – (ang. *protein kinase B*) – kinaza białkowa B

ATG – (ang. *Autophagy-related proteins*) – białka powiązane z autofagią

BAK – (ang. *BCL-2 homologous antagonist*) – homologiczny antagonista BCL-2

Bax – (ang. *BCL-2-like protein 4*) – białko z rodziny Bcl-2 4

BCL-2 – (ang. *B-cell lymphoma 2*) – białko z rodziny Bcl-2

BCL-xL – (ang. *B-cell lymphoma extra-large*) – wielkocząsteczkowe białko z rodziny Bcl-2

cisPt – (ang. *cisplatin*) – cisplatyna

COVID-19 – (ang. *coronavirus disease-2019*) – choroba wywołana przez SARS-CoV-2

ERK – (ang. *extracellular signal-regulated kinase*) – kinaza regulowana sygnałem zewnątrzkomórkowym

FAK – (ang. *focal adhesion kinase*) – główna kinaza adhezyjna

GC-MS – (ang. *gas chromatography-mass spectrometry*) – chromatografia gazowa sprzężona ze spektrometrem masowym

IL-1β – (ang. *Interleukin 1β*) – interleukina-1β

IL-8 – (ang. *Interleukin 8*) – interleukina-8

IL-10 – (ang. *Interleukin 10*) – interleukina-10

LC-PDA-MS – (ang. *liquid chromatography-photodiode array-mass spectrometry*) – chromatografia cieczowa sprzężona z detektorem fotodiodowym i spektrometrem masowym

LC3B – (ang. *light chain 3 protein B*) – białko lekkiego łańcucha 3 B

MTT – (ang. *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide*) – bromek 3-(4,5-dimetylotiazol-2-ylo)-2,5-difenylotetrazoliowy

NF-κB – *nuclear factor kappa-light-chain-enhancer of activated B cells* – kompleks białkowy działający jako czynnik transkrypcyjny

PBMC – (ang. *peripheral blood mononuclear cells*) – mononuklearne komórki krwi obwodowej

PI3K – (ang. *phosphoinositide 3 kinase*) – kinaza 3-fosfatydyloinozytolu

SH1 – ekstrakt metanolowy z nasion *Scorzonera hispanica*

SH4 – frakcja eterowa ekstraktu z nasion *Scorzonera hispanica*

SH11 – frakcja chloroformowa ekstraktu z nasion *Scorzonera hispanica*

TNF-α – (ang. *tumor necrosis factor α*) – czynnik martwicy nowotworu α

2. Wprowadzenie

Rak piersi jest jedną z najczęstszych chorób nowotworowych występujących u kobiet na całym świecie. W 2018 r. raka piersi zdiagnozowano u 2,1 miliona pacjentów, a 627 000 zgonów było spowodowanych tym właśnie rodzajem nowotworu złośliwego. Rak piersi jest przyczyną największej liczby zgonów spowodowanych nowotworem wśród kobiet [1]. Skuteczna terapia tego nowotworu stanowi istotny element zdrowia publicznego na całym świecie. Pandemia COVID-19 znacząco wpłynęła na diagnostykę i leczenie pacjentów z nowotworami piersi, toteż ostatnie raporty statystyczne dotyczące nowotworów dotyczą roku 2018. Szacuje się, że w 2022 roku, w samych tylko Stanach Zjednoczonych diagnozę raka piersi usłyszy przeszło 290 tysięcy pacjentów, a dla niemal 44 tysięcy osób będzie on w tym roku przyczyną śmierci [2].

Obecnie stosowane metody leczenia powodują szereg poważnych skutków ubocznych i mimo początkowego sukcesu prowadzonej terapii przeciwnowotworowej, w znaczącej liczbie przypadków następują nawroty choroby. Nierzadko jest to spowodowane wytworzeniem w komórkach oporności na stosowane leki [3]. Opracowywanie nowych metod terapeutycznych o zwiększonej skuteczności i wysokim profilu bezpieczeństwa w leczeniu raka piersi wciąż pozostaje wyzwaniem dla badaczy na całym świecie.

2.1. Cele molekularne w terapii przeciwnowotworowej

Jednym z najbardziej pożądanych efektów terapeutycznych nowych substancji o aktywności przeciwnowotworowej jest ich zdolność do indukcji apoptozy w komórkach nowotworowych [4]. Apoptoza jest procesem komórkowym odgrywającym znaczącą rolę na każdym etapie życia organizmu. W warunkach fizjologicznych apoptoza odpowiada m.in. za prawidłową wymianę komórek nabłonka jelita czy inwolucję grasicy po okresie pokwitania. Nieprawidłowe funkcjonowanie systemu regulującego apoptozę prowadzi do wystąpienia stanów patologicznych, takich jak: śmierć komórek w chorobach neurodegeneracyjnych czy w odpowiedzi na promieniowanie [5]. Nieprawidłowa regulacja procesu apoptozy skutkuje również indukcją procesu nowotworowego. Komórki nowotworowe unikają apoptozy poprzez zaburzenie równowagi w ekspresji białek pro- (np. Bad, Puma, Bax) i przeciwapoptotycznych (BCL-2, BCL-xL), ograniczenie funkcjonowania kaspaz inicjatorowych (kaspaza-2, -8, -9, -10) oraz wykonawczych (kaspaza-3, -6, -7), a także upośledzenie szlaków sygnałowych receptorów śmierci [6–9]. Ukierunkowanie działania nowych substancji aktywnych na mechanizmy oporności na

apoptozę w komórkach nowotworowych może zwiększyć efektywność terapii oraz ograniczyć skutki uboczne [5].

Innym mechanizmem programowanej śmierci komórki jest autofagia, obserwowana we wszystkich komórkach eukariotycznych. Autofagia, uczestnicząc w wewnątrzkomórkowej degradacji zbędnych lub uszkodzonych białek oraz elementów cytoplazmy, zapewnia homeostazę i zapobiega akumulacji niepożądanych składników we wnętrzu komórki [10]. Autofagia, w przeciwieństwie do nekrozy i apoptozy, nie zawsze jest jednoznaczna ze śmiercią komórki. W warunkach hipoksji czy pod wpływem chemioterapii, inicjacja autofagii może stać się dla komórki strategią przetrwania [11]. Ze względu na swoją rolę w zachowaniu homeostazy komórki, autofagia przyczynia się do zachowania integralności genomu oraz supresji nowotworów [12]. Inhibicja procesu autofagii może być skutecznym narzędziem do pokonywania oporności na leki, a także ułatwiać indukcję apoptozy w komórkach nowotworowych [13].

Mimo że apoptoza i autofagia ulegają regulacji przez inne mechanizmy, niekiedy oba te procesy występują w komórce w odpowiedzi na określone czynniki. Procesy apoptozy i autofagii mogą następować po sobie, indukcja jednego może prowadzić do aktywacji drugiego, a także oba te procesy mogą występować jednocześnie [14,15]. Do białek łączących apoptozę i autofagię należą m.in. białka z rodziny ATG, BCL-2, oraz kaspazy [13].

Związek stanu zapalnego z procesem nowotworowym zaobserwowano w wielu rodzajach raka, również w raku piersi [16]. Mimo że dokładny mechanizm zainicjowania procesu nowotworowego pozostaje niejasny, sugeruje się, że stan zapalny może przyczyniać się zarówno do wywołania, jak i progresji nowotworu, angiogenezy oraz powstawania przerzutów w organizmie [17].

Jedną z głównych cytokin wiążących stan zapalny z nowotworem piesi jest czynnik martwicy nowotworu α (TNF-α). TNF-α jest prozapalną cytokiną obecną w mikrośrodowisku guza, biorącą udział we wszystkich etapach jego rozwoju, od proliferacji komórek, przez tworzenie przerzutów aż do występowania nawrotów. U pacjentów z rakiem piersi obserwuje się podwyższone stężenie TNF-α zarówno w miejscu guza, jak również w surowicy, a jego zwiększony poziom koreluje ze złośliwym i inwazyjnym fenotypem raka piersi. Mimo że TNF-α może wykazywać zarówno pro- jak i przeciwnowotworowe właściwości, pierwotną odpowiedzią komórkową na TNF-α jest inhibicja apoptozy oraz udział w wywoływaniu nabytej oporności komórek na chemioterapię [18].

Inną prozapalną chemokiną związaną z rozwojem raka piersi jest interleukina-8 (IL-8), której zwiększoną ekspresję obserwuje się w wielu typach raka piersi. IL-8 również bierze udział w angiogenezie i stymuluje proliferację komórek nowotworowych [19]. Badania wykazały, że terapia ukierunkowana na inhibicję IL-8 może uwrażliwiać komórki nowotworowe na chemioterapeutyki [20]. W świetle dostępnych doniesień, można wysnuć wniosek, że pożądanym efektem terapii przeciwnowotworowej jest aktywność przeciwzapalna w komórkach raka piersi, wywołana przez obniżenie stężenia cytokin prozapalnych.

2.2. Surowce roślinne jako źródło związków przeciwnowotworowych

Odkrywanie nowych substancji o właściwościach przeciwnowotworowych nie ogranicza się do konwencjonalnej syntezy organicznej. W latach 1981–2019, spośród 247 związków o aktywności przeciwnowotworowej, zatwierdzonych przez agencje leków na świecie, zaledwie 29 to substancje w całości zsyntetyzowane w laboratoriach. Pozostałe niemal 90% stanowią preparaty naturalne (pochodzenia biologicznego takie jak peptydy i białka wyizolowane z organizmów zwierzęcych, ale także rejestrowane w ostatniej dekadzie preparaty roślinne), lub takie, których synteza laboratoryjna oparta była na farmakoforze pochodzenia naturalnego [21]. Pierwsze stosowane klinicznie związki o działaniu przeciwnowotworowym wyizolowane z roślin to alkaloidy winkrystyna i winblastyna, pozyskane z barwinka różowego (*Catharanthus roseus* G. Don, Apocynaceae). Związki te posłużyły później jako punkt wyjścia do syntezy nowych pochodnych o działaniu cytotoksycznym [22].

Rozmaite rośliny lecznicze są przedmiotem badań jako źródło związków biologicznie aktywnych [23]. Ocena potencjału przeciwnowotworowego roślin znanych w medycynie ludowej jest przedmiotem zainteresowania naukowców, zwłaszcza w aspekcie ich molekularnego mechanizmu działania. Aktywność biologiczna produktów pochodzenia roślinnego wobec raka piersi jest badana na całym świecie, z uwzględnieniem ich wpływu na proliferację, apoptozę, cykl komórkowy, migrację oraz ekspresję białek związanych ze szlakami sygnałowymi wewnątrz komórki [24–27].

2.3. Aktywność biologiczna gatunków z rodzaju *Scorzonera* L.

Scorzonera L. (Asteraceae) (pol. wężymord) jest rodzajem obejmującym niemal 200 gatunków [28], występujących na terenie Europy, Azji a także północnych krańców Afryki [29]. W rejonach pustynnych, niektóre gatunki roślin z rodzaju *Scorzonera* wykorzystywane są jako pasza dla zwierząt hodowlanych [30]. Inne natomiast, jak *S. sau-*

taghyz Lipsch. & G.G. Bosse, stanowią źródło wykorzystywanego przemysłowo kauczuku [31].

W medycynie ludowej Eurazji i północnej Afryki rośliny z rodzaju wężymord odgrywają szczególną rolę. W Turcji różne gatunki, w tym *S. laciniata* L., *S. latifolia* (Fisch i Mey.) DC oraz *S. tomentosa* L., są stosowane w terapii ze względu na swoje przypuszczalne właściwości lecznicze, w tym działanie przeciwcukrzycowe, przeciwbólowe, przeciwgorączkowe, przeciwreumatyczne, obniżające ciśnienie krwi, a także hemostatyczne [32–34]. Mongolska oraz chińska medycyna tradycyjna wykorzystuje gatunki *S. divaricata* Turcz. and *S. pseudodivaricata* Lipsch. do leczenia chorób pasożytniczych, biegunki czy gorączki wywołanej infekcjami bakteryjnymi i wirusowymi, a nawet w przypadku guzów żołądka [35]. Do innych zastosowań roślin z rodzaju *Scorzonera* w medycynie ludowej należą: w Indiach leczenie żółtaczki [36], w Libii przeciwdziałanie bólom wątroby [37], a nawet stosowanie ich jako antidotum na jad węża w Algierii [38]. Co warte podkreślenia, uważa się, że nazwa rodzaju pochodzi od dawnego przypuszczenia, że gatunki z rodzaju *Scorzonera* posiadają zdolność leczenia ukąszeń węży (wł. *scorzone* – wąż, żmija) [39].

Rośliny należące do rodzaju *Scorzonera* stały się przedmiotem zainteresowania ze względu na zawartość w nich związków biologicznie czynnych, w tym pochodnych kwasów fenolowych, terpenoidów, lignanów, flawonoidów i innych [40]. Zarówno ekstrakty, jak i związki wyizolowane z części nadziemnych i podziemnych gatunków z rodzaju wężymord, były materiałem badawczym w licznych analizach ich aktywności biologicznej. W oparciu o przekazy pochodzące z medycyny ludowej, gatunki *Scorzonera* są współcześnie badane pod kątem ich właściwości przeciwbólowych [32, 41–43], przeciwzapalnych [44–46], przeciwdrobnoustrojowych [47–50] czy antyoksydacyjnych [30, 51–53]. Prowadzone są również badania wpływu roślin rodzaju wężymord na proces gojenia ran [54–57] oraz ich potencjału cytotoksycznego wobec komórek nowotworowych [49, 58–62].

Scorzonera hispanica L. (wężymord czarny korzeń, skorzonera) jest wieloletnią rośliną uprawną [63]. W tradycyjnej medycynie europejskiej korzenie wężymordu były stosowane w leczeniu przeziębień, a także jako środek mukolityczny czy pobudzający apetyt [35, 64]. Obecnie korzenie skorzonery są cenionym warzywem uprawianym w Europie. Wcześniejsze badania nad tym gatunkiem wykazały, że nadziemne części rośliny zawierają flawonoidy, kwas kawowy oraz jego pochodne [65, 66]. W częściach podziemnych stwierdzono obecność lignanów, seskwiterpenów, kwasu kawowego i jego

pochodnych, a także inuliny [64, 65, 67]. Korzenie skorzonery również zostały przebadane jako potencjalne źródło substancji o właściwościach cytotoksycznych wobec komórek nowotworowych. W badaniach przeprowadzonych przez Granicę i wsp. [65], wyizolowany z korzenia wężymordu lignan (-)–syringarezynol wykazał cytotoksyczność wobec komórek szpiczaka mnogiego linii NCI-H92 oraz MM.1S, a także raka jelita grubego SW480. Związek ten został uprzednio przebadany pod kątem aktywności biologicznej wobec linii komórkowych raka piersi, jelita grubego, białaczki oraz szpiczaka, w których wykazywał działanie cytotoksyczne [68, 69]. Co więcej, dwa seskwiterpenoidy wyizolowane z korzenia skorzonery (puligluton oraz ester metylowy kwasu 1-oxo-bisabola-(2,10*E*)-dieno-12-karboksylowego) wykazywały działanie cytotoksyczne wobec komórek raka jelita grubego, natomiast nie stwierdzono działania toksycznego wobec prawidłowych komórek krwi obwodowej (PBCM) w badanych stężeniach (do 50 μM). Inne gatunki wężymordu również były analizowane jako źródło związków o potencjale przeciwnowotworowym. W badaniach przeprowadzonych przez węgierski zespół badaczy, ekstrakt chloroformowy w korzeni gatunku *S. austriaca* Willd wykazywał silne właściwości cytotoksyczne wobec komórek raka szyjki macicy (HeLa), raka skóry (A431) oraz raka piersi (MCF-7) [60]. Wyizolowane z korzeni *S. austriaca* dimeryczne guaianolidy (biguaianoskorzolidy A i B), a także laktony seskwiterpenowe, działały cytotoksycznie na komórki białaczki szpikowej linii K562 [61,70]. Znaczącą aktywność przeciwnowotworową wykazuje również gatunek *Scorzonera divaricata*. Triterpeny i seskwiterpeny, wyizolowane z ekstraktu etanolowego z korzeni rośliny, wykazywały aktywność cytotoksyczną wobec komórek raka szyjki macicy linii HeLa, raka wątroby linii HepG2 i SMMC-7721 oraz białaczki linii HL60 [62]. Części nadziemne roślin gatunku *S. divaricata* również stanowią źródło związków chemicznych o znaczącym potencjale przeciwnowotworowym. Największą toksyczność związków wyizolowanych w badaniu zaobserwowano wobec komórek HeLa, HepG2 oraz K562 [49].

Rośliny z rodzaju *Scorzonera* są również przedmiotem badań w kontekście ich aktywności przeciwzapalnej. Liczne gatunki, w tym *S. latifolia* i *S. tomentosa*, wykazywały działanie hamujące ekspresję prozapalnych cytokin TNF-α i IL-1β [45]. Z działaniem przeciwzapalnym wiąże się aktywność przeciwbólowa. W badaniach na myszach stwierdzono, że ekstrakty z części podziemnych gatunków *S. latifolia* i *S. tomentosa* wykazują istotne działanie łagodzące ból u badanych zwierząt [42].

3. Cel pracy

Nieustającym wyzwaniem dla zespołów badawczy na całym świecie jest poszukiwanie nowych substancji o aktywności przeciwnowotworowej. Znaczącego potencjału terapeutycznego upatruje się w produktach pochodzących z roślin. Produkty metabolizmu licznych gatunków roślin wykazują działanie cytotoksyczne wobec komórek nowotworowych a także są punktem wyjścia w syntezie innych związków o dużej aktywności biologicznej.

W ramach rozprawy doktorskiej przeprowadzono przegląd literatury dotyczącej rodzaju *Scorzonera* w kontekście występowania w medycynie ludowej. Dokonano również analizy składu fitochemicznego oraz oceny aktywności biologicznej produktów otrzymanych z roślin rodzaju wężymord, z uwzględnieniem zróżnicowanych kierunków badań nad potencjałem terapeutycznym – wpływu na proces gojenia ran, aktywności antyoksydacyjnej, a także właściwości przeciwnowotworowych, przeciwzapalnych, przeciwbólowych, przeciwdrobnoustrojowych czy hepatoprotekcyjnych (**Publikacja I**).

Celem rozprawy doktorskiej było otrzymanie oleju, ekstraktów i frakcji z nasion *Scorzonera hispanica*, a także ocena ich aktywności biologicznej wobec komórek raka piersi: estrogenozależych komórek MCF-7 i estrogenoniezależnych komórek linii MDA-MB-231. Dokonano analizy jakościowej i ilościowej składu chemicznego oleju oraz ekstraktów i frakcji z nasion *S. hispanica* z wykorzystaniem technik GC-MS oraz LC-PDA-MS. Przeprowadzona została również ocena aktywności biologicznej otrzymanych produktów z nasion skorzonery wobec komórek raka piersi linii MCF-7 oraz MDA-MB-231, a także komórek prawidłowych – fibroblastów skóry ludzkiej. W kolejnym etapie badań wybrano trzy produkty o najbardziej obiecującej aktywności cytotoksycznej i dokonano pogłębionej analizy ich molekularnego mechanizmu działania w komórkach raka piersi MCF-7. Oceniono ich wpływ na proces biosyntezy DNA, a także zdolność do indukcji procesu apoptozy w komórkach nowotworowych. Następnie zbadano wpływ wybranych ekstraktów i frakcji na ekspresję białek związanych z apoptozą i autofagią (BCL-2, Bax, ATG5, LC3B) oraz białka FAK, odpowiedzialnego za adhezję i migrację komórek. Dokonano oceny wpływu wybranych ekstraktów i frakcji na stężenie białek uczestniczących w komórkowych szlakach sygnałowych (kinazy Akt i ERK1/2) oraz stężenie wybranych cytokin prozapalnych (IL-8, TNF-α) i przeciwzapalnych (IL-10) (**Publikacja II**).

4. Realizacja celów naukowych – materiały, metody badawcze, wyniki

4.1. Surowiec roślinny, przygotowanie ekstraktów, frakcji i oleju z nasion *Scorzonera hispanica*

Nasiona *Scorzonera hispanica* zostały zakupione w styczniu 2020 roku od firmy W. Legutko Przedsiębiorstwo Hodowlano-Nasienne Sp. z o.o., z siedzibą w Jutrosinie, woj. wielkopolskie (nr partii 68347).

Ekstrakty przygotowano w dwóch oddzielnych procedurach. Pierwsza procedura polegała na ekstrakcji surowca wspomaganej ultradźwiękami. Każdy z ekstraktów przygotowano poprzez pięciokrotne wytrawianie 15 g surowca 100 ml ekstrahentu (metanolu, 50% metanolu, wody oraz 70% acetonu) w 40 °C. Po odparowaniu rozpuszczalnika, uzyskano następujące ekstrakty: metanolowy (**SH1**; wydajność 9,1%), 50% metanolowy (**SH2**; wydajność 10,5%), wodny (**SH3**; wydajność 21,3%) oraz 70% acetonowy (*v*/*v*) (**SH8**; wydajność 27,9%). Druga procedura ekstrakcyjna polegała na wykorzystaniu aparatu Soxhleta. Rozdrobniony surowiec (90 g) poddano ekstrakcji ciągłej benzyną (**SH9**; 3 l × 25 h, wydajność 15,1%), następnie chloroformem (**SH10**; 3,5 l × 25 h, wydajność 3,2%). Po ekstrakcji surowiec wysuszono i ekstrahowano pod chłodnicą zwrotną kolejno metanolem (26 × 1,5 l) i 50% metanolem (5 × 1,5 l) każdorazowo przez 1 godzinę. Połączone ekstrakty odparowano i zawieszono w wodzie. Następnie, dokonano wyczerpującej ekstrakcji ciecz-ciecz rozpuszczalnikami o rosnącej polarności: chloroformem (**SH11**; 40 × 150 ml, wydajność 0,43%), eterem dietylowym (**SH4**; 59 × 50 ml, wydajność 0,37%), octanem etylu (**SH5**; 60 × 150 ml, wydajność 0,63%) i *n*-butanolem (**SH6**; 34 × 150 ml, wydajność 1,28%). Pozostałość wodną po frakcjonowaniu przefiltrowano i potraktowano jako dodatkową frakcję **SH7** (wydajność 1,58%). Otrzymane ekstrakty i frakcje poddano liofilizacji.

W celu uzyskania oleju z nasion *S. hispanica*, dokonano trzykrotnego tłoczenia na zimno porcji nasion (150 g, 35 °C). Następnie produkt odwirowano i oddzielono od osadu. Uzyskany w ten sposób olej oznaczono jako **SH12** (wydajność 3,2%).

4.2. Metody badawcze

4.2.1. Analiza fitochemiczna produktów z nasion *Scorzonera hispanica*

Analizy składu chemicznego ekstraktów, frakcji i oleju z nasion *Scorzonera hispanica* dokonano metodami chromatograficznymi. Do analizy fitochemicznej ekstraktów i frakcji **SH1**-**SH8** użyto techniki LC-PDA-MS. Technika GC-MS została

wykorzystana do oceny składu lipofilnych produktów pozyskanych z surowca (**SH1**, **SH9**-**SH12**).

4.2.2. Ocena potencjału przeciwnowotworowego nasion *Scorzonera hispanica*

Do wstępnego skriningu aktywności przeciwnowotworowej produktów z nasion *S. hispanica* **SH1**-**SH12** wobec komórek raka piersi MCF-7 oraz MDA-MB-231 wykorzystano metodę Carmichaela z użyciem soli tetrazolowej MTT oraz oceniono wpływ badanych ekstraktów, frakcji oraz oleju na proces biosyntezy DNA poprzez pomiar [^{3}H]-tymidyny wbudowanej do DNA komórek. Na podstawie uzyskanych wyników, do dalszych badań wybrano 3 najbardziej aktywne produkty (**SH1**, **SH4**, **SH11**) w stężeniach odpowiadających wartościom IC_{25} oraz IC_{50} uzyskanych w teście MTT. Cisplatyna (cisPt) została użyta jako związek referencyjny w stężeniach 50 i 100 μM.

Dokonano oceny wpływu badanego ekstraktu i frakcji (**SH1**, **SH4**, **SH11**) na proces indukcji apoptozy w komórkach raka piersi MCF-7 wykorzystując technikę cytometrii przepływowej z użyciem zestawu Annexin V Binding Apoptosis Detection Kit II (ThermoFisher, USA).

Dokonano oceny ekspresji wybranych białek związanych z procesem apoptozy (Bax, BCL-2) i autofagii (LC3B, ATG5), a także biorącej udział w migracji, adhezji i apoptozie komórek kinazy FAK, metodą Western blot.

Zbadano wpływ ekstraktu **SH1** i frakcji **SH4** i **SH11** na stężenie białek uczestniczących w szlakach sygnałowych komórki – kinazy białkowej B (Akt) i kinazy regulowanej sygnałem zewnątrzkomórkowej ERK1/2, a także wybranych cytokin prozapalnych: czynnika martwicy nowotworów α (TNF-α) i interleukiny-8 (IL-8), oraz przeciwzapalnej interleukiny-10 (IL-10). Powyższe badania wykonano techniką immunoenzymatyczną ELISA.

4.3. Wyniki

4.3.1. Analiza fitochemiczna produktów z nasion *Scorzonera hispanica*

Analizy składu chemicznego ekstraktów i frakcji **SH1**-**SH8** dokonano przy użyciu techniki LC-PDA-MS. Potwierdzono obecność w ekstraktach i frakcjach kwasów fenolowych, kwasu *p*-kumarowego, a także flawonoidów (luteoliny i apigeniny) zarówno w wolnej, jak i związanej formie. Analiza ilościowa wykazała, że frakcja eterowa **SH4** charakteryzuje się najbardziej bogatym we flawonoidy i pochodne kawoilochinowe składem.

Do oceny lipofilnych produktów otrzymanych z nasion *Scorzonera hispanica* (**SH1**, **SH9**-**SH12**) wykorzystano technikę GC-MS. Wykazano, że głównymi składnikami oleju **SH12** są kwasy tłuszczowe (linolenowy, palmitynowy, oleinowy) oraz β-sitosterol. Ekstrakt metanolowy **SH1** charakteryzował się znaczną ilością D-chiro-inozytolu oraz kwasu kawowego. Najbardziej znaczącą część składu produktu otrzymanego poprzez ekstrakcję benzyną **SH9** stanowił kwas linolenowy. Głównym związkiem wchodzącym w skład ekstraktu chloroformowego **SH10** były kwas linolenowy oraz glicerol. Dominującym składnikiem frakcji chloroformowej ekstraktu metanolowego **SH11** był 3,4-dimetoksycynamonian metylu. We frakcji tej stwierdzono również znaczące ilości kwasu ferulowego i linolenowego.

4.3.2. Ocena potencjału przeciwnowotworowego nasion *Scorzonera hispanica*

Aktywność cytotoksyczną produktów pozyskanych z nasion *Scorzonera hispanica* (**SH1**-**SH12**) wobec komórek raka piersi MCF-7 i MDA-MB-231, a także prawidłowych komórek fibroblastów skóry ludzkiej, oznaczono za pomocą testu MTT. Zaobserwowano, iż frakcja chloroformowa **SH11** wykazywała najsilniejsze działanie cytotoksyczne wobec komórek nowotworowych. Wartości IC_{50} zaobserwowane dla **SH11** wynosiły 399,18 μg/ml i 781,26 μg/ml odpowiednio dla komórek linii MCF-7 i MDA-MB-231. Ekstrakt metanolowy **SH1** i frakcja eterowa **SH4** wykazywały aktywność jedynie wobec komórek MCF-7 – wartości IC_{50} dla **SH1** i **SH4** wyniosły odpowiednio 847,72 μg/ml i 626,01 μg/ml. Pozostałe produkty (**SH2**-**SH3**, **SH5**-**SH10, SH12**) nie wykazały aktywności cytotoksycznej wobec komórek raka piersi, ani komórek prawidłowych w stężeniach 0,01-1 mg/ml.

W następnym etapie dokonano oceny wpływu ekstraktu **SH1** i frakcji **SH4** i **SH11** na proces biosyntezy DNA poprzez pomiar ilości radioaktywnej [^{3}H]-tymidyny wbudowywanej do DNA komórek linii MCF-7 i MDA-MB-231. Wykazano, że inhibicja procesu biosyntezy DNA w komórkach odbywa się w sposób proporcjonalny do stężenia ekstraktu czy frakcji. Największą aktywność antyproliferacyjną zaobserwowano dla frakcji chloroformowej **SH11** (wartości IC_{50}: 293,64 μg/ml i 265,05 μg/ml odpowiednio dla komórek linii MCF-7 i MDA-MB-231). Pozostałe produkty, **SH1** i **SH4** również wykazały zbliżoną aktywność antyproliferacyjną dla komórek obu linii. W przypadku **SH1**, wartość IC_{50} w komórkach MCF-7 i MDA-MB-231 wyniosła odpowiednio 943,23 μg/ml i 863,21 μg/ml. Dla frakcji eterowej **SH4** wartości IC_{50} zaobserwowane w teście wyniosły: dla MCF-7 – 630,52 μg/ml i MDA-MB-231 – 648,61 μg/ml.

Molekularny mechanizm działania produktów **SH1**¸ **SH4** i **SH11** w komórkach raka piersi może być związany z ich zdolnością do indukcji procesu apoptozy w komórkach. W celu potwierdzenia tej hipotezy, potencjalne działanie proapoptotyczne produktów z nasion *S. hispanica* wobec komórek estrogenozależnego raka piersi MCF-7 zostało zbadane techniką cytometrii przepływowej, z wykorzystaniem aneksyny V. Po 24 h inkubacji z ekstraktem **SH1** i frakcjami **SH4** i **SH11**, a także cisplatyną jako związkiem referencyjnym, we wszystkich badanych próbach zaobserwowano zależny od stężenia wzrost liczby komórek wczesno- i późnoapoptotycznych. Najbardziej wyraźny efekt został zaobserwowany dla frakcji chloroformowej **SH11** po 24-godzinnej inkubacji w stężeniu 200 μg/ml. Średnio 32,5% komórek znalazło się we wczesnej lub późnej fazie apoptozy, w stężeniu wyższym (400 μg/ml) odsetek ten wzrósł do 53,4%. 24-godzinna inkubacja z frakcją **SH4** w stężeniach 600 μg/ml i 800 μg/ml skutkowała indukcją apoptozy w odpowiednio 39,9% i 49,6% komórek MCF-7. W przypadku ekstraktu metanolowego **SH1**, efekt proapoptotyczny był mniej wyraźny – po inkubacji z ekstraktem metanolowym w stężeniach 600 i 800 μg/ml indukcja apoptozy została zaobserwowana w 11,5% i 13,4% komórek. Inkubacja z cisplatyną skutkowała wzrostem udziału komórek ulegających apoptozie do 19,7% (stężenie 50 μM) i 26,6% (stężenie 100 μM). W żadnej z analizowanych próbek, odsetek komórek nekrotycznych nie przekroczył 2%, co sugeruje, że to apoptoza, nie nekroza jest dominującym mechanizmem cytotoksyczności badanych produktów z nasion *S. hispanica*.

Aby sprawdzić, czy indukcja apoptozy przebiega wewnętrznym szklakiem mitochondrialnym, dokonano oceny ekspresji białek BCL-2 i Bax pod wpływem ekstraktu i frakcji **SH1, SH4** i **SH11** metodą Western blot. Bax jest białkiem przyspieszającym apoptozę, którego ekspresję reguluje białko p53. Niezablokowany przez BCL-2 Bax powoduje obniżenie potencjału błonowego mitochondrium, co prowadzi do powstania na jego błonie porów, przez które uwalniany jest cytochrom c, inicjujący kaskadę kaspaz, doprowadzając do śmierci komórki [71]. BCL-2 natomiast pełni podwójną funkcję: hamuje aktywność nie tylko proapoptotycznych białek Bax i BAK, ale także kluczowej dla procesu autofagii Bekliny 1 [72]. Wykazano, że wszystkie badane produkty (**SH1**, **SH4** i **SH11**) wywołują obniżenie ekspresji BCL-2 i wzrost ekspresji białka Bax. Największą inhibicję ekspresji BCL-2 zaobserwowano dla ekstraktu metanolowego **SH1**, natomiast w przypadku białka Bax, inkubacja z frakcją chloroformową **SH11** skutkowała największym wzrostem jego ekspresji.

Obniżenie ekspresji BCL-2 może sugerować, że produkty **SH1**, **SH4** i **SH11** wpływają również na indukcję autofagii w komórkach. W celu oceny tego zjawiska, zbadano ekspresję dwóch kluczowych w tym procesie białek: uczestniczącego w procesie formowania pre-autofagosomu białka ATG5 oraz uznawanego za sztandarowy marker autofagii białka LC3B. Metodą Western blot wykazano zwiększenie ekspresji ATG5 i LC3B w komórkach raka piersi MCF-7. Spośród badanych produktów z nasion *S. hispanica*, najbardziej wyraźny wzrost ekspresji białek zaangażowanych w proces autofagii ATG5 i LC3B zaobserwowano dla frakcji **SH11**.

Zbadano również wpływ ekstraktu i frakcji **SH1, SH4** i **SH11** na istotne białka odpowiedzialne za proliferację i przeżycie komórek, takie jak kinazy Akt i ERK1/2. Metodą Western blot oceniono ekspresję białka FAK, którego aktywacja wpływa zarówno na szlak sygnałowy PI3K/Akt, jak również ERK1/2. Kinaza FAK ulega nadekspresji w wielu typach raka piersi [73]. Dodatkowo, techniką ELISA oznaczono stężenie fosforylowanego białka Akt (p-Akt) i ERK1/2 (p-ERK1/2) w lizatach komórkowych linii MCF-7. Akt hamuje apoptozę przez m.in. inaktywację białek FOX i Bad oraz inaktywację NF-κB [74]. Szacuje się, że średnio 30% ludzkich typów raka piersi charakteryzuje się deregulacją szlaku sygnałowego ERK1/2 [75]. Zahamowanie fosforylacji zarówno Akt jak i ERK1/2 prowadzi do indukcji apoptozy w komórkach, w tym w komórkach raka piersi MCF-7 [76,77]. W analizie Western blot wykazano, że produkty **SH1**, **SH4** i **SH11** obniżają ekspresję kinazy FAK. W ocenie wpływu ekstraktu i frakcji z nasion *S. hispanica* na stężenie p-Akt i p-ERK1/2 wykorzystano technikę ELISA. Zaobserwowano, że wszystkie badane produkty obniżają stężenie zarówno p-Akt, jak i p-ERK1/2 w sposób zależny od stężenia. Największą zdolność do hamowania stężenia wspomnianych białek odnotowano dla frakcji chloroformowej **SH11** – w stężeniu 400 μg/ml spadek był kilku- a nawet kilkunastokrotny w stosunku do kontroli (dla p-Akt: z 2,43 U/ml w kontroli do 0,09 U/ml w przypadku **SH11**; dla p-ERK1/2: z 150,33 pg/ml w kontroli do 38,67 pg/ml dla **SH11**) Uzyskane wyniki potwierdzają zdolność ekstraktu **SH1** i frakcji **SH4** i **SH11** do indukcji procesu apoptozy na szlaku mitochondrialnym w komórkach raka piersi MCF-7.

Na przestrzeni ostatnich lat, proces progresji nowotworów coraz częściej kojarzony jest z procesem zapalnym, w tym z aktywnością cytokin prozapalnych i przeciwzapalnych. W związku z tym dokonano oceny wpływu ekstraktu i frakcji **SH1**, **SH4** i **SH11** na stężenie wybranych cytokin prozapalnych (TNF-α, IL-8) i cytokiny przeciwzapalnej IL-10. IL-8 jest chemokiną prozapalną, która odgrywa istotną rolę w szlakach sygnałowych, w tym zaangażowanych w angiogenezę, proliferację i przerzuty w nowotworach. Działanie

hamujące IL-8 na szlaki sygnalizacyjne jest pożądanym efektem działania środków terapeutycznych w leczeniu nowotworów [78]. TNF-α, który jest obecny w mikrośrodowisku guza, bierze udział w rozwoju i przerzutowaniu raka piersi. TNF-α odgrywa podwójną rolę w raku piersi – może sprzyjać zarówno apoptozie, jak i proliferacji komórek różnych linii *in vitro*. Jednakże, pierwotną odpowiedzią komórkową na TNF-α jest zwiększona proliferacja i tworzenie przerzutów. Dlatego też, w oparciu o dane przedkliniczne przypuszcza się, że antagoniści TNF-α mogą hamować metastazę raka piersi [18]. Interleukina-10, podobnie jak TNF-α może wykazywać działanie zarówno pro- jak i przeciwnowotworowe. Z jednej strony, duże stężenie IL-10 jest postrzegane jako dobra prognoza w przypadku pacjentów we wczesnych stadiach inwazyjnego raka piersi, z drugiej natomiast, obecność IL-10 i BCL-2 w mikrośrodowisku guza może odzwierciedlać jego zwiększoną agresywność [79,80]. Wykazano, że wszystkie trzy badane produkty z nasion *S. hispanica* (**SH1**, **SH4** i **SH11**) wykazują działanie przeciwzapalne w komórkach raka piersi MCF-7. Najbardziej wyraźną aktywność badanych produktów zaobserwowano wobec IL-8. Jej stężenie w lizatach komórek linii MCF-7 po inkubacji z produktami **SH1**, **SH4** i **SH11** uległo zależnemu od stężenia obniżeniu z 32,58 pg/ml w komórkach kontrolnych do 4,00-5,69 pg/ml w badanych próbach. Ekstrakt i frakcje z nasion *S. hispanica* wywołały również nieznaczne obniżenie stężenia TNF-α, jednak tylko po 24-godzinnej inkubacji z **SH4** w stężeniu 800 μg/ml zaobserwowano istotną statystycznie różnicę z wartościami kontrolnymi. Odnotowano istotny statystycznie, zależny od dawki, wzrost stężenia IL-10 w lizatach komórkowych linii MCF-7. Największy wpływ na stężenie IL-10 w porównaniu z kontrolą (71,84 pg/ml) zaobserwowano dla **SH4**, gdzie stężenie IL-10 osiągnęło wartości 92,55 pg/ml (600 μg/ml) i 111,10 pg/ml (800 μg/ml).

Na podstawie wyników uzyskanych w trakcie badań zawartych w niniejszej rozprawie doktorskiej, można stwierdzić, że nasiona *Scorzonera hispanica* są obiecującym surowcem roślinnym o szerokim spektrum potencjalnych zastosowań, jako zarówno nowe źródło oleju, jak i substancji biologicznie czynnych o charakterze przeciwnowotworowym, które mogą w przyszłości znaleźć zastosowanie w terapii wspomagającej leczenie raka piersi. Niemniej jednak, dalsze pogłębione badania, zwłaszcza nad metabolizmem i bezpieczeństwem składników aktywnych otrzymanych produktów, są konieczne, aby móc w pełni ocenić ich potencjał biologiczny.

5. Wnioski

1. Jakościowa i ilościowa analiza LC-PDA-MS ekstraktów i frakcji otrzymanych z nasion *Scorzonera hispanica* wykazała obecność aktywnych biologicznie związków z grupy flawonoidów (w tym luteoliny i apigeniny) oraz szeregu pochodnych kwasu chinowego (w tym kwasy kawoilochinowe, dikawoilochinowe i trikawoilochinowe).
2. Analiza GC-MS ekstraktów i frakcji z nasion *S. hispanica* wykazała wysoką zawartość kwasów tłuszczowych (linolenowego i palmitynowego), a także 3,4-dimetoksycynamonianu metylu, D-chiro-inozytolu i kwasu kawowego.
3. Olej z nasion *S. hispanica* charakteryzuje się wysoką zawartością nienasyconych kwasów tłuszczowych oraz fitosteroli, w tym β-sitosterolu, i nie wykazuje właściwości cytotoksycznych w stężeniach do 1 mg/ml.
4. Ekstrakt metanolowy **SH1** oraz frakcje: eterowa **SH4** i chloroformowa **SH11** wykazują zdolność do indukcji mitochondrialnego szlaku apoptozy, wpływają na ekspresję białek związanych z procesem apoptozy (BCL-2, Bax) i autofagii (ATG5, LC3B) oraz wykazują działanie hamujące na ekspresję kinazy Akt oraz ERK1/2 w komórkach raka piersi MCF-7.
5. Ekstrakt **SH1** i frakcje **SH4** i **SH11** wykazują działanie przeciwzapalne poprzez inhibicję cytokin prozapalnych IL-8 i TNF-α i zwiększanie stężenia cytokiny przeciwzapalnej IL-10 w komórkach raka piersi linii MCF-7.
6. Nasiona *S. hispanica* są obiecującym źródłem związków bioaktywnych, które potencjalnie mogą znaleźć zastosowanie w terapii raka piersi.

6. Literatura

1. World Health Organization *World Cancer Report: Cancer Research for Cancer Prevention*; Wild, C.P., Weiderpass, E., Stewart, B.W., Eds.; International Agency for Research on Cancer, 2020; Vol. 199; ISBN 978-92-832-0447-3.
2. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer Statistics, 2022. *CA Cancer J Clin* **2022**, *72*, 7–33, doi:10.3322/caac.21708.
3. Czarnomysy, R.; Surażyński, A.; Muszynska, A.; Gornowicz, A.; Bielawska, A.; Bielawski, K. A Novel Series of Pyrazole-Platinum(II) Complexes as Potential Anti-Cancer Agents That Induce Cell Cycle Arrest and Apoptosis in Breast Cancer Cells. *J Enzyme Inhib Med Chem* **2018**, *33*, 1006–1023, doi:10.1080/14756366.2018.1471687.
4. Pfeffer, C.M.; Singh, A.T.K. Apoptosis: A Target for Anticancer Therapy. *Int J Mol Sci* 2018, *19*.
5. Wong, R.S.Y. Apoptosis in Cancer: From Pathogenesis to Treatment. *J Exp Clin Cancer Res* **2011**, *30*, doi:10.1186/1756-9966-30-87.
6. Fulda, S. Evasion of Apoptosis as a Cellular Stress Response in Cancer. *Int J Cell Biol* **2010**, *2010*, doi:10.1155/2010/370835.
7. Dewson, G.; Kluck, R.M. Bcl-2 Family-Regulated Apoptosis in Health and Disease. *Cell Health Cytoskelet* **2010**, *2*, 9–22, doi:10.2147/chc.s6228.
8. Fink, S.L.; Cookson, B.T. Apoptosis, Pyroptosis, and Necrosis: Mechanistic Description of Dead and Dying Eukaryotic Cells. *Infect Immun* **2005**, *73*, 1907–1916, doi:10.1128/iai.73.4.1907-1916.2005.
9. Fernald, K.; Kurokawa, M. Evading Apoptosis in Cancer. *Trends Cell Biol* **2013**, *23*, 620–633, doi:10.1016/j.tcb.2013.07.006.
10. Polewska, J. Autophagy – Molecular Mechanism, Apoptosis and Cancer. *Postępy Hig Med Dosw* **2012**, *66*, 921–936, doi:10.5604/17322693.1021109.
11. Dereń-Wagemann, I.; Kiełbiński, M.; Kuliczkowski, K. Autofagia – Proces o Dwóch Obliczach. *Acta Haematol Pol* **2013**, *44*, 383–391, doi:10.1016/j.achaem.2013.05.003.
12. Andrade-Tomaz, M.; de Souza, I.; Rocha, C.R.R.; Gomes, L.R. The Role of Chaperone-Mediated Autophagy in Cell Cycle Control and Its Implications in Cancer. *Cells 2020, Vol. 9, Page 2140* **2020**, *9*, 2140, doi:10.3390/cells9092140.
13. Buzun, K.; Gornowicz, A.; Lesyk, R.; Bielawski, K.; Bielawska, A. Autophagy Modulators in Cancer Therapy. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22115804.

14. Mariño, G.; Niso-Santano, M.; Baehrecke, E.H.; Kroemer, G. Self-Consumption: The Interplay of Autophagy and Apoptosis. *Nat Rev Mol Cell Biol* **2014**, *15*, 81–94, doi:10.1038/NRM3735.
15. Chen, Q.; Kang, J.; Fu, C. The Independence of and Associations among Apoptosis, Autophagy, and Necrosis. *Signal Transduct Target Ther* **2018**, *3*, doi:10.1038/S41392-018-0018-5.
16. Lee, H.-M.; Lee, H.-J.; Chang, J.-E. Inflammatory Cytokine: An Attractive Target for Cancer Treatment. *Biomedicines* **2022**, *10*, 2116, doi:10.3390/biomedicines10092116.
17. Grivennikov, S.I.; Greten, F.R.; Karin, M. Immunity, Inflammation, and Cancer. *Cell* **2010**, *140*, 883–899, doi:10.1016/J.CELL.2010.01.025.
18. Cruceriu, D.; Baldasici, O.; Balacescu, O.; Berindan-Neagoe, I. The Dual Role of Tumor Necrosis Factor-Alpha (TNF-α) in Breast Cancer: Molecular Insights and Therapeutic Approaches. *Cellular Oncology* 2020, *43* doi:10.1007/s13402-019-00489-1.
19. Mishra, A.; Suman, K.H.; Nair, N.; Majeed, J.; Tripathi, V. An Updated Review on the Role of the CXCL8-CXCR1/2 Axis in the Progression and Metastasis of Breast Cancer. *Mol Biol Rep* **2021**, *48*, 6551–6561, doi:10.1007/S11033-021-06648-8.
20. Shi, Z.; Yang, W.M.; Chen, L.P.; Yang, D.H.; Zhou, Q.; Zhu, J.; Chen, J.J.; Huang, R.C.; Chen, Z.S.; Huang, R.P. Enhanced Chemosensitization in Multidrug-Resistant Human Breast Cancer Cells by Inhibition of IL-6 and IL-8 Production. *Breast Cancer Res Treat* **2012**, *135*, 737–747, doi:10.1007/S10549-012-2196-0.
21. Newman, D.J.; Cragg, G.M. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J Nat Prod* **2020**, *83*, 770–803, doi:10.1021/acs.jnatprod.9b01285.
22. Cragg, G.M.; Pezzuto, J.M. Natural Products as a Vital Source for the Discovery of Cancer Chemotherapeutic and Chemopreventive Agents. *Med Princ Pract* **2016**, *25 Suppl 2*, 41–59, doi:10.1159/000443404.
23. Khan, M.I.; Bouyahya, A.; Hachlafi, N.E.L.; Menyiy, N. El; Akram, M.; Sultana, S.; Zengin, G.; Ponomareva, L.; Shariati, M.A.; Ojo, O.A.; et al. Anticancer Properties of Medicinal Plants and Their Bioactive Compounds against Breast Cancer: A Review on Recent Investigations. *Environ Sci Pollut Res Int* **2022**, doi:10.1007/S11356-021-17795-7.

24. Abutaha, N.; Nasr, F.A.; Mohammed, A.Z.; Semlali, A.H.; Al-Mekhlafi, F.A.; Wadaan, M.A. Calendula Arvensis L. as an Anti-Cancer Agent against Breast Cancer Cell Lines. *Mol Biol Rep* **2019**, *46*, 2187–2196, doi:10.1007/S11033-019-04672-3.
25. Chun, J.; Song, K.; Kim, Y.S. Sesquiterpene Lactones-Enriched Fraction of Inula Helenium L. Induces Apoptosis through Inhibition of Signal Transducers and Activators of Transcription 3 Signaling Pathway in MDA-MB-231 Breast Cancer Cells. *Phytotherapy research* **2018**, *32*, 2501–2509, doi:10.1002/ptr.6189.
26. Engel, N.; Falodun, A.; Kühn, J.; Kragl, U.; Langer, P.; Nebe, B. Pro-Apoptotic and Anti-Adhesive Effects of Four African Plant Extracts on the Breast Cancer Cell Line MCF-7. *BMC Complement Altern Med* **2014**, *14*, 1–13, doi:10.1186/1472-6882-14-334.
27. Lang, S.J.; Schmiech, M.; Hafner, S.; Paetz, C.; Steinborn, C.; Huber, R.; Gaafary, M. El; Werner, K.; Schmidt, C.Q.; Syrovets, T.; et al. Antitumor Activity of an Artemisia Annua Herbal Preparation and Identification of Active Ingredients. *Phytomedicine* **2019**, *62*, doi:10.1016/j.phymed.2019.152962.
28. Zaika, M.A.; Kilian, N.; Jones, K.; Krinitsina, A.A.; Nilova, M. V; Speranskaya, A.S.; Sukhorukov, A.P. *Scorzonera* Sensu Lato (*Asteraceae, Cichorieae*) - Taxonomic Reassessment in the Light of New Molecular Phylogenetic and Carpological Analyses. *PhytoKeys* **2020**, *137*, 1–85, doi:10.3897/phytokeys.137.46544.
29. Duran, A.; Hamzaoğlu, E. A New Species of *Scorzonera* L. (*Asteraceae*) from South Anatolia, Turkey. *Biologia (Bratisl)* **2004**, *59*, 47–50.
30. Wang, Y.; Edrada-Ebel, R.; Tsevegsuren, N.; Sendker, J.; Braun, M.; Wray, V.; Lin, W.; Proksch, P. Dihydrostilbene Derivatives from the Mongolian Medicinal Plant *Scorzonera radiata*. *J Nat Prod* **2009**, *72*, 671–675, doi:10.1021/np800782f.
31. Buranov, A.U.; Elmuradov, B.J. Extraction and Characterization of Latex and Natural Rubber from Rubber-Bearing Plants. *J Agric Food Chem* **2010**, *58*, 734–743, doi:10.1021/jf903096z.
32. Bahadır Acıkara, Ö.; Citoğlu Gülçin, S.; Dall'Acqua, S.; Özbek, H.; Cvačka, J.; Žemlička, M.; Šmejkal, K. Bioassay-Guided Isolation of the Antinociceptive Compounds Motiol and β-Sitosterol from *Scorzonera latifolia* Root Extract. *Pharmazie* **2014**, *69*, 711–714, doi:10.1691/ph.2014.3920.
33. Karakaya, S.; Polat, A.; Aksakal, Ö.; Sümbüllü, Y.Z.; İncekara, Ü. Ethnobotanical Study of Medicinal Plants in Aziziye District (Erzurum, Turkey). *Turk J Pharm Sci* **2020**, *17*, 211–220, doi:10.4274/tjps.galenos.2019.24392.

34. Yaldiz, G.; Koca Çalişkan, U.; Aka, C. In Vitro Screening of Natural Drug Potentials for Mass Production. *Not Bot Horti Agrobot Cluj Napoca* **2017**, *45*, 292–300, doi:10.15835/nbha45110397.
35. Tsevegsuren, N.; Edrada, R.A.; Lin, W.; Ebel, R.; Torre, C.; Ortlepp, S.; Wray, V.; Proksch, P. Biologically Active Natural Products from Mongolian Medicinal Plants *Scorzonera divaricata* and *Scorzonera pseudodivaricata*. *J Nat Prod* **2007**, *70*, 962–967, doi:10.1021/np070013r.
36. Sharma, J.; Gairola, S.; Gaur, R.D.; Painuli, R.M. The Treatment of Jaundice with Medicinal Plants in Indigenous Communities of the Sub-Himalayan Region of Uttarakhand, India. *J Ethnopharmacol* **2012**, *143*, 262–291, doi:10.1016/j.jep.2012.06.034.
37. Auzi, A.R.A.; Hawisa, N.T.; Sherif, F.M.; Sarker, S.D. Neuropharmacological Properties of *Launaea resedifolia*. *Revista Brasileira de Farmacognosia* **2007**, *17*, 160–165, doi:10.1590/S0102-695X2007000200004.
38. Harkati, B.; Salah, A.; Bayet, C.; Laouer, H.; Dijoux-Franca, M.-G. Evaluation of Antioxidant Activity, Free Radical Scavenging and CUPRAC of Two Compounds Isolated from *Scorzonera undulata* Ssp. *deliciosa*. *Adv Environ Biol* **2013**, *7*, 591–594.
39. Quattrocchi, U. *CRC World Dictionary of Plant Names*; CRC Press, 1999; ISBN 0-8493-2678-8.
40. Lendzion, K.; Gornowicz, A.; Bielawski, K.; Bielawska, A. Phytochemical Composition and Biological Activities of *Scorzonera* Species. *Int J Mol Sci* **2021**, *22*, 5128, doi:10.3390/ijms22105128.
41. Bahadır, Ö.; Citoğlu, G.S.; Smejkal, K.; Dall'Acqua, S.; Ozbek, H.; Cvacka, J.; Zemlicka, M. Analgesic Compounds from *Scorzonera latifolia* (Fisch. and Mey.) DC. *J Ethnopharmacol* **2010**, *131*, 83–87, doi:10.1016/j.jep.2010.06.003.
42. Bahadır, Ö.; Saltan, H.G.; Özbek, H. Antinociceptive Activity of Some *Scorzonera* L. Species. *Turk J Med Sci* **2012**, *42*, 861–866, doi:10.3906/sag-1012-1320.
43. Akkol, E.K.; Bahadir Acikara, Ö.; Süntar, I.; Ergene, B.; Saltan Çitoğlu, G. Ethnopharmacological Evaluation of Some *Scorzonera* Species: In Vivo Anti-Inflammatory and Antinociceptive Effects. *J Ethnopharmacol* **2012**, *140*, 261–270, doi:10.1016/j.jep.2012.01.015.
44. Bahadır-Acıkara, Ö.; Özbilgin, S.; Saltan-İşcan, G.; Dall'Acqua, S.; Rjašková, V.; Özgökçe, F.; Suchý, V.; Šmejkal, K. Phytochemical Analysis of *Podospermum* and

Scorzonera n-Hexane Extracts and the HPLC Quantitation of Triterpenes. *Molecules* **2018**, *23*, doi:10.3390/molecules23071813.

45. Bahadır Acikara, Ö.; Hošek, J.; Babula, P.; Cvačka, J.; Budešínský, M.; Dračinský, M.; Saltan İşcan, G.; Kadlecová, D.; Ballová, L.; Šmejkal, K. Turkish *Scorzonera* Species Extracts Attenuate Cytokine Secretion via Inhibition of NF-KB Activation, Showing Anti-Inflammatory Effect *in Vitro*. *Molecules* **2016**, *21*, E43, doi:10.3390/molecules21010043.
46. Şahin, H.; Sarı, A.; Özsoy, N.; Özbek Çelik, B.; Koyuncu, O. Two New Phenolic Compounds and Some Biological Activities of *Scorzonera pygmaea* Sibth. & Sm. Subaerial Parts. *Nat Prod Res* **2020**, *34*, 621–628, doi:10.1080/14786419.2018.1493585.
47. Boussaada, O.; Saidana, D.; Chriaa, J.; Chraif, I.; Mahjoub, M.A.; Mighri, Z.; Daami, M.; Helal, A.N. Chemical Composition and Antimicrobial Activity of Volatile Components of *Scorzonera undulata*. *Journal of Essential Oil Research* **2008**, *20*, 358–362, doi:10.1080/10412905.2008.9700030.
48. Abdelkader, H.B.; Salah, K.B.H.; Liouane, K.; Boussaada, O.; Gafsi, K.; Mahjoub, M.A.; Aouni, M.; Hellal, A.N.; Mighri, Z. Antimicrobial Activity of *Rhaponticum acaule* and *Scorzonera undulata* Growing Wild in Tunisia. *Afr J Microbiol Res* **2010**, *4*, 1954–1958.
49. Wu, Q.X.; He, X.F.; Jiang, C.X.; Zhang, W.; Shi, Z.N.; Li, H.F.; Zhu, Y. Two Novel Bioactive Sulfated Guaiane Sesquiterpenoid Salt Alkaloids from the Aerial Parts of *Scorzonera divaricata*. *Fitoterapia* **2017**, *124*, 113–119, doi:10.1016/j.fitote.2017.10.011.
50. Sweidan, A.; El-Mestrah, M.; Kanaan, H.; Dandache, I.; Merhi, F.; Chokr, A. Antibacterial and Antibiofilm Activities of *Scorzonera mackmeliana*. *Pak J Pharm Sci* **2020**, *33*, 199–206.
51. Yang, Y.-J.; Liu, X.; Wu, H.-R.; He, X.-F.; Bi, Y.-R.; Zhu, Y.; Liu, Z.-L. Radical Scavenging Activity and Cytotoxicity of Active Quinic Acid Derivatives from *Scorzonera divaricata* Roots. *Food Chem* **2013**, *138*, 2057–2063, doi:10.1016/j.foodchem.2012.10.122.
52. Nasseri, M.A.; Bigy, S.S.; Allahresani, A.; Malekaneh, M. Assessment of Antioxidant Activity, Chemical Characterization and Evaluation of Fatty Acid Compositions of *Scorzonera paradoxa* Fisch and C.A. Mey. *Jundishapur J Nat Pharm Prod* **2015**, *10*, doi:10.17795/jjnpp-19781.

53. Milella, L.; Bader, A.; de Tommasi, N.; Russo, D.; Braca, A. Antioxidant and Free Radical-Scavenging Activity of Constituents from Two *Scorzonera* Species. *Food Chem* **2014**, *160*, 298–304, doi:10.1016/j.foodchem.2014.03.097.
54. Küpeli Akkol, E.; Acikara, O.B.; Süntar, I.; Citolu, G.S.; Kele, H.; Ergene, B. Enhancement of Wound Healing by Topical Application of *Scorzonera* Species: Determination of the Constituents by HPLC with New Validated Reverse Phase Method. *J Ethnopharmacol* **2011**, *137*, 1018–1027, doi:10.1016/j.jep.2011.07.029.
55. Küpeli Akkol, E.; Šmejkal, K.; Kurtul, E.; Ilhan, M.; Güragac, F.T.; Çitoğlu, G.S.; Acıkara, Ö.B.; Cvačka, J.; Buděšínský, M. Inhibitory Activity of *Scorzonera latifolia* and Its Components on Enzymes Connected with Healing Process. *J Ethnopharmacol* **2019**, *245*, doi:10.1016/j.jep.2019.112168.
56. Süntar, I.; Bahadır Acıkara, Ö.; Saltan Çitoğlu, G.; Keleş, H.; Ergene, B.; Akkol, E.K. In Vivo and in Vitro Evaluation of the Therapeutic Potential of Some Turkish *Scorzonera* Species as Wound Healing Agent. *Curr Pharm Des* **2012**, *18*, 1421–1433, doi:10.2174/138161212799504867.
57. Bahadır Acıkara, Ö.; Ilhan, M.; Kurtul, E.; Šmejkal, K.; Akkol, E.K. Inhibitory Activity of *Podospermum canum* and Its Active Components on Collagenase, Elastase and Hyaluronidase Enzymes. *Bioorg Chem* **2019**, *93*, doi:10.1016/j.bioorg.2019.103330.
58. Zidorn, C.; Ellmerer-Müller, E.P.; Stuppner, H. Tyrolobibenzyls - Novel Secondary Metabolites from *Scorzonera humilis*. *Helv Chim Acta* **2000**, *83*, 2920–2925, doi:10.1002/1522-2675(20001108)83:11<2920::AID-HLCA2920>3.0.CO;2-5.
59. Wang, B.; Li, G.-Q.; Guan, H.; Yang, L.; Tong, G. A New Erythrodiol Triterpene Fatty Ester from *Scorzonera mongolica*. *Yao Xue Xue Bao* **2009**, *44*, 1258–1261.
60. Csupor-Löffler, B.; Hajdú, Z.; Réthy, B.; Zupkó, I.; Máthé, I.; Rédei, T.; Falkay, G.; Hohmann, J. Antiproliferative Activity of Hungarian *Asteraceae* Species against Human Cancer Cell Lines. Part II. *Phytotherapy Research* **2009**, *23*, 1109–1115, doi:10.1002/ptr.2755.
61. Zhu, Y.; Wu, Q.X.; Hu, P.Z.; Wu, W.S. Biguaiascorzolides A and B: Two Novel Dimeric Guaianolides with a Rare Skeleton, from *Scorzonera austriaca*. *Food Chem* **2009**, *114*, 1316–1320, doi:10.1016/j.foodchem.2008.11.009.
62. Yang, Y.-J.; Yao, J.; Jin, X.-J.; Shi, Z.-N.; Shen, T.-F.; Fang, J.-G.; Yao, X.-J.; Zhu, Y. Sesquiterpenoids and Tirucallane Triterpenoids from the Roots of *Scorzonera divaricata*. *Phytochemistry* **2016**, *124*, 86–98, doi:10.1016/j.phytochem.2016.01.015.

63. Strzelecka, Halina.; Kowalski, J. *Encyklopedia Zielarstwa i Ziołolecznictwa*; 1st ed.; Wydawnictwo Naukowe PWN: Warszawa, 2000; ISBN 8301131322.
64. Zidorn, C.; Ellmerer-Müller, E.P.; Stuppner, H. Sesquiterpenoids from *Scorzonera hispanica* L. *Pharmazie* **2000**, *55*, 550–551.
65. Granica, S.; Lohwasser, U.; Jöhrer, K.; Zidorn, C. Qualitative and Quantitative Analyses of Secondary Metabolites in Aerial and Subaerial of *Scorzonera hispanica* L. (Black Salsify). *Food Chem* **2015**, *173*, 321–331, doi:10.1016/j.foodchem.2014.10.006.
66. Granica, S.; Zidorn, C. Phenolic Compounds from Aerial Parts as Chemosystematic Markers in the *Scorzonerinae* (*Asteraceae*). *Biochem Syst Ecol* **2015**, *58*, 102–113, doi:10.1016/j.bse.2014.11.005.
67. Petkova, N. Characterization of Inulin from Black Salsify (*Scorzonera hispanica* L.) for Food and Pharmaceutical Purposes. *Asian Journal of Pharmaceutical and Clinical Research* **2018**, *11*, 221–225, doi:10.22159/ajpcr.2018.v11i12.28262.
68. Park, B.Y.; Oh, S.R.; Ahn, K.S.; Kwon, O.K.; Lee, H.K. (-)-Syringaresinol Inhibits Proliferation of Human Promyelocytic HL-60 Leukemia Cells via G1 Arrest and Apoptosis. *Int Immunopharmacol* **2008**, *8*, 967–973, doi:10.1016/j.intimp.2008.02.012.
69. Jeong, Y.H.; Chung, S.Y.; Han, A.R.; Sung, M.K.; Jang, D.S.; Lee, J.; Kwon, Y.; Lee, H.J.; Seo, E.K. P-Glycoprotein Inhibitory Activity of Two Phenolic Compounds, (-)-Syringaresinol and Tricin from *Sasa borealis*. *Chem Biodivers* **2007**, *4*, 12–16, doi:10.1002/cbdv.200790001.
70. Zhu, Y.; Hu, P.Z.; He, Z.W.; Wu, Q.X.; Li, J.; Wu, W.S. Sesquiterpene Lactones from *Scorzonera austriaca*. *J Nat Prod* **2010**, *73*, 237–241, doi:10.1021/np900378c.
71. Edlich, F. BCL-2 Proteins and Apoptosis: Recent Insights and Unknowns. *Biochem Biophys Res Commun* **2018**, *500*, 26–34, doi:10.1016/j.bbrc.2017.06.190.
72. Marquez, R.T.; Xu, L. Bcl-2:Beclin 1 Complex: Multiple, Mechanisms Regulating Autophagy/Apoptosis Toggle Switch. *Am J Cancer Res* **2012**, *2*, 214.
73. Luo, M.; Guan, J.L. Focal Adhesion Kinase: A Prominent Determinant in Breast Cancer Initiation, Progression and Metastasis. *Cancer Lett* **2010**, *289*, doi:10.1016/j.canlet.2009.07.005.
74. Dan, H.C.; Cooper, M.J.; Cogswell, P.C.; Duncan, J.A.; Ting, J.P.Y.; Baldwin, A.S. Akt-Dependent Regulation of NF-KB Is Controlled by MTOR and Raptor in Association with IKK. *Genes Dev* **2008**, *22*, 1490–1500, doi:10.1101/gad.1662308.

75. Whyte, J.; Bergin, O.; Bianchi, A.; McNally, S.; Martin, F. Key Signalling Nodes in Mammary Gland Development and Cancer. Mitogen-Activated Protein Kinase Signalling in Experimental Models of Breast Cancer Progression and in Mammary Gland Development. *Breast Cancer Res* **2009**, *11*, doi:10.1186/bcr2361.
76. Kello, M.; Takac, P.; Kubatka, P.; Kuruc, T.; Petrova, K.; Mojzis, J. Oxidative Stress-Induced DNA Damage and Apoptosis in Clove Buds-Treated MCF-7 Cells. *Biomolecules* **2020**, *10*, doi:10.3390/biom10010139.
77. Chun, J.; Han, L.; Xu, M.Y.; Wang, B.; Cheng, M.S.; Kim, Y.S. The Induction of Apoptosis by a Newly Synthesized Diosgenyl Saponin through the Suppression of Estrogen Receptor-α in MCF-7 Human Breast Cancer Cells. *Arch Pharm Res* **2014**, *37*, 1477–1486, doi:10.1007/s12272-013-0279-z.
78. Waugh, D.J.J.; Wilson, C. The Interleukin-8 Pathway in Cancer. *Clin Cancer Res* **2008**, *14*, 6735–6741, doi:10.1158/1078-0432.ccr-07-4843.
79. Dennis, K.L.; Blatner, N.R.; Gounari, F.; Khazaie, K. Current Status of IL-10 and Regulatory T-Cells in Cancer. *Curr Opin Oncol* **2013**, *25*, 637, doi:10.1097/cco.0000000000000006.
80. Chang, C.M.; Lam, H.Y.P.; Hsu, H.J.; Jiang, S.J. Interleukin-10: A Double-Edged Sword in Breast Cancer. *Tzu Chi Med J* **2021**, *33*, 203–211, doi:10.4103/tcmj.tcmj_162_20.

7. Streszczenie w języku polskim i angielskim

Streszczenie

Wężymord czarny korzeń (*Scorzonera hispanica* L.) (Asteraceae) jest wieloletnią rośliną uprawną, hodowaną w Europie i południowej Syberii. W medycynie ludowej Europy skorzonera stosowana była w leczeniu przeziębienia oraz jako środek pobudzający apetyt czy mukolityk przy chorobach płuc. Analizy części nadziemnych i podziemnych rośliny wykazały obecność licznych substancji o potencjale biologicznym, w tym związków o właściwościach cytotoksycznych wobec komórek nowotworowych.

Celem niniejszej rozprawy doktorskiej była analiza jakościowa i ilościowa oleju oraz ekstraktów i frakcji z *S. hispanica* (**SH1**-**SH12**) z wykorzystaniem technik GC-MS oraz LC-PDA-MS. Dokonano oceny aktywności biologicznej otrzymanych produktów z nasion skorzonery wobec komórek raka piersi linii MCF-7 oraz MDA-MB-231, a także komórek prawidłowych – fibroblastów skóry ludzkiej. Następnie dokonano analizy molekularnego mechanizmu działania trzech najbardziej aktywnych produktów (ekstraktu metanolowego **SH1** oraz frakcji eterowej **SH4** i chloroformowej **SH11**) w komórkach raka piersi. Oceniono ich wpływ na proces biosyntezy DNA, a także zdolność do indukcji procesu apoptozy w komórkach. Dokonano oceny wybranych produktów na ekspresję białek związanych z apoptozą i autofagią. Następnie, zbadano wpływ wybranych ekstraktów i frakcji na stężenie białek uczestniczących w komórkowych szlakach sygnałowych oraz stężenie wybranych cytokin pro- i przeciwzapalnych.

Analizy LC-PDA-MS i GC-MS wykazały obecność aktywnych biologicznie związków z grupy flawonoidów oraz szereg pochodnych kwasu chinowego, a także wysoką zawartość kwasów tłuszczowych (linolenowego i palmitynowego) oraz 3,4-dimetoksycynamonianu metylu, D-chiro-inozytolu i kwasu kawowego. Uzyskane w toku pracy wyniki wykazały, że **SH1**, **SH4** i **SH11** posiadają zdolność do indukcji apoptozy i autofagii w komórkach raka piersi linii MCF-7. Otrzymane produkty powodowały obniżenie stężenia kinazy Akt oraz ERK1/2 w komórkach. Dodatkowo, wykazano ich przeciwzapalne działanie na komórki nowotworowe.

Uzyskane wyniki mogą sugerować, że nasiona *S. hispanica* są obiecującym źródłem związków o potencjalnym zastosowaniu w terapii raka piersi. Niemniej jednak, konieczne są dalsze pogłębione badania, zwłaszcza nad aktywnością składników najbardziej aktywnych ekstraktów i frakcji, a także ich metabolizmem i bezpieczeństwem.

Summary

Black salsify (*Scorzonera hispanica* L.) (Asteraceae) is a perennial cultivated plant, grown in Europe and southern Siberia. In European folk medicine, black salsify was used to treat colds, as an appetite stimulant or for lung diseases. Today, its roots are a valued vegetable. Analyses of the aerial and subaerial parts of the plant have revealed the presence of numerous substances with biological potential, including compounds with cytotoxic properties against cancer cells.

The aim of this dissertation was to qualitatively and quantitatively evaluate the composition of oil and extracts and fractions from *S. hispanica* (**SH1**-**SH12**) using GC-MS and LC-PDA-MS techniques. Biological activity of the products obtained *Scorzonera* seeds against breast cancer cells of the MCF-7 and MDA-MB-231 lines, as well as normal cells – human skin fibroblasts was evaluated. Subsequently, the molecular mechanism of action of the three most active products – methanolic extract (**SH1**) and ether (**SH4**) and chloroform (**SH11**) fractions – in breast cancer cells was evaluated. The effects on DNA biosynthesis were assessed, as well as the ability to induce the apoptosis in MCF-7 cells. Then, the influence of **SH1**, **SH4**, and **SH11** on the expression of proteins related to apoptosis and autophagy was examined. In the next step, the effects of selected extracts and fractions on the concentration of proteins involved in cellular signaling pathways and the concentration of selected pro- and anti-inflammatory cytokines were evaluated.

LC-PDA-MS and GC-MS analyses revealed the presence of biologically active compounds, including flavonoids and quinic acid derivatives, as well as a high content of fatty acids (linolenic and palmitic acids) Notable amounts of methyl 3,4-dimethoxycinnamate, D-chiro-inositol and caffeic acid were reported as well. The results obtained in the course of the work showed that **SH1**, **SH4**, and **SH11** exhibit the ability to induce apoptosis and autophagy in breast cancer cells. The obtained products also caused a decrease in Akt and ERK1/2 concentrations in MCF-7 cells. In addition, their anti-inflammatory effect on cancer cells in vitro was demonstrated.

The results may suggest that *S. hispanica* seeds are a promising source of compounds with potential applications in breast cancer therapy. Nevertheless, further in-depth studies are needed, especially on the activity of the phytocomponents of extracts and fractions, as well as their metabolism and safety.

9. Publikacja I: Phytochemical Composition and Biological Activities of *Scorzonera* Species

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Review

Phytochemical Composition and Biological Activities of *Scorzonera* Species

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Abstract: The genus *Scorzonera* comprises nearly 200 species, naturally occurring in Europe, Asia, and northern parts of Africa. Plants belonging to the *Scorzonera* genus have been a significant part of folk medicine in Asia, especially China, Mongolia, and Turkey for centuries. Therefore, they have become the subject of research regarding their phytochemical composition and biological activity. The aim of this review is to present and assess the phytochemical composition, and bioactive potential of species within the genus *Scorzonera*. Studies have shown the presence of many bioactive compounds like triterpenoids, sesquiterpenoids, flavonoids, or caffeic acid and quinic acid derivatives in extracts obtained from aerial and subaerial parts of the plants. The antioxidant and cytotoxic properties have been evaluated, together with the mechanism of anti-inflammatory, analgesic, and hepatoprotective activity. *Scorzonera* species have also been investigated for their activity against several bacteria and fungi strains. Despite mild cytotoxicity against cancer cell lines in vitro, the bioactive properties in wound healing therapy and the treatment of microbial infections might, in perspective, be the starting point for the research on *Scorzonera* species as active agents in medical products designed for miscellaneous skin conditions.

Keywords: *Scorzonera*; biological activity; herbal medicine; phytochemical composition; *Asteraceae*

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1. Introduction

Scorzonera L. is a genus in the *Cichorieae* tribe of the *Asteraceae* family. It is spread mostly in central and southern parts of Europe, Eurasia, and Africa in arid areas [1,2]. Numerous species are endemic to Anatolia (Turkey) [1,3–11], Mongolia [12–15], and China [16–18] The genus comprises approximately 180–190 species [19], including *S. hispanica*, whose roots are a valued vegetable, with the taste similar to asparagus [20], and *S. tau-saghyz* (a species of interest in terms of obtaining natural rubber) [21]. Several *Scorzonera* species are a source of feed for farming animals in arid regions [15]. Typically, plants within the *Scorzonera* genus are perennial herbs characterized by the presence of a caudex or tuber. Biennial plants or dwarf subshrubs are rare [19]. Plants within the genus *Scorzonera* are reported to contain flavonoids [12,13,22–24], phenolic acid derivatives [8,13,25,26], triterpenoids [18,23,27–31], sesquiterpenoids [14,17,20,32–34], dihydroisocoumarins [7,35–37], and other bioactive compounds. *Scorzonera* species have been commonly used as medicinal plants in European and Asian herbal therapy for ages. In Turkey, they are known as hemostatic agents, as well as, when used externally, as plasters in the process of wound healing [38]. The plants are also present in folk medicine as a remedy for hypertension, atherosclerosis, or kidney dysfunction [39].

This review aims to present the phytochemical composition of *Scorzonera* species, including the compounds characteristic for the genus, as well as novel compounds, which have not been previously isolated from *Asteraceae*. Extraction methods have been briefly summarized as well. A summary of available data regarding the use of *Scorzonera* in folk

medicine has also been included. Phytochemical composition and ethnopharmacological reports lead to the third part of this paper, the assessment of biological activities of natural products (extracts, fractions, and pure compounds) obtained from species within the genus *Scorzonera.* To our best knowledge, this is the first comprehensive review of the current findings in the field of phytochemistry and bioactivity of *Scorzonera* species. The assessment of biological activity in vitro and in vivo is the first step in the development of new plant-derived products and those play a substantial role in healthcare [40]. Some novel natural medicines are under clinical trials [41], others have been approved in therapy [42,43]. In this paper, a summary of the results of in vitro and in vivo studies has been made, as well as an attempt to evaluate their significance and therapeutic potential.

The search strategy for this review involved browsing results for terms '*Scorzonera*' and 'biological activity' or 'bioactive', '*Scorzonera*' and 'phytochemistry' 'phytochemical composition' in the following databases: Reaxys, PubMed, and ScienceDirect. The search was limited to the years 2000–2021, with three studies published before the year 2000 included in this review [44–46].

2. *Scorzonera* in Traditional Medicine

Genera within the family *Asteraceae* have been present in folk medicine across Europe, Asia, and northern Africa. That includes species within the *Scorzonera* genus, which are a significant part of Turkish traditional medicine in the therapy of arteriosclerosis, kidney disorders, wounds, rheumatism, but also as antidiabetic, antihypertensive, and antinociceptive medications [5]. The leaves of *S. latifolia* (Fisch and Mey.) DC., applied topically, act as plaster and prevent nausea. Turkish folk medicine uses latex obtained from *S. latifolia* to treat infertility and as an anthelmintic and pain-relieving medication [3,6,38,47]. Roots of *S. tomentosa* L. are believed to have hemostatic properties when ingested [38]. Aerial parts of *S. laciniata* L. are known as antipyretic, antipyogenic, antiatherosclerotic, antidiabetic, antirheumatic, and blood pressure-lowering agents in folk therapy [39]. Turkish folk medicine uses *S. phaeopappa* Boiss., *S. sosnowskyii* Lipsch., and *S. mirabilis* Lipsch. for headaches. *S. mollis* Biela is used as a diuretic and against kidney stones [48]. In Algerian traditional medicine, *S. undulata* ssp. *deliciosa* is a part of the treatment of snake bites [49]. Mongolian folk remedies for various ailments include *Scorzonera* species as well. There are reports for the use of *S. pseudodivaricata* Lipsch. as antipyretic in viral and bacterial infections, anti-diarrheal and diuretic agents, as well as for the treatment of lung edema and diseases caused by parasite infections. Aerial and subaerial parts of *S. divaricata* Turcz. are used to treat ulcers and stomach tumors. [14]. Leaves and shoots of *S. divaricata* are also present in the folk medicine of India in the therapy of jaundice [50]. Traditional Chinese medicine uses *S. mongolica* Maxim. root to reduce fever and treat carbuncle mastitis, as well as an antineoplastic agent [31]. Roots of *Scorzonera hispanica* L., currently cultivated and eaten as a vegetable, were formerly used in European folk medicine as a mucolytic agent in pulmonary diseases, appetite stimulator, and to defeat a cold. [14,32]. Tibetan folk medicine has used *S. austriaca* Willd. for the treatment of carbuncle, inflammation, and fever [17,28]. *Scorzonera radiata* Fisch. is a Mongolian traditional remedy for bacterial and viral infection-induced fever, poisonous ulcers, and as a lactation-inducing and diuretic agent [15]. In Libya, *Scorzonera resedifolia* L. is known as a folk medication for liver pain [51].

3. Phytochemical Composition of *Scorzonera* Species

Species within the *Scorzonera* genus are a source of flavonoid aglycones and glycosides, phenolic acids and their derivatives, lignans, triterpenoids, sesquiterpenoids, dihydroisocoumarins, bibenzyl derivatives, as well other compounds [4,10,14,27,35,36,52–55].

3.1. Scorzonera acuminata Boiss.

The samples of *Scorzonera acuminata* aerial parts and roots were collected in the northern part of Anatolia, Turkey. In the study by Süntar et al. [9], plant samples were extracted with

20% aqueous methanol. The study by Bahadır-Acıkara et al. [27] includes a phytochemical analysis of *n*-hexane extracts of *S. acuminata* aerial parts and roots.

A 20% aqueous methanol extract of the aerial parts of *S. acuminata* was reported to contain chlorogenic acid, rutin, and cyranoside [9]. Another study reports the presence of α-amyrin, lupeol, and lupeol acetate in the *n*-hexane extract [27].

In the aqueous methanol extract from the roots, chlorogenic acid and trace amounts of rutin were found [9]. An *n*-hexane extract contained lupeol, lupeol acetate, and α-amyrin [27].

3.2. Scorzonera aristata Rameond ex DC.

Samples of *S. aristata* were collected in Northern Italy [26,55,56]. The leaves from the specimen in the study by Jehle et al. [56] were extracted with methanol and subsequently with a mixture of methanol, acetone and water (3/1/1, *v*/*v*/*v*), the roots were first treated with the mixture of methanol, acetone, and water (3/1/1, *v*/*v*/*v*), then were extracted with methanol. The subaerial parts of the samples in the study by Zidorn et al. [55] were mixed with a stock solution and sonicated with methanol, then HPLC was performed. A study by Granica and colleagues [26] involved extraction of aerial parts of the species with 50% aqueous methanol and the HPLC analysis of obtained extracts.

From the aerial part extracts of the plant, flavonoids (rutin, isoorientin, and quercetin 3-*O*-glucoside) and caffeic acid derivatives (chlorogenic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid) were isolated [56]. In the 50% aqueous methanol extract, the presence of chlorogenic acid, 4-*O*-caffeoylquinic acid, 1,5-*O*-dicaffeoylquinic acid, and rutin, as well as apigenin derivative and luteolin derivative [26].

From subaerial parts, 3,5-dicaffeoylquinic acid and caffeic acid methyl ester were isolated, along with the following triterpenes: lupeol, magnificol, and 3α-hydroxyolean-5-ene [56]. The presence of chlorogenic acid and 3,5-dicaffeoylquinic acid was previously reported in the study by Zidorn et al. [55].

3.3. Scorzonera aucheriana DC.

Samples of *S. aucheriana* were collected in central Turkey and aerial parts were extracted with methanol at room temperature [7,29].

Investigation on aerial parts of the plant led to the isolation of dihydroisocoumarins and dihydroisocoumarin derivatives (scorzopygmaecoside, scorzocreticoside II, isoscorzopygmaecoside, scorzoaucherioside I and II), quinic acid derivatives (3,5-*O*-dicaffeoyl-*epi*-quinic acid and 3,5-*O*-dicaffeoylquinic acid), and 3,4-dihydroxyphenyl caffeate [7]. In another study, chlorogenic acid derivatives (methyl 1-(2-methylcyclopropyl-1-carbonyloxy) chlorogenate and 3,4-bis[(3′,4-dioxo-1′,3′,5′,6′-tetrahydrospiro[cyclohexa-2,5-diene-1,4′-cyclopenta[*c*]-furan]-1′-yl)]chlorogenic acid), triterpenoids (taraxasterol, taraxasterol acetate, taraxasterol oleate, lupeol, lupeol acetate and ptiloepoxide) and β-sitosterol were isolated from the methanol extract of *S. aucheriana* aerial parts [29].

3.4. Scorzonera austriaca Willd.

Samples of *S. austriaca* were collected in the northeast [24] and central [17,33] parts of China.

Herbs of *S. austriaca* were extracted with 70% aqueous ethanol. The extract was reported to contain the following flavonoid glycosides and flavonoid glycoside derivatives: 5,7,4′-trihydroxyflavone 6-*C*-(2″-*O*-β-D-glucopyranosyl β-D-glucopyranoside), 5,7,3′,4′-tetrahydroxyflavone 6-*C*-(2″-*O*-β-D-glucopyranosyl β-D-glucopyranoside), quercetin 3-*O*-rutinoside, 5,7,4′-trihydroxyflavone 6-*C*-β-D-glucopyranoside, 3′-methoxy-5,7,4′-trihydroxyflavone 6-*C*-β-D-glucopyranoside, 5,7,4′-trihydroxyflavone 8-*C*-(6″-*O*-*trans*-caffeoyl β-D-glucopyranoside and 5,7,3′,4′-tetrahydroxyflavone 8-*C*-(6″-*O*-*trans*-caffeoyl β-D-glucopyranoside [24].

Roots of the plant samples collected in central China were extracted with acetone and guaianolides (biguaiascorzolide A and biguaiascorzolide B) were isolated [17]. Before that discovery, in 2004, Li et al. [16] isolated a sesquiterpene lactone (3β,11α-dihydroxy-4β-methyl-guaia-10 (14)-en-12, 6α-olide) from the acetone extract of *S. austriaca* roots. Other identified

in the root acetone extract sesquiterpenoids were: scorzoaustriacoside, scorzoaustriacin, scorzoaustriacin 3-*O*-β-D-glucoside, 4-*epi*-dihydroestafiatol, 14-isovaleroxyscorzoaustricin, 14-isovaleroxyscorzoaustricin sulfate, zaluzanin C, glucozaluzanic C, dehydrocostus lactone, 11β,13-dihydrozaluzanin and diacetoxyisolippidiol [33]. In the study by Wu et al. from 2011 [28], the following compounds were isolated from the acetone root extract: oleanane-type triterpenes (3β-acetoxyglutin-5(10)-en-6-oxo, glutinol, β-amyrin-3-(3′-methylbutanonate), β-amyrin 3-acetyl, 3β-acetyl-11α,12α-oxidotaraxerol), ursane-type triterpenes (α-amyrin 3-acetyl, α-amyrin 3-acetyl-11-oxo, D-friedours-14-en-3β-acetyl-11α,12α-epoxy, taraxasterol, and ψ-taraxasteryl 3(3′-methyl-butanonate)), lupeol, (23*Z*)-cycloart-23-ene-3β, 25-dihydroxy, 9β,19-cyclolanostane-24-en-3-*oxo*, and steroids (β-sitosterol, β-stigmasterol, stigmast-4-en-3-one, stigmast-3β,5α,6β-trihydroxy, and β-sitosterol 3-β-D-glucoside).

3.5. Scorzonera baetica (Boiss.) Boiss.

Aerial parts of plant samples, collected in Spain, were extracted with 50% aqueous methanol at room temperature and the extracts were analyzed using HPLC.

The aerial part 50% methanol extract was reported to contain caffeoylquinic acid derivatives (3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, chlorogenic acid, 1,5-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid), flavonoid glycosides (orientin, isoorientin, vitexin, isovitexin, cyranoside), and flavonoid diglycosides [26].

3.6. Scorzonera cana (C.A. Meyer) Hoffm. var. alpina (Boiss.) Chamb.

Plant samples were collected in the north-central part of Turkey and extracted with 20% aqueous methanol at room temperature.

In the extract of the aerial parts, rutin and notable amounts of chlorogenic acid were reported present.

The presence of chlorogenic acid was detected in the root extract [9].

3.7. Scorzonera cana (C.A. Meyer) Hoffm. var. jacquiniana (W. Koch) Chamb.

Plant samples were collected in central Turkey [9,27]. Aerial and subaerial parts were separated and extracted with *n*-hexane [27] and 20% aqueous methanol [9].

Triterpenoids present in the *n*-hexane extract of the aerial parts were taraxasteryl acetate, lupeol, lupeol acetate, and α-amyrin [27]. Compounds present in the aqueous methanol extract were: chlorogenic acid, rutin, hyperoside, luteolin 7-glucoside, and trace amounts of apigenin [9].

In the root aqueous methanol extracts, chlorogenic acid was found. [9]. There were also reports on the presence of α-amyrin. taraxasteryl acetate, lupeol, and lupeol acetate in the *n*-hexane extract [27].

3.8. Scorzonera cinerea Boiss.

Samples of *S. cinerea* collected in central Turkey were extracted with *n*-hexane at room temperature.

In the extract of the aerial parts of *S. cinerea,* the following triterpenoids were detected: lupeol, lupeol acetate, taraxasteryl acetate, 3β-hydroxy-fern-7-en-6-one-acetate, α-amyrin, and olean-12-en-11-one-3-acetyl.

In the root extract of the plant, taraxasteryl acetate, lupeol, lupeol acetate, and α-amyrin were detected [27].

3.9. Scorzonera cretica Willd.

Samples of *S. cretica* were collected on Crete, Greece. The whole plant was subjected to extraction with dichloromethane and subsequently with methanol.

From the dichloromethane extract, dihydroisocoumarin (scorzocreticin), dihydroisocoumarins glycosides (scorzocreticoside I, scorzocreticoside II), and 3-*O*-β-D-glucopyranosylsitosterol were isolated. Triterpenoids (lupeol, lupeol acetate, lupenone, germanicol, german-

icol acetate, germanicone, taraxasterol, taraxasterol acetate, oleanol, oleanol acetate) were isolated from the methanol extract of the plant [37].

3.10. Scorzonera crispatula Boiss.

Plant samples (aerial parts) were collected in England [44] and Spain. Samples from Spain were extracted at room temperature with a mixture of methanol and water (1:1, *v*/*v*) and subjected to HPLC [26]. Samples collected in England were extracted with ethanol [44].

In the *S. crispatula* aerial part hydromethanolic extract, the presence of caffeic acid derivatives (3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, chlorogenic acid, 1,5-*O*-dicaffeoylquinic acid. 3,5-*O*-dicaffeoylquinic acid and 4,5-*O*-dicaffeoylquinic acid), flavonoid aglycones (quercetin and luteolin) flavonoid C-glycosides (isoorientin and isovitexin), and several flavonoid diglycosides was reported [26]. In the ethanol extract of *S. crispatula*, luteolin and quercetin were detected.

3.11. Scorzonera divaricata Turcz.

Samples of *S. divaricata* were collected in Mongolia [14] and central China [34,57–59]. Aerial parts were extracted with methanol at room temperature [14,34], then with methanol at 65 °C [34]. Subaerial parts were subjected to extraction with 95% aqueous ethanol [57–59].

From the aerial part methanol extract, feruloylpodospermic acid A and feruloylpodospermic acid B were isolated and the presence of known compounds (scopoletin, chlorogenic acid, isovitexin 4′-O-glucoside, isovitexin 2′-O-xyloside, kaempferol 3-O-rutinoside, apigenin) was detected [14]. The aerial part methanol extract was used to isolate the novel compounds sulfoscorzonin D and sulfoscorzonin E. Apart from sulfoscorzonins, the following compounds were isolated: benzoic acid derivatives (methyl-3,4-dihydroxybenzoate, *m*-hydroxybenzoic acid), coumarin derivatives (scopoletin, 7-hydroxycoumarin), flavonoid aglycones (diosmetin, luteolin, tricin, 7,3′,4′-trihydroxyflavonol, 5,7-dihydroxy-8-methoxyflavone, 5,7-dihydroxy-6-methoxyflavone), phenolic acid derivatives (*trans*-caffeic acid, *trans*-*p*-hydroxycoumaric acid, 4-hydroxy-3-methoxyphenyl ferulate), sesquiterpenoids (glucozaluzanin C, 1β,4α-dihydroxy-5α,6β,7α,11β*H*-eudermn-12,6-olide), steroids ((22*E*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol, ergosta-3β,5α,6β-trialcohol, stigma-5-en-3-*O*-β-glucoside), triterpenoids (oleanolic acid and lup-20(29)-ene-3β,28-diol), sacrolide A, and vomifoliol [34].

From the subaerial part ethanolic extract, the following compounds were isolated: phenolic acids and their derivatives ((–)–1,4-di-*O*-feruloyl-3-*O*-dihydrocaffeoylquinic acid, (–)–1-O-feruloyl-4-O-dihydrocaffeoylquinic acid (–)–3,5-di-O-feruloylquinic acid, (–)–1-*O*-feruloyl-3-*O*-dihydrocaffeoylquinic acid, (–)–1-*O*-feruloyl-5-*O*-dihydrocaffeoylquinic acid, 3-*O*-feruloylquinate, butyl 3-*O*-feruloylquinate, caffeic acid, dihydrocaffeic acid and its methyl, ethyl and n-butyl esters), triterpenoids (scorzodivaricin A, scorzodivaricin B and scorzodivaricin C, scorzodivaricin D, 23(Z)-3β-acetoxy-25-hydroxy-tirucalla-7,23-diene, 23(*Z*)-3β,25-dihydroxy-tirucalla-7,23-diene, 23(*Z*)-3β,25-dihydroxy-tirucalla-7,23-diene, 20(*R*)-3β-acetoxy21-hydroxy-24(31)-methylene-dammarane and oleanolic acid), sesquiterpenoids (sulfoscorzonin A, sulfoscorzonin B, sulfoscorzonin C, and 10(*Z*)-1-oxo-bisabola-2,10-dien-13-al), steroids (5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol, stigmast-4-en-6β-ol-3-one 6β-hydroxystigmastan-4-en-3-one, 3β-hydroxystigmast-5-en-7-one, 5,6α-epoxy-5α-stigmastan-3β-ol, 7β-hydroxysitosterol, 7α-hydroxysitosterol, β-sitosterol, and β-daucosterol), benzene derivatives (vanillin, vanillic acid 4-*O*-β-D-glucoside, vanillic acid 1-*O*-β-D-glucopyranosyl ester, tachioside, syringic acid ethyl ester, and 3,4-dimenthoxy-3′-hydroxy propiophenone), fatty acids (pinellic acid, linoleic acid, and palmitic acid), coumarin derivatives (scopolin and scopoletin), and a lignan (pinoresinol) [57–59].

3.12. Scorzonera eriophora DC.

Samples of *S. eriophora* aerial and subaerial parts were collected in Turkey and extracted at room temperature with 20% aqueous methanol [9] and *n*-hexane [27].

Chlorogenic acid was detected in aqueous methanol extracts of both aerial parts and roots. The aerial part extract was also reported to contain luteolin and luteolin 7-

glycoside [9]. The *n*-hexane extracts of aerial and subaerial parts both contained taraxasteryl acetate, lupeol, lupeol acetate Additionally, 3β-hydroxy-fern-7-en-6-one-acetate was reported to be present in the *n*-hexane root extract [27].

3.13. Scorzonera graminifolia L.

Aerial parts of the plant, collected in England, were extracted with ethanol and reported to contain quercetin and luteolin [44].

3.14. Scorzonera hieraciifolia Hayek

Samples of the plant were collected in central Turkey. Aerial and subaerial parts were separated, and aerial parts were extracted with ethanol at room temperature and fractioned. Then isolation of compounds was performed.

From the subaerial part ethanol extract, the following compounds were isolated: quinic acid derivatives (5-*O*-feruloyl quinic acid methyl ester, 1,5-di-*O*-feruloylquinic acid, chlorogenic acid methyl ester, 3-*O*-caffeoylquinic acid methyl ester, 1,3-di-*O*-caffeoylquinic acid methyl ester, 3,5-di-*O*-caffeoylquinic acid methyl ester, and 4,5-di-*O*-caffeoylquinic acid methyl ester), caffeic acid, and 3-(4′-hydroxyphenyl)-2-propenoic acid (4″-carboxyl)-phenyl ester [8].

3.15. Scorzonera hirsuta L.

Samples of *S. hirsuta* aerial parts, collected from the University of Reading (Reading, UK), were extracted with alcohol and the extract was reported to contain flavonoid aglycones: kaempferol, luteolin, and quercetin [44].

3.16. Scorzonera hispanica L.

In the studies by Granica et al. [20,26], plant samples were collected in Germany, subaerial parts for the quantitative analyses were purchased in Austria and Warsaw. In the study by Zidorn et al. [32], plant samples from Belgium were used. In the study by Petkova [60], the plant was harvested in Bulgaria.

For the isolation and identification of major constituents of *S. hispanica* subaerial parts, maceration with ethyl acetate was carried out. The phenolic compounds in aerial and subaerial parts were quantified using a modification of a method described by Zidorn et al. [61] with a mixture of methanol/acetone/water (3:1:1) [20]. For the elucidation of inulin content, the roots were extracted with water via microwave-assisted extraction [60].

In the aerial part aqueous methanol extract, the following compounds were detected: flavonoid glycosides (isoorientin, hyperoside, isoquercitrin, miquelianin), luteolin di-*C*-glycoside (*C*-hexoside, *C*-pentoside), quercetin, caffeic acid, and caffeic acid derivatives (chlorogenic acid, 4-*O*-caffeoylquinic acid 1,5-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 1,5-*O*-dicaffeoylquinic acid, 3,5-*O*-dicafeoylquinic acid, 4,5-*O*-dicaffeoylquinic acid) [20,26].

From the methanol extract of *S. hispanica* subaerial parts, plugitone, ixerioside D, and 3-*O*-angeloyl-11β,13-dihydrodesacylcynaropicrin-8β-D-glucoside were isolated [32].

Subaerial part ethyl acetate extract was reported to contain a lignan ((-)-syringaresinol), octadecadienoic acids (linoleic acid, 9-hydroxyocta-(10*E*,12*E*)-decadienoic acid, 8-13-*oxo*-(9*Z*,11*E*)-octadecadienoic acid, 9-*oxo*-(10*E*,12*Z*)-octadecadienoic acid, 13-*oxo*-(9*E*,11*E*)-octadecadienoic acid, and 9-*oxo*-(10*E*,12*E*)-octadecadienoic acid), and sesquiterpenoids (1-*oxo*-bisabola-(2,10*E*)-diene-12-carboxylic acid, 1-*oxo*-bisabola-(2,10*E*)-diene-12-ol, plitostemonol, puliglutone, 1-*oxo*-bisabola-(2,10*E*)-diene-12-carboxylic acid methyl ester, 2,9-epoxycurcumen-12-al, and ixerisoside D) [20].

Caffeic acid and caffeic acid derivatives (chlorogenic acid, 4-*O*-caffeoylquinic acid, 1,5-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, and 4,5-*O*-dicaffeoylquinic acid) were identified in subaerial part samples as well [20]. Notable amounts of inulin (over 20% of dry plant material) were identified in the roots of *S. hispanica* [60].

3.17. Scorzonera humilis L.

Samples of *S. humilis* were collected in Austria and subaerial parts of the plant were extracted with methanol [53–55].

From the methanol extract, tyrolobibenzyls were isolated via column chromatography [53–55]. Tyrolobibenzyls A, B, and C were isolated and identified in the study from 2000, together with a lignin—pinoresinol-1-yl β-D-glucopyranoside [54]. In the study from 2002, the structure of tyrolobibenzyl D was elucidated [53]. A year later, the structure of two novel tyrolobibenzyls (E and F) was identified and the presence of chlorogenic acid and 3,5-dicaffeoylquinic acid was detected [55].

3.18. Scorzonera incisa DC.

Samples of the plant were collected in Turkey and aerial and subaerial parts were extracted separately at room temperature with 20% aqueous methanol [5] and *n*-hexane [27].

The aerial part *n*-hexane extract was reported to contain triterpenes: lupeol, lupeol acetate, α-amyrin, and taraxasteryl acetate [27]. Additionally, the presence of rutin, cyranoside, and chlorogenic acid was detected in the aqueous methanol extract [5].

In the root extracts, the presence of chlorogenic acid was detected in the aqueous methanol extract [5]. Triterpenoids (lupeol and lupeol acetate, taraxasteryl acetate, and olean-12-en-11-one-3-acetyl) were reported present in the *n*-hexane root extract [5,27].

3.19. Scorzonera judaica Eig.

The roots of the plant were collected in Jordan and subsequently extracted with *n*-hexane, chloroform, a mixture of chloroform and methanol (9:1), and methanol. Then, the isolation of compounds was carried out.

From the chloroform root extract, 4α-hydroxypinoresinol, hydrangenol, and scorzotomentosin were isolated.

The $CHCl_3$:MeOH (9:1) extract was reported to contain 3*S*-hydrangenol 4′-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside, hydrangenol 4′-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, 2-hydroxy-6-[2-(4-hydroxyphenyl)-2-*oxo*-ethyl]benzoic acid, *E*-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)dihydrofuran-2-one, *Z*-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone, 4-[β-D-glucopyranosyl)hydroxy]-pinoresinol, hydrangenol 8-*O*-β-D-glucopyranoside, hydramacrophyllol A, hydramacrophyllol B, 4α-hydroxypinoresinol, hydrangenol 4′-*O*-β-D-glucopyranoside, thunberginol F, and hydrangenol.

From the methanol extract, the following compounds were isolated: 3*S*-hydrangenol 4′-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside, hydrangenol 4′-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, thunberginol F 7-*O*-β-D-glucopyranoside, 2-hydroxy-6-[2-(4-hydroxyphenyl)-2-*oxo*-ethyl]benzoic acid, 2-hydroxy-6-[2-(3,4-dihydroxyphenyl)-2-*oxo*-ethyl] benzoic acid, 2-hydroxy-6-[2-(3,4-dihydroxyphenyl-5-methoxy)-2-oxoethyl]benzoic acid, hydrangeic acid 4′-*O*-β-D-glucopyranoside, 4-[β-D-glucopyranosyl)hydroxy]-pinoresinol, hydrangenol 8-*O*- β-D-glucopyranoside, and hydrangenol 4′-*O*-β-D-glucopyranoside [25].

3.20. Scorzonera laciniata L. ssp. laciniata

Plant samples were collected in the north-western [9,27] and eastern parts of Turkey. [22]. Aerial and subaerial parts were separated and extracted with 20% aqueous methanol [9] and *n*-hexane [27]. In the study by Erden et al. [22], the extraction was carried out using methanol, a mixture of hexane and isopropyl alcohol (3:2, *v*/*v*), water, and a mixture of HNO_3:H_2SO_4:H_2O_2 (10:1:1, *v*/*v*/*v*).

In the aerial part 20% aqueous methanol extract, chlorogenic acid and luteolin 7-glucoside, as well as trace amounts of rutin were reported [9]. Myricetin, kaempferol, and trace amounts of morin and quercetin were present in the methanol extract. Phytosterols (ergosterol, stigmasterol, and β-sitosterol) and vitamins D and K were identified in the hexane/isopropyl alcohol (3:2, *v*/*v*) extract and notable amounts of potassium were identified in the extract obtained with the mixture of HNO_3:H_2SO_4:H_2O_2 [22]. The presence of

lupeol, lupeol acetate, taraxasteryl acetate, and α-amyrin in the *n*-hexane aerial part extract has been reported as well [27].

Root aqueous methanol extract contains chlorogenic acid [9]. In the *n*-hexane extract of the roots, triterpenoids (taraxasteryl acetate, lupeol, lupeol acetate, and α-amyrin) were detected [27].

3.21. *Scorzonera latifolia (Fisch. and Mey.) DC.*

Samples of *S. latifolia* were collected in eastern [5,27] and central-eastern Turkey [10,22]. Aerial and subaerial parts were separated and extracted with 20% aqueous methanol [5], and *n*-hexane [27]. The study by Erden et al. [22] reports extraction with methanol, a mixture of hexane and isopropyl alcohol (3:2, *v*/*v*), water, and a mixture of HNO_3:H_2SO_4:H_2O_2 (10:1:1, *v*/*v*/*v*).

Aerial part methanol extract of *S. latifolia* was reported to contain flavonoid aglycones: myricetin, quercetin, kaempferol, and morin. Ergosterol, stigmasterol, and β-sitosterol were also reported present in the hexane:isopropyl alcohol (3:2, *v*/*v*) extract from the aerial parts of the plant, together with retinol and vitamins D, E, and K [22]. Triterpenoids identified in aerial part *n*-hexane extract were: taraxasteryl acetate, 3β-hydroxy-fern-7-en-6-one-acetate, lupeol, lupeol acetate, and α-amyrin [27]. The aqueous methanol extract from the aerial parts was reported to contain chlorogenic acid, hyperoside, and luteolin 7-glucoside [5].

In the roots, the following triterpenoids are reported to be present in the *n*-hexane extract: taraxasteryl acetate, lupeol, lupeol acetate, β-hydroxy-fern-7-en-6-one-acetate, and olean-12-en-11-one-3-acetyl [27]. Moreover, chlorogenic acid was detected in the 20% aqueous methanol extract [5].

3.22. *Scorzonera mirabilis Lipsch.*

Samples of the plant were collected in the city of Van, eastern Turkey, aerial parts and roots were separated in extracted at room temperature with *n*-hexane.

Both aerial and subaerial part *n*-hexane extracts of *S. mirabilis* contain taraxasteryl acetate, lupeol, and lupeol acetate [27].

3.23. *Scorzonera mollis Bieb. ssp. szowitsii (DC.) Chamb.*

Plants samples were collected in north-central Turkey [5,27]. Aerial and subaerial parts were separated and extracted at room temperature using *n*-hexane [27] and 20% aqueous methanol [5].

In the aqueous methanol extract of aerial parts, chlorogenic acid, rutin, hyperoside, and cyranoside were detected [5]. The presence of taraxasteryl acetate, lupeol, lupeol acetate, and α-amyrin was reported in *n*-hexane extracts from both aerial and subaerial parts of the plant [27].

Aqueous methanol root extract was reported to contain chlorogenic acid [5].

3.24. *Scorzonera papposa DC.*

Samples were collected in Jordan. Aerial parts and roots were separated, dried, and sequentially macerated with *n*-hexane, chloroform, a mixture of chloroform and methanol (9:1), and methanol.

From the aerial part methanol extract, the following compounds were isolated: (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid, a mixture of (6-*cis*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid and (6-*cis*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-ribono-*c*-lactone, (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid methyl ester, (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-(5-acetyl)-2-deoxy-D-riburonic acid, isoorientin, orientin, isoschaftoside, and swertiajaponin. From the $CHCl_3$:MeOH extract, (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid, a mixture of (6-*cis*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid and (6-*cis*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-ribono-c-lac-

tone, and (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-(5-acetyl)-2-deoxy-D-riburonic acid were isolated.

The root methanol extract was reported to contain thunberginol G [62].

3.25. Scorzonera parviflora Jacq.

Aerial and subaerial parts of the plant, collected in central Turkey, were separated and extracted at room temperature using *n*-hexane [27] and 20% aqueous methanol [5].

Aerial part *n*-hexane extract of *S. parviflora* was reported to contain the following triterpenoids: taraxasteryl acetate, lupeol, and lupeol acetate [27]. The aqueous methanol extract contained chlorogenic acid, hyperoside, and cynaroside [5].

In the roots, chlorogenic acid was detected in the aqueous methanol extract [5]. Taraxasteryl acetate, lupeol, and lupeol acetate were the main components of the *n*-hexane extract [27].

3.26. Scorzonera pseudodivaricata Lipsch.

Samples for the analysis were collected in Mongolia. Aerial and subaerial parts of the plant were separated, then aerial parts were macerated with methanol at room temperature.

Aerial part extract of the plant is reported to contain isochlorogenic acid A, cynaroside, isovitexin 2″-*O*-xyloside, luteolin, luteolin 5-*O*-glucoside, platyphylloside, scopoletin, scorzoneric acid and scorzonerin [14].

3.27. Scorzonera pusilla Pall.

In the aerial parts, collected in Reading (England), the presence of quercetin and luteolin was reported [44].

3.28. Scorzonera pygmaea Sibth. and Sm.

Plant samples (subaerial parts) were collected in Turkey. The dried and powdered subaerial parts were macerated in ethanol.

From the subaerial part methanol extract, the following compounds were isolated: 3,5-dicaffeoylquinic acid, chlorogenic acid, chlorogenic acid methyl ester, cudrabibenzyl A, scorzocreticoside I scorzocreticoside II, scorzonerol, scorzopygmaecoside, and thunberginol C [35].

3.29. Scorzonera radiata Fisch.

Samples of *S. radiata* aerial parts were collected in Mongolia and macerated with methanol at room temperature [12,13,15].

From the aerial part methanol extract of *S. radiata,* scorzodihydrostibenes A-E were isolated [15]. Apart from that, the presence of 3,5-dicaffeoyl-*epi*-quinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, 5-*p*-coumaroylquinic acid (*cis* and *trans*), chlorogenic acid, isoorientin, kaempferol 3-*O*-rutinoside, macroantoin F, macroantoin G, quinic acid, rutin, and violanthin was detected [12], Moreover, scorzonerin A, scorzonerin B, and 4,5-dicaffeoyl-*epi*-quinic acid were isolated [13].

3.30. Scorzonera suberosa C. Koch ssp. suberosa

Samples of the plant were collected in the central part of Turkey. Aerial and subaerial parts were separated and extracted with *n*-hexane at room temperature [27]. In the study by Erden et al. [22], the solvents used for extraction were methanol, a mixture of hexane and isopropyl alcohol (3:2, *v*/*v*), water, and a mixture of HNO_3:H_2SO_4:H_2O_2 (10:1:1, *v*/*v*/*v*).

Aerial parts and roots are reported to contain taraxasteryl acetate, lupeol, and lupeol acetate [27]. Myricetin, morin, and quercetin were found in the methanol extract, vitamins D, E, and K, retinol, and phytosterols: β-sitosterol, ergosterol, and stigmasterol were detected in the hexane/isopropyl alcohol extract. Sodium and potassium were also reported present (extraction using the mixture of HNO_3:H_2SO_4:H_2O_2) [22].

3.31. Scorzonera sublanata Lipsch.

Samples of *S. sublanata* were collected in Turkey and aerial parts and roots were separated [9,27]. The extraction was carried out using *n*-hexane [27] and 20% aqueous methanol [9].

Aerial part aqueous methanol extract contains chlorogenic acid and hyperoside [9]. In the *n*-hexane extract, the presence of lupeol, lupeol acetate, and taraxasteryl acetate was reported [27].

Root aqueous methanol extract was reported to contain chlorogenic acid [9]. The *n*-hexane extract is reported to contain taraxasteryl acetate, 3β-hydroxy-fern-7-en-6-one-acetate, lupeol, and lupeol acetate [27].

3.32. Scorzonera tomentosa L.

Samples of *S. tomentosa* were collected in Turkey [5,27,36]. Aerial parts and roots were separated and extracted using *n*-hexane [27], and 20% aqueous methanol [5]. Subaerial parts were extracted with methanol at room temperature [36].

Aerial part *n*-hexane extract is reported to contain lupeol, lupeol acetate, and taraxasteryl acetate [27]. In the aqueous ethanol extract, chlorogenic acid, hyperoside, and cyranoside were detected [5].

In the roots, the presence of taraxasteryl acetate, 3β-hydroxy-fern-7-en-6-one-acetate, olean-12-en-11-one-3-acetyl, lupeol, lupeol acetate, and α-amyrin was detected in the n-hexane extract [27]. The aqueous methanol extract was also reported to contain chlorogenic acid [5], (±)-scorzotomentosin, (-)-,scorzotomentosin, (-)-scorzotomentosin 4′-O-β-glucoside, (±)-scorzophtalide, scorzoerzincanin, (±)-hydrangenol, (-)-hydrangenol 4′-O-glucoside, (±)-hydramacrophyllol A, and (±)-hydramacrophyllol B [36].

3.33. Scorzonera trachysperma Guss.

Aerial parts of *S. trachysperma* samples, collected in Italy, were extracted with 50% aqueous methanol and subjected to HPLC.

The analysis revealed that the aerial part methanol:water extract contains chlorogenic acid, *cis*-chlorogenic acid, cryptochlorogenic acid, isochlorogenic acid A, isochlorogenic acid C, 3,5-dicaffeoylquinic acid, isoorientin, luteolin, and apigenin diglycosides, and luteolin [26].

3.34. Scorzonera undulata ssp. alexandrina Boiss.

Samples of the plant were collected in Algeria and the whole plant was macerated with petroleum ether.

Lupeol, 24-methylenecycloartanol, 3-*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-β-sitosterol, daucosterol, and apigenin were isolated from *S. undulata* ssp. *alexandrina* whole plant petroleum ether extract [23].

3.35. Scorzonera undulata ssp. deliciosa (Guss.) Marie

Plant samples were collected in Algeria and subaerial parts were macerated in dichloromethane, then the isolation of compounds was performed.

From the roots of the plant, the following compounds were isolated: verbascoside, galangustin [49], cichoriin, β-amyrin acetate, β-sitosterol, stigmasterol methyl oleanate, ethyl ursolate [63].

3.36. Scorzonera veratrifolia Fenzl.

The samples used for the studies on *S. veratrifolia* were collected in eastern Turkey. The subaerial parts were separated from the aerial parts and extracted with methanol at room temperature [30,64].

Subaerial parts of *S. veratrifolia* contain the following triterpenes: α-amyrin acetate, α-amyrinone, β-amyrin acetate, β-amyrinone, β-amyrin, ψ-taraxasterol, ψ-taraxasterol acetate, fern-7-en-3-one, germanicol, germanicol acetate, germanicone, lupenone, lupeol, lu-

peol acetate, taraxasterol, and taraxasterol acetate, as well as β-sitosterol [30]. The presence of chlorogenic acid, chlorogenic acid methyl ester, isochlorogenic acid A, cryptochlorogenic acid, 4,5-dicaffeoylquinic acid, together with scorzoveratrin and scorzoveratrozit has also been reported [64].

3.37. Scorzonera villosa Scop. ssp. villosa

Samples of *S. villosa* were collected in Slovenia. Aerial parts were extracted with 50% aqueous methanol at room temperature and HPLC analysis was performed.

The extract of aerial parts was reported to contain 1,5-*O*-dicaffeoylquinic acid, 3,5-*O*-dicafeoylquinic acid, 5-*O*-caffeoylquinic acid, chlorogenic acid, cryptochlorogenic acid, apigenin 7-*O*-glucuronide, apigenin di-*C*-glycoside, apigetrin, cyranoside, hyperoside, isochlorogenic acid C, isoorientin, isoquercitrin, luteolin, luteolin 7-*O*-glucuronide, and vitexin [26].

Compounds present in aerial parts of species within the genus *Scorzonera*, together with the concentration in the dry plant matter and the solvent used in the process of extraction (if available in the literature) are listed in Table 1. The phytochemical composition of subaerial parts of species belonging to the genus, with concentrations and solvents used, is presented in Table 2. Compounds isolated from the whole plants are presented in Table 3. As was presented in Tables 1 and 2, subaerial parts of the species within the genus *Scorzonera* are reported to contain a greater diversity of triterpenoid ad phenolic acid derivatives. This could be explained by the fact that a larger range of solvents was used for the extraction of phytochemicals from subaerial parts. The aerial parts; however, are reported to contain notably more different flavonoids. This is an anticipated outcome because, as flavonoid compounds are involved in biochemical processes within the whole plant, they are significant for the activities related to exposure to external factors (e.g., UV radiation or attracting pollinators) [65,66]. Another reason is the fact that only aerial parts of *Scorzonera* species were thoroughly assessed for flavonoid content in a study by Granica et al. [26]. Based on the research included in this review, the steroid, coumarin, and dihydroisocoumarin content seems similar in both aerial and subaerial parts. The species that are most investigated in the greatest number of papers are *S. divaricata* [14,34,57–59], *S. hispanica* [20,26,32,45,46,60], *S. latifolia* [3–6,27,47,67–71], and *S. radiata* [12,13,15].

Table 1. Compounds present in aerial parts of *Scorzonera* species.

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Benzoic acid derivatives				
Methyl-3,4-dihydroxybenzoate	*S. divaricata*	0.6 μg/g	methanol	[34]
3-Hydroxybenzoic acid	*S. divaricata*	0.32 μg/g	methanol	[34]
Coumarins and coumarin glycosides				
Scopoletin	*S. divaricata*	3 μg/g	methanol	[34]
	S. pseudodivaricata	N/D	methanol	[14]
Hydrangenol 8-O-glucoside	*S. latifolia*	N/D	20% aqueous methanol	[10]
7-Hydroxycoumarin	*S. divaricata*	0.13 μg/g	methanol	[34]
Dihydroisocoumarins and dihydroisocoumarins glycosides				
(3S)-6-[O-β-d-glucopyranosyl-(6→1)-O-β-d-apiofuranosyl]-8-hydroxy-3-(4-methoxyphenyl)-3,4-dihydro-1*H*-isochromen-1-one (*iso*-scorzopygmaecoside)	*S. aucheriana*	19.208 μg/g	methanol	[7]
(3S)-6-{*O*-β-d-glucopyranosyl-[(4→2)-O-glyceryl)]-(6→1)-O-β-d-apiofuranosyl}-8-hydroxy-3-(4-methoxyphenyl)-3,4-dihydro-1*H*-isochromen-1-one (scorzoaucherioside I)	*S. aucheriana*	49.67 μg/g	methanol	[7]
(3S,3′ R)-8-{*O*-β-d-glucopyranosyl-[(4→2)-*O*-glyceryl)]-(6→1)-*O*-β-d-apiofuranosyl}-3-(4-methoxyphenyl)-6-{[3-(4-methoxyphenyl)-1-oxo-8-[*O*-β-d-glucopyranosyl-(6→1)-O-α-l-rhamnopyranosyl-(4→1)-O-β-d-glucopyran-osyl]-3,4-dihydro-1*H*-isochromen-6-yl]oxy}-3,4-dihydro-1*H*-isochromen-1-one (scorzoaucherioside II)	*S. aucheriana*	11.165 μg/g	methanol	[7]
Scorzopygmaecoside	*S. aucheriana*	9.724 μg/g	methanol	[7]
Scorzocreticoside II	*S. aucheriana*	5.642 μg/g	methanol	[7]
Flavonoids				
Flavonoid aglycones				
5,7-dihydroxy-6-methoxyflavone	*S. divaricata*	0.56 μg/g	methanol	[34]
5,7-dihydroxy-8-methoxyflavone	*S. divaricata*	2.01 μg/g	methanol	[34]
7,3′,4′-trihydroxyflavonol	*S. divaricata*	0.23 μg/g	methanol	[34]
Apigenin	*S. divaricata*	N/D	methanol	[44]
	S. laciniata	N/D	ethanol	[14]
Apigenin derivative	*S. austriaca*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. baetica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
Diosmetin	*S. divaricata*	1.7 μg/g	methanol	[34]
Kaempferol	*S. hirsuta*	N/D	ethanol	[44]
	S. laciniata	3.55 ± 0.78 μg/g	methanol	[22]
	S. latifolia	0.62 ± 0.11 μg/g	methanol	[22]

Table 1. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Luteolin	*S. crispatula*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. divaricata	0.21 µg/g	methanol	[34]
	S. graminifolia	N/D	ethanol	[44]
	S. hirsuta	N/D	ethanol	[44]
	S. laciniata	N/D	ethanol	[44]
	S. mollis	N/D	ethanol	[44]
	S. pseudodivaricata	N/D	methanol	[14]
	S. pussila	N/D	ethanol	[44]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one (morin)	*S. laciniata*	0.17 ± 0.01 µg/g	methanol	[22]
	S. latifolia	0.23 ± 0.04 µg/g	methanol	[22]
	S. suberosa	0.91 ± 0.83 µg/g	methanol	[22]
Myricetin	*S. laciniata*	4.45 ± 0.9 µg/g	methanol	[22]
	S. latifolia	16.16 ± 0.92 µg/g	methanol	[22]
	S. suberosa	3.12 ± 1.02 µg/g	methanol	[22]
Quercetin	*S. austriaca* var. *angustifolia*	N/D	ethanol	[44]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. graminifolia	N/D	ethanol	[44]
	S. hirsuta	N/D	ethanol	[44]
	S. hispanica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. laciniata	0.17 ± 0.01 µg/g	methanol	[22]
	S. latifolia	0.65 ± 0.15 µg/g	methanol	[22]
	S. mollis	N/D	ethanol	[44]
	S. pussila	N/D	ethanol	[44]
	S. suberosa	6.54 ± 1.16 µg/g	methanol	[22]
Quercetin derivative	*S. aristata*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
Tricin	*S. divaricata*	0.16 µg/g	methanol	[34]
Unknown flavonoid	*S. hispanica*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
Flavonoid *C*-glycosides				
3′-methoxy-5,7,4′-trihydroxyflavone 6-C-β-D-glucopyranoside	*S. austriaca*	10 µg/g	70% aqueous ethanol	[24]
5,7,3′,4′-tetrahydroxyflavone 8-C-(6″-*O*-*trans*-caffeoyl β-D-glucopyranoside)	*S. austriaca*	100 µg/g	70% aqueous ethanol	[24]
5,7,3′,4′-tetrahydroxyflavone 6-C-(2″-*O*-β-D-glucopyranosyl β-D-glucopyranoside)	*S. austriaca*	15 µg/g	70%aqueous ethanol	[24]
5,7,4′-trihydroxyflavone 6-C-(2″-*O*-β-D-glucopyranosyl β-D-glucopyranoside)	*S. austriaca*	15 µg/g	70% aqueous ethanol	[24]
5,7,4′-trihydroxyflavone 6-*C*-β-D-glucopyranoside	*S. austriaca*	15 µg/g	70% aqueous ethanol	[24]
5,7,4′-trihydroxyflavone 8-*C*-(6″-*O*-*trans*-caffeoyl β-D-glucopyranoside)	*S. austriaca*	30 µg/g	70% aqueous ethanol	[24]
5,7-dihydroxy-2-(4-hydroxyphenyl)-6-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-8-[(2*S*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]chromen-4-one (violanthin)	*S. radiata*	N/D	methanol	[13]
7-methylisoorientin	*S. latifolia*	6.4 µg/g	methanol	[4]
7-*O*-methylapigenin 6-C-β-*D*-glucopyranoside (swertisin)	*S. latifolia*	N/D	20% aqueous methanol	[10]
	S. tomentosa	N/D	20% aqueous methanol	[10]
Apigenin 3-C-α-L6-rhamnopyranosyl-8-C-β-D-glucopyranoside (scorzonerin B)	*S. radiata*	7.334 µg/g	methanol	[13]

Table 1. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Apigenin 6-C-glucoside (isovitexin)	*S. baetica*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
Apigenin 6-C-β-D-galactopyranosyl-8-C-α-L-6-rhamnopyranoside (scorzonerin A)	*S. radiata*	30.667 µg/g	methanol	[13]
Apigenin 8-C-glucoside (vitexin)	*S. baetica*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
Apigenin di-C-glycoside (C-pentoside, C-hexoside)	*S. crispatula*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
Apigenin di-C-glycoside (di-C-hexoside)	*S. baetica*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
5,7-dihydroxy-2-(4-hydroxyphenyl)-8-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-6-[(2*S*,3*R*,4*S*,5*S*)-3,4,5-trihydroxyoxan-2-yl]chromen-4-one (isoschaftoside)	*S. papposa*	3.334 µg/g	methanol	[62]
	S. aristata	24.815 µg/g	methanol methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
Luteolin 6-C-glucoside (isoorientin)	*S. austriaca*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. baetica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. latifolia	9 µg/g	methanol	[4]
	S. papposa	48.667 µg/g	methanol	[62]
	S. radiata	N/D	methanol	[13]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
Luteolin 8-C-glucoside (orientin)	*S. aristata*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. baetica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. papposa	6.667 µg/g	methanol	[62]
Luteolin di-C-glycoside (C-hexoside, C-pentoside)	*S. crispatula*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
Swertiajaponin	*S. papposa*	3.667 µg/g	methanol	[62]
Flavonoid *O*-glycosides and *O*-glucuronides				
Apigenin 7-*O*-glucoside (apigetrin)	*S. villosa*	N/D	methanol:water (1:1, *v*/*v*)	[26]
Apigenin 7-*O*-glucuronide	*S. trachysperma*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
Kaempferol 3-*O*-rutinoside	*S. divaricata*	N/D	methanol	[14]
	S. radiata	N/D	methanol	[13]
Luteolin 5-*O*-glucoside	*S. pseudodivaricata*	N/D	methanol	[14]

Table 1. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Luteolin 7-*O*-glucoside (cynaroside)	*S. acuminata*	9.583 ± 0.203 µg/mg	20% aqueous methanol	[9]
	S. baetica,	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. cinerea	81.14 ± 0.62 µg/mg	20% aqueous methanol	[5]
	S. incisa	12.08 ± 0.1 µg/mg	20% aqueous methanol	[5]
	S. latifolia	629.23 ± 3.53 µg/mg	20% aqueous methanol	[5]
	S. mollis ssp. *szowitsii*	107.43 ± 0.09 µg/mg	20% aqueous methanol	[5]
	S. parviflora	51.80 ± 0.71 µg/mg	20% aqueous methanol	[5]
	S. pseudodivaricata	N/D	methanol	[14]
	S. tomentosa	47.81 ± 0.50 µg/mg	20% aqueous methanol	[5]
Luteolin 7-*O*-glucuronide	*S. villosa*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	
Quercetin 3-*O*-arabinofuranoside (avicularin)	*S. austriaca*	N/D	methanol:water (1:1, *v*/*v*)	[26]
Quercetin 3-*O*-galactoside (hyperoside)	*S. cinerea*	124.22 ± 0.56 µg/mg	20% aqueous methanol	[5]
	S. hispanica	11.41 ± 0.05 mg/g 11.35 ± 0.15 mg/g	methanol:acetone:water (3:1:1)	[20]
	S. latifolia	305.71 ± 1.70 µg/mg	20% aqueous methanol	[5]
	S. mollis ssp. *szowitsii*	39.46 ± 0.03 µg/mg	20% aqueous methanol	[5]
	S. parviflora	9.71 ± 0.51 µg/mg	20% aqueous methanol	[5]
	S. tomentosa	94.54 ± 0.33 µg/mg	20% aqueous methanol	[5]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
Quercetin 3-*O*-glucoside (isoquercitrin)	*S. aristata*	40.37 µg/g	methanol methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	6.41 ± 0.02 mg/g 6.91 ± 0.01 mg/g	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
Quercetin 3-*O*-glucuronide (miquelianin)	*S. villosa*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	N/D 13.87 ± 0.10 mg/g 15.29 ± 0.25 mg/g	methanol:water (1:1, *v*/*v*) methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[26] [20]
Quercetin 3-*O*-rhamnoglucoside (rutin)	*S. acuminata*	597.335 ± 1.104 µg/mg	20% aqueous methanol methanol	[9]
	S. aristata	36.667 µg/g	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. incisa	198.81 ± 0.18 µg/mg	20% aqueous methanol	[5]
	S. mollis ssp. *szowitsii*	26.32 ± 0.04 µg/mg	20% aqueous methanol	[5]
	S. radiata	N/D	methanol	[13]
	S. suberosa	15.38 ± 3.27 µg/g	methanol	[22]
Quercetin 3-*O*-α-rhamnopyranosyl-(1→6)-β-D-galactopyranoside	*S. latifolia*	2.267 µg/g	methanol	[4]
Quercetin 3-*O*-β-apiofuranosyl-(1‴→2″)-β-D-glucopyranoside	*S. latifolia*	11.53 µg/g	methanol	[4]
Quercetin 3-*O*-β-D-glucoside	*S. latifolia*	N/D	20% aqueous methanol	[10]
	S. tomentosa	N/D	20% aqueous methanol	
Quercetin *O*-mallonylhexoside	*S. aristata*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	2.65 ± 0.05 µg/mg 2.83 ± 0.01 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]

Table 1. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Flavonoid *O*-*C*-glycosides				
Apigenin *O*-*C*-glycoside (*O*-hexoside, *C*-hexoside)	*S. trachysperma*	N/D	methanol:water (1:1, *v*/*v*)	[26]
Apigenin O-C-glycoside (*O*-pentoside, *C*-hexoside)	*S. crispatula*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
Isovitexin 2′-*O*-xyloside	*S. divaricata*	N/D	methanol	[14]
Isovitexin 2″-*O*-xyloside	*S. pseudodivaricata*	N/D	methanol	[14]
Isovitexin 4′-*O*-glucoside	*S. divaricata*	N/D	methanol	[14]
Luteolin *O*-*C*-glycoside (*O*-hexoside, *C*-hexoside)	*S. austriaca*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. baetica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
Luteolin *O*-*C*-glycoside (*O*-pentoside, *C*-hexoside)	*S. aristata*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
Macrolides				
Sacrolide A	*S. divaricata*	0.6 μg/g	methanol	[34]
Organic acids/Phenolic acids and their derivatives				
1,5-*O*-dicaffeoylquinic acid	*S. baetica*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	3.95 ± 0.11 μg/mg 6.59 ± 0.17 μg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
3,4-bis[(3′,4-dioxo-1′,3′,5′,6′-tetrahydrospiro[cyclohexa-2,5-diene-1,4′-cyclopenta[c]-furan]-1′-yl)]chlorogenic acid	*S. aucheriana*	7.443 μg/g	methanol	[29]
3,4-*O*-dihydroxyphenyl caffeate	*S. aucheriana*	7.323 μg/g	methanol	[7]
3,5-*O*-dicaffeoyl quinic acid	*S. pseudodivaricata*	N/D	methanol	[14]
3,5-*O*-dicaffeoyl-*epi*-quinic acid	*S. radiata*	N/D	methanol	[12,13]
	S. aucheriana	6.243 μg/g	methanol	[7]
3,5-*O*-dicaffeoylquinic acid methyl ester (macroantoin G)	*S. radiata*	N/D	methanol	[13]
	S. aristata	23.334 μg/g	methanol methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
3,5-*O*-dicaffeoylquinic acid (isochlorogenic acid A)	*S. austriaca*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. baetica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	35.15 ± 0.61 μg/mg 19.23 ± 0.58 μg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. radiata	N/D	methanol	[13]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]

Table 1. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
3-*O*-caffeoylquinic acid (chlorogenic acid)	*S. acuminata*	372.128 ± 0.961 µg/mg	20% aqueous methanol methanol	[9]
	S. aristata	9.259 µg/g	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. baetica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. cinerea	266.51 ± 1.51 µg/mg	20% aqueous methanol	[5]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. divaricata	N/D	methanol	[14]
	S. hispanica	85.49 ± 1.49 µg/mg 75.83 ± 1.01 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. incisa	569.19 ± 1.62 µg/mg	20% aqueous methanol	[5]
	S. latifolia	652.32 ± 2.48 µg/mg	20% aqueous methanol	[5]
	S. mollis ssp. *szowitsii*	1032.16 ± 2.05 µg/mg	20% aqueous methanol	[5]
	S. parviflora	444.77 ± 2.78 µg/mg	20% aqueous methanol	[5]
	S. radiata	N/D	methanol	[13]
	S. tomentosa	268.75 ± 1.72 µg/mg	20% aqueous methanol	[5]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
3-*O*-feruloyl-1,4-di-O-dihydrocaffeoylquinic acid (feruloylpodospermic acid B)	*S. divaricata*	23.438 µg/g	methanol	[14]
3-*O*-feruloyl-1,5-di-*O*-dihydrocaffeoylquinic acid (feruloylpodospermic acid A)	*S. divaricata*	82.031 µg/g	methanol	[14]
4,5-dicaffeoyl-*epi*-quinic acid	*S. radiata*	10.333 µg/g	methanol	[13]
4,5-dicaffeoyl-*epi*-quinic acid methyl ester (macroantoin F)	*S. radiata*	N/D	methanol	[13]
4,5-*O*-dicaffeoylquinic acid (isochlorogenic acid C)	*S. aristata*	13.33 µg/g	methanol methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. baetica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	5.42 ± 0.01 µg/mg 3.14 ± 0.15 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. radiata	N/D	methanol	[13]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
4-hydroxy-3-methoxyphenyl ferulate	*S. divaricata*	0.13 µg/g	methanol	[34]
4-*O*-caffeoylquinic acid (cryptochlorogenic acid)	*S. aristata*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. austriaca	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. baetica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	2.99 ± 0.03 µg/mg 3.99 ± 0.04 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]
5-*O*-caffeoylquinic acid (*cis*-chlorogenic acid)	*S. baetica*	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. crispatula	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. hispanica	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. trachysperma	N/D	methanol:water (1:1, *v*/*v*)	[26]
	S. villosa	N/D	methanol:water (1:1, *v*/*v*)	[26]

Table 1. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
5-*p*-coumaroylquinic acid (*cis*)	*S. radiata*	N/D	methanol	[12]
5-*p*-coumaroylquinic acid (*trans*)	*S. radiata*	N/D	methanol	[12,13]
Methyl 1-(2-methylcyclopropyl-1-carbonyloxy)chlorogenate	*S. aucheriana*	7.683 µg/g	methanol	[29]
Quinic acid	*S. radiata*	N/D	methanol	[12,13]
Trans-caffeic acid	*S. divaricata*	1.8 µg/g	methanol	[34]
Trans-*p*-hydroxy coumaric acid	*S. divaricata*	1 µg/g	methanol	[34]
Sesquiterpenoids				
1β,4α- dihydroxy-5α,6β,7α,11βH-eudermn-12,6-olide	*S. divaricata*	0.038 µg/g	methanol	[34]
5-(1-(2-*O*-hexanoyl)-β-D-glucopyranosyloxy)-2-hydroxy-3-[4-(4-hydroxyphenyl)-2-oxobutyl]benzoic acid (scorzoneric acid)	*S. pseudodivaricata*	14.5 µg/g	methanol	[14]
8*R*-matricarinyl 3-[4-(1-β-D-glucopyranosyloxy)phenyl]propanoate (scorzonerin)	*S. pseudodivaricata*	260 µg/g	methanol	[14]
Glucozaluzanin C	*S. divaricata*	1.56 µg/g	methanol	[34]
Sulfoscorzonin D	*S. divaricata*	1.3 µg/g	methanol	[34]
Sulfoscorzonin E	*S. divaricata*	1.25 µg/g	methanol	[34]
Steroids				
(22*E*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol	*S. divaricata*	0.76 µg/g	methanol	[34]
Ergosta-3β,5α,6β-trialcohol	*S. divaricata*	0.7 µg/g	methanol	[34]
Ergosterol	*S. laciniata*	2.44 ± 0.11 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
	S. latifolia	3.22 ± 0.09 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
	S. suberosa	3.06 ± 0.41 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
Stigma-5-en-3-*O*-β-glucoside	*S. divaricata*	3.5 µg/g	methanol	[34]
Stigmasterol	*S. laciniata*	21.67 ± 1.1 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
	S. latifolia	30.76 ± 1.19 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
	S. suberosa	10.80 ± 0.54 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
β-Sitosterol	*S. aucheriana*	11.765 µg/g	methanol	[29]
	S. laciniata	4.26 ± 0.34 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
	S. latifolia	35.55 ± 1.71 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
	S. suberosa	50.75 ± 3.15 µg/g	hexane/isopropanol (3:2, *v*/*v*)	[22]
Triterpenoids				
3β-hydroxy-fern-7-en-6-one-acetate	*S. latifolia*	18 ± 1 µg/g	*n*-hexane	[27]
Lup-20(29)-ene3β,28-diol	*S. divaricata*	3 µg/g	methanol	[34]

Table 1. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Lupeol	*S. acuminata*	327 ± 5 µg/g	*n*-hexane	[27]
	S. aucheriana	9.724 µg/g	methanol	[29]
	S. cana var. *jacquiniana*	932 ± 2 µg/g	*n*-hexane	[27]
	S. cinerea	1174 ± 16 µg/g	*n*-hexane	[27]
	S. eriophora	228 ± 6 µg/g	*n*-hexane	[27]
	S. incisa	1090 ± 2 µg/g	*n*-hexane	[27]
	S. laciniata ssp. *laciniata*	932 ± 2 µg/g	*n*-hexane	[27]
	S. latifolia	1538 ± 1 µg/g	*n*-hexane	[27]
	S. mirabilis	954 ± 14 µg/g	*n*-hexane	[27]
	S. mollis ssp. *szowitsii*	321 ± 1 µg/g	*n*-hexane	[27]
	S. parviflora	649 ± 6 µg/g	*n*-hexane	[27]
	S. suberosa ssp. *suberosa*	1005 ± 17 µg/g	*n*-hexane	[27]
	S. sublanata	169 ± 1 µg/g	*n*-hexane	[27]
	S. tomentosa	509 ± 2 µg/g	*n*-hexane	[27]
Lupeol acetate	*S. acuminata*	67 ± 1 µg/g	*n*-hexane	[27]
	S. aucheriana	5.642 µg/g	methanol	[29]
	S. cana var. *jacquiniana*	535 ± 4 µg/g	*n*-hexane	[27]
	S. cinerea	839 ± 6 µg/g	*n*-hexane	[27]
	S. eriophora	368 ± 1 µg/g	*n*-hexane	[27]
	S. incisa	236 ± 9 µg/g	*n*-hexane	[27]
	S. laciniata ssp. *laciniata*	892 ± 2 µg/g	*n*-hexane	[27]
	S. latifolia	607 ± 1 µg/g	*n*-hexane	[27]
	S. mirabilis	998 ± 13 µg/g	*n*-hexane	[27]
	S. mollis ssp. *szowitsii*	149 ± 7 µg/g	*n*-hexane	[27]
	S. parviflora	594 ± 5 µg/g	*n*-hexane	[27]
	S. suberosa ssp. *suberosa*	312 ± 4 µg/g	*n*-hexane	[27]
	S. sublanata	302 ± 1 µg/g	*n*-hexane	[27]
	S. tomentosa	411 ± 1 µg/g	*n*-hexane	[27]
Oleanolic acid	*S. divaricata*	1.5 µg/g	methanol	[34]
Ptiloepoxide	*S. aucheriana*	14.646 µg/g	methanol	[29]
Taraxasterol	*S. aucheriana*	30.972 µg/g	methanol	[29]
	S. aucheriana	5.552 µg/g	methanol	[29]
Taraxasteryl acetate/Taraxasterol acetate	*S. cana* var. *jacquiniana*	81 ± 3 µg/g	*n*-hexane	[9]
	S. cinerea	417 ± 11 µg/g	*n*-hexane	[27]
	S. eriophora	545 ± 5 µg/g	*n*-hexane	[27]
	S. incisa	280 ± 10 µg/g	*n*-hexane	[27]
	S. laciniata ssp. *laciniata*	69 ± 5 µg/g	*n*-hexane	[27]
	S. latifolia	1062 ± 2 µg/g	*n*-hexane	[27]
	S. mirabilis	1262 ± 728 µg/g	*n*-hexane	[27]
	S. mollis ssp. *szowitsii*	263 ± 4 µg/g	*n*-hexane	[27]
	S. parviflora	433 ± 2 µg/g	*n*-hexane	[27]
	S. suberosa ssp. *suberosa*	535 ± 4 µg/g	*n*-hexane	[27]
	S. sublanata	4981 ± 2 µg/g	*n*-hexane	[27]
	S. tomentosa	376 ± 13 µg/g	*n*-hexane	[27]
Taraxasterol oleate	*S. aucheriana*	36.255 µg/g	methanol	[29]

Table 1. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
α-Amyrin	*S. acuminata*	1102 ± 6 µg/g	*n*-hexane	[27]
	S. cana var. *jacquiniana*	442 ± 5 µg/g	*n*-hexane	[27]
	S. cinerea	309 ± 2 µg/g	*n*-hexane	[27]
	S. incisa	644 ± 2 µg/g	*n*-hexane	[27]
	S. laciniata ssp. *laciniata*	209 ± 3 µg/g	*n*-hexane	[27]
	S. latifolia	827 ± 2 µg/g	*n*-hexane	[27]
	S. mollis ssp. *szowitsii*	246 ± 8 µg/g	*n*-hexane	[27]
Vomifoliols				
Vomifoliol	*S. divaricata*	0.7 µg/g	methanol	[34]

Compound concentration was taken directly from literature or it was calculated, dividing the mass of the isolated compound by the mass of plant material used for extraction; N/D—no data was available in the literature.

Table 2. Compounds present in subaerial parts of *Scorzonera* species.

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Coumarins and coumarin derivatives				
Scopoletin	*S. divaricata*	2.546 µg/g	95% aqueous ethanol	[57]
Scopolin	*S. divaricata*	16.364 µg/g	95% aqueous ethanol	[57]
Coumarin *O*-β-glycoside (cichoriin)	*S. undulata* ssp. *deliciosa*	12.99 µg/g	dichloromethane	[63]
	S. cana var. *jacquiniana*	6.667 µg/g	methanol	[72]
Scorzonerol	*S. pygmaea*	0.857 µg/g	ethanol	[35]
Dihydroisocoumarins and dihydroisocoumarin glycosides				
(-)-Hydrangenol 4′-*O*-glucoside	*S. tomentosa*	20.101 µg/g	methanol	[36]
	S. judaica	14.286 µg/g	chloroform:methanol (9:1)	[25]
			methanol	
(-)-Scorzotomentosin 4′-*O*-β-glucoside	*S. tomentosa*	158.29 µg/g	methanol	[36]
	S. latifolia	N/D	methanol:water (8:2)	[73]
(±)-Hydrangenol	*S. tomentosa*	82.915 µg/g	methanol	[36]
	S. judaica	51.143 µg/g	chloroform	[25]
			chloroform:methanol (9:1)	
(3RS)-3,4-dihydro-3-(4-hydroxyphenyl)-8-methoxy-1*H*-2-benzopyran-1-one ((±)-scorzotomentosin)	*S. tomentosa*	216.08 µg/g	methanol	[36]
	S. judaica	8.714 µg/g	chloroform	[25]
8-*O*-[α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl]scorzocreticin (scorzocreticoside II)	*S. pygmaea*	21.429 µg/g	ethanol	[35]
8-*O*-β-D-glucopyranosylscorzocreticin (scorzocreticoside I)	*S. pygmaea*	14.286 µg/g	ethanol	[35]
Hydrangenol 4′-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside	*S. judaica*	22.143 µg/g	chloroform:methanol (9:1)	[25]
			methanol	
3S-hydrangenol 4′-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside	*S. judaica*	48.571 µg/g	chloroform:methanol (9:1)	[25]
			methanol	
Hydrangenol 8-*O*-β-D-glucopyranoside	*S. judaica*	14.143 µg/g	chloroform:methanol (9:1)	[25]
			methanol	
Scorzopygmaecoside	*S. pygmaea*	14.286 µg/g	ethanol	[35]
Thunberginol C	*S. pygmaea*	4.714 µg/g	ethanol	[35]
Thunberginol F	*S. judaica*	7.429 µg/g	chloroform:methanol (9:1)	[25]
Thunberginol G	*S. papposa*	38.4 µg/g	methanol	[62]

Table 2. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Fatty acids				
Linoleic acid	*S. divaricata*	1.091 µg/g	95% aqueous ethanol	[57]
	S. hispanica	7.143 µg/g	ethyl acetate	[20]
9*S*,12*S*,13*S*-trihydroxy-10*E*-octadecenoate	*S. divaricata*	0.546 µg/g	95% aqueous ethanol	[57]
Palmitic acid	*S. divaricata*	0.727 µg/g	95% aqueous ethanol	[57]
Flavonoid aglycones				
Galangustin	*S. undulata* ssp. *deliciosa*	15.464 µg/g	dichloromethane	[49,63]
Flavonoid *C*-glycosides				
Luteolin 6-*C*-glucoside (isoorientin)	*S. cana* var. *jacquiniana*	21.667 µg/g	methanol	[72]
Luteolin 8-*C*-glucoside (orientin)	*S. cana* var. *jacquiniana*	20 µg/g	methanol	[72]
Apigenin 8-*C*-glucoside (vitexin)	*S. cana* var. *jacquiniana*	7.133 µg/g	methanol	[72]
Flavonoid O-glycosides				
Apigenin 7-*O*-β-glucoside	*S. cana* var. *jacquiniana*	18.333 µg/g	methanol	[72]
Luteolin 7-*O*-β-glucoside	*S. cana* var. *jacquiniana*	12.55 µg/g	methanol	[72]
Apigenin 7-*O*-β-rutinoside	*S. cana* var. *jacquiniana*	25 µg/g	methanol	[72]
Hydroquinone derivatives				
Arbutin	*S. cana* var. *jacquiniana*	13.333 µg/g	methanol	[72]
6′-*O*-caffeoylarbutin	*S. cana* var. *jacquiniana*	10.5 µg/g	methanol	[72]
Lignans				
Pinoresinol-1-yl-β-D-glucopyranoside	*S. humilis*	90.147 µg/g	methanol	[54]
(–)–Syringaresinol	*S. hispanica*	9.643 µg/g	ethyl acetate	[20]
Pinoresinol	*S. divaricata*	0.909 µg/g	95% aqueous ethanol	[57]
4-[β-D-glucopyranosyl)hydroxy]-pinoresinol (pinoresinol-4-*O*-glucoside)	*S. judaica*	15.857 µg/g	chloroform:methanol (9:1) methanol	[25]
4α-hydroxypinoresinol	*S. judaica*	26.429 µg/g	chloroform chloroform:methanol (9:1)	[25]
Organic acids/Phenolic acids and their derivatives				
(–)–1,4-di-*O*-feruloyl-3-*O*-dihydrocaffeoylquinic acid	*S. divaricata*	5.363 µg/g	95% aqueous ethanol	[58]
(–)–1-*O*-feruloyl-3-*O*-dihydrocaffeoylquinic acid	*S. divaricata*	N/D	95% aqueous ethanol	[58]
(–)–1-*O*-feruloyl-4-*O*-dihydrocaffeoylquinic acid	*S. divaricata*	0.909 µg/g	95% aqueous ethanol	[58]
(–)–1-*O*-feruloyl-5-*O*-dihydrocaffeoylquinic acid	*S. divaricata*	N/D	95% aqueous ethanol	[58]
(–)–3,5-di-*O*-feruloylquinic acid	*S. divaricata*	1.818 µg/g	95% aqueous ethanol	[58]
1,3-di-*O*-caffeoylquinic acid methyl ester	*S. hieraciifolia*	N/D	ethanol	[8]
1,5-di-*O*-feruloylquinic acid	*S. hieraciifolia*	N/D	ethanol	[8]
1,5-*O*-dicaffeoylquinic acid	*S. hispanica*	2.26–12.72 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
13-*Oxo*-(9*E*,11*E*)-octadecadienoic acid	*S. hispanica*	3.929 µg/g	ethyl acetate	[20]
13-*Oxo*-(9*Z*,11*E*)-octadecadienoic acid	*S. hispanica*	2.679 µg/g	ethyl acetate	[20]
2-Hydroxy-6-[2-(3,4-dihydroxyphenyl)-2-*oxo*-ethyl]benzoic acid	*S. judaica*	5 µg/g	methanol	[25]
2-Hydroxy-6-[2-(3,4-dihydroxyphenyl-5-methoxy)-2-oxoethyl]benzoic acid	*S. judaica*	3.714 µg/g	methanol	[25]

Table 2. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
2-Hydroxy-6-[2-(4-hydroxyphenyl)-2-oxo-ethyl]benzoic acid	*S. judaica*	17.143 µg/g	chloroform:methanol (9:1)	[25]
3-(4′-Hydroxyphenyl)-2-propenoic acid (4″-carboxyl)-phenyl ester	*S. hieraciifolia*	N/D	methanol ethanol	[8]
3,5-di-*O*-caffeoylquinic acid	*S. aristata*	13.735 µg/g	methanol:acetone:water (3/1/1, *v*/*v*/*v*) methanol	[56]
	S. hispanica	1.04–52.13 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. humilis	N/D	methanol	[55]
	S. latifolia	12.5 µg/g	methanol	[67]
	S. pygmaea	3.214 µg/g	ethanol	[35]
3,5-dicaffeoylquinic acid methyl ester (macroantoin G)	*S. cana* var. *jacquiniana*	10 µg/g	methanol	[72]
	S. hieraciifolia	N/D	ethanol	[8]
3-*O*-caffeoylquinic acid methyl ester	*S. hieraciifolia*	N/D	ethanol	[8]
4,5-dicaffeoylquinic acid (isochlorogenic acid C)	*S. hispanica*	2.46–4.59 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. latifolia	2.5 µg/g	methanol	[67]
	S. veratrifolia	25.667 µg/g	methanol	[64]
4,5-di-*O*-caffeoylquinic acid methyl ester	*S. latifolia*	25 µg/g	methanol	[67]
	S. hieraciifolia	N/D	ethanol	[8]
4-Hydroxybenzoic acid 4-(6-*O*-α-rhamnopyranosyl-β-glucopyranosyl) benzyl ester	*S. cana* var. *jacquiniana*	20.433 µg/g	methanol	[72]
4-*O*-caffeoylquinic acid (cryptochlorogenic acid)	*S. hispanica*	0.52–0.93 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
5-*O*-feruloyl quinic acid methyl ester	*S. hieraciifolia*	N/D	ethanol	[8]
9-Hydroxyocta-(10*E*,12*E*)-decadienoic acid	*S. hispanica*	N/D	ethyl acetate	[20]
9-*Oxo*-(10*E*,12*E*)-octadecadienoic acid	*S. hispanica*	1.071 µg/g	ethyl acetate	[20]
9-*Oxo*-(10*E*,12*Z*)-octadecadienoic acid	*S. hispanica*	N/D	ethyl acetate	[20]
Butyl 3-*O*-feruloylquinate	*S. divaricata*	2.909 µg/g	95% aqueous ethanol	[58]
	S. divaricata	2.909 µg/g	95% aqueous ethanol	[57]
Caffeic acid	*S. hieraciifolia*	N/D	ethanol	[8]
	S. hispanica	0.13–2.47 µg/g	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. latifolia	4.58 µg/g	Methanol	[67]
Caffeic acid methyl ester	*S. aristata*	56.627 µg/g	methanol:acetone:water (3/1/1, *v*/*v*/*v*) methanol	[56]
Chlorogenic acid	*S. cinerea*	412.89 ± 0.55 µg/mg	20% aqueous methanol	[5]
	S. hispanica	3.80–43.82 µg/mg	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[20]
	S. humilis	N.D	methanol	[55]
	S. incisa	141.49 ± 0.20 µg/mg	20% aqueous methanol	[5]
	S. latifolia	1246.78 ± 3.20 µg/mg	20% aqueous methanol	[5]
	S. mollis ssp. *szowitsii*	159.25 ± 0.24 µg/mg	20% aqueous methanol	[5]
	S. parviflora	509.96 ± 6.64 µg/mg	20% aqueous methanol	[5]
	S. pygmaea	3.43 µg/g	ethanol	[35]
	S. tomentosa	734.72 ± 1.04 µg/mg	20% aqueous methanol	[5]
	S. veratrifolia	61.857 µg/g	methanol	[64]

Table 2. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Chlorogenic acid methyl ester	*S. hieraciifolia*	N/D	ethanol	[8]
	S. latifolia	3.75 µg/g	methanol	[67]
	S. pygmaea	10 µg/g	ethanol	[35]
	S. veratrifolia	31.667 µg/g	methanol	[64]
Cryptochlorogenic acid	*S. veratrifolia*	11 µg/g	methanol	[64]
Dihydrocaffeic acid	*S. divaricata*	1.091 µg/g	95% aqueous ethanol	[57]
Dihydrocaffeic acid ethyl ester (ethyl dihydrocaffeate)	*S. divaricata*	1.091 µg/g	95% aqueous ethanol	[57]
Dihydrocaffeic acid methyl ester (methyl dihydrocaffeate)	*S. divaricata*	0.909 µg/g	95% aqueous ethanol	[57]
Dihydrocaffeic acid n-butyl ester (propyl dihydrocaffeate)	*S. divaricata*	7.273 µg/g	95% aqueous ethanol	[57]
E-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)dihydrofuran-2-one	*S. judaica*	9.714 µg/g	chloroform:methanol (9:1)	[25]
Hydrangeic acid 4′-*O*-β-D-glucopyranoside	*S. judaica*	3.857 µg/g	methanol	[25]
Methyl 3-O-feruloylquinate	*S. divaricata*	0.727 µg/g	95% aqueous ethanol	[58]
Protocatechuic acid	*S. cana* var. *jacquiniana*	8.5 µg/g	methanol	[72]
Z-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone	*S. judaica*	4.571 µg/g	chloroform:methanol (9:1)	[25]
Phtalides				
(3*RS*)-3-[(*SR*)-hydroxy(4-hydroxyphenyl)-methyl]-7-methoxy-2-benzofuran-1(3*H*)-one ((±)-scorzophthalide)	*S. tomentosa*	7.538 µg/g	methanol	[36]
(±)-hydramacrophyllol A	*S. judaica*	3.571 µg/g	chloroform:methanol (9:1)	[25]
	S. tomentosa	31.91 µg/g	methanol	[36]
(±)-hydramacrophyllol B	*S. tomentosa*	43.97 µg/g	methanol	[36]
	S. judaica	6.429 µg/g	chloroform:methanol (9:1)	[25]
(±)-3-(4-hydroxybenzyl)-7-hydroxyphthalide (scorzoveratrin)	*S. latifolia*	25.83 µg/g	methanol	[67]
	S. veratrifolia	65 µg/g	methanol	[64]
Scorzoveratrin 4′-O-β-glucoside	*S. latifolia*	8.833 µg/g	methanol	[67]
3-(4-β-glucopyranosyloxybenzyl)-7-methoxyphthalide (scorzoveratrozit)	*S. latifolia*	50 µg/g	methanol	[67]
	S. veratrifolia	333.333 µg/g	methanol	[64]
Polysaccharides				
Inulin	*S. hispanica*	226.4 µg/g	water	[60]
Sesquiterpene lactones				
(3*aS*,6*aR*,8*S*,9*aR*,9*bS*)-3,6,9-trimethylidene-8-[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3a,4,5,6a,7,8,9a,9b-octahydroazuleno[4,5-b]furan-2-one (glucozaluzanin C)	*S. austriaca*	N/D	acetone	[33]
(3*aS*,6*aR*,8*S*,9*aR*,9*bS*)-8-hydroxy-3,6,9-trimethylidene-3*a*,4,5,6*a*,7,8,9*a*,9*b*-octahydroazuleno[4,5-*b*]furan-2-one (zaluzanin C)	*S. austriaca*	N/D	acetone	[33]
(3*aS*,6*aR*,9*aR*,9*bS*)-3,6,9-trimethylidene-3*a*,4,5,6*a*,7,8,9*a*,9*b*-octahydroazuleno[4,5-*b*]furan-2-one (dehydrocostus lactone)	*S. austriaca*	N/D	acetone	[33]
11β,13-dihydrozaluzanin C	*S. austriaca*	N/D	acetone	[33]
14-isovaleroxyscorzoaustricin	*S. austriaca*	4.286 µg/g	acetone	[33]
14-isovaleroxyscorzoaustricin sulfate	*S. austriaca*	7.143 µg/g	acetone	[33]
4-*epi*-dihydroestafiatol	*S. austriaca*	4.286 µg/g	acetone	[33]
Biguaiascorzolide A	*S. austriaca*	5.714 µg/g	acetone	[17]
Biguaiascorzolide B	*S. austriaca*	0.857 µg/g	acetone	[17]
Diacetoxyisolippidiol	*S. austriaca*	N/D	acetone	[33]

Table 2. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Scorzoaustriacin	*S. austriaca*	7.143 µg/g	acetone	[33]
Scorzoaustriacin 3-*O*-β-D-glucoside	*S. austriaca*	10 µg/g	acetone	[33]
Scorzoaustriacoside	*S. austriaca*	5.714 µg/g	acetone	[33]
Sesquiterpenoids				
(1*R*,5*S*,6*S*,7*R*,8*S*)-8-sulfoxyguaia-4(15),10(14),11(13)-trine-6,12-olide (sulfoscorzonin A)	*S. divaricata*	0.546 µg/g	95% aqueous ethanol	[59]
(1*R*,5*S*,6*S*,7*R*,8*S*,13*S*)-8-sulfoxy-13-L-prolineyl-guaia-4(15),10(14)-dien-6a,12-olide (sulfoscorzonin C)	*S. divaricata*	7.273 µg/g	95% aqueous ethanol	[59]
(1*R*,5*S*,6*S*,7*R*,8*S*,13*S*)-8-sulfoxy-13-pyridyl-guaia-4(15),10(14)-dien-6,12-olide (sulfoscorzonin B)	*S. divaricata*	0.723 µg/g	95% aqueous ethanol	[59]
10(*Z*)-1-oxo-bisabola-2,10-dien-13-al	*S. divaricata*	1.455 µg/g	95% aqueous ethanol	[59]
1-*Oxo*-bisabola-(2,10*E*)-diene-12-al (puliglutone)	*S. hispanica*	21.786 µg/g	ethyl acetate	[20]
1-*Oxo*-bisabola-(2,10*E*)-diene-12-carboxylic acid	*S. hispanica*	14.286 µg/g	ethyl acetate	[20]
1-*Oxo*-bisabola-(2,10*E*)-diene-12-carboxylic acid methyl ester	*S. hispanica*	10.714 µg/g	ethyl acetate	[20]
1-*Oxo*-bisabola-(2,10*E*)-diene-12-ol	*S. hispanica*	3.929 µg/g	ethyl acetate	[20]
1-*Oxo*-bisabola-2-ene-12-ol (ptilostemonol)	*S. hispanica*	7.143 µg/g	ethyl acetate	[20]
2,9-Epoxycurcumen-12-al	*S. hispanica*	4.643 µg/g	ethyl acetate	[20]
Ixerisoside D	*S. hispanica*	7.021 µg/g	methanol	[32]
3β,11α-dihydroxy-4β-methyl-guaia-10(14)-en-12,6α-olide	*S. austriaca*	N/D	acetone	[16]
Steroids				
3β-hydroxystigmast-5-en-7-one	*S. divaricata*	1.455 µg/g	95% aqueous ethanol	[57]
3β-hydroxyl-5α,8α-epidioxyergosta-6,22-diene (5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol)	*S. divaricata*	3.636 µg/g	95% aqueous ethanol	[57]
5,6α-epoxy-5α-stigmastan-3β-ol	*S. divaricata*	1.273 µg/g	95% aqueous ethanol	[57]
Stigmast-3β, 5α, 6β-trihydroxy	*S. austriaca*	2.837 µg/g	acetone	[28]
Stigmast-3β, 7β-dihydroxyl-5-ene (7β-hydroxysitosterol)	*S. divaricata*	0.909 µg/g (mixture)	95% aqueous ethanol	[57]
Stigmast-3β,7α-dihydroxyl-5-ene (7α-hydroxysitosterol)	*S. divaricata*		95% aqueous ethanol	[57]
Stigmast-4-en-3-one	*S. austriaca*	2.257 µg/g	acetone	[28]
Stigmast-4-en-6β-ol-3-one (6β-hydroxystigmastan-4-en-3-one)	*S. divaricata*	1.636 µg/g	95% aqueous ethanol	[57]
Stigmasterol	*S. undulata* ssp. *deliciosa*	N/D	dichloromethane	[63]
β-Sitosterol	*S. austriaca,*	3.286 µg/g	acetone	[28]
	S. divaricata	6.182 µg/g	95% aqueous ethanol	[57]
	S. latifolia	75.714 µg/g	methanol	[3,6]
	S. undulata ssp. *deliciosa*	N/D	dichloromethane	[63]
	S. veratrifolia	N/D	methanol	[30]
β-Sitosterol 3-*O*-β-D-glucoside (β-daucosterol)	*S. austriaca*	4.314 µg/g	acetone	[28]
	S. divaricata	3.636 µg/g	95% aqueous ethanol	[57]
β-Stigmasterol	*S. austriaca*	1.971 µg/g	acetone	[28]
Triterpenoids				
(23Z)-Cycloart-23-en-3β,25-dihydroxy	*S. austriaca*	2.943 µg/g	acetone	[28]
(3*S*,10*R*,13*S*,14*S*,17*S*,20*S*,24*R*)-(23*E*)-3-acetoxy-25-hydroperoxy-tirucalla-7,23-diene (scorzodivaricin C)	*S. divaricata*	0.909 µg/g	95% aqueous ethanol	[59]
(3*S*,10*R*,13*S*,14*S*,17*S*,20*S*,24*R*)-3β-hydroxyl-24-hydroperoxy-24-vinyl-tirucalla-8-ene (scorzodivaricin D)	*S. divaricata*	1.091 µg/g	95% aqueous ethanol	[59]
(3*S*,5*R*,10*R*,13*S*,14*S*,17*R*,20*S*,24*R*)-3-acetoxy-24-hydroxyl-tirucalla-7,25-diene (scorzodivaricin B)	*S. divaricata*	0.909 µg/g	95% aqueous ethanol	[59]
(6*S*,7*R*)-10,11,13-trihydroxy-bisabola-2-en-1-one (scorzodivaricin A)	*S. divaricata*	0.727 µg/g	95% aqueous ethanol	[59]
23(Z)-3β, 25-dihydroxy-tirucalla-7,23-diene	*S. divaricata*	3.091 µg/g	95% aqueous ethanol	[59]
23(Z)-3β-acetoxy-25-hydroxy-tirucalla-7,23-diene	*S. divaricata*	1.273 µg/g	95% aqueous ethanol	[59]

Table 2. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
3α-hydroxyolean-5-ene	*S. aristata*	19.036 µg/g	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
3β-acetoxyglutin-5(10)-en-6-oxo	*S. austriaca*	4.086 µg/g	methanol acetone	[28]
3β-acetyl-11α,12α-oxidotaraxerol	*S. austriaca*	3.3171 µg/g	acetone	[28]
3-β-hydroxy-fern-7-en-6-one-acetate	*S. cinerea*	65 ± 1 µg/g	*n*-hexane	[27]
	S. eriophora	20 ± 1 µg/g	*n*-hexane	[27]
	S. latifolia	50 ± 1 µg/g	*n*-hexane	[27]
		7.143 µg/g	methanol	[47]
	S. sublanata	35 ± 1 µg/g	*n*-hexane	[27]
	S. tomentosa	47 ± 1 µg/g	*n*-hexane	[27]
3-β-hydroxy-fern-8-en-7-one-acetate	*S. latifolia*	N/D	methanol	[73]
9β,19-cyclolanostane- 24-en-3-oxo	*S. austriaca*	7.314 µg/g	acetone	[28]
Amyrin β-acetate	*S. undulata* ssp. *deliciosa*	10.103 µg/g	dichloromethane	[49]
D-friedours-14-en-3β-acetyl-11α,12α-epoxy	*S. austriaca*	2.229 µg/g	acetone	[28]
Fern-7-en-3-one	*S. veratrifolia*	N/D	methanol	[30]
Fern-7-en-3-β-ol (motiol)	*S. latifolia*	6.714 µg/g	methanol	[3,6]
Fern-7-en-3-β-one	*S. latifolia*	32.143 µg/g	methanol	[6]
Germanicol	*S. veratrifolia*	N/D	methanol	[30]
Germanicol acetate	*S. veratrifolia,*	N/D	methanol	[30]
Germanicone	*S. veratrifolia*	N/D	methanol	[30]
Glutinol	*S. austriaca*	4.4861 µg/g	acetone	[28]
Lupenone	*S. veratrifolia*	N/D	methanol	[30]
Lupeol	*S. acuminata*	512 ± 1 µg/g	*n*-hexane	[27]
	S. aristata	N/D	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
	S. austriaca	2.7431 µg/g	methanol acetone	[28]
	S. cana var. *jacquiniana*	932 ± 2 µg/g	*n*-hexane	[27]
	S. cinerea	1073 ± 6 µg/g	*n*-hexane	[27]
	S. eriophora	244 ± 7 µg/g	*n*-hexane	[27]
	S. incisa	283 ± 2 µg/g	*n*-hexane	[27]
	S. laciniata ssp. *laciniata*	447 ± 2 µg/g	*n*-hexane	[27]
	S. latifolia	213 ± 2 µg/g	*n*-hexane	[27]
	S. mirabilis	224 ± 1 µg/g	*n*-hexane	[27]
	S. mollis ssp. *szowitsii*	282 ± 11 µg/g	*n*-hexane	[27]
	S. parviflora	132 ± 4 µg/g	*n*-hexane	[27]
	S. suberosa ssp. *suberosa*	342 ± 4 µg/g	*n*-hexane	[27]
	S. sublanata	415 ± 1 µg/g	*n*-hexane	[27]
	S. tomentosa	564 ± 2 µg/g	*n*-hexane	[27]
	S. veratrifolia	N/D	methanol	[30]

Table 2. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Lupeol acetate	*S. acuminata*	297 ± 1 µg/g	*n*-hexane	[27]
	S. cana var. *jacquiniana*	4273 ± 12 µg/g	*n*-hexane	[27]
	S. cinerea	3645 ± 8 µg/g	*n*-hexane	[27]
	S. eriophora	2195 ± 7 µg/g	*n*-hexane	[27]
	S. incisa	736 ± 10 µg/g	*n*-hexane	[27]
	S. laciniata ssp. *laciniata*	3212 ± 13 µg/g	*n*-hexane	[27]
	S. latifolia	2261 ± 94 µg/g	*n*-hexane	[27]
	S. mirabilis	1356 ± 2 µg/g	*n*-hexane	[27]
	S. mollis ssp. *szowitsii*	1244 ± 1 µg/g	*n*-hexane	[27]
	S. parviflora	711 ± 3 µg/g	*n*-hexane	[27]
	S. suberosa ssp. *suberosa*	1261 ± 5 µg/g	*n*-hexane	[27]
	S. sublanata	3920 ± 8 µg/g	*n*-hexane	[27]
	S. tomentosa	2502 ± 7 µg/g	*n*-hexane	[27]
	S. veratrifolia	N/D	methanol	[30]
Magnificol	*S. aristata*	N/D	methanol:acetone:water (3/1/1, *v*/*v*/*v*)	[56]
Methyl oleanate	*S. undulata* ssp. *deliciosa*	N/D	methanol	[63]
Methyl ursolate	*S. undulata* ssp. *deliciosa*	N/D	dichloromethane	[63]
Olean-12-en-11-one-3-acetyl	*S. cinerea*	115 ± 1 µg/g	dichloromethane	[63]
	S. incisa	151 ± 1 µg/g	*n*-hexane	[27]
	S. latifolia	8 µg/g	*n*-hexane	[27]
		135 ± 1 µg/g	methanol	[47]
	S. tomentosa	187 ± 1 µg/g	*n*-hexane	[27]
Oleanolic acid	*S. divaricata*	1.818 µg/g	*n*-hexane	[27]
Taraxasterol	*S. austriaca*	3.371 µg/g	95% aqueous ethanol	[59]
	S. veratrifolia	N/D	acetone	[28]
Taraxasterol acetate/Taraxasteryl acetate	*S. cana* var. *jacquiniana*	719 ± 3 µg/g	methanol	[30]
	S. cinerea	2171 ± 6 µg/g	*n*-hexane	[27]
	S. eriophora	3212 ± 17 µg/g	*n*-hexane	[27]
	S. incisa	1191 ± 5 µg/g	*n*-hexane	[27]
	S. laciniata ssp. *laciniata*	276 ± 3 µg/g	*n*-hexane	[27]
	S. latifolia,	4201 ± 16 µg/g	*n*-hexane	[27]
	S. mirabilis	2099 ±4 µg/g	*n*-hexane	[27]
	S. mollis ssp. *szowitsii*	3791 ± 14 µg/g	*n*-hexane	[27]
	S. parviflora	811.96 ± 4 µg/g	*n*-hexane	[27]
	S. suberosa ssp. *suberosa*	2340 ± 6 µg/g	*n*-hexane	[27]
	S. sublanata	4981 ± 2 µg/g	*n*-hexane	[27]
	S. tomentosa	3168 ± 12 µg/g	*n*-hexane	[27]
	S. veratrifolia	N/D	methanol	[30]
Taraxasteryl myristate	*S. latifolia*	142.85 µg/g	methanol	[6]
Urs-12-en-11-one-3-acetyl	*S. latifolia*	N/D	methanol	[10]
α-Amyrin	*S. acuminata*	1646 ± 10 µg/g	*n*-hexane	[27]
	S. cana var. *jacquiniana*	920 ± 11 µg/g	*n*-hexane	[27]
	S. cinerea	3221 ± 13 µg/g	*n*-hexane	[27]
	S. laciniata ssp. *laciniata*	146 ± 4 µg/g	*n*-hexane	[27]
	S. mollis ssp. *szowitsii*	609 ± 6 µg/g	*n*-hexane	[27]
	S. tomentosa	969 ± 11 µg/g	*n*-hexane	[27]

Table 2. *Cont.*

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
α-Amyrin acetate	*S. veratrifolia*	N/D	methanol	[30]
α-Amyrin-3-acetyl	*S. austriaca*	2.857 µg/g	acetone	[28]
α-Amyrin-3-acetyl-11-oxo	*S. austriaca*	2.971 µg/g	acetone	[28]
α-Amyrinone	*S. veratrifolia*	N/D	methanol	[30]
β-Amyrin acetate	*S. veratrifolia*	N/D	methanol	[30]
β-Amyrinone	*S. veratrifolia*	N/D	methanol	[30]
β-Amyrin	*S. veratrifolia*	N/D	methanol	[30]
β-Amyrin acetate	*S. undulata* ssp. *deliciosa*	10.103 µg/g	dichloromethane	[63]
β-Amyrin-3- acetyl	*S. austriaca*	3.2 µg/g	acetone	[28]
β-Amyrin-3(3′-methylbutanoate)	*S. austriaca*	2.857 µg/g	acetone	[28]
ψ-Taraxasterol	*S. veratrifolia*	N/D	methanol	[30]
ψ-Taraxasterol acetate	*S. veratrifolia*	N/D	methanol	[30]
ψ-Taraxasteryl-3 (3′-methyl-butanonate)	*S. austriaca*	2.8 µg/g	acetone	[28]
Tyrolobibenzyls				
β-D-glucopyranosyl 4-[2-(4-hydroxyphenyl)ethyl]benzofuran-2-carboxylate (tyrolobibenzyl A)	*S. humilis*	701.258 µg/g	methanol	[54]
β-D-glucopyranosyl 5-hydroxy-4-[2-(4-hydroxyphenyl)ethyl]benzofuran-2-carboxylate (tyrolobibenzyl B)	*S. humilis*	829.141 µg/g	methanol	[54]
1-{3-(β-D-glucopyranosyloxy)-6-hydroxy-2-[2-(4-hydroxyphenyl)ethyl]phenyl}ethanone (tyrolobibenzyl C)	*S. humilis*	489.518 µg/g	methanol	[54]
1‴→6″-β-D-apiofuranosyl-β-D-glucopyranosyl 4-[2-(4-hydroxyphenyl)ethyl]benzofuran-2-carboxylate (tyrolobibenzyl D)	*S. humilis*	223.594 µg/g	methanol	[53]
6-*O*-β-D-glucosyl derivative of tyrolobibenzyl C (tyrolobibenzyl E)	*S. humilis*	78.189 µg/g	methanol	[55]
5-*O*-glucosyl derivative of tyrolobibenzyl B (tyrolobibenzyl F)	*S. humilis*	6.859 µg/g	methanol	[55]
Other compounds				
Verbascoside (acteoside)	*S. undulata* ssp. *deliciosa*	72.165 µg/g	dichloromethane	[49,63]
Methyl-β-D-fructofuranoside	*S. divaricata*	3.273 µg/g	95% aqueous ethanol	[57]
1-monolinolein (glycerol 1–9′,12′-octadecadienoate)	*S. divaricata*	2.364 µg/g	95% aqueous ethanol	[57]
2-[(*E*)-2-(4-hydroxyphenyl)ethenyl]-6-methoxybenzoic acid (scorzoerzincanin)	*S. tomentosa*	105.528 µg/g	methanol	[36]

Compound concentration was taken directly from literature or it was calculated, dividing the mass of the isolated compound by the mass of plant material used for extraction; N/D—no data was available in the literature.

Table 3. Compounds isolated from *Scorzonera* whole plants.

Compounds	*Scorzonera* Species	Concentration	Type of Solvent	References
Dihydroisocoumarins and dihydroisocoumarin derivatives				
6,8-dihydroxy-3-(4methoxyphenyl)isochroman-1-one (scorzocreticin)	*S. cretica*	13.334 µg/g	methanol	[37]
8-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]scorzocreticin (scorzocreticoside II)	*S. cretica*	48.2 µg/g	methanol	[37]
8-O-β-D-glucopyranosylscorzocreticin (scorzocreticoside I)	*S. cretica*	23.334 µg/g	methanol	[37]
Flavonoid aglycones				
Apigenin	*S. undulata* ssp. *alexandrina*	9 µg/g	petroleum ether	[23]
Steroids				
3-*O*-β-D-glucopyranosylsitosterol	*S. cretica*	N/D	methanol	[37]
3-O-(6-O-acetyl-β-D-glucopyranosyl)β-sitosterol	*S. undulata* ssp. *alexandrina*	6 µg/g	petroleum ether	[23]
Daucosterol	*S. undulata* ssp. *alexandrina*	12 µg/g	petroleum ether	[23]
Triterpenoids				[37]
24-methylenecycloartanol	*S. undulata* ssp. *alexandrina*	4 µg/g	petroleum ether	[23]
Germanicol	*S. cretica*	N/D	dichloromethane	[37]
Germanicol acetate	*S. cretica*	N/D	dichloromethane	[37]
Germanicone	*S. cretica*	N/D	dichloromethane	[37]
Lupenone	*S. cretica*	N/D	dichloromethane	[37]
Lupeol	*S. cretica*	N/D	dichloromethane	[37]
	S. undulata ssp. *alexandrina*	5 µg/g	petroleum ether	[23]
Lupeol acetate	*S. cretica*	N/D	dichloromethane	[37]
Oleanol	*S. cretica*	N/D	dichloromethane	[37]
Oleanol acetate	*S. cretica*	N/D	dichloromethane	[37]
Taraxasterol	*S. cretica*	N/D	dichloromethane	[37]
Taraxasterol acetate/Taraxasteryl acetate	*S. cretica*	N/D	dichloromethane	[37]

Compound concentration was taken directly from literature or it was calculated, dividing the mass of the isolated compound by the mass of plant material used for extraction; N/D—no data was available in the literature.

4. Biological Activity

The biological activity of species within the *Scorzonera* genus is the subject of research due to their presence in folk medicine in Eurasia and northern Africa. In Mongolia, *S. divaricata* and *S. pseudodivaricata* play a significant role in herbal therapy. *S. divaricata* is used to treat fever and poisonous ulcers or even malignant stomach neoplasia. *S. pseudodivaricata* is a folk remedy for digestive problems, parasites, or lung edema [14].

Species that belong to the *Scorzonera* genus are reported to be the source of numerous bioactive compounds. Researchers evaluate their potential as antioxidant [58,59,62,74], anti-inflammatory [27,68,75], and pain-relieving agents [6,70], as well as their cytotoxicity against cancer cell lines [20,28,59] and wound healing properties [4,5]. Biological activities of *Scorzonera* species in vitro are summarized in Figure 1.

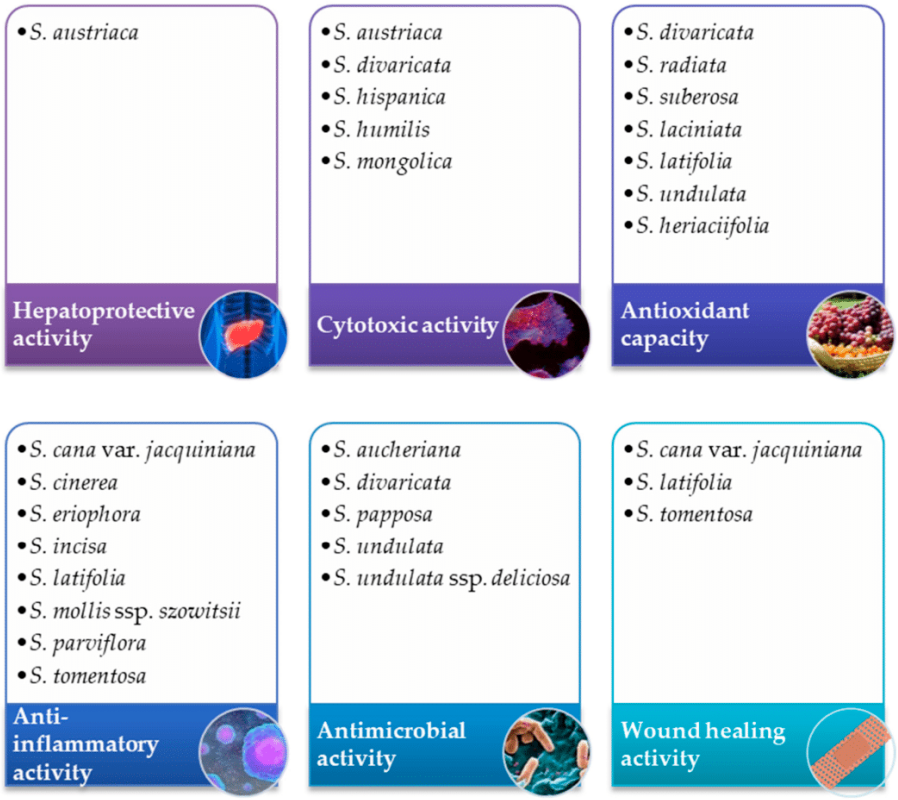

Figure 1. Biological activity of species within the genus *Scorzonera* evaluated in vitro. Species were assigned to sections according to their activity investigated in in vitro tests, described in Sections 4.1–4.7.

4.1. Cytotoxic Activity

Cytotoxicity is the primary characteristic of compounds and substances in terms of their qualification as therapeutic agents and is the toxicity that a certain factor causes in live cells [76]. High cytotoxicity against rapidly dividing cancer cells in vitro is the basis for further research on their bioactivity (e.g., necrosis, autophagy, or apoptosis induction), low cytotoxicity on the other hand is desired in the development of drugs that are not intended to induce death in cells. In this review, the cytotoxicity of extracts and compounds obtained and isolated from *Scorzonera* species against cancer cell lines was presented.

The first reported attempt to evaluate the antineoplastic activity of *Scorzonera* species in vitro was made in 2000 by Zidorn and colleagues [54]. The biological activity of compounds isolated from a methanol extract from *S. humilis* subaerial parts (together with newly isolated tyrolobibenzyls) was assessed and none influenced the DNA biosynthesis

in the GTB and HL60 human leukemia cell lines at the concentration range of 0.25–4.00 µM. In their further research, tyrolobibenzyls D was isolated from the extract. It was assayed for cytotoxicity against the P388 (mouse leukemia) cell line along with previously obtained tyrolobibenzyls A-C and their peracetyl derivatives. In the assay, only Tylorobibenzyl D exhibited low cytotoxic activity with IC_{50} (half-maximal inhibitory concentration) of 25 µg/mL. The cytotoxicity of crude extracts was assayed as well and no activity was observed up to the point where the concentration reached 0.5 mg/mL. The EtOAc fraction of the crude extract exhibited cytotoxic properties with IC_{50} value at the concentration of 95 µg/mL. In the research, the antimicrobial activity of tyrolobibenzyls and their derivatives was evaluated but none was active against neither bacteria nor fungi. The DPPH assay did not reveal any significant radical scavenging properties of tyrolobibenzyls and the compounds were able to inhibit the activity of COX-1 (cyclooxygenase 1) to an insignificant degree [53].

In the study from 2009, Wang and colleagues [31] obtained two triterpene fatty esters: erythrodiol and 3β-tetradecanoyl erythrodiol from a methanol extract of *Scorzonera mongolica* whole plants. The isolated compounds were then assayed for their cytotoxicity towards 3 cancer cell lines (P388 mouse leukemia cell line, A549 human lung cancer cell line, and Bel-7402 human hepatocellular carcinoma cell line) and both esters exhibited cytotoxic activity against A549 lung cancer cells (in the concentration of 50 µg/mL, the compounds induced the cell growth inhibition by 66.8% and 69.8%).

Two out of five congeners (Scorzodihydrostilbenes A and B) isolated from the methanol extract from the aerial parts of *Scorzonera radiata* Fisch. were tested in the MTT cytotoxicity assay but at a concentration of 10 µg/mL, neither displayed cytotoxic activity toward mouse lymphoma cell line (L5175Y) [15]. The concentration (10 µg/mL) converted to µM is 21.53 µM for Scorzodihydrostilbene A and 20.90 µM for Scorzodihydrostilbene B. When compared to a study from 2007, dihydrostilbenes isolated from a *Bulbophyllum odoratissimum* Lindl., low toxicity of *Scorzonera radiata* Fisch. is even more notable, compounds in the mentioned study were toxic towards SGC-7901 (human gastric cancer), KB (nasopharyngeal carcinoma), and HT-1080 (fibrosarcoma) cell lines with IC_{50} values of 5.50–9.20 µM for SGC-7901 and KB lines and 25.50–40 µM for HT-1080 line [77].

A screening study on cytotoxic activity of several species of *Asteraceae* genus present in Hungary, including *Scorzonera austriaca* Willd, was carried out in 2009. In a cytotoxicity assay, the most active against human cell lines: A431 (skin epidermoid carcinoma), HeLa (cervix adenocarcinoma), and MCF-7 (breast adenocarcinoma) was a chloroform root extract of *S. austriaca* IC_{50} values of the extract were: 4.71 µg/mL for A431 line, 6.42 µg/mL for HeLa line and 5.52 µg/mL for MCF-7 line. It was more active than the extracts obtained using other solvents, as well as leaf extracts from the same plant—at a concentration of 10 µg/mL the antiproliferative activity of *S. austriaca* root chloroform was at 86.32% for A431 cell line, 77.27% for HeLa cell line, and 83.79% for MCF-7 cell line. Other *S. austriaca* extracts obtained in the study did not influence the proliferation of those cell lines by more than 48.11% [78]. Those results can be compared to a 2018 research, in which leaf chloroform extract from another species within the *Asteraceae* family, *Anvillea garcinii* (Burm.f.) DC., exhibited antiproliferative properties against MCF-7 and HeLa cell lines with IC_{50} of 24.50 µg/mL for MCF-7 and 12 µg/mL for HeLa [79]. Another *Asteraceae* family member, *Pulicaria undulata* (Forssk.) Oliver., was evaluated as a potential source of cytotoxic agents. Whole plant chloroform extract turned out to have cytotoxic properties with the IC_{50} value of 16.4 µg/mL for MCF-7 cell line, 3.01 µg/mL for HepG2, and 7.4 µg/mL for HCT-116 cell lines. Those values were compared to cisplatin used as a positive control in the study (IC_{50} of cisplatin was 3.68–4.51 µg/mL) [80]. However, in the study from 2011, Bader et al. isolated nine new phenolic compounds and nine known phenolic derivatives from *Scorzonera judaica* root extracts. The newly obtained compounds were assayed for their cytotoxic activity toward human lymphocyte T cells, as well as MCF-7 and HeLa cell lines. Compounds did not exhibit cytotoxic activity in concentrations below 100 µM [25].

Zhu and colleagues [17] discovered that two new dimeric guaianolides (biguaiascorzolides A and B) are present in an acetone extract from *Scorzonera austriaca* roots. In the study, biguaianoscorzolide A was acetylated and the derivative's cytotoxicity against adriamycin-resistant myelogenous leukemia (K562/ADM) cell line and gastric carcinoma (BGC-823) cell line was measured. The compound's activity towards K562/ADM cells (IC_{50} = 39.8 μM) was more significant than towards the MGC-803 cell line (IC_{50} > 100 μM), which suggests that the compound's cytotoxic activity might depend on the type of tumor cells. In a continuation of the study, Zhu and colleagues [33] elucidated the presence of six sesquiterpene lactones in acetone and ethanol *S. austriaca* root extracts (scorzoaustriacoside, scorzoaustriacin, scorzoaustriacin 3-*O*-β-D-glucoside, 4-*epi*-dihydroestafiatol, 14-isovaleroxyscorzoaustricin, and 14-isovaleroxyscorzoaustricin sulfate). The cytotoxic activity against four cancer cell lines (K562, K562/ADM, BGC-823, and Hep-G2) of scorzoaustriacin, scorzoaustriacin 3-*O*-β-D-glucoside, 14-isovaleroxyscorzoaustricin, and 14-isovaleroxyscorzoaustricin sulfate was assayed and only scorzoaustriacin was reported cytotoxic towards K562 (human myelogenous leukemia) cell line (IC_{50} = 11.3 μM).

In the study by Granica et al. [20], (–)-syringaresinol was isolated from the ethyl acetate extract of *Scorzonera hispanica* subaerial parts. It was then reported that (–)-syringaresinol was cytotoxic towards NCI and MMS-1 myeloma cell lines and exhibited moderate activity against SW-480 colon cancer cells. Moreover, the compound's cytotoxicity was reported in peripheral blood mononuclear cells. In the previous research on (–)-syringaresinol, it was reported to exhibit the ability to induce apoptosis and arrest the G_1 phase in the HL-60 human leukemia cell line [81]. Jeong et al. [82] found out that (–)-syringaresinol inhibits P-glycoprotein in MCF-7/ADR human breast cancer cell line and enhances the cytotoxic activity of daunomycin.

In a phytochemical study on *Scorzonera divaricata* root ethanol extract, one of the isolated compounds, a tirucallane terpene ((3*S*,5*R*,10*R*,13*S*,14*S*,17*R*,20*S*,24*R*)-3-acetoxy-24-hydroxyl-tirucalla-7,25-dienem, named scorzodivaricin B), exhibited a cytotoxic activity towards HeLa, HepG2, HL60 and SMMC-7721 (human cervical cancer, human liver cancer human leukemia cancer and hepatocellular carcinoma, accordingly) cell lines with IC_{50} values between 24.4 ± 3.6 μM and 66.7 ± 5.2 μM. Cisplatin used as a positive control in the study exhibited higher activity with IC_{50} within the range of 7.45 ± 0.9–12.8 ± 2.4 μM [59].

Wu and colleagues [34] evaluated five compounds isolated from *Scorzonera divaricata* aerial parts for their potential activity against cancer cell lines (HepG2, HeLa, and K562). Sulfoscorzonin E exhibited cytotoxicity similar to 5-FU. IC_{50} values of sulfoscorzonin E were 4.21 μg/mL (10.59 μM) for HepG2, 8.15 μg/mL (20.5 μM) for HeLa, and 6.53 μg/mL (16.43 μM) for K562 cell line. Sacrolide A was active against HeLa and HepG2 cells (IC_{50} = 6.28 μg/mL = 20.16 μM for HeLa and 3.56 μg/mL = 11.43 μM for HepG2 lines). In the study, sulfoscorzonin D, glucozaluzanin C, and 1β,4α-dihydroxy-5α,6β,7α,11β*H*-eudermn-12,6-olide did not exhibit any significant cytotoxic activity at the concentration below 80 μg/mL (141.92–298.32 μM).

4.2. Anti-Inflammatory Activity

For centuries, suppression of inflammatory response has been an observed effect of various medicinal plants. Ethnopharmacological reports give examples of plants extracts able to combat the process of inflammation in human bodies and; therefore, novel plant-derived products are investigated for their anti-inflammatory activity [83].

A study carried out by Bahadır-Acıkara et al. in 2018 [27] revealed that *n*-hexane extracts from roots and aerial parts of eleven *Scorzonera* species (*S. acuminata*, *S. cinerea*, *S. eriophora*, *S. incisa*, *S. latifolia*, *S. mirabilis*, *S. mollis* ssp. *szowitsii*, *S. parviflora*, *S. suberosa* ssp. *suberosa* and *S. tomentosa*) contained significant amounts of triterpenes, including taraxasteryl acetate, lupeol, and lupeol acetate. In general, root extracts were notably richer in analyzed triterpenes, except for lupeol, whose concentration in aerial parts of *S. incisa*, *S. latifolia*, *S. mirabilis*, *S. parviflora*, and *S. suberosa* was approximately three to seven times higher than in root extracts (approximately 0.9–1.5 mg/g). The results from the study

correlate with previously observed anti-inflammatory and pain-relieving properties of lupeol [84,85].

The evaluation of the anti-inflammatory properties of *Scorzonera pygmaea* subaerial parts was conducted in 2018 by measuring COX (cyclooxygenase) inhibition. The inhibitory activity of ethanol extract and its fractions against COX-1 and COX-2 (cyclooxygenase 2) was low [35]. That is on the contrary to the study of Bahadır Acıkara et al. from 2015 [10], where it has been observed that extracts from other *Scorzonera* species (*S. cana* var. *jacquiniana, S. cinerea, S. eriophora, S. incisa, S. latifolia, S. mollis* ssp. *szowitsii, S. parviflora,* and *S. tomentosa)* have an inhibitory effect on pro-inflammatory cytokines (TNF-α (tumor necrosis factor α) and IL-1β (interleukin 1β)) production and NF-κB (nuclear factor kappa B) nuclear translocation in macrophages. However, it might suggest that the anti-inflammatory activity of *S. pygmaea* could be evaluated by the measurement of the inhibitory activity against pro-inflammatory cytokines, as they induce COX production [35].

4.3. Analgesic Activity

Pain is an experience known to nearly every animal. There are cases where pain requires medical intervention. Although pain may indicate injury of organs, nervous system-derived pain should be relieved beforehand to prevent the deterioration of the quality of the patient's life. Morphine, a well-known analgesic, was isolated from opium 200 years ago [86]. Nowadays other natural products are assayed for their pain-relieving activity.

Scorzonera latifolia is a plant endemic to Turkey, whose roots are used as a pain-reducing and anthelmintic agent in Turkish folk medicine [4]. An in vivo study on the properties of a methanol extract from *S. latifolia* roots showed that *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and water fractions indeed exhibit analgesic activity on mice in the dose of 50 mg/kg in the tail-flick test. Taraxasteryl mirystate and taraxasteryl acetate present in one of the extracts were active in the dose of 10 mg/kg in both writhing test and flick-tail test. The general antinociceptive properties of *S. latifolia* are reported to be significant. Such outcome of the study can be a result of the synergy of the extract's components [6]. The study was extended to four *Scorzonera* species in 2012. It was then reported that *S. tomentosa, S. latifolia,* and *S. mollis* ssp. *szowitsii* all possess analgesic properties in the writhing test and tail-flick test (the dose was 100 mg/kg) [70].

4.4. Hepatoprotective Activity

The liver plays a significant role in the metabolism and detoxication of the human body. Because of its importance, liver diseases are one of the greatest threats to people's lives. Herbal medicine has used plants as preventive agents for hepatic problems for ages [87]. Based on that knowledge, in vitro and in vivo investigations are conducted to assess the hepatoprotective potential of plants, including the ones with the *Scorzonera* genus.

4.4.1. In Vitro Assays

A study from 2016 investigated the hepatoprotective properties of *Scorzonera austriaca*. The plant is used in folk medicine to treat hepatitis B in China. Xie et al. [24] isolated flavonoid glycosides and their derivatives from *Scorzonera austriaca* herb ethanolic extract. Having measured the concentration of ALT (alanine aminotransferase) in CCl_4-treated rat hepatocytes, it was reported that two flavonoid glycoside derivatives, 5,7,41-trihydroxyflavone 8-*C*-(6″-*O*-*trans*-caffeoyl β-D-glucopyranoside) and 5,7,31,41-tetrahydroxyflavone 8-*C*-(6″-*O*-*trans*-caffeoyl β-D-glucopyranoside), present in herbs of *S. austriaca* have hepatoprotective properties. That conclusion confirmed the validity of the use of the plant in the treatment of hepatitis B in the traditional medicine of China.

4.4.2. In Vivo Assays

The in vivo assays of a *Scorzonera alexandrina* hydroethanolic extract from aerial and subaerial parts revealed that the extract caused a reduction in glucose concentration in

rat's blood, as well as the ALT, and total protein levels in doses of 200 and 400 mg/kg. The extract also exhibited hepatoprotective, and anti-ulcerogenic effects in rats [75].

Hepatoprotective activities of the roots of several *Scorzonera* species (*S. cana* var. *jacquiniana*, *S. latifolia*, *S. mollis* ssp. *szowitsii*, *S. parviflora*, *S. tomentosa*), together with compounds isolated from the *S. latifolia* root extract (chlorogenic acid, hydrangenol-8-O-β-glucoside, and scorzotomentosin-4′-O-β-glucoside) were evaluated in a preclinical in vivo study from 2017. The tests were aiming to elucidate the extract's influence on counteracting CCl_4-induced liver damage in rats. Although the influence of the extract and compounds on the ALT and AST (aspartate transaminase) levels was insignificant, the histological condition of animal livers was notably better in most samples (except for hydrangenol-8-*O*-β-glucoside and scorzotomentosin-4′-*O*-β-glucoside—treated groups). What is interesting in terms of future clinical research is the fact that chlorogenic acid was the most active compound in the treatment of acute carbon tetrachloride-induced liver toxicity [73].

4.5. Antimicrobial Activity

Folk medicine has been treating microbial infections for centuries. Along with the decrease in bacteria's susceptibility to antibiotics, the need for novel antimicrobial drugs is increasing. Plants have been a source of folk medications in the treatment of infectious diseases before the concept of infectious agents emerged [88]. The activity against microbes could also be used to substitute synthetic additives in food to prevent foodborne diseases induced by pathogenic bacteria [89] Several species within the genus *Scorzonera* have been investigated as a source of products with antimicrobial potential.

Volatile oil distilled from aerial parts of *Scorzonera undulata* ssp. *deliciosa* was assessed as an antibacterial agent against Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Micrococcus luteus*) and Gram-negative (*Salmonella typhimurium*, *Escherichia coli*, and *Pseudomonas aeruginosa*) bacteria strains and it was reported more active towards Gram-positive strains with MIC (minimal inhibitory concentration) values of 0.5 mg/mL for *S. aureus* and *M. luteus* and 0.8 mg/mL for *S. epidermidis*, *S. typhimurium*, and *E. coli*. MBC (minimal bactericidal concentration) was not determined for any Gram-negative strain. The authors suggest that greater activity against Gram-positive bacteria strains could be caused by easier penetration through the lipophilic cell membranes by hydrophobic ingredients of the oil. Unfortunately, no reference compound was assessed together with the oil, thus it is difficult to compare those results with any known antibacterial substances [90].

Antibacterial properties of *S. undulata* were assayed in 2010 by Abdelkader and colleagues [91]. The study showed that ethyl acetate fraction of the aerial part methanol extract exhibited antibacterial properties against *P. aeruginosa*, *S. aureus*, *E. faecalis*, *C. freundei*, and *P. mirabilis* with MIC exceeding 1 mg/mL. The petroleum ether fraction; however, was active against *P. aeruginosa*, *S. aureus*, and *C. freundei*. Fractions obtained from the roots had a narrower spectrum of activity, but the petroleum ether fraction exhibited a stronger antimicrobial potential against *S. aureus* with a MIC of 500 μg/mL.

Bactericidal properties of compounds isolated from a *Scorzonera divaricata* aerial parts petroleum ether/diethyl ether/methanol extract were evaluated in a study by Wu and colleagues in 2018 [34]. Sulfoscorzonin D, a new, rare pyrrolidine salt alkaloid obtained in the study, exhibited more potent activity against *Clostridium perfingens* than ampicillin, its activity was similar to the activity of erythromycin and streptomycin. Sacrolide A was more effective against Newman WT than streptomycin (12.5 to 25 μg/mL) and similarly effective to levofloxacin (12.5 μg/mL). Other compounds which were reported to possess antibacterial properties (*B. megaterum*, *C. perfingens*, Newman WT, and *E. coli*) were oleanolic acid, lup-20(29)-ene3β,28-diol, (22E)-5α,8α-epidioxyergosta-6,22-dien-3β-ol, ergosta-3β,5α,6β-trialcohol, and diosmetin (MIC values were 12.5–100 μg/mL).

A comprehensive study on endemic to Lebanon species *Scorzonera mackmeliana* in terms of the plant's antibacterial and antibiofilm properties was carried out by Sweidan and colleagues in 2020 [92]. The authors conducted a phytoanalysis of the constituents of water and ethanol extracts of the whole plant as well as its particular parts (flowers,

stems, leaves, roots) and determined their activity against Gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacterial strains. The highest activities in inhibition of the bacteria strains were observed in two water extracts: the ones obtained from the stems and the whole plant. For the stem water extract, the inhibitory effect was observed in four out of five tested strains, the lowest MIC values were reported in *P. aeruginosa* (48.98 mg/mL), *S. aureus* (48.98 mg/mL), and *S. epidermidis* (48.98 mg/mL). For the water extract from the whole plant, it was proven active against *S. epidermidis*, *P. aeruginosa,* and *E. coli* in the concentration of 122.25 mg/mL for each strain. MBC was determined for only two extracts and those were root ethanol extract (for *S. epidermidis* MBC = 284.35 mg/mL) and water flower extract (for *P. aeruginosa*, MBC = 160.85 mg/mL). Those values; however, are notably high, compared to the literature data available for other plant water and ethanol extracts. MIC and MBC values for *Cinnamomum impressicostatum* stem bark extract against MRSA strain were 19.53 and 39.06 µg/mL, respectively. For *Cinnamomum porrectum* stem bark extract, it was 2.5 (MIC) and 5 mg/mL (MBC) [93]. In the *Asteraceae* family, water extracts from *Sonchus erzincanicus* aerial parts exhibit antibacterial properties against *Staphylococcus aureus*, *Escherichia coli*, and *Proteus mirabilis* with the MIC value of 1.25 mg/mL [94]. In the mentioned study on *S. mackmeliana;* however, an interesting pattern of the activity against biofilm formation was observed—with the concentration decrease, greater destruction of the biofilm occurred. It was also observed that flower and stem water extracts and ethanol leaf, flower, and whole plant extracts exhibited the most potent activity in eradicating bacterial biofilm, with MBEC (minimal biofilm eradication concentration) values of 0.1–2.2 mg/mL, causing 84–98% biofilm eradication. For water extracts, the presence of coumarin was suspected to be the active factor and in ethanol extracts, terpenoids were major constituents and; therefore, they are thought to be responsible for the antibiofilm effect [92].

The antibacterial and antifungal activity of the aerial parts and root extracts from *S. papposa* was investigated in a recent study by Mohammed and colleagues [95]. The extracts were toxic for bacteria at the concentrations of 50–800 µg/mL and their antifungal effectiveness was observed at 50–100 µg/mL. The reference compounds (ampicillin, amikacin, ciprofloxacin, fluconazole, and amphotericin B) on the other hand were active at notably lower concentrations (1.56–3.12 µg/mL). In general, samples collected in Turkey were slightly more effective against both bacteria and fungi (50 µg/mL against *Pseudomonas aeruginosa*), although all tested extracts can potentially be used as mild nature-derived antimicrobial agents.

In a study on antimicrobial activities of compounds isolated from aerial parts of *Scorzonera aucheriana* it was observed that scorzoaucherioside II, iso-scorzopygmaecoside, and 3,4-dihydroxyphenyl caffeate possess strong anti-tuberculosis activity with MIC of 21.2 µg/mL, 25.6 µg/mL, and 145 µg/mL, respectively. 3,4-Dihydroxyphenyl caffeate and scorzoaucherioside I were reported active against Gram-negative bacteria strain *Pseudomonas aeruginosa* (MIC = 290 and 377.5 µg/mL, respectively). *Enterococcus faecalis*, a Gram-positive strain, was reported sensitive to scorzopygmaecoside and scorzocreticoside II, where MIC values were 135 and 200 µg/mL, respectively [7].

4.6. Wound Healing Activity

Wounds defined as a disruption of tissue do not pose a threat unless the blood loss is significant. They can; however, be a gateway for pathogenic infections which is much more dangerous for the patient. The acceleration of wound recovery includes infection prevention and the promotion of the natural healing process. Medicinal plants are suspected to possess both those qualities and are considered potentially effective in the therapy of wound healing [96].

4.6.1. In Vitro Assays

A study carried out by Küpeli Akkol and colleagues in 2019 [4] reports that ethyl acetate and chloroform fractions of a methanol extract from aerial parts of *S. latifolia* have

wound healing properties in vitro, which are a result of the inhibitory effect on collagenase and elastase enzymes activity. None of the fractions influenced the activity of hyaluronidase to any considerable degree.

In a study on the extract from *S. cana* var. *jacquiniana* aerial parts (stems, leaves, and flowers), their activity against matrix metalloproteinases (collagenase, hyaluronidase, and elastase) was evaluated. A methanol extract was partitioned into chloroform, petroleum ether, ethyl acetate, and water fraction. From the ethyl acetate fraction, eleven compounds were isolated (3,5-dicaffeoylquinic acid methyl ester, 4-hydroxy-benzoic acid 4-(6-*O*-α-rhamnopyranosyl-β-glucopyranosyl) benzyl ester, 6′-*O*-caffeoylarbutin, apigenin 7-*O*-β-glucoside, apigenin 7-*O*-β-rutinoside, arbutin, cichoriin, isoorientin, luteolin 7-O-β-glucoside, orientin, protocatechuic acid, and vitexin) and all obtained samples were tested for their enzyme-inhibitory properties. In the hyaluronidase inhibition assay, the extracts, fractions, and isolated compounds exhibited only a mild inhibitory effect at the concentration of 100 µg/mL (not exceeding 30% for luteolin 7-*O*-β-glucoside), compared to the positive control—Tannic acid—Which inhibited the hyaluronidase activity by approximately 75%. The methanol extract was observed to possess a potent inhibitory effect on both elastase and collagenase activity (51.7% inhibition for elastase and 35.7% for collagenase, in the concentration of 100 µg/mL). Moreover, several compounds isolated from the extract were significantly active against collagenase (apigenin 7-O-β-glucoside, apigenin 7-*O*-β-rutinoside, and isoorientin) and elastase (apigenin 7-*O*-β-glucoside, luteolin 7-*O*-β-glucoside, and apigenin 7-*O*-β-rutinoside) as well. In light of those results, the authors suggested that flavonoids present in methanol extract were the main wound-healing agents and that the synergy between components of the extract contributes to the inhibitory effect [72].

4.6.2. In Vivo Assays

In 2011, a study by Küpeli Akkol et al. [5] on the promotion of the process of wound healing in mice took place. Researchers obtained hydroethanolic extracts from the aerial parts and roots of several *Scorzonera* species (*S. cinerea*, *S. latifolia*, *S. incisa*, *S. mobilis*, *S. mollis* ssp. *szowitsii*, *S. tomentosa*). Most promising results in the assays carried out with wound models were observed with ointments made with the extracts obtained from *S. latifolia*, *S. mollis* ssp. *Szowitsii,* and *S. tomentosa* aerial parts. Those three extracts were the most active in the hydroxyproline level enhancement as wells as in terms of skin remodeling. Moreover, the *S. latifolia* aerial part extracts were reported to have anti-inflammatory properties in vivo, with an inhibitory value of 23.5% at the dose of 100 mg/kg.

A study from 2012, carried out on mice, reports wound healing properties of several *Scorzonera* species. Aqueous methanolic extracts from the aerial parts of *S. cana var. jacquiniana*, *S. eriophora,* and *S. acuminata* caused the contraction of wound area by up to 46.27% on day 12 in the circular excision wound model. Ointments containing extracts of *S. cana* (C.A. Mey.) Hoffm. var. *jacquiniana* (W. Koch) Chamb. and *S. eriophora* DC. aerial parts, when applied topically on the linear incision wound models, caused an increase in the activity of anti-hyaluronidase and significant enhancement of hydroxyproline level in the regenerated tissue [9].

Figure 2 summarizes literature data regarding the biological activity of *Scorzonera* species in vivo.

4.7. Antioxidant Capacity

Exposure to reactive species in the environment may have a negative impact on humans and animals. The balance between oxidants and antioxidants is becoming more difficult to maintain [97].; therefore, the need for antioxidant agents is growing and, because of the potentially harmful effect of synthetic antioxidants, the attention seems to be currently directed towards naturally occurring antioxidants found in plants [98]. The products from species belonging to the genus *Scorzonera* have been assayed as antioxidant agents as well.

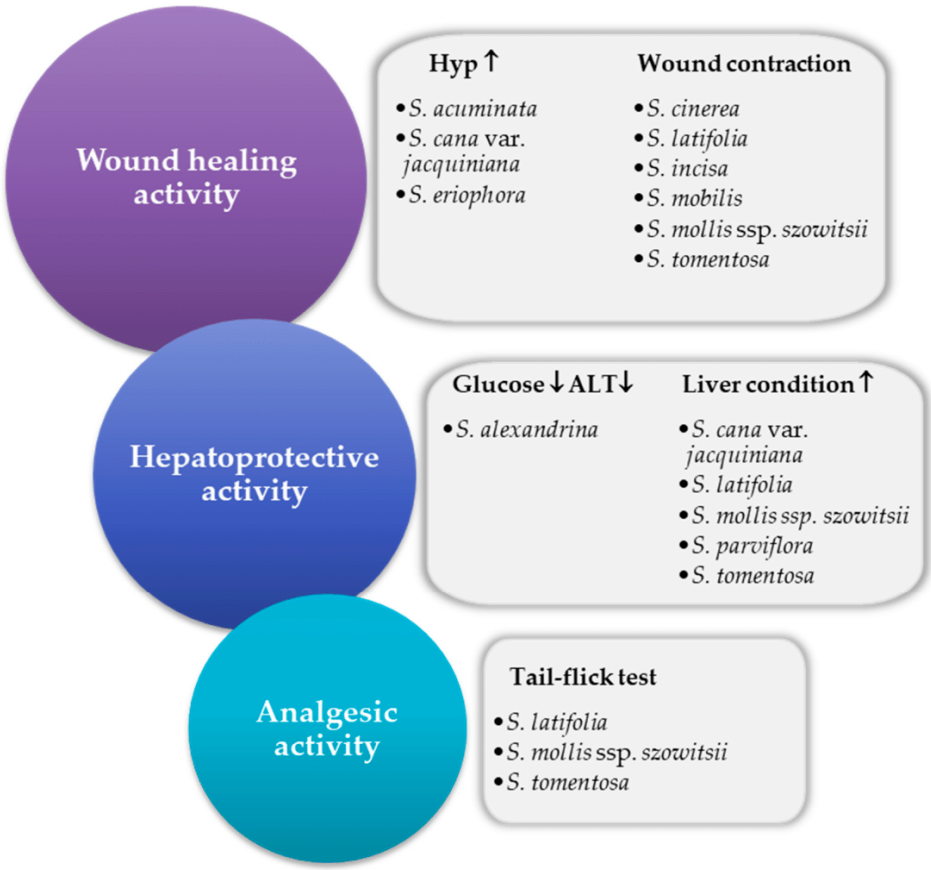

Figure 2. Biological activity of species within the genus *Scorzonera* evaluated in vivo. Hyp↑: Increase in the hydroxyproline level; Glucose↓: Decrease in the glucose level; ALT↓: Decrease in the alanine transaminase level; Liver condition↑: Improvement in the liver condition.

In the DPPH radical scavenging assay compounds isolated from the ethyl acetate fraction of the methanol extract from *Scorzonera divaricata* and *Scorzonera pseudodivaricata* aerial parts (ferulopodospermic acid A and B) did exhibit a strong antioxidant activity, more potent than chlorogenic acid used as a reference compound in the study. The IC_{50} values ferulopodospermic acid A and B were 36.36 and 34.24 µmol/mL, respectively, compared to the IC_{50} of chlorogenic acid, which was 67.92 µmol/mL [14]. The study was continued with five compounds obtained from a *Scorzonera radiata* aerial part MeOH extract were tested for their radical-scavenging activity in the DPPH assay. Scorzodihydrostilbenes A and E exhibited a higher activity level than resveratrol, well-known for its antioxidant activity, used as a reference in the study. The IC_{50} values were 105.51 µM for scorzodihydrostilbene A, 102.60 µM for scorzodihydrostilbene B, and 149.52 µM for resveratrol [15]. Although the difference in the scale of IC_{50} values might seem interesting, as IC_{50} in *S. divaricata* and *S. pseudodivaricata* was given in µmol/mL, whereas for *S. radiata* it was presented in µM (µmol/L), more informative is how those results correspond to reference compounds used in both studies.

In the assessment of antioxidant activity, acteoside isolated from a methanolic extract from *Scorzonera undulata* ssp. *deliciosa* roots was reported to possess similar antiradical power to Trolox used as a standard in the DPPH test (IC_{50} values were 0.16 ± 0.02 mg/mg DPPH for acteoside and 0.2 ± 0.01 mg/mg DPPH for Trolox). The Trolox Equivalent Antioxidant Capacity (TEAC) value of acteoside in the DPPH assay was 1.25. In the CUPRAC (cupric

reducing antioxidant capacity) assay acteoside was slightly less active than the reference compound, rutin (TEAC = 3.16 for rutin compared to TEAC = 2.4 for acteoside) [49].

Nasseri et al. [52] evaluated the chemical composition and the radical scavenging activity of *Scorzonera paradoxa* root and leaf ethanol/water extracts. Leaf extracts turned out to be a more potent antioxidant with an IC_{50} value of 18.81 mg/mL, compared to the roots (IC_{50} = 88.9 mg/mL). This may be due to higher levels of phenolic compounds, flavonoids and tannins reported in the study. The authors also made an assessment of the fatty acids composition of the plant samples and based on the data obtained in the study of chemical composition and antioxidant properties, it was suggested that *S. paradoxa* might be successful as an antidiabetic agent. Those results and IC_{50} values correspond with a study from 2013, when Erden and colleagues [22] investigated the antioxidant properties of methanol extracts obtained from the leaves of three *Scorzonera* species (*S. suberosa*, *S. laciniata,* and *S. latifolia*). Those properties were examined in the DPPH assay and exhibited a concentration-dependent antioxidant activity with IC_{50} values of 29.36 mg/mL for *S. latifolia*, 42.33 mg/mL for *S. suberosa,* and 77.07 mg/mL for *S. laciniata*.

In 2013 Milella et al. [62] measured the antioxidant properties of pure compounds isolated from methanol extracts from aerial parts and roots of *Scorzonera papposa*. The authors assessed the antioxidant activity of the compounds obtained from *S. judaica* in Bader's previous study from 2011 [25] as well. Four out of nine compounds isolated from *S. papposa* extracts were previously unknown. In the study, the antioxidant activity was measured in four different assays: the DPPH assay, the FRAP (ferric reducing antioxidant power) assay, the BCB (β-Carotene bleaching) assay, and the TPC (total phenolic content) assay. It has been observed that the antioxidant capacity of particular compounds depends on the method. The authors suggested that the antioxidant activity of the compounds found in the extract is a result of the synergistic effect of their combination. In the study, a new concept for presenting the antioxidant capacity of compounds—Relative Antioxidant Capacity Index (RACI)—Was applied [62]. Briefly, the parameter is used to integrate the data from several methods for the assessment of the antioxidant activity, where each method is assigned equal weight. RACI can take positive or negative values [99].

Yang and colleagues [59] assessed the antioxidant properties of several compounds isolated from an ethanol *Scorzonera divaricata* root extract. In the ABTS antioxidant capacity assay, two compounds obtained in the study—(*1R*,*5S*,*6S*,*7R*,*8S*)-8-sulfoxyguaia-4(15),10(14),11(13)-trine-6,12-olide (sulfoscorzonin A) and (1*R*,*5S*,*6S*,*7R*,*8S*,13*S*)-8-sulfoxy-13-L-prolineyl-guaia-4(15),10(14)-dien-6a,12-olide (sulfoscorzonin C)—Were reported to be moderately active in radical scavenging. The SC_{50} (half-maximal scavenging concentration) values were equal to 32.88 μM for sulfoscorzonin A and 24.86 μM for sulfoscorzonin C.

A recent study on *Scorzonera papposa* was a comparison of the antioxidant and antimicrobial activity of ethanol extracts from aerial parts and roots of *S. papposa* from Iraq and Turkey. It has been observed that samples from Iraq exhibited a higher level of TAS (Total Antioxidant Status) and a lower level of TOS (Total Oxidant Status) than samples collected in Turkey. Therefore, the OSI (Oxidative Stress Index—The TAS/TOS ratio) parameter in the samples from Iraq was lower than in the samples from Turkey. Compared to the reported TAS and TOS values of other plant species (i.e., *Calendula officinalis* L., *Rhus coriaria* L. var. *zebaria*, Shahbaz and *Mentha longifolia* L.), the extracts from the aerial parts of *S. papposa* obtained in the study exhibited a notable antioxidant activity [95].

5. Conclusions

Aerial and subaerial parts of species within the *Scorzonera* genus have been the subject of research regarding their phytochemical composition as well as their therapeutic potential. In many European and Asian cultures, *Scorzonera* species are commonly used in folk medicine,; therefore, modern phytoanalyses and biological studies have been carried out to verify the bioactive activities of the plants. Due to the presence of numerous bioactive compounds, including flavonoid aglycones and glycosides, triterpenoids, sesquiterpenoids, quinic acid, and caffeic acid derivatives, in the studied plant material, *Scorzonera* species

are considered a potential source of antioxidant agents. Although the reported cytotoxicity of *Scorzonera* extract against cancer cell lines so far was insignificant, they exhibit other bioactive properties, potentially applicable not only in the therapy of pain, inflammation, and microbial infections, but also as an enhancement of the effectiveness of the wound healing process. It should be pointed out that a promising direction of further research on the genus *Scorzonera* is the investigation of their activity towards normal cell lines, especially skin cells, to assess their potential as wound-healing and skincare active agents.

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Abbreviations

ALT	alanine transaminase
AST	aspartate transaminase
BCB	beta-carotene bleaching
CCl_4	carbon tetrachloride
$CHCl_3$	chloroform
COX-1	cyclooxygenase 1
COX-2	cyclooxygenase 2
CUPRAC	cupric reducing antioxidant capacity
FRAP	ferric reducing antioxidant activity
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
HNO_3	nitric acid
Hyp	hydroxyproline
IC_{50}	half-maximal inhibitory concentration
IL-1β	interleukin 1β
MBC	minimal bactericidal concentration
MIC	minimal inhibitory concentration
NF-κB	nuclear factor kappa B
OSI	oxidative stress index
RACI	relative antioxidant capacity index
SC_{50}	half-maximal scavenging concentration
TAS	total antioxidant status
TEAC	Trolox equivalent antioxidant capacity
TNF-α	tumor necrosis factor α
TOS	total oxidant status
TPC	total phenolic content

References

1. Duran, A.; Hamzaoğlu, E. A new species of *Scorzonera* L. (*Asteraceae*) from South Anatolia, Turkey. *Biologia* **2004**, *59*, 47–50.
2. Karaer, F.; Celep, F. Rediscovery of *Scorzonera amasiana* Hausskn. and Bornm.—A threatened endemic species in Turkey. *Bangladesh J. Bot.* **2007**, *36*, 139–144. [CrossRef]
3. Bahadir-Acikara, Ö.; Citoğlu-Gülçin, S.; Dall'Acqua, S.; Özbek, H.; Cvačka, J.; Žemlička, M.; Šmejkal, K. Bioassay-guided isolation of the antinociceptive compounds motiol and β-sitosterol from *Scorzonera latifolia* root extract. *Pharmazie* **2014**, *69*, 711–714. [CrossRef]
4. Küpeli-Akkol, E.; Šmejkal, K.; Kurtul, E.; Ilhan, M.; Güragac, F.T.; Çitoğlu, G.S.; Acıkara, Ö.B.; Cvačka, J.; Buděšínský, M. Inhibitory activity of *Scorzonera latifolia* and its components on enzymes connected with healing process. *J. Ethnopharmacol.* **2019**, *245*. [CrossRef]

5. Küpeli-Akkol, E.; Acikara, O.B.; Süntar, I.; Citolu, G.S.; Kele, H.; Ergene, B. Enhancement of wound healing by topical application of *Scorzonera* species: Determination of the constituents by HPLC with new validated reverse phase method. *J. Ethnopharmacol.* **2011**, *137*, 1018–1027. [CrossRef]
6. Bahadır, Ö.; Citoğlu, G.S.; Smejkal, K.; Dall'Acqua, S.; Ozbek, H.; Cvacka, J.; Zemlicka, M. Analgesic compounds from *Scorzonera latifolia* (Fisch. and Mey.) DC. *J. Ethnopharmacol.* **2010**, *131*, 83–87. [CrossRef] [PubMed]
7. Erik, İ.; Yaylı, N.; Coşkunçelebi, K.; Makbul, S.; Karaoğlu, Ş.A. Three new dihydroisocoumarin glycosides with antimicrobial activities from *Scorzonera aucheriana*. *Phytochem. Lett.* **2021**, *43*, 45–52. [CrossRef]
8. Sarı, A.; Şahin, H.; Özsoy, N.; Özbek Çelik, B. Phenolic compounds and in vitro antioxidant, anti-inflammatory, antimicrobial activities of *Scorzonera hieraciifolia* Hayek roots. *S. Afr. J. Bot.* **2019**, *125*, 116–119. [CrossRef]
9. Süntar, I.; Bahadır-Acıkara, Ö.; Saltan-Çitoğlu, G.; Keleş, H.; Ergene, B.; Küpeli Akkol, E. In vivo and in vitro evaluation of the therapeutic potential of some Turkish *Scorzonera* species as wound healing agent. *Curr. Pharm. Des.* **2012**, *18*, 1421–1433. [CrossRef]
10. Bahadır-Acikara, Ö.; Hošek, J.; Babula, P.; Cvačka, J.; Budešínský, M.; Dračinský, M.; Saltan İşcan, G.; Kadlecová, D.; Ballová, L.; Šmejkal, K. Turkish *Scorzonera* species Extracts attenuate cytokine secretion via inhibition of NF-κB activation, showing anti-inflammatory effect in vitro. *Molecules* **2016**, *21*, 43. [CrossRef]
11. Coşkunçelebi, K.; Makbul, S.; Gültepe, M.; Okur, S.; Güzel, M.E. A conspectus of *Scorzonera* s.l. in Turkey. *Turk. J. Bot.* **2015**, *39*, 76–87. [CrossRef]
12. Tsevegsuren, N.; Proksch, P.; Wang, Y.; Davaakhuu, G. Bioactive phenolic acids from *Scorzonera radiata* Fisch. *Mong. J. Chem.* **2014**, *12*, 78–84. [CrossRef]
13. Wang, Y.; Wray, V.; Tsevegsuren, N.; Lin, W.; Proksch, P. Phenolic compounds from the Mongolian medicinal plant *Scorzonera radiata*. *Z. Naturforsch. Sect. C J. Biosci.* **2012**, *67*, 135–143. [CrossRef]
14. Tsevegsuren, N.; Edrada, R.A.; Lin, W.; Ebel, R.; Torre, C.; Ortlepp, S.; Wray, V.; Proksch, P. Biologically active natural products from Mongolian medicinal plants *Scorzonera divaricata* and *Scorzonera pseudodivaricata*. *J. Nat. Prod.* **2007**, *70*, 962–967. [CrossRef]
15. Wang, Y.; Edrada-Ebel, R.; Tsevegsuren, N.; Sendker, J.; Braun, M.; Wray, V.; Lin, W.; Proksch, P. Dihydrostilbene derivatives from the mongolian medicinal plant *Scorzonera radiata*. *J. Nat. Prod.* **2009**, *72*, 671–675. [CrossRef]
16. Li, J.; Wu, Q.X.; Shi, Y.P.; Zhu, Y. A new sesquiterpene lactone from *Scorzonera austriaca*. *Chin. Chem. Lett.* **2004**, *15*, 1309–1310.
17. Zhu, Y.; Wu, Q.X.; Hu, P.Z.; Wu, W.S. Biguaiascorzolides A and B: Two novel dimeric guaianolides with a rare skeleton, from *Scorzonera austriaca*. *Food Chem.* **2009**, *114*, 1316–1320. [CrossRef]
18. Wang, B.; Li, G.Q.; Qiu, P.J.; Guan, H.S. Two new olean-type triterpene fatty esters from *Scorzonera mongolica*. *Chin. Chem. Lett.* **2007**, *18*, 708–710. [CrossRef]
19. Zaika, M.A.; Kilian, N.; Jones, K.; Krinitsina, A.A.; Nilova, M.V.; Speranskaya, A.S.; Sukhorukov, A.P. *Scorzonera* sensu lato (*Asteraceae*, *Cichorieae*)—Taxonomic reassessment in the light of new molecular phylogenetic and carpological analyses. *PhytoKeys* **2020**, *137*, 1–85. [CrossRef]
20. Granica, S.; Lohwasser, U.; Jöhrer, K.; Zidorn, C. Qualitative and quantitative analyses of secondary metabolites in aerial and subaerial of *Scorzonera hispanica* L. (black salsify). *Food Chem.* **2015**, *173*, 321–331. [CrossRef]
21. Buranov, A.U.; Elmuradov, B.J. Extraction and characterization of latex and natural rubber from rubber-bearing plants. *J. Agric. Food Chem.* **2010**, *58*, 734–743. [CrossRef] [PubMed]
22. Erden, Y.; Kırbağ, S.; Yılmaz, Ö. Phytochemical composition and antioxidant activity of some *Scorzonera* species. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.* **2013**, *83*, 271–276. [CrossRef]
23. Benabdelaziz, I.; Haba, H.; Lavaud, C.; Benkhaled, M. Triterpenoids and flavonoid from *Scorzonera undulata* ssp. alexandrina. *Int. J. Chem. Biochem. Sci.* **2014**, *5*, 1–5.
24. Xie, Y.; Guo, Q.S.; Wang, G.S. Flavonoid glycosides and their derivatives from the herbs of *Scorzonera austriaca* Willd. *Molecules* **2016**, *21*, 803. [CrossRef]
25. Bader, A.; de Tommasi, N.; Cotugno, R.; Braca, A. Phenolic compounds from the roots of jordanian viper's grass, *Scorzonera judaica*. *J. Nat. Prod.* **2011**, *74*, 1421–1426. [CrossRef]
26. Granica, S.; Zidorn, C. Phenolic compounds from aerial parts as chemosystematic markers in the *Scorzonerinae* (*Asteraceae*). *Biochem. Syst. Ecol.* **2015**, *58*, 102–113. [CrossRef]
27. Bahadır-Acıkara, Ö.; Özbilgin, S.; Saltan-İşcan, G.; Dall'Acqua, S.; Rjašková, V.; Özgökçe, F.; Suchý, V.; Šmejkal, K. Phytochemical analysis of *Podospermum* and *Scorzonera n*-hexane extracts and the HPLC quantitation of triterpenes. *Molecules* **2018**, *23*, 1813. [CrossRef]
28. Wu, Q.X.; Su, Y.B.; Zhu, Y. Triterpenes and steroids from the roots of *Scorzonera austriaca*. *Fitoterapia* **2011**, *82*, 493–496. [CrossRef] [PubMed]
29. Erik, İ.; Coşkunçelebi, K.; Makbul, S.; Yayli, N. New chlorogenic acid derivatives and triterpenoids from *Scorzonera aucheriana*. *Turk. J. Chem.* **2021**, *45*, 199–209. [CrossRef]
30. Çetin, B.; Şahin, H.; Sarı, A. Triterpenoids from *Scorzonera veratrifolia* Fenzl. *Istanbul J. Pharm.* **2019**, *48*, 23–27. [CrossRef]
31. Wang, B.; Li, G.-Q.; Guan, H.; Yang, L.; Tong, G. A new erythrodiol triterpene fatty ester from *Scorzonera mongolica*. *Yao Xue Xue Bao* **2009**, *44*, 1258–1261. [PubMed]
32. Zidorn, C.; Ellmerer-Müller, E.P.; Stuppner, H. Sesquiterpenoids from *Scorzonera hispanica* L. *Pharmazie* **2000**, *55*, 550–551. [PubMed]

33. Zhu, Y.; Hu, P.Z.; He, Z.W.; Wu, Q.X.; Li, J.; Wu, W.S. Sesquiterpene lactones from *Scorzonera austriaca*. *J. Nat. Prod.* **2010**, *73*, 237–241. [CrossRef]
34. Wu, Q.X.; He, X.F.; Jiang, C.X.; Zhang, W.; Shi, Z.N.; Li, H.F.; Zhu, Y. Two novel bioactive sulfated guaiane sesquiterpenoid salt alkaloids from the aerial parts of *Scorzonera divaricata*. *Fitoterapia* **2017**, *124*, 113–119. [CrossRef] [PubMed]
35. Şahin, H.; Sarı, A.; Özsoy, N.; Özbek Çelik, B.; Koyuncu, O. Two new phenolic compounds and some biological activities of *Scorzonera pygmaea* Sibth. and Sm. subaerial parts. *Nat. Prod. Res.* **2020**, *34*, 621–628. [CrossRef] [PubMed]
36. Sarı, A.; Zidorn, C.; Ellmerer, E.P.; Özgökçe, F.; Ongania, K.H.; Stuppner, H. Phenolic compounds from *Scorzonera tomentosa* L. *Helv. Chim. Acta* **2007**, *90*, 311–317. [CrossRef]
37. Paraschos, S.; Magiatis, P.; Kalpoutzakis, E.; Harvala, C.; Skaltsounis, A.L. Three new dihydroisocoumarins from the Greek endemic species *Scorzonera cretica*. *J. Nat. Prod.* **2001**, *64*, 1585–1587. [CrossRef]
38. Karakaya, S.; Polat, A.; Aksakal, Ö.; Sümbüllü, Y.Z.; İncekara, Ü. Ethnobotanical study of medicinal plants in aziziye district (Erzurum, Turkey). *Turk. J. Pharm. Sci.* **2020**, *17*, 211–220. [CrossRef]
39. Yaldiz, G.; Koca Çalişkan, U.; Aka, C. In vitro screening of natural drug potentials for mass production. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2017**, *45*, 292–300. [CrossRef]
40. De Smet, P.A.G.M. The Role of Plant-Derived Drugs and Herbal Medicines in Healthcare. *Drugs* **1997**, *54*, 801–840. [CrossRef]
41. Saklani, A.; Kutty, S.K. Plant-derived compounds in clinical trials. *Drug Discov. Today* **2008**, *13*. [CrossRef] [PubMed]
42. Li, F.S.; Weng, J.K. Demystifying traditional herbal medicine with modern approaches. *Nat. Plants* **2017**, *3*, 1–7. [CrossRef] [PubMed]
43. Patridge, E.; Gareiss, P.; Kinch, M.S.; Hoyer, D. An analysis of FDA-approved drugs: Natural products and their derivatives. *Drug Discov. Today* **2016**, *21*, 204–207. [CrossRef] [PubMed]
44. Rees, S.; Harborne, J. Flavonoids and other phenolics of Cichorium and related members of the *Lactuceae* (*Compositae*). *Bot. J. Linn. Soc.* **1984**, *89*, 313–319. [CrossRef]
45. Bryanskii, O.V.; Tolstikhina, V.V.; Zinchenko, S.V.; Semenov, A.A. A sesquiterpene glucoside from cultivated cells of *Scorzonera hispanica*. *Chem. Nat. Compd.* **1992**, *28*, 556–560. [CrossRef]
46. Tolstikhina, V.V.; Bryanskii, O.V.; Syrchina, A.I.; Semenov, A.A. Chemical composition of a culture of tissue of *Scorzonera hispanica*. *Chem. Nat. Compd.* **1988**, *24*, 655. [CrossRef]
47. Bahadır-Acıkara, Ö.; Saltan-Çitoğlu, G.; Dall'Acqua, S.; Šmejkal, K.; Cvačka, J.; Žemlička, M. A new triterpene from *Scorzonera latifolia* (Fisch. and Mey.) DC. *Nat. Prod. Res.* **2012**, *26*, 1892–1897. [CrossRef]
48. Yildirim, B.; Terzioglu, Ö.; Özgökçe, F.; Türközü, D. Ethnobotanical and pharmacological uses of some plants in the districts of Karpuzalan and Adigüzel (Van-Turkey). *J. Anim. Vet. Adv.* **2008**, *7*, 873–878.
49. Harkati, B.; Salah, A.; Bayet, C.; Laouer, H.; Dijoux-Franca, M.-G. Evaluation of antioxidant activity, free radical scavenging and CUPRAC of two compounds isolated from *Scorzonera undulata* ssp. deliciosa. *Adv. Environ. Biol.* **2013**, *7*, 591–594.
50. Sharma, J.; Gairola, S.; Gaur, R.D.; Painuli, R.M. The treatment of jaundice with medicinal plants in indigenous communities of the Sub-Himalayan region of Uttarakhand, India. *J. Ethnopharmacol.* **2012**, *143*, 262–291. [CrossRef]
51. Auzi, A.R.A.; Hawisa, N.T.; Sherif, F.M.; Sarker, S.D. Neuropharmacological properties of *Launaea resedifolia*. *Rev. Bras. Farmacogn.* **2007**, *17*, 160–165. [CrossRef]
52. Nasseri, M.A.; Bigy, S.S.; Allahresani, A.; Malekaneh, M. Assessment of antioxidant activity, chemical characterization and evaluation of fatty acid compositions of *Scorzonera paradoxa* Fisch and C. A. Mey. *Jundishapur J. Nat. Pharm. Prod.* **2015**, *10*. [CrossRef]
53. Zidorn, C.; Spitaler, R.; Ellmerer-Müller, E.P.; Perry, N.B.; Gerhäuser, C.; Stuppner, H. Structure of tyrolobibenzyl D and biological activity of tyrolobibenzyls from *Scorzonera humilis*. *Z. Naturforsch. Sect. C J. Biosci.* **2002**, *57*, 614–619. [CrossRef]
54. Zidorn, C.; Ellmerer-Müller, E.P.; Stuppner, H. Tyrolobibenzyls—Novel secondary metabolites from *Scorzonera humilis*. *Helv. Chim. Acta* **2000**, *83*, 2920–2925. [CrossRef]
55. Zidorn, C.; Ellmerer, E.P.; Sturm, S.; Stuppner, H. Tyrolobibenzyls E and F from *Scorzonera humilis* and distribution of caffeic acid derivatives, lignans and tyrolobibenzyls in European taxa of the subtribe *Scorzonerinae* (*Lactuceae*, *Asteraceae*). *Phytochemistry* **2003**, *63*, 61–67. [CrossRef]
56. Jehle, M.; Bano, J.; Ellmerer, E.; Zidorn, C. Natural products from *Scorzonera aristata* (*Asteraceae*). *Nat. Prod. Commun.* **2010**, *5*, 725–727. [CrossRef]
57. Meng, X.H.; Yang, Y.J.; Gong, Y.; Zhu, Y. Chemical constituents of the roots of *Scorzonera divaricata* and their chemotaxonomic significance. *Biochem. Syst. Ecol.* **2020**, *93*, 104135. [CrossRef]
58. Yang, Y.-J.; Liu, X.; Wu, H.-R.; He, X.-F.; Bi, Y.-R.; Zhu, Y.; Liu, Z.-L. Radical scavenging activity and cytotoxicity of active quinic acid derivatives from *Scorzonera divaricata* roots. *Food Chem.* **2013**, *138*, 2057–2063. [CrossRef]
59. Yang, Y.-J.; Yao, J.; Jin, X.-J.; Shi, Z.-N.; Shen, T.-F.; Fang, J.-G.; Yao, X.-J.; Zhu, Y. Sesquiterpenoids and tirucallane triterpenoids from the roots of *Scorzonera divaricata*. *Phytochemistry* **2016**, *124*, 86–98. [CrossRef] [PubMed]
60. Petkova, N. Characterization of inulin from black salsify (*Scorzonera hispanica* L.) for food and pharmaceutical purposes. *Asian J. Pharm. Clin. Res.* **2018**, *11*, 221–225. [CrossRef]
61. Zidorn, C.; Gottschlich, G.; Stuppner, H. Chemosystematic investigations on phenolics from flowerheads of Central European taxa of *Hieracium sensu lato* (*Asteraceae*). *Plant Syst. Evol.* **2002**, *231*, 39–58. [CrossRef]

62. Milella, L.; Bader, A.; de Tommasi, N.; Russo, D.; Braca, A. Antioxidant and free radical-scavenging activity of constituents from two *Scorzonera* species. *Food Chem.* **2014**, *160*, 298–304. [CrossRef] [PubMed]
63. Harkati, B.; Akkal, S.; Bayat, C.; Laouer, H.; Dijoux Franca, M.G. Secondary metabolites from *Scorzonera undulata* ssp. deliciosa (Guss.) Maire (Asteraceae) and their antioxidant activities. *Rec. Nat. Prod.* **2010**, *4*, 171–175.
64. Sarı, A. Two new 3-benzylphthalides from *Scorzonera veratrifolia* Fenzl. *Nat. Prod. Res.* **2010**, *24*, 56–62. [CrossRef]
65. Scarano, A.; Chieppa, M.; Santino, A. Looking at flavonoid biodiversity in horticultural crops: A colored mine with nutritional benefits. *Plants* **2018**, *7*, 98. [CrossRef]
66. Agati, G.; Azzarello, E.; Pollastri, S.; Tattini, M. Flavonoids as antioxidants in plants: Location and functional significance. *Plant Sci.* **2012**, *196*, 67–76. [CrossRef]
67. Sarı, A. Phenolic compounds from *Scorzonera latifolia* (Fisch. and Mey.) DC. *Nat. Prod. Res.* **2012**, *26*, 50–55. [CrossRef]
68. Bahadır-Acıkara, Ö.; Küpeli-Akkol, E.; Süntar, I.; Ergene, B.; Saltan-Çitoğlu, G.; Çoban, T. Assessment of anti-inflammatory and free radical scavenger activities of selected *Scorzonera* species and determination of active components. *Int. J. Pharmacogn. Phytochem. Res.* **2014**, *6*, 492–498.
69. Turan, M.; Kordali, S.; Zengin, H.; Dursun, A.; Sezen, Y. Macro and micro mineral content of some wild edible leaves consumed in Eastern Anatolia. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2003**, *53*, 129–137. [CrossRef]
70. Bahadır, Ö.; Saltan, H.G.; Özbek, H. Antinociceptive activity of some *Scorzonera* L. species. *Turk. J. Med. Sci.* **2012**, *42*, 861–866. [CrossRef]
71. Bahadır-Acıkara, Ö.; Smejkal, K.; Cvačka, J.; Buděšínský, M.; Dračínský, M.; Saltan, G. Secondary metabolites from *Scorzonera latifolia* roots. *Planta Med.* **2015**, *81*. [CrossRef]
72. Bahadır-Acıkara, Ö.; Ilhan, M.; Kurtul, E.; Šmejkal, K.; Küpeli Akkol, E. Inhibitory activity of *Podospermum canum* and its active components on collagenase, elastase and hyaluronidase enzymes. *Bioorg. Chem.* **2019**, *93*. [CrossRef] [PubMed]
73. Özbek, H.; Bahadir, O.; Keskin, I.; Kırmızı, N.İ.; Yigitbasi, T.; Sayin Sakul, A.; Iscan, G. Preclinical evaluation of *Scorzonera* sp. root extracts and major compounds against acute hepatotoxicity induced by carbon tetrachloride. *Indian J. Pharm. Sci.* **2017**, *79*. [CrossRef]
74. Erden, Y.; Kırbağ, S. Chemical and biological activities of some *Scorzonera* species: An *in vitro* study. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.* **2015**, *85*, 319–326. [CrossRef]
75. Donia, A.E.R.M. Phytochemical and pharmacological studies on *Scorzonera alexandrina* Boiss. *J. Saudi Chem. Soc.* **2016**, *20*, S433–S439. [CrossRef]
76. Mukherjee, P.K. Bioassay-Guided Isolation and Evaluation of Herbal Drugs. In *Quality Control and Evaluation of Herbal Drugs*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 515–537.
77. Zhang, W.G.; Zhao, R.; Ren, J.; Ren, L.X.; Lin, J.G.; Liu, D.L.; Wu, Y.L.; Yao, X.S. Synthesis and anti-proliferative *in vitro* activity of two natural dihydrostilbenes and their analogues. *Arch. Pharm.* **2007**, *340*, 244–250. [CrossRef]
78. Csupor-Löffler, B.; Hajdú, Z.; Réthy, B.; Zupkó, I.; Máthé, I.; Rédei, T.; Falkay, G.; Hohmann, J. Antiproliferative activity of Hungarian *Asteraceae* species against human cancer cell lines. Part II. *Phyther. Res.* **2009**, *23*, 1109–1115. [CrossRef]
79. Perveen, S.; Al-Taweel, A.M.; Yusufoglu, H.S.; Fawzy, G.A.; Foudah, A.; Abdel-Kader, M.S. Hepatoprotective and cytotoxic activities of *Anvillea garcinii* and isolation of four new secondary metabolites. *J. Nat. Med.* **2018**, *72*, 106–117. [CrossRef]
80. Alshehri, K.M.; Ghobashy, M.O.I. Antitumor, antimicrobial activities and phytochemicals constituent of different extracts of *Pulicaria undulata* (Forssk.) Oliver. grown naturally in Saudi Arabia. *Int. J. Res. Pharm. Sci.* **2020**, *11*, 4889–4901. [CrossRef]
81. Park, B.Y.; Oh, S.R.; Ahn, K.S.; Kwon, O.K.; Lee, H.K. (-)-Syringaresinol inhibits proliferation of human promyelocytic HL-60 leukemia cells via G1 arrest and apoptosis. *Int. Immunopharmacol.* **2008**, *8*, 967–973. [CrossRef]
82. Jeong, Y.H.; Chung, S.Y.; Han, A.R.; Sung, M.K.; Jang, D.S.; Lee, J.; Kwon, Y.; Lee, H.J.; Seo, E.K. P-glycoprotein inhibitory activity of two phenolic compounds, (-)-syringaresinol and tricin from *Sasa borealis*. *Chem. Biodivers.* **2007**, *4*, 12–16. [CrossRef] [PubMed]
83. Tasneem, S.; Liu, B.; Li, B.; Choudhary, M.I.; Wang, W. Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents. *Pharmacol. Res.* **2019**, *139*, 126–140. [CrossRef] [PubMed]
84. Wal, P.; Wal, A.; Sharma, G.; Rai, A. Biological activities of lupeol. *Syst. Rev. Pharm.* **2011**, *2*, 96–103. [CrossRef]
85. Lucetti, D.L.; Lucetti, E.C.P.; Bandeira, M.A.M.; Veras, H.N.H.; Silva, A.H.; Leal, L.K.A.M.; Lopes, A.A.; Alves, V.C.C.; Silva, G.S.; Brito, G.A.; et al. Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated from *Himatanthus drasticus* (Mart.) Plumel. *J. Inflamm.* **2010**, *7*, 60. [CrossRef] [PubMed]
86. Shilpi, J.A.; Uddin, S.J. Analgesic and antipyretic natural products. In *Annual Reports in Medicinal Chemistry*; Elsevier: Amsterdam, The Netherlands, 2020; Volume 55, pp. 435–458.
87. Köngül-Şafak, E. Plant extracts with putative hepatoprotective activity. In *Influence of Nutrients, Bioactive Compounds, and Plant Extracts in Liver Diseases*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 227–257.
88. Lal, M.; Chandraker, S.K.; Shukla, R. Antimicrobial properties of selected plants used in traditional Chinese medicine. In *Functional and Preservative Properties of Phytochemicals*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 119–143.
89. Gutiérrez-del-Río, I.; Fernández, J.; Lombó, F. Plant nutraceuticals as antimicrobial agents in food preservation: Terpenoids, polyphenols and thiols. *Int. J. Antimicrob. Agents* **2018**, *52*, 309–315. [CrossRef]
90. Boussaada, O.; Saidana, D.; Chriaa, J.; Chraif, I.; Mahjoub, M.A.; Mighri, Z.; Daami, M.; Helal, A.N. Chemical composition and antimicrobial activity of volatile components of *Scorzonera undulata*. *J. Essent. Oil Res.* **2008**, *20*, 358–362. [CrossRef]

91. Abdelkader, H.B.; Salah, K.B.H.; Liouane, K.; Boussaada, O.; Gafsi, K.; Mahjoub, M.A.; Aouni, M.; Hellal, A.N.; Mighri, Z. Antimicrobial activity of *Rhaponticum acaule* and *Scorzonera undulata* growing wild in Tunisia. *Afr. J. Microbiol. Res.* **2010**, *4*, 1954–1958.
92. Sweidan, A.; El-Mestrah, M.; Kanaan, H.; Dandache, I.; Merhi, F.; Chokr, A. Antibacterial and antibiofilm activities of *Scorzonera mackmeliana*. *Pak. J. Pharm. Sci.* **2020**, *33*, 199–206.
93. Buru, A.S.; Pichika, M.R.; Neela, V.; Mohandas, K. In vitro antibacterial effects of *Cinnamomum* extracts on common bacteria found in wound infections with emphasis on methicillin-resistant *Staphylococcus aureus*. *J. Ethnopharmacol.* **2014**, *153*, 587–595. [CrossRef]
94. Mavi, A.; Yiğit, N.; Yiğit, D.; Kandemir, A. Antioxidant and antimicrobial activity of Turkish endemic *Sonchus erzincanicus* extracts. *Turk. J. Biol.* **2011**, *35*, 243–250. [CrossRef]
95. Mohammed, F.; Şabik, A.; Akgül, H.; Sevindik, M. Antioxidant and Antimicrobial activity of *Scorzonera papposa* collected from Iraq and Turkey. *Kahramanmaraş Sütçü İmam Üniv. Tarım Doğa Dergisi* **2020**, *23*, 1114–1118. [CrossRef]
96. Yadav, S.; Mishra, A.P.; Kumar, S.; Negi, A.; Maurya, V.K. Herbal wound healing agents. In *Preparation of Phytopharmaceuticals for the Management of Disorders*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 169–184.
97. Khuda-Bukhsh, A.R.; Saha, S.K.; Das, S.; Saha, S.S. Molecular approaches toward targeted cancer therapy with some food plant products: On the role of antioxidants and immune microenvironment. In *Cancer*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 191–202.
98. Olszowy, M. What is responsible for antioxidant properties of polyphenolic compounds from plants? *Plant Physiol. Biochem.* **2019**, *144*, 135–143. [CrossRef] [PubMed]
99. Petrovic, M.; Suznjevic, D.; Pastor, F.; Veljovic, M.; Pezo, L.; Antic, M.; Gorjanovic, S. Antioxidant capacity determination of complex samples and individual phenolics-multilateral approach. *Comb. Chem. High Throughput Screen.* **2016**, *19*, 58–65. [CrossRef] [PubMed]

10. Publikacja II: LC-PDA-MS and GC-MS Analysis of *Scorzonera hispanica* Seeds and Their Effects on Human Breast Cancer Cell Lines

Publikacja II

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Article

LC-PDA-MS and GC-MS Analysis of *Scorzonera hispanica* Seeds and Their Effects on Human Breast Cancer Cell Lines

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Abstract: *Scorzonera hispanica* is an herbaceous perennial cultivated in Central and Southern Europe. This study aimed to qualitatively and quantitatively evaluate the composition of oil, extracts, and fractions (**SH1-SH12**) obtained from *S. hispanica* seeds. Furthermore, an evaluation of biological activities in breast cancer cell lines was also performed. GC-MS analysis revealed that the primary components of the seed oil (**SH12**) were fatty acids and β-sitosterol. In the evaluation of extracts (**SH1-SH3**, **SH8-SH10**) and fractions (**SH4-SH7**, **SH11**) composition, the presence of apigenin, derivatives of *p*-coumaric and caffeic acids, was reported. In the biological assays, methanolic extract (**SH1**), diethyl ether (**SH4**), and chloroform (**SH11**) fractions exhibited cytotoxicity toward cells. The highest activity was observed for fatty acids- and 3,4-dimethoxycinnamate-rich **SH11** (IC_{50}: 399.18 µg/mL for MCF-7, 781.26 µg/mL for MDA-MB-231). **SH11** was also observed to induce apoptosis in MCF-7 cells (52.4%). **SH1**, **SH4**, and **SH11** attenuate signaling pathways and affect the expression of apoptosis-, autophagy-, and inflammation-related proteins. **SH12** was non-toxic toward either cancer or normal cell lines in concentrations up to 1 mg/mL. The results suggest that *S. hispanica* seeds exhibit a wide range of potential uses as a source of oil and bioactive compounds for complementary therapy of breast cancer.

Keywords: *Scorzonera*; seeds; polyphenols; LC-PDA-MS; GC-MS; breast cancer; biological activity

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1. Introduction

Scorzonera L. (Asteraceae) is a genus comprising approximately 200 plants, growing across Europe, Asia, and northern Africa [1,2]. In desert regions, some *Scorzonera* species are used as forage [3]. An species endemic to Central Asia, *S. tau-saghyz* Lipsch. and Bosse, is cultivated as a rubber-bearing plant [4]. In traditional medicine, plants of the genus *Scorzonera* play a particular role, including their antidiabetic, analgesic, or antipyretic activities [5–8]. *Scorzonera* species have also been a subject of interest in terms of their content of bioactive compounds [9]. The cytotoxic [10,11], anti-inflammatory [12–14], and wound healing [15,16] activities of extracts from *Scorzonera* species, in addition to isolated compounds, were evaluated in multiple in vitro and in vivo studies.

Scorzonera hispanica L. (black salsify) syn.: *Pseudopodospermum hispanicum* (L.) Zaika, Sukhor. and N. Kilian (Asteraceae) is a perennial plant, spread across Europe and southern Siberia [17]. In the traditional medicine of Europe, *S. hispanica* roots were used to treat colds, stimulate appetite, and as a mucolytic agent in lung diseases [8,18]. In modern times, black

salsify root is a valued vegetable. Previous studies on the species have indicated that aerial parts of the plant contain flavonoids, in addition to caffeic acid and its derivatives [17,19]. In the aerial parts, the presence of lignans, sesquiterpenoids, caffeic acid derivatives, and inulin was reported [17,18,20]. (−)-Syringaresinol, isolated from the roots of black salsify [17], was previously observed to exhibit cytotoxicity to several carcinoma cell lines, including breast cancer [21,22]. No previous reports on the composition or biological activity of the seeds of *S. hispanica* are available in the literature. To our best knowledge, this is the first attempt to evaluate the phytochemical profile and bioactivity of these products obtained from *S. hispanica* seeds.

The aim of this study was to obtain and elucidate the components of oil, extracts, and fractions obtained from the seeds of *S. hispanica* and their activities against two human mammary carcinoma cell lines in addition to normal cells (human skin fibroblasts). The GC-MS analysis and cytotoxicity assessment of the oil were aimed to evaluate the seeds as a novel plant oil source. Six extracts and five fractions using various methods were obtained and their phytochemical profiles using LC-PDA-MS and GC-MS techniques, in addition to their influence on viability and DNA biosynthesis in the mentioned cell lines, were evaluated. The effect of the three most promising products on apoptosis induction in the MCF-7 cell line was assessed. Then, the expression of apoptosis- and autophagy-related proteins using the Western blot technique was investigated. The influence of selected extracts on the concentration of proteins participating in cell signaling pathways and their anti-inflammatory potential was also assessed. As the anticancer activity of *S. hispanica* seeds is yet to be elucidated, we investigated their effect on the concentration of phosphorylated extracellular signal-regulated kinases 1 and 2 (ERK 1/2), in addition to phosphorylated protein kinase B (p-Akt), as previous clinical studies have indicated a correlation between the expression of those two proteins in breast cancer patients. Coexpression of p-Akt and p-Erk 1/2 was reported as a potential predictor of a reduced disease-free survival time for patients diagnosed in the early stage of breast cancer [23]. Therefore, inhibition of those two proteins involved in cell signaling pathways leads to cell death and is the desired effect of anticancer agents. As focal adhesion kinase (FAK) is involved in cell migration, adhesion, and apoptosis, and regulates PI3K/Akt cell signaling pathway [24,25], we assessed the influence of **SH1**, **SH4**, and **SH11** on the expression of phosphorylated FAK (p-FAK) in MCF-7 breast cancer cells. Finally, ERK 1/2 and Akt both lead to the inhibition of the expression of pro-apoptotic Bad protein which inhibits the activity of anti-apoptotic BCL-2. BCL-2 in turn blocks the expression of Bax [26]. Additionally, BCL-2 prevents Beclin-1 from initiating the process of autophagy [27]. Apoptosis and autophagy often occur simultaneously in the cell [28]. Hence, to investigate the influence of the assessed extract and fractions on autophagy in breast cancer cells, we evaluated the expression of ATG5 and LC3B proteins. In addition to the apoptosis-autophagy investigation, we assessed the influence of **SH1**, **SH4**, and **SH11** on pro- (IL-8, TNF-α) and anti-inflammatory (IL-10) cytokines. As IL-8 and TNF-α are associated with cancer progression and metastasis [29,30], the inhibitory effect of the extracts on those cytokines was anticipated. Interleukin-10, which is generally considered to possess anti-inflammatory properties, plays a dual role in breast cancer. It can exert both pro-tumor and anti-tumor activity [31,32]. Therefore, we investigated how **SH1**, **SH4**, and **SH11** affect the concentration of IL-10 in MCF-7 cells.

2. Results

2.1. GC-MS Analysis of SH1, SH9-SH12

The GC-MS analysis of **SH1**, **SH9-SH11** revealed that the dominating groups of compounds for **SH1** were carbohydrates (54.6% relative content; with sucrose as the main constituent) and organic polyols (15.8%; main constituent: D-chiro-inositol). Another interesting group present in **SH1** was phenolic compounds, with caffeic acid as the primary phenolic acid detected in the sample. The presence of quinic acid was also reported in **SH1**. For **SH9** and **SH12**, 41.7% and 62.2% of the relative composition were fatty acids, with most being linoleic acid. Fatty acid esters, with butyl 9,12-octadecadienoate and conjugated

linoleic acid esters, were 18.8% of relative extract **SH9** composition. Noteworthily, **SH9** and **SH12** were observed to contain a notable amount of phytosterols like β-sitosterol, 5α-stigmast-7-en-3β-ol, and stigmasterol. **SH10** relatively consisted of 44.2% fatty acids, with linoleic acid (LA), oleic acid (OA), and palmitic acid (PA) as the primary fatty acids. Glycerol was 15.7% of the total phytochemicals detected in **SH10**. **SH11** relatively consisted of 33% fatty acids (linoleic acid, conjugated linoleic acid, oleic, and palmitic acids) and 21.16% methyl 3,4-dimethoxycinnamate. The primary components of **SH12** were fatty acids (61.8%; including 27.2% linoleic acid) and phytosterols (31.4%; main constituent: β-sitosterol—21.9%). Notable amounts of α-tocopherol and α-amyrin were also observed. Campesterol, 2,3-butanediol, and 3-hexanol were detected only in **SH12**. Noteworthily, the ratio of fatty acids (LA:OA:PA) in **SH1** and **SH10-SH12** remained similar (approximately 2.5:1.1:1), with a prevailing share of linoleic acid. The greatest similarities in the LA:OA:PA ratio were observed between **SH1** and **SH10** and between **SH11** and **SH12**. All compounds identified in **SH1**, **SH9-SH12** are listed in Table 1.

Table 1. GC-MS analysis of compound groups identified in **SH1**, **SH9**-**SH12**.

Compounds	Analytical Parameters				Relative Composition, %				
	RI^{Exp}	RI^{Lit}	Target Ions, m/z	M^+	SH1	SH9	SH10	SH11	SH12
Organic Acids					**11.2**	**41.7**	**53.0**	**41.0**	**62.2**
Fatty acids					**7.5**	**41.7**	**44.2**	**33.0**	**61.8**
Linoleic acid (LA, 18:2), mono-TMS	2220	2215	73 (100), 75 (99), 337 (86), 67 (62), 81 (59)	352	3.7	38.7	18.8	9.9	27.2
Oleic acid (OA, 18:1), mono-TMS	2225	2220	339 (100), 73 (93), 117 (92), 75 (86), 129 (76)	354	1.4		9.0	6.5	16.0
Palmitic acid (PA, 16:0), mono-TMS	2054	2052	313 (100), 117 (91), 73 (71), 75 (51), 132 (42)	328	1.2		6.9	5.9	15.7
Conjugated linoleic acid (CLA, 18:2), mono-TMS			73 (100), 75 (83), 117 (62), 129 (50), 105 (48)	352	0.3	2.1	0.4	2.4	
Aliphatic monocarboxylic acids					**0.1**		**1.4**	**1.6**	**0.4**
Hexanoic acid (6:0), mono-TMS	1078	1071	75 (100), 173 (87), 73 (85), 117 (38), 131 (15)	188	0.02		0.8	0.8	0.4
Nonanoic acid (9:0), mono-TMS	1366	1358	73 (100), 75 (76), 215 (74), 117 (61), 129 (25)	230			0.2	0.4	
Octanoic acid (8:0), mono-TMS	1269	1262	73 (100), 75 (86), 201 (84), 117 (60), 129 (23)	216	0.03		0.2	0.2	
Aliphatic dicarboxylic acid					**1.2**		**6.1**	**2.2**	
Glutaric acid, di-TMS	1388	1394	73 (100), 147 (85), 131 (41), 103 (23), 59 (16)	276	0.3		4.1	0.4	
Azelaic acid, di-TMS	1810	1812	73 (100), 75 (78), 317 (53), 201 (41), 129 (33)	332			1.0	0.7	
Malic acid, tri-TMS	1513	1510	73 (100), 147 (70), 233 (26), 245 (16), 133 (12)	350	0.6				
Sebacic acid, di-TMS	1907	1905	73 (100), 75 (76), 331 (65), 215 (36), 129 (34)				0.3	0.4	
Aromatic acids					**0.03**		**0.3**	**0.7**	
Benzoic acid, mono-TMS	1247	1248	179 (100), 147 (81), 105 (69), 135 (48), 77 (46)	194	0.03		0.1	0.2	
Phenylacetic acid, mono-TMS	1300	1302	73 (100), 75 (32), 164 (19), 91 (17), 193 (16)	208				0.2	
Salicylic acid, di-TMS	1518	1513	73 (100), 267 (46), 147 (29), 103 (24), 75 (19)				0.2		
Aliphatic hydroxy acids					**0.6**		**0.6**	**0.6**	
L (+)-Lactic acid, di-TMS	1073	1073	73 (100), 147 (77), 117 (62), 198 (18), 191 (15)		0.1		0.2		
β-Lactic acid, di-TMS	1155	1145	147 (100), 73 (31), 177 (18), 119 (17), 148 (15)		0.03		0.2		
Glycolic acid, di-TMS	1086	1085	147 (100), 73 (76), 148 (17), 66 (16), 133 (11)		0.05			0.2	
2-Isopropyl-3-ketobutyrate, di-O-TMS	1461	1463	73 (100), 273 (80), 147 (42), 155 (31), 183 (22)	288				0.25	
Glyceric acid, tri-TMS	1351	1350	73 (100), 147 (69), 189 (39), 292 (31), 199 (27)		0.18			0.17	
Aromatic hydroxy acids					**1.8**		**0.2**	**2.0**	
Quinic acid, penta-TMS	1899	1902	73 (100), 345 (60), 147 (32), 75 (27), 255 (25)		1.5		0.1		
Shikimic acid, tetra-TMS	1844	1845	252 (100), 73 (88), 204 (83), 131 (56), 103 (36)					1.2	
Hydroxybenzoic acid, di-TMS	1623	1623	73 (100), 267 (42), 193 (33), 282 (32), 103 (13)	282	0.02			0.5	

Table 1. *Cont.*

Compounds	Analytical Parameters				Relative Composition, %				
	RI^{Exp}	RI^{Lit}	Target Ions, m/z	M^+	SH1	SH9	SH10	SH11	SH12
α,β-Unsaturated carboxylic acids					**0.1**		**0.1**	**0.9**	
Cinnamic acid, mono-TMS	1546	1549	205 (100), 131 (81), 103 (54), 161 (50), 73 (47)	220				0.4	
E-*p*-Coumaric acid, di-TMS	1947	1947	73 (100), 293 (81), 219 (87), 308 (69), 249 (45)	308	0.1		0.1	0.4	
Organic esters, carbonyl compounds					**0.5**	**20.1**	**3.8**	**27.9**	
Fatty acid esters					**0.3**	**18.8**	**2.7**	**4.3**	
Methyl linolelaidate	2095	2095	73 (100), 67 (56), 81 (48), 95 (33), 55 (33)	280	0.1		0.5	0.9	
9-Octadecenoic acid, 18-TMS, methyl ester	2434	2435	73 (100), 225 (55), 75 (42), 130 (27), 369 (19)	384	0.2		0.7	0.3	
Butyl 9,12-octadecadienoate	2470	2478	67 (100), 81 (84), 55 (62), 95 (58), 79 (56)	336		8.8			
Octadecadienoic acid (CLA), ester	2474	-	55 (100), 67 (76), 81 (71), 69 (60), 95 (56)	356		5.7			
α,β-Unsaturated carboxylic esters					**0.04**			**22.8**	
Cinnamic acid, 3,4-di-TMS, methyl ester	2020	2018	219 (100), 238 (58),73 (41), 220 (17), 339 (16)	338	0.04			21.16	
Cinnamic acid, methyl ester	1856	1858	252 (100), 73 (76), 179 (71), 166 (64), 209 (64)					1.19	
Carbonyl compounds					**0.2**	**1.3**	**1.1**	**0.8**	
2,4-Decadienal, (E, E)-	1316	1315	81 (100), 41 (16), 67 (13), 83 (11), 55 (10)	152	0.2	0.5	0.9		
Benzaldehyde, 3,5-dimethoxy-4-[(TMS)oxy]-	1708	1711	224 (100), 239 (43), 223 (29), 254 (27), 73 (25)	254				0.2	
2′,4′-Dihydroxyacetophenone, di-TMS, ether	1703	1709	73 (100), 194 (94),70 (61), 281 (53), 44 (52)	296				0.2	
2,4-Decadienal, (E, Z)-	1292	1291	81 (100), 41 (23), 83 (18), 67 (18), 55 (15)	152		0.3			
2-Decennial, E-	1260	1262	70 (100), 55 (88), 41 (82), 43 (74), 83 (68)			0.2			
Organic alcohols, diols, polyols					**16.3**	**0.8**	**19.5**	**4.1**	**0.2**
Organic alcohols					**0.3**	**0.8**	**0.1**	**4.1**	**0.2**
5-Allyl-1-methoxy-2,3-dihydroxybenzene, di-TMS, ether	1953	1950	73 (100), 324 (91), 293 (56), 394 (39), 204 (38)	324	0.1			3.7	
3-Heptene, 4-ol, mono-TMS	981	986	171 (100), 172 (17), 73 (10), 173 (8), 78 (7)	186	0.2				
2-Phenylethanol, mono-TMS	1229	1227	73 (100), 103 (42), 179 (40), 75 (35), 77 (21)	194			0.1		
Z,E-2,13-Octadecadien-1-ol	2071	2076	99 (100), 67 (68), 55 (66), 79 (59), 81 (58)			0.5			
3-Hexanol, mono-TMS	998	994	75 (100), 159 (95), 73 (46), 103 (21), 77 (17)						0.2
Diols					**0.1**		**1.6**		**0.3**
Ethylene glycol, di-TMS	998	992	147 (100), 73 (41), 191 (15), 148 (15), 103 (13)		0.05		0.9		

Table 1. *Cont.*

Compounds	Analytical Parameters				Relative Composition, %				
	RI^{Exp}	**RI^{Lit}**	**Target Ions, m/z**	**M^+**	**SH1**	**SH9**	**SH10**	**SH11**	**SH12**
Propylene glycol, di-TMS	1014	1013	117 (100), 147 (66), 73 (64), 66 (11), 148 (10)		0.02		0.5		
2,3-Butanediol, di-TMS, rac	1049	1049	147 (100), 73 (97), 174 (24), 262 (18), 77 (17)						0.3
Polyols					**15.8**		**17.8**		
Glycerol, tri-TMS	1296	1295	147 (100), 73 (95), 205 (83), 117 (40), 103 (32)		2.1		15.7		
Inositol, Hexa-OTMS, D-chiro-	1999	1996	318 (100), 305 (85), 73 (75), 217 (71), 147 (53)	612	7.2				
D-(+)-Arabitol, Penta-TMS	1759	1760	73 (100), 217 (92), 147 (56), 103 (48), 205 (43)		2.0		0.3		
Carbohydrates					**54.6**		**5.4**	**0.1**	
Sucrose, octa-OTMS	2712	2712	361 (100), 73 (56), 117 (40), 362 (33), 147 (21)		18.0		2.9		
Maltose, octa-TMS, methyloxime (isomer 2)	2731	2733	73 (100), 361 (88), 217 (75), 289 (64), 147 (36)		5.4				
α-D-Fructofuranose, penta-TMS	1845	1843	73 (100), 217 (89), 147 (34), 437 (26), 218 (17)		3.8		0.3		
β-D-Fructofuranose, penta-TMS	1856	1854	217 (100), 73 (71), 437 (31), 147 (30), 218 (21)		3.0				
Phosphorous/Organophosphorous compounds					**1.6**		**10.8**	**3.8**	
Phosphoric acid, tri-TMS	1292	1285	299 (100), 300 (25), 73 (21), 314 (17), 301 (14)	314	0.9		8.2	3.1	
Phosphoric acid, di-TMS monomethyl ester	1192	-	241 (100), 242 (17), 133 (13), 73 (12), 211 (11)	256	0.01		1.3	0.3	
Phosphoric acid, di-TMS, 2,3-di[(TMS)oxy]propyl ester	1799	1793	73 (100), 357 (56), 299 (48), 147 (36), 129 (21)		0.8		0.3		
Phenols					**3.1**		**0.8**	**6.5**	
E-Ferulic acid, di-TMS	2102	2104	73 (100), 338 (89), 323 (48), 322 (42), 309 (39)	338	0.1		0.2	4.2	
Caffeic acid, tris-TMS	2158	2159	396 (100), 219 (94), 73 (65), 397 (36), 381 (24)	396	2.7				
Vanillin, mono-TMS	1538	1545	194 (100), 193 (51), 209 (47), 224 (27), 73 (24)	224			0.1	0.5	
Vanillic acid, di-TMS	1777	1776	297 (100), 267 (71), 73 (68), 312 (56), 223 (54)	312	0.04		0.1	0.3	
Sterols					**1.4**	**13.5**	**2.3**	**3.3**	**31.4**
β-Sitosterol, mono-TMS	3348	3342	129 (100), 357 (56),73 (58), 396 (54), 81 (47)	486	0.7	8.0	1.9	1.3	21.9
Stigmasterol, mono-TMS	3290	3286	55 (100), 83 (91), 81 (78), 73 (75), 67 (67)	484	0.2	1.1	0.5	0.7	2.6
5α-Stigmast-7-en-3β-ol	3359	3355	414 (100), 255 (85), 55 (53), 81 (50), 43 (46)	414		1.6			
5α-Stigmast-7-en-3β-ol, mono-TMS	3401	3401	73 (100), 255 (92), 487 (79), 147 (45), 229 (20)	486					4.8
Campesterol, mono-TMS	3253	3251	73 (100), 129 (62), 343 (36), 147 (30), 382 (23)	472					2.1
Amino acids					**3.0**		**0.6**		**0.3**
L-Proline, di- TMS	1303	1302	142 (100), 73 (28), 143 (14), 147 (7), 216 (5)	259	0.9		0.2		
Pyroglutamic acid, di-TMS	1532	1524	156 (100), 73 (60), 147 (28), 157 (13), 217 (13)	273	0.5		0.3		

Table 1. *Cont.*

Compounds	Analytical Parameters				Relative Composition, %				
	RI^{Exp}	RI^{Lit}	Target Ions, m/z	M^+	SH1	SH9	SH10	SH11	SH12
Threonine, tri-TMS	1406	1408	73 (100), 218 (60), 219 (54), 117 (41), 147 (30)		0.3				
Glycerolipids					**0.9**	**3.3**	**1.8**	**4.5**	
2-Monolinolenin, di-TMS	2776	2780	129 (100), 73 (97), 147 (60), 103 (48), 67 (41)	498	0.5		1.1	2.1	
2-Monoolein, di-TMS	2742	2744	103 (100), 73 (84), 129 (79), 67 (43), 55 (35)		0.2		0.5	1.5	
Glycerol 1-monolinolate	2688	2697	67 (100), 81 (88), 55 (74), 95 (62), 79 (56)	354		2.8			
Tocopherols					**0.2**	**2.6**	**0.2**		**2.4**
(+)-α-Tocopherol, OTMS-	3152	3156	502 (100), 73 (80), 237 (68), 55 (51), 67 (41)	502	0.2		0.2		2.4
α-Tocopherol	3130	3130	165 (100), 430 (86), 164 (31), 431 (28), 166 (12)	430		2.3			
α-Tocopheryl acetate	3141	3132	165 (100), 55 (67), 430 (61), 67 (59), 81 (56)			0.3			
Nucleosides					**1.0**		**0.2**		
Cytidine, 2′,3′,5′-tri-TMS ether	2822	2811	73 (100), 217 (69), 147 (34), 147 (28), 151 (24)	459	0.5				
5-Methyluridine, tri-TMS derivative	2429	2428	73 (100), 217 (81), 75 (34), 55 (30), 67 (25)	474	0.2		0.2		
Uridine, 2′,3′,5′-tri-OTMS	2461	2469	73 (100), 217 (62), 103 (23), 259 (21), 147 (21)	460	0.2				
Terpenoids					**0.2**	**6.2**			**0.2**
Monoterpenes, Monoterpenoids						**0.6**			
p-Menthane, trans-	984	978	97 (100), 55 (67), 41 (20), 96 (20), 57 (19)	140		0.2			
Carvone	1241	1242	82 (100), 54 (38), 108 (36), 93 (36), 107 (25)	150		0.2			
Camphor	1143	1143	95 (100), 81 (86), 67 (64), 152 (59), 55 (54)	152		0.1			
Triterpenes					**0.2**	**5.4**			**2.0**
α-Amyrin, mono-TMS	3384	3382	218 (100), 73 (35), 189 (25), 190 (20), 219 (19)	498	0.2				2.0
α-Amyrin	3376	3376	218 (100), 207 (22), 95 (22), 135 (22), 203 (22)	426		4.2			
β-Amyrin	3330	3337	218 (100), 203 (48), 207 (26), 55 (24), 81 (23)	426		1.2			
Sesquiterpenes, Sesquiterpenoids						**0.2**			
α-Longipinene	1363	1360	41 (100), 55 (96), 43 (87), 91 (78), 44 (71)	204		0.1			
β-E-Caryophyllene	1415	1416	41 (100), 91 (99), 105 (93), 55 (91), 79 (89)	204		0.1			
Hydrocarbons						**4.8**			

Table 1. *Cont.*

Compounds	Analytical Parameters				Relative Composition, %				
	RI^{Exp}	RI^{Lit}	Target Ions, m/z	M^+	SH1	SH9	SH10	SH11	SH12
Aliphatic hydrocarbons						3.9			
Alicyclic hydrocarbons						0.6			
Aromatic hydrocarbons						0.3			
Flavonoids								**0.4**	
Other compounds					**1.2**	**1.1**	**0.8**	**1.9**	**0.4**
Non-identified compounds					**3.5**	**6.0**	**2.3**	**6.5**	**0.6**

2.2. LC-PDA-MS Characterization of SH1-SH8

2.2.1. Qualitative Analysis

Qualitative evaluation of the extracts and fractions confirmed free phenolic acids in the composition (7), (2, 4–5, 10, 13–14, 16, 18, and 20), and *p*-coumaric acid (3, 12). The flavonoids were represented in free (22, 24) and bound form (15, 19). All 24 compounds listed in Table 2 were present in the extracts and fractions, displaying selectivity to the corresponding solvent, as indicated in Figures S1 and S2 (Supplementary Materials).

Table 2. LC-PDA-TOF/MS qualitative analysis of extracts and fractions of *S. hispanica* seeds.

No	Retention Time [min]	UV λ max [nm]	[M-H]-[m/z]	Compound Name
1	13.46	290 sh, 325	250, 300, **310**	unknown
2	18.23	290 sh, 326	**353**	5-CQa [S]
3	19.16	290 sh, 340	**339**	3-p-CoumQa
4	21.61	250, 290 sh, 325	191, **353**, 705	3-CQa [S]
5	22.45	290 sh, 325	**353**	4-CQa [S]
6	23.06	295 sh, 325	**292**	unknown
7	23.27	295 sh, 326	**179**	CA [S]
8	23.91	325	**306**	unknown
9	24.89	325	**530**	unknown
10	28.65	310	**367**	methylated 4-CQa
11	31.83	295 sh, 325	**435**	unknown
12	45.38	300 sh, 325	133, 161, 387, **549**	p-CoumQA derivatives
13	48.73	295 sh, 325	147, 353, **515**	3,5-dCQa [S]
14	50.26	295 sh, 325	353, **515**	cis-3,5-dCQa
15	52.39	265, 338	269, **445**	apigenin 7-O-glucuronide [S]
16	53.47	295 sh, 325	353, **515**	4,5-dCQa [S]
17	57.16	295 sh, 325	507	unknown
18	57.47	295 sh, 320	529	methylated-diCQa
19	58.42	265 sh, 338	268, 459	luteolin 7-O-glucuronide
20	58.76	295 sh, 320	339, **529**	methylated-diCQa
21	59.26	295 sh, 328	437	unknown
22	59.58	265 sh, 338	285	luteolin [S]
23	60.26	295 sh, 325	353, 515, **677**	triCQa
24	63.17	265, 340	151, **269**	apigenin (A) [S]

[S]—comparisons with chemical standards were made, sh—value on the deflection of the UV spectrum, bold—most abundant ion.

2.2.2. Quantitative Analysis

The quantitative assessment of apigenin and caffeoylquinic derivatives in **SH1-SH8** is presented in Table 3.

Table 3. Assessment of apigenin and caffeoylquinic derivatives content in extracts (SH1-SH3, SH8) and fractions (SH4-SH7) of *S. hispanica* seeds.

No.	Content mg Per g of Extract/Fraction [a]							
	SH1	**SH2**	**SH3**	**SH4**	**SH5**	**SH6**	**SH7**	**SH8**
2	nd	blq	nd	nd	nd	blq	blq	blq
4	3.80 ± 0.03	12.37 ± 0.18	blq	blq	nd	54.34 ± 0.13	6.23 ± 0.06	13.39 ± 0.18
5	nd	nd	nd	nd	2.90 ± 0.10	2.25 ± 0.03	blq	nd
7	nd	nd	1.16 ± 0.03	20.97 ± 0.07	nd	nd	nd	nd
10	nd	nd	nd	4.87 ± 0.07	nd	nd	nd	nd
14	7.75 ± 0.04	13.57 ± 0.19	blq	36.54 ± 0.2	242.00 ± 0.20	13.54 ± 0.13	nd	20.54 ± 0.33
15	nd	nd	nd	5.55 ± 0.7	nd	nd	nd	nd

Table 3. *Cont.*

No.	Content mg Per g of Extract/Fraction [a]							
	SH1	**SH2**	**SH3**	**SH4**	**SH5**	**SH6**	**SH7**	**SH8**
16	3.96 ± 0.17	8.48 ± 0.09	nd	nd	nd	48.18 ± 0.41	nd	10.36 ± 0.12
17	blq	blq	nd	16.82 ± 0.49	55.6 ± 0.20	10.31 ± 0.13	nd	blq
19	nd	nd	nd	1.60 ± 0.04	nd	nd	nd	nd
21	nd	nd	nd	blq	nd	nd	nd	nd
24	blq	blq	nd	13.98 ± 0.37	6.00 ± 0.10	nd	nd	0.80 ± 0.29
25	1.98 ± 0.09	1.10 ± 0.15	blq	5.90 ± 0.11	nd	nd	nd	blq
Total CQa	**11.55**	**25.95**	**1.16**	**100.33**	**306.5**	**80.44**	**6.23**	**34.73**

[a]—content expressed as mean with standard deviation; blq—below the limit of quantification; nd—not detected.

The chemical structures of the main components of **SH1**-**SH12** detected and identified in GC-MS and LC-PDA-MS analyses are presented in Figure 1.

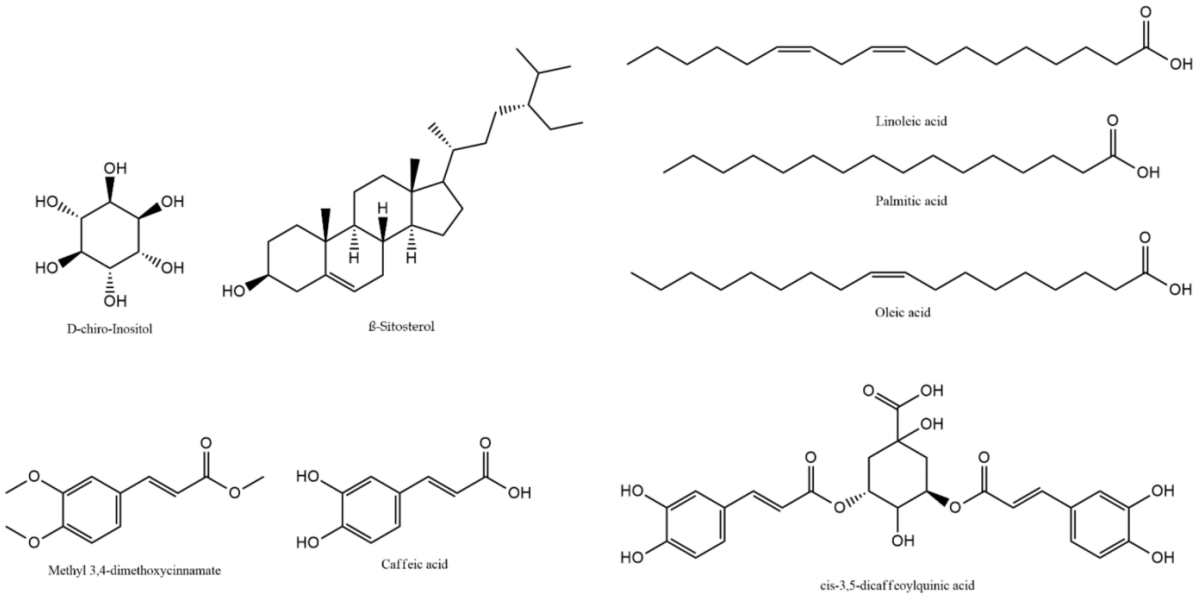

Figure 1. Structures of major compounds (D-chiro-inositol, β-sitosterol, methyl 3,4-dimethoxycinnamate, caffeic acid, cis-3,5-dicaffeoylquinic acid, linoleic acid, palmitic acid, oleic acid) identified in **SH1**-**SH12**.

2.3. Cell Viability Assay

A preliminary cell viability test indicated that three (**SH1**, **SH4**, and **SH11**) out of the eleven obtained extracts and fractions from *S. hispanica* seeds displayed cytotoxicity against MCF-7 and MDA-MB-231 human mammary carcinoma cell lines. The remaining extracts and fractions (**SH2-3**, **SH5-SH10**) and **SH12** did not exhibit any cytotoxicity toward either breast cancer cell lines or normal skin fibroblast cells at concentrations up to 1000 µg/mL.

Figure 2A presents the cytotoxic activity of **SH1**, **SH4,** and **SH11** against **MCF-7** cells. Figure 2B portrays the cytotoxicity of the extracts in MDA-MB-231 cells. The greatest cytotoxic activity was observed for **SH11**. IC_{50} values for the tested cell lines were 399.18 ± 54.15 µg/mL for MCF-7 and 781.26 ± 21.43 µg/mL for MDA-MB-231. **SH1** and **SH4** was active only in MCF-7 cells with IC_{50} values of 847.72 ± 69.25 µg/mL, 626.01 ± 5.07 µg/mL, respectively. Data obtained from the phytochemical analysis indicate that **SH1**, **SH4,** and **SH11** were characterized by the greatest content of potentially bioactive compounds and therefore their influence on the process of cell proliferation was

evaluated. In a previous study by the research team, cisPt—a reference compound in this study—inhibited the growth of 50% of breast cancer cells at concentrations of 93 ± 2 μM for MCF-7 and 82 ± 2 μM for MDA-MB-231 [33].

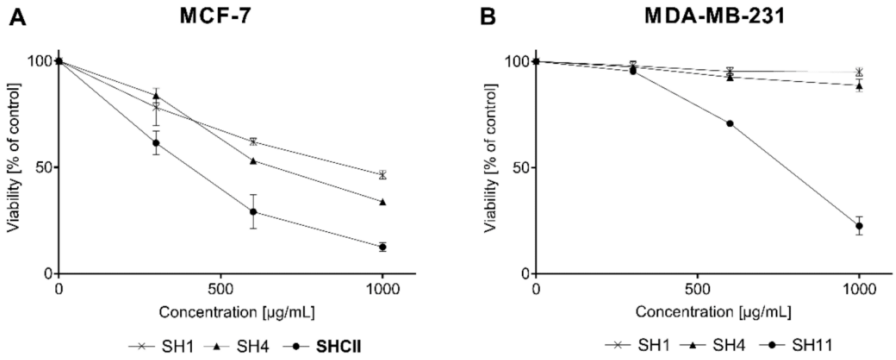

Figure 2. The influence of **SH1**, **SH4**, and **SH11** on the viability of MCF-7 (**A**) and MDA-MB-231 (**B**) cell lines after 24 h of incubation with increasing concentrations of the given extract and fractions (300–1000 μg/mL). Values are presented as mean ± SD from three independent experiments performed in duplicate.

2.4. DNA Biosynthesis Assay

To confirm the results obtained in the preliminary cytotoxicity assay, the effect of **SH1**, **SH4**, and **SH11** on [^{3}H]-thymidine incorporation in breast cancer cells was evaluated. The results are presented in Figure 3.

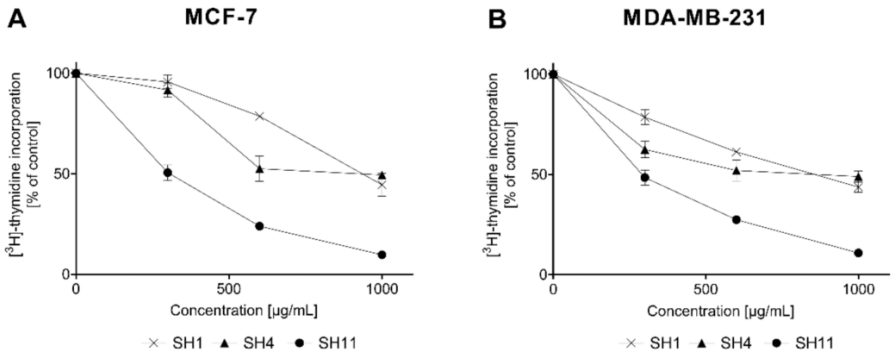

Figure 3. The effect of **SH1**, **SH4**, and **SH11** on the process of DNA biosynthesis in MCF-7 (**A**) and MDA-MB-231 (**B**) cell lines after 24 h of incubation with increasing concentrations of the given extract and fractions (300–1000 μg/mL). Values are presented as mean ± SD from three independent experiments performed in duplicate.

The results obtained in the DNA biosynthesis assay indicate that **SH11** was similarly effective as a proliferation inhibitor in both cell lines, with IC_{50} of 293.64 ± 16.61 μg/mL (MCF-7) and 265.05 ± 25.44 μg/mL (MDA-MB-231). The reference compound cisPt was previously reported to reduce the incorporation of [^{3}H]-thymidine by 50% at 98 ± 2 and 86 ± 2 μM for MCF-7 and MDA-MB-231 cells, respectively [33]. Table 4 summarizes all IC_{50} values obtained in the assay.

Table 4. The influence of **SH1**, **SH4**, and **SH11** on the DNA biosynthesis in MCF-7 and MDA-MB-231 cell lines.

Sample Name	IC_{50} for MCF-7 [μg/mL]	IC_{50} for MDA-MB-231 [μg/mL]
SH1	943.23 ± 55.5	863.21 ± 35.81
SH4	630.52 ± 64.96	648.61 ± 182.62
SH11	293.64 ± 16.61	265.05 ± 25.44

Results are presented as mean IC_{50} values ± SD from three independent experiments performed in duplicate.

2.5. Annexin V/PI Binding Assay

To examine whether the molecular mechanism of cytotoxicity of **SH1**, **SH4,** and **SH11** in MCF-7 cells was associated with their ability to induce apoptosis, an analysis of Annexin V/PI binding was performed.

All extracts, in addition to cisPt used as a reference, were applied at concentrations that are approximately IC_{25} and IC_{50} values evaluated in the preliminary cytotoxicity assays. The results obtained in the performed assay reveal that **SH1**, **SH4,** and **SH11** induce the apoptosis process in MCF-7 cells in a concentration-dependent manner. Figure 4 indicates that the greatest pro-apoptotic activity was exhibited by **SH11**; 24 h incubation with the extract at a concentration of 200 μg/mL resulted in the detection of 32.5% of early and late apoptotic cells. At a higher concentration (400 μg/mL), the total percentage of apoptotic cells increased to 53.4%. For **SH4**, 39.9% of apoptotic cells were detected after incubation with 600 μg/mL, and 49.6% for 800 μg/mL. **SH1** did not exhibit a similarly strong pro-apoptotic effect. Incubation for 24 h with the extract resulted in the detection of 11.5% and 13.4% of apoptotic cells for concentrations of 600 and 800 μg/mL, respectively. For cisPt, 19.7% of total apoptotic cells after incubation with 50 μM and 26.6% of early and late apoptotic cells for a 100 μM concentration of the compound were detected. The number of necrotic cells did not exceed 2% in the analyzed samples, which suggests that apoptosis, not necrosis, is the dominant mechanism of cytotoxicity for **SH1**, **SH4**, and **SH11**.

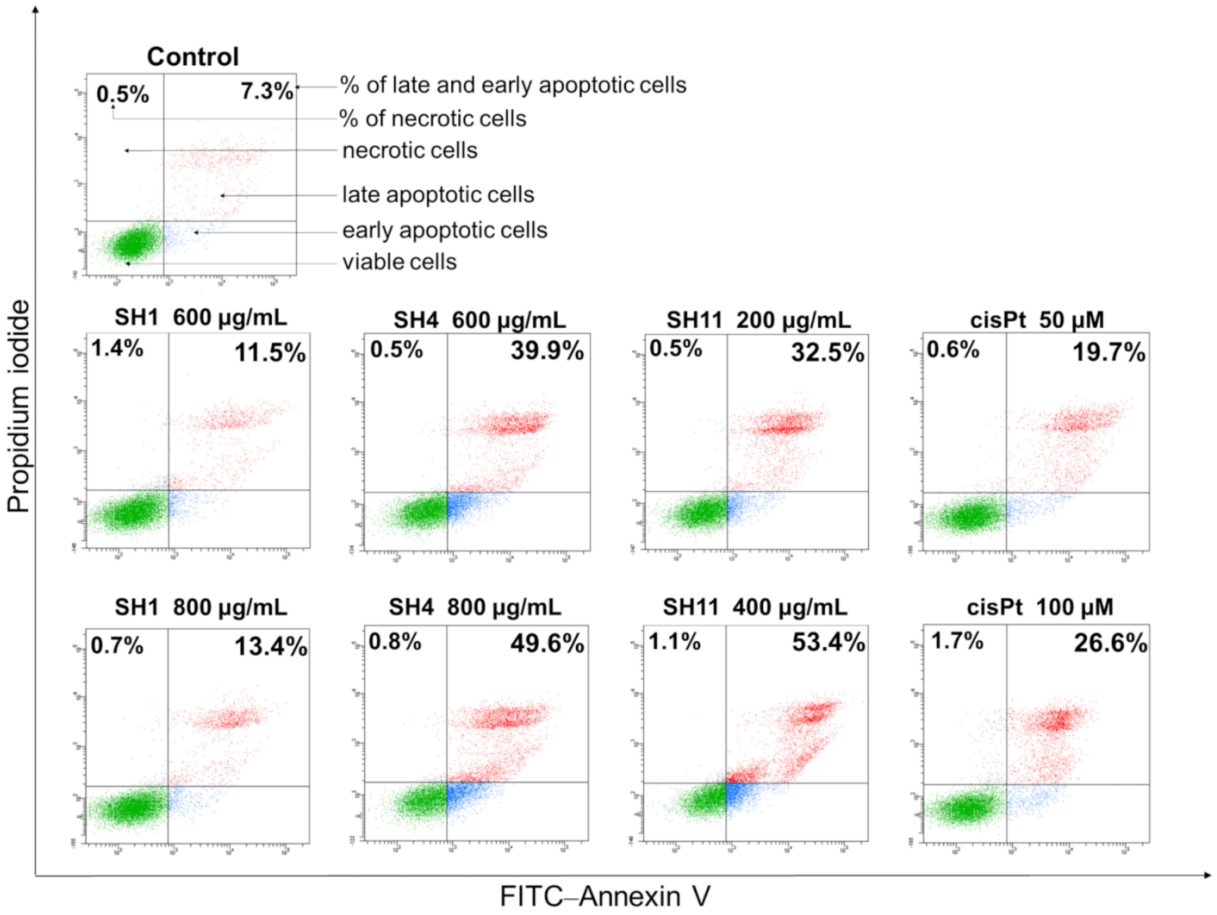

Figure 4. Apoptosis induction in MCF-7 breast cancer cells after 24-h incubation with **SH1**, **SH4**, **SH11**, and cisPt as a reference. The tested concentrations were 600 and 800 µg/mL for **SH1** and **SH4**, 200 and 400 µg/mL for **SH11**, and 50 and 100 µM for cisPt. The number of total early and late apoptotic cells, in addition to the number of necrotic cells, are the mean percentage from three experiments performed in duplicate.

2.6. Western Blot Evaluation of the Expression of Apoptosis- and Autophagy-Related Protein

A deeper investigation of the pro-apoptotic and pro-autophagic effects of **SH1**, **SH4**, and **SH11** in MCF-7 cells was performed. To assess how the extract and fractions affect the expression of proteins involved in the processes of apoptosis and autophagy (BCL-2, Bax, ATG5, and LC3B), in addition to phosphorylated focal adhesion kinase (p-FAK), the Western blot technique was used.

In the Western blot analyses, extracts and cisPt were applied to MCF-7 cells at concentrations that corresponded to the approximate IC_{50} values determined in the viability and DNA biosynthesis assays—for **SH1** and **SH4**: 800 µg/mL; **SH11**: 400 µg/mL. CisPt used as reference was applied at 100 µM. Results of the Western blot analyses are presented in Figure 5. **SH1**, **SH4**, and **SH11**, in addition to cisPt, were observed to exhibit the ability to inhibit the expression of pro-survival protein BCL-2 and increased the expression of apoptosis-accelerating protein Bax. For BCL-2, the greatest inhibition was observed for **SH1**. After 24 h of incubation with **SH4** and **SH11**, the attenuative activity on BCL-2 expression was observed as well. CisPt decreased the concentration of BCL-2 to a degree comparable to **SH11**. For the apoptosis regulator Bax, enhanced expression was observed in all the examined samples. The most potent activity was observed for **SH11**—the intensity of the band increased the most compared with the untreated control cells. The reference drug

cisPt caused a similar effect on Bax expression. **SH1** and **SH4** enhanced Bax expression; **SH1**, **SH4**, **SH11,** and cisPt all increased the concentration of autophagy-related proteins ATG5 and LC3B. Enhancement of ATG5 expression was significant. The intensity of the **SH11** band was doubled in comparison with the control band. **SH1** and **SH4** increased the expression of ATG5 to a notable degree as well. For cisPt, the greatest enhancement in band density was observed. The expression of autophagy marker LC3B was intensified in all the analyzed samples. **SH1**, **SH4,** and **SH11** all increased the expression of LC3B compared with the untreated control cells. The reference drug cisPt increased the expression of the protein to the greatest degree, by approximately 50%. Significant inhibition of p-FAK expression was observed in all assessed extracts, in addition to cisPt. The most significant inhibitory effect was observed for cisPt and **SH11**—the relative intensity of the bands was below 50% compared with the control band for both samples.

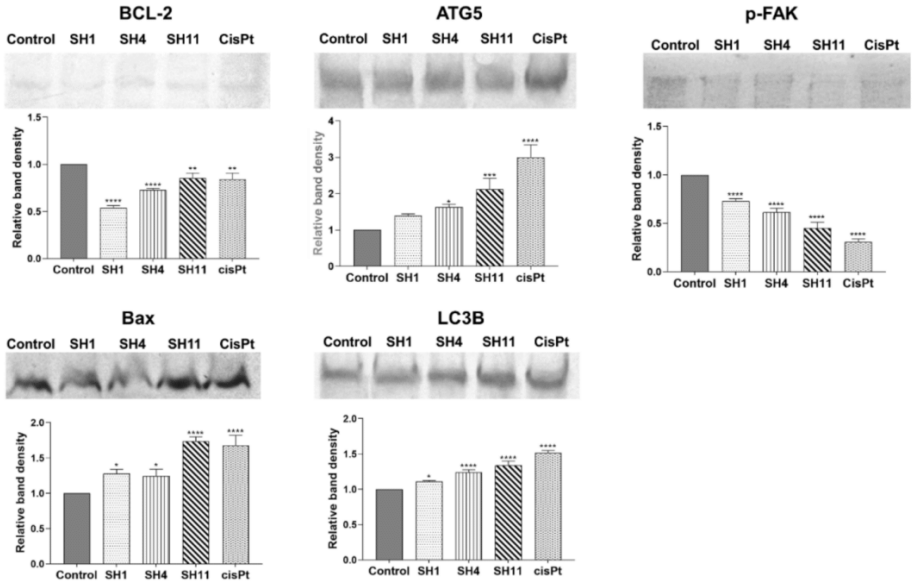

Figure 5. Western blot analyses of BCL-2, Bax, ATG5, LC3B, and p-FAK expression in MCF-7 cells after 24-h incubation with **SH1**, **SH4**, **SH11**, and cisPt. The tested concentrations were 800 µg/mL for **SH1** and **SH4**, 400 µg/mL for **SH11**, and 100 µM for cisPt. Results are presented as mean optical density ± SD from three measurements. Statistical significance was calculated using one-way ANOVA with Bonferroni multiple comparison test. Differences were considered statistically significant at * ($p \leq 0.05$), ** ($p \leq 0.005$),*** ($p \leq 0.0005$), and **** ($p \leq 0.0001$).

2.7. Influence of SH1, SH4, and SH11 on the Expression of Proteins Related to Cell Survival and Proliferation

An inhibitory effect on phosphorylated Akt (p-Akt) was observed in all the examined samples. As demonstrated in Figure 6, the most significant decrease was observed for **SH11**—from 2.43 U/mL in untreated control cells to 0.25 U/mL for 200 µg/mL and 0.9 U/mL for 400 µg/mL. After incubation with **SH4** at 600 and 800 µg/mL, the concentration of p-Akt was lowered to 0.43 and 0.32 U/mL, respectively. **SH1** caused a decline in p-Akt concentration to 0.94 and 0.81 U/mL, respectively. Incubation of cells with cisPt used as reference resulted in the detection of 1.63 and 0.34 U p-Akt/mL for 50 and 100 µM, respectively.

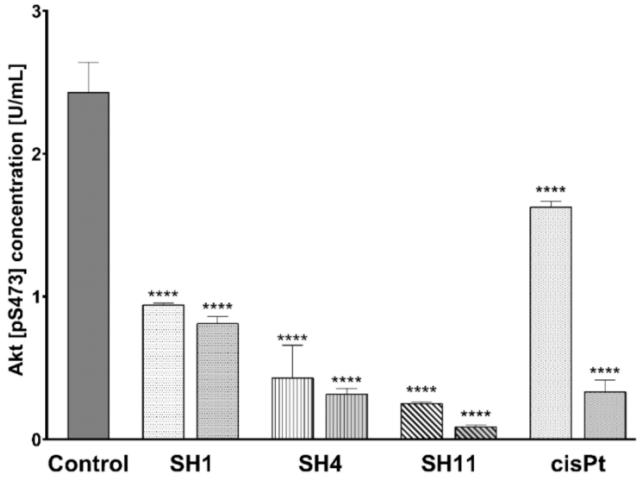

Figure 6. Concentrations of Akt [pS473] in MCF-7 human breast cancer cells after 24-h incubation with **SH1** and **SH4** at concentrations of 600 μg/mL and 800 μg/mL, **SH11** at 200 μg/mL and 400 μg/mL, and cisPt at 50 μM and 100 μM. Results are presented as mean ± SD from three experiments performed in duplicate. Statistical significance was calculated using one-way ANOVA with Bonferroni multiple comparison test. Differences were considered statistically significant at **** ($p \leq 0.0001$).

Figure 7 presents a dose-dependent, inhibitory effect of all the analyzed *S. hispanica* extracts and fractions on the concentration of phosphorylated ERK 1/2 (p-ERK 1/2) in MCF-7 cells. In the untreated control cells, the concentration of p-ERK 1/2 was 150.33 pg/mL. The most significant decrease in p-ERK 1/2 expression was observed in **SH11**—84.33 pg/mL at 200 μg/mL and 38.67 pg/mL at 400 μg/mL. 24 h incubation with **SH4** resulted in the reduction of p-ERK 1/2 concentration to 93 pg/mL at 600 μg/mL, and 80.67 pg/mL at 800 μg/mL **SH4**. For **SH1**, the concentration of p-ERK 1/2 declined to 128 pg/mL and 126.33 pg/mL for the lower and the higher concentrations, respectively. The reference drug cisPt inhibited p-ERK 1/2 expression to 122.67 pg/mL at 50 μM and 81.33 pg/mL at 100 μM cisPt.

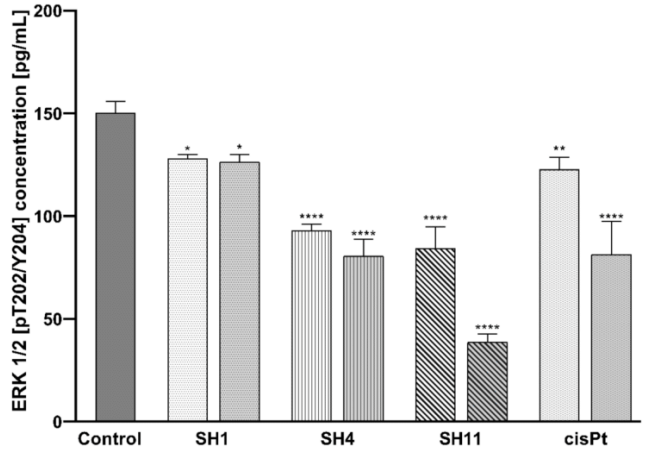

Figure 7. Concentrations of ERK 1/2 [pT202/Y204] in MCF-7 cells after 24-h incubation with **SH1**, **SH4**, **SH11**, and cisPt. The tested concentrations were 600 μg/mL and 800 μg/mL for **SH1** and **SH4**, 200 μg/mL and 400 μg/mL for **SH11**, and 50 μM and 100 μM for cisPt. Results are presented as mean ± SD from three independent experiments performed in duplicate. Statistical significance was calculated using one-way ANOVA with Bonferroni multiple comparison test. Differences were considered statistically significant at * ($p \leq 0.05$), ** ($p \leq 0.005$), and **** ($p \leq 0.0001$).

2.8. Influence of SH1, SH4, and SH11 on the Concentration of TNF-α, Interleukin-8, and Interleukin-10

The effect of **SH1**, **SH4**, and **SH11** on the concentrations of pro-inflammatory cytokines tumor necrosis factor α (TNF-α) and interleukin-8 (IL-8), in addition to anti-inflammatory interleukin-10 (IL-10), in MCF-7 cells was investigated.

As demonstrated in Figure 8A, the inhibitory activity towards TNF-α was observed for all three extracts. For **SH1**, the concentration of this cytokine was reduced from 36.72 pg/mL in the control cells to 34.82 pg/mL and 32.99 pg/mL at the concentrations of 600 µg/mL and 800 µg/mL, respectively. At 600 and 800 µg/mL concentrations of **SH4**, the TNF-α concentration decreased to 34.46 and 31.10 pg/mL, respectively. For **SH11**, the observed concentrations of TNF-α were 35.66 pg/mL for 200 µg/mL and 33.93 pg/mL for 400 µg/mL **SH4**. As it is portrayed in Figure 8B, all three extracts caused a notable inhibition of IL-8 concentration. The inhibitory activity of **SH1**, **SH4**, and **SH11** was more significant in comparison with the control than for TNF-α. The 24-h incubation with **SH1** at 600 and 800 µg/mL resulted in a decrease in IL-8 concentration from 32.58 pg/mL in the control cells to 5.69 and 4.87 pg/mL, respectively. The analysis indicated that **SH4** decreased the concentration of IL-8 to a more notable degree with 4.48 pg/mL for 600 µg/mL and 4.00 pg/mL for 800 µg/mL. Incubation with **SH11** led to a decline in IL-8 concentration to 5.16 pg/mL for 200 µg/mL and 4.21 pg/mL for 400 µg/mL. Figure 8C illustrates that the enhancement of IL-10 concentration was observed for **SH1**, **SH4**, and **SH11**. In comparison with control cells (71.84 pg/mL), the greatest increase in the concentration of IL-10 was detected for **SH4** (92.55 pg/mL and 111.10 pg/mL for 600 and 800 µg/mL, respectively). **SH1** and **SH11** caused a similar enhancement of IL-10 concentration. 24 h incubation with 600 µg/mL **SH1** resulted in the detection of IL-10 in the concentration of 84.31 pg/mL For 800 µg/mL, the concentration of IL-10 was 89.93 pg/mL. For **SH11** at 200 and 400 µg/mL, the concentration of the cytokine went up to 84.63 and 87.26 pg/mL, respectively.

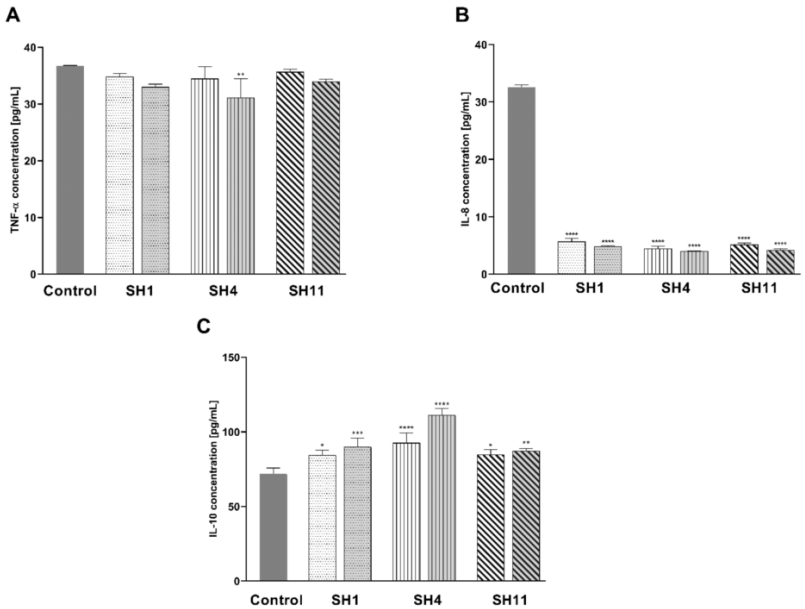

Figure 8. Concentrations of pro-inflammatory cytokines TNF-α (**A**) and IL-8 (**B**), and anti-inflammatory cytokine IL-10 (**C**) in MCF-7 human breast cancer cells after 24-h incubation with **SH1** and **SH4** at concentrations of 600 µg/mL and 800 µg/mL and **SH11** at concentrations of 200 µg/mL and 400 µg/mL. Results are presented as mean ± SD from three experiments performed in duplicate. Statistical significance was calculated using one-way ANOVA with Bonferroni multiple comparison test. Differences were considered statistically significant at **** ($p \leq 0.0001$), *** ($p \leq 0.0005$), ** ($p \leq 0.005$), and * ($p \leq 0.05$).

3. Discussion

Plant-based medicinal products can have varied effects on cancer patients, including influence on the activity of hormones and enzymes, stimulation of immune cells, or alleviating the side effects of treatment [34]. In Europe, breast cancer patients are frequent users of phytotherapy as complementary medicine along with their standard therapy [35,36]. Plant products are reported to be applied to bring physical and emotional comfort, relieve the side effects of therapy, avert tumor relapse, and improve the patient's immune system [37]. Anti-breast cancer activity of various medicinal plants, in addition to phytochemicals isolated from them, was reported in multiple studies. The activity of some of them was not only proven in in vitro studies but also clinical trials regarding their anticancer properties were designed and conducted [38].

The chemical composition of the seeds of *S. hispanica* has not been previously reported in the literature. However, there are reports on the evaluation of the phytochemical composition of aerial and subaerial parts of the plant. While the dominant groups of compounds in the aerial part extracts are flavonoids and phenolic acids, the subaerial parts contain mostly phenolic acids, steroids, terpenoids, and fatty acids [9]. Linoleic acid (LA) was the primary fatty acid of **SH1** and **SH9**-**SH12**. The presence of LA was also reported in *S. hispanica* subaerial part ethyl acetate extract [18]. The presence of caffeoylquinic acid derivatives, including CA, 3-CQa, 4-CQa, 4,5-dCQa, and 3,5-dCQa was reported in the subaerial parts as well [17]. Those compounds have been reported in **SH1**-**SH8** as well, particularly in **SH4**.

A significant amount of β-sitosterol in the oil obtained from the *S. hispanica* seeds (**SH12**) indicated that the oil might possess health-promoting properties, as β-sitosterol is known to lower cholesterol levels, increase the activity of vitamin D, or even possess anti-breast cancer properties [39,40]. The notable amount of unsaturated fatty acids (44.8% of all constituents, 72.5% of all fatty acids) in **SH12** suggests that it can be utilized in the food industry. Unsaturated fatty acids must be delivered with food, as humans are not able to synthesize those compounds [41].

Although the yield of oil pressing was not as efficient as other oilseeds, such as lemon (*Citrus limon* L., Rutaceae) or pumpkin (*Cucurbita pepo* L., Cucurbitaceae) (approx. 33–37%), [42,43], **SH12** is an interesting product in other aspects, including attractive composition and lack of cytotoxicity at high concentrations. In the wild, *S. hispanica* grows in a warm steppe environment but is easy to cultivate in temperate climates. When cultivated, the plant is characterized by a high tolerance for low temperatures and requires extensive exposure to sunlight. Additionally, the oil pressure procedure is uncomplicated, and the yield might be improved by optimization of the process conditions in the future.

Out of 12 products obtained in this study, 3 were cytotoxic toward breast cancer cells—methanolic extract **SH1**, and fractions of methanolic extracts diethyl ether (**SH4**) and chloroform (**SH11**). Phytochemical analysis of **SH1** revealed that the primary constituents of the extract are carbohydrates. Although little is understood about the anticancer activity of sucrose (which was the primary carbohydrate in the extract), the pro-apoptotic activity might be a result of interactions between the remaining components, including inositol, CA, and QA. Phosphorylated inositol—inositol hexaphosphate—was cytotoxic toward mammary carcinoma cells and its synergy with doxorubicin and tamoxifen was observed [44]. The attenuative activity of CA on multi-drug resistance in cancer cells, including breast cancer cells, was reported as well. CA was observed to modify the estrogen receptors of MCF-7 cells [45,46]. This might suggest that the CA present in **SH1** sensitizes the cells to other extract components, and therefore enhances its activity in MCF-7 cells.

The major phytocomponents of **SH4** were phenolic acids, including CA, 4,5-dCQa, and a flavonoid apigenin (A). Phenolic acids have been reported in several papers as anti-breast cancer agents in vitro [47,48]. Apigenin, which was the major flavonoid in **SH4,** was reported to be selectively cytotoxic toward MCF-7 cells [49]. A combination of apigenin, CA, and 4,5-dCQa present in **SH4** might be responsible for its selective cytotoxicity in MCF-7 observed in this study.

The greatest inhibitory activity on the growth of breast cancer cells was observed for **SH11**. This fraction contained notable amounts of LA, conjugated linoleic acid (CLA), and cinnamic acid derivatives. All those compounds were previously observed to decrease the viability of breast cancer cells [50–52].

Based on preliminary viability tests and phytochemical composition, a series of assays was performed to investigate the molecular mechanism of the activity of those three products from *S. hispanica* seeds in breast cancer cells in vitro.

To assess the influence of **SH1**, **SH4**, and **SH11** on the cellular signaling pathways, the concentrations of proteins crucial in the pathways associated with cell survival: phosphorylated Akt and ERK 1/2, in addition to the expression of phosphorylated focal adhesion kinase (p-FAK), were evaluated in MCF-7 cells. The PI3K/Akt- and ERK 1/2-mediated pathways are essential for cell survival and proliferation. Phosphorylated Akt inhibits apoptosis by, among others, the inactivation of FOX proteins or BAD and the upregulation of NF-κB activation [53]. Inhibition of PI3K/Akt signaling via a decrease of phosphorylated Akt activates BAX and therefore promotes apoptosis in cells, including MCF-7 cells [54]. Approximately three out of ten human breast cancers are reported to be characterized by dysregulations in the ERK 1/2 cell signaling pathway [51]. Attenuation of ERK 1/2 phosphorylation leads to the initiation of apoptosis via the mitochondrial pathway [55]. FAK is a kinase whose expression promotes both PI3K/Akt and ERK 1/2 signaling pathways. Additionally, FAK is considered a crucial mediator, overexpressed in many breast cancer types. FAK promotes tumorigenesis and progression of breast cancer [56]. Disruption of Akt and ERK 1/2 phosphorylation caused by fruit-derived polyphenols was suggested to be related to apoptosis induction in breast carcinoma cells [57]. In this study, it was indicated that the phytochemicals present in *S. hispanica* seeds, particularly in **SH11**, inhibit the concentrations of phosphorylated Akt, FAK, and ERK 1/2 and therefore suppress their pro-survival activity in breast cancer cells. This correlated with the observations from the Annexin V binding assay, where 24-h incubation with **SH11** induced apoptosis in over 50% of cells. This may be due to the high content of LA, CLA, and dimethyl cinnamate. A *Cinnamomum cassia* (L.) J. Presl (Lauraceae) ethanolic extract, where major components were cinnamic acid and derivatives, decreased the viability, and promoted apoptosis in carcinoma cells [58]. In the literature, plant extracts rich in cinnamic acid derivatives was observed to activate apoptotic pathways in MCF-7 cells [50]. Additionally, LA was previously reported as pro-apoptotic for breast cancer cells via the ERK 1/2-mediated pathway [51]. CLA exhibits pro-apoptotic activity on MCF-7 cells via the intrinsic pathway [52]. However, the oil obtained from the seeds in this study did not exhibit any toxicity towards the cells, even at the highest concentration (1 mg/mL), although over 27% of its relative composition was LA. This might suggest that the cytotoxicity of **SH11** was caused by compounds other than LA or that the activity against cancer cells was an effect of synergy between the constituents of **SH11**.

To confirm the ability of **SH1**, **SH4**, and **SH11** to induce apoptosis on the mitochondrial pathway, their influence on the expression of proteins involved in the process of apoptosis, BCL-2 and Bax, was assessed. BCL-2 is a regulatory protein involved in the mitochondrial pathway of apoptosis, characterized by inhibitory activity on pro-apoptotic proteins—Bax and BAK [59]. Inhibition of BCL-2 expression leads to an increase in Bax and BAK concentrations, which consequently initiates apoptosis on the intrinsic pathway [60]. Previously, downregulation of BCL-2 and upregulation of Bax concentrations in MCF-7 cells were observed for extracts of *Cassia fistula* Linn. (Fabaceae) In the phytochemical analysis, the authors demonstrated that in *n*-butanol extract, the primary component was inositol [61]. In this study, the greatest inhibitory activity on BCL-2 was exhibited by **SH1**, which contains notable amounts of inositol. However, the greatest expression of Bax was observed in **SH11**, in which dimethyl cinnamate was one of the major constituents. Cinnamic acid derivatives were reported to possess cytotoxic and pro-apoptotic activity in cancer cells [62].

Along with apoptosis, the pro-autophagic activity of **SH1**, **SH4**, and **SH11** in MCF-7 cells was assessed by analyzing ATG5 and LC3B expression after exposure to the assessed products. Autophagy is a process of degradation of redundant or faulty cytoplasm components, in response to, among others, a deficiency in nutrients or chemotherapy. Autophagy can promote either cell survival or death. Autophagy and apoptosis may be induced in the cell simultaneously. In this case, the activation of both pathways leads to cell death [28]. In this study, it was demonstrated that **SH1**, **SH4**, and **SH11** affected the expression of proteins involved in autophagy—ATG5 and LC3B. Interactions between ATG5 and BCL-X_L in an autophagic cell indirectly promote apoptosis [63]. LC3B, involved in the formation of autophagosomes, is considered one of the most used autophagy markers [64]. Previously, the pro-autophagic activity of plant-derived products in breast cancer cells was reported [65]. Additionally, extract from the roots of *Bryonia multiflora* L. (Cucurbitaceae) enhanced the expression of LC3B in breast cancer cell lines. Interestingly, the major components of the extract were phenolic acids, including cynarine (1,5-di-caffeoylquinic acid), *p*-coumaric acid, and *trans*-ferulic acid [66]. Those and the derived compounds were present in **SH1**, **SH4**, and **SH11**, investigated in this study.

Progression of cancers, including breast cancers, involves pro-inflammatory cytokines as well [67]. Therefore, the effect of **SH1**, **SH4**, and **SH11** on the expression of IL-8 and TNF-α was assessed. IL-8 is a proinflammatory chemokine that is a significant factor in signaling pathways, including the ones involved in angiogenesis, proliferation, and metastasis in tumors. Inhibitory activity on IL-8 signaling is a desired effect of therapeutic agents in cancer treatment [29]. In the literature, inhibition of IL-8 concentration in breast cancer cells after exposure to plant-derived products was reported in several papers [68,69]. In the present study, all three examined *S. hispanica* seed extracts and fractions lowered the concentration of IL-8 in MCF-7 cell lysates to a significant degree (by approximately 90%). No previous studies on the anti-inflammatory activities of *S. hispanica* have been reported in the literature.

Independently, the influence of **SH1**, **SH4**, and **SH11** on another pro-inflammatory cytokine, TNF-α, was assessed. Present in the microenvironment of the tumor, it takes part in the development and metastasis of breast cancer, in addition to its relapse. TNF-α plays a dual role in breast cancer—it can promote apoptosis and proliferation in different breast cancer cell lines, however, the original cellular response to TNF-α is increased proliferation and induction of breast cancer metastasis. Therefore, TNF-α antagonists are suspected to suppress metastasis based on the results of preclinical research [30]. The in vitro investigation of the anti-inflammatory properties of several *Scorzonera* species present in Turkey revealed that aqueous methanolic extracts from the aerial parts of the plants can inhibit TNF-α production in LPS-treated leukemia cells [70]. Costantini and colleagues [71] reported that a hydrophilic fraction of the oil from pomegranate [(*Punica granatum* L. (Lythraceae)] seeds caused a decrease in viability and TNF-α concentration in breast cancer cell lines, but no significant impact on apoptosis was discovered. This study demonstrated that the extract and fractions from *S. hispanica* seeds inhibit TNF-α production in the cells, but only **SH4** at the higher concentration exhibits a statistically significant inhibitory activity. Contrary to the study, all the assessed products (**SH1**, **SH4**, **SH11**) exhibited pro-apoptotic properties.

Scorzonera hispanica seeds yielded biologically active products, particularly **SH11**. According to the chemical characterization and biological studies performed for the purpose of this study, the seeds from *S. hispanica* can be a material of wide interest, with potential applicability in the field the breast cancer treatment.

4. Materials and Methods

4.1. Chemicals and Equipment

Hexane, BSTFA:TMCS (99:1) (N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylsilyl chloride), a C7-C40 *n*-alkanes calibration standard, DMSO (dimethyl sulfoxide), MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide)), TRIS (2-amino-

2-(hydroxymethyl)-1,3-propanediol), and SDS (sodium dodecyl sulfate) were purchased from Sigma-Aldrich (St Louis, MO, USA). For LC-MS analysis, acetonitrile Optima (ACN) (Fisher Chemical, Loughborough, UK) and ultrapure water, freshly prepared using the system POLWATER DL3-100 system (Kraków, Poland), were used. The phase modifier formic acid (FA) was ordered from Merck. The standards apigenin (A) and 3-caffeoylquinic acid (3-CQa), 4-caffeoylquinic acid (4-CQa), 5-caffeoylquinic acid (5-CQa), 3,5-di-caffeoylquinic acid (3,5-dCQa), and 4,5-di-caffeoylquinic acid (4,5-dCQa) used for the LC-MS analysis were purchased from BIOKOM (Janki, Poland). Caffeic acid (CA) was purchased from Carl Roth (Karlsruhe, Germany), while apigenin 7-*O*-glucuronide and luteolin (purity > 98%) were previously isolated in the Department of Pharmacognosy of the Medical University of Bialystok, Poland [72,73]. Extraction of the analyzed plant material was assisted by ultrasound generated by an ultrasonic bath (Sonic-5, Polsonic, Warsaw, Poland). Extracts and fractions were filtered and concentrated to dryness under vacuum (BÜCHI system (Flawil, Switzerland)) at a controlled temperature ($40 \pm 2^{\circ}C$) and subjected to lyophilization using Lymph-Lock 1.0 (LABCONCO, Kansas City, MO, USA) vacuum concentrator until a constant weight was obtained. The seed oil was pressed using a Wartmann oil press (Ronic, Lodz, Poland). All samples were centrifuged in an MPW-380R centrifuge (MPW Med Instruments, Warsaw, Poland). Analysis of the chemical composition of the samples was performed using an Agilent Infinity 1260 liquid chromatography system coupled with a 6230 MS/TOF mass spectrometer (Agilent, Santa Clara, CA, USA). Separation was performed on Kinetex XB-C18 column (150 × 2.1 mm, 1.7 µm) (Phenomenex, Torrance, CA, USA). The 7890A GC System coupled with a Q mass spectrometer (5975C VL MSD) (Agilent Technologies, Palo Alto, CA, USA) was used for the GC-MS analysis of samples. Cell lines (MCF-7, MDA-MB-231, and human skin fibroblasts) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's modification of eagle medium (DMEM), 1% streptomycin/penicillin mixture, phosphate-buffered saline (PBS) without calcium and magnesium, and 0.05% trypsin with 0.02% EDTA were purchased from Corning (Kennebunk, ME, USA), 10% FBS (fetal bovine serum) was purchased from Eurx (Gdansk, Poland). Hydrogen chloride (HCl) and sodium chloride (NaCl) were purchased from POCH (Gliwice, Poland). Sodium hydroxide (NaOH) and trichloroacetic acid (TCA) were purchased from Stanlab (Lublin, Poland). [^{3}H]-thymidine (7 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA, USA). Tween 20 and non-fat dairy milk were purchased from BIO-RAD (Warsaw, Poland). Primary and secondary antibodies for Western blot analyses were purchased from Cell Signaling Technology (Davers, MA, USA). Round 100 mm plates and 6-well plates for adherent cell culture were purchased from Sarstedt (Nümbrecht, Germany). UV-VIS Helios Gamma Spectrophotometer (Unicam/ThermoFisher Scientific Inc., Waltham, MA, USA) was used to measure the absorbance in the cell viability assay. Radioactivity in the DNA biosynthesis assay was measured in TRI-CARB 1900TR Liquid Scintillation Counter (Packard, Perkin Elmer, Inc., San Jose, CA, USA). BD Annexin V: FITC Apoptosis Detection Kit II, (ThermoFisher Scientific Inc., Waltham, MA, USA). The analysis of Annexin V: FITC was performed with a BD FACSCanto II flow cytometer (BD Biosciences Systems, San Jose, CA, USA) using FACSDiva software (version 6.1.3, BD Biosciences Systems, San Jose, CA, USA). LKB 2117 Multiphor II Electrophoresis Unit (LKB, Stockholm, Sweden) was used to perform electrophoresis. Images of the nitrocellulose membranes were captured using Bioanalytical Imaging System Azure 280 (Azure Biosystems Inc., Dublin, CA, USA). Analysis of the images was performed with ImageJ (version 1.53, National Institute of Health, Bethesda, MD, USA). High sensitivity ELISA kit for the analysis of Akt [pS473] concentration was purchased from Invitrogen (ThermoFisher Scientific, Waltham, MA, USA). ELISA kits for the quantification of ERK 1/2 [pT202/Y204], IL-8, IL-10, and TNF-α, in addition to Sigmafast NBT/BCIP solution, were purchased from Abcam (Cambridge, UK).

4.2. Plant Material, Preparation of Extracts, Fractions, and Oil

S. hispanica seeds were purchased from W. Legutko (batch number 68347; Jutrosin, Poland). For the preparation of the extracts and fractions, *S. hispanica* seeds were broken into pieces using an electric mill. Powdered seeds (15 g, each) were then treated with ultrasound-enhanced extraction for 5 × 15 min. at 40 °C using 100 mL of solvent for each time. Finally, elimination of the solvent yielded the extracts: **SH1** (methanol) (9.1%), **SH2** (50% methanol) (10.5%), **SH3** (water) (21.3%), and **SH8** (70% acetone (*v*/*v*)) (27.9%). In addition, the fractured seeds (90 g) were continuously extracted with petrol (**SH9**; 3 L × 25 h) (15.1%), then chloroform (**SH10**; 3.5 L × 25 h) (3.2%) using the Soxhlet apparatus. Then, the cleaned source was etched with methanol (1.5 L × 26) and 50% methanol (*v*/*v*, 1.5 L × 5) for 1 h each time. The combined alcoholic extracts were suspended in water and subjected to fractioning with solvents of increasing polarity: chloroform (**SH11**; 40 × 150 mL) (0.43%), diethyl ether (**SH4**; 59 × 150 mL) (0.37%), ethyl acetate (**SH5**; 60 × 150 mL) (0.63%), and *n*-butanol (**SH6**; 34 × 150 mL) (1.28%). Water residue was filtered and treated as an additional fraction named **SH7** (1.58%). Fractions **SH1-SH8** were freeze-dried. Cold pressing the seeds (150 g, triplicate, at 35 °C) provided the oil (**SH12**). The crude oil was then centrifuged (2000 rpm, 10 min, at 25 °C) and then separated from the precipitate. The pressing procedure yielded 4.8 mL of the oil (3.2%).

4.3. GC-MS Analysis of SH1, SH9-SH12

For GC-MS analysis, 15 mg of **SH9** was diluted three times with hexane. However, to prepare the **SH1**, **SH10-SH12** samples, derivatization to trimethylsilyl (TMS) derivatives was performed. For this purpose, 200 µL of BSTFA:TMCS (99:1) was mixed with 15 mg of the dry residue of the samples. The reaction mixture was then sealed and heated at 80 °C for 30 min. The **SH9** and TMS derivatives of **SH1, SH10**, and **SH11** were analyzed on a GC System coupled with a Q mass spectrometer with a source of electron ionization (EI) (the energy of ionization was 70 eV). Chromatographic separation was performed on an HP-5ms capillary column (internal diameter: 0.25 mm, film thickness: 0.25 µm, length: 30 m, Agilent Technologies), equipped with electronic pressure control and a split/splitless injector. The helium flow rate through the column was 1 mL min^{-1} in constant flow mode. The injector (300 °C) worked in split mode (split ratio 1:10). The injection volume was 1 µL. The initial temperature of the column was 40 °C, increased by 3 °C/min until 300 °C was reached, and maintained at 300 °C for 15 min. The MSD detector acquisition parameters were as follows: transfer line temperature—300 °C; and the MS source temperature—230 °C. Detection was performed in full scan mode from 40 to 850 amu [74]. Subsequently to integration, the calculation of the fraction of separated components in the total ion current (TIC) was performed.

4.4. Identification of the Chemical Composition of SH1, SH9-SH12

Both mass spectral data and the calculated retention indices (RI) were utilized in the identification of the compounds. The calculation of linear-temperature-programmed RI was done from the equation:

$$RIx = 100n + 100\frac{t_{R(x)} - t_{R(n)}}{t_{R(n+1)} + t_{R(n)}}$$

where $t_{R(x)}$ is the retention time of the analyzed compound (x) and $t_{R(n)}$ and $t_{R(n+1)}$ are retention times of *n*-alkanes leaving the chromatographic column before and after the under consideration.

Therefore, the dichloromethane solution of C_7-C_{40} *n*-alkanes was previously separated under the above-mentioned conditions. The MS libraries used were Wiley and NIST [75]. The MS library was searched using a probability-based matching algorithm. Other literature was used to identify individuals [76–80]. The percentage of individual

component relative number was presented as percent peak area relative to total peak area (%) (semiquantitative analysis).

4.5. LC-PDA-MS Conditions

Separation and qualitative evaluation of the extracts were performed on a C18 column using a liquid chromatograph. The qualitative and quantitative assessments were done under the following conditions: eluent A and B: UPW and ACN with 0.1% FA, respectively, flow rate: 300 µL/min; thermostat temperature 25 ± 0.8 °C; chromatogram wavelength 325 and 340 nm, UV-Vis spectrum at range 190–500 nm, injection: 1 µL. The gradient starts from 5 min of the 5% B starting condition and forms two isocrats—18% B between 15–40 min and 65% between 72–80 min with corresponding increments. Equilibration was 10 min. The MS/TOF conditions were as follows: the flow of dying and shielding—12 L/min at 350 °C. The nebulizer pressure was set at 45 psi, the capillary voltage at 2500 V with nozzle voltage 1000 V for negative ion mode. The acquisition was set at 120–1700 m/z controlled by Mass Hunter Data Acquisition 10.1. Data analysis was performed in Mass Hunter Qualitative b10.0 with a ChemStation integrator.

4.6. LC-PDA-MS Optimization and Validation

4.6.1. Preparation of Standard Solutions and Samples

The 5CQa and A were prepared in 50% MeOH, then filtered through a 0.45-µm PVDF membrane. Final solutions were achieved through the serial dilution of stock solution in volumetric flasks with the initial phase position. The working concentration range was 0.5–100 µg/mL and 2.5–100 µg/mL for 5CQa and A, respectively. Samples were prepared by carefully making aliquots, dissolving, centrifuging, and diluting in the initial mixture of phases to 1 mg/mL.

4.6.2. Chromatographic Optimization

Separation optimization allowed for the separation of substances confirmed using PDA and MS detectors. The linearity of the detector operation was assessed with a satisfactory result. Limits of detection (LOD) and quantification (LOQ) were plotted from the value of dividing the standard error of the response by slope. For LOD, multiplication by 3.3 was assumed, and for LOQ, 10 times this value. The validation process meets the ICH standards [81] and the parameters are summarized in Table 5.

Table 5. Validation parameters for CQAs and A derivatives analysis by LC-MS.

Parameter	5CQa	A
Linear Range [µg/mL]	0.5–100	2.5–100
r^2 (n = 6)	0.9998	0.9995
Regression Equation [a]	$y = 19.692x + 10.238$	$y = 11.494x + 7.493$
LOD [µg/mL]	0.64	0.91
LOQ [µg/mL]	1.92	2.76
Accuracy [%]	101.45 ± 4.47	101.77 ± 6.59
Intraday precision (%CV) (N = 6)	1.28	0.97
Interday precision (%CV) (N = 9)	1.82	0.87

[a]—the value for y corresponds to the peak area and x to the concentration, respectively.

4.7. Cell Culture

The cell culture medium was DMEM (10% FBS and a 1% streptomycin/penicillin mixture were added to the medium). Cell culture was maintained in 100 mm plates and placed in an incubator in the proper conditions: 37 °C, 5% CO_2, and 90% humidity. After the achievement of desired confluence (approximately 85%), the cells were detached from the plate using PBS and 0.05% trypsin with 0.02% EDTA. Then, the cells, suspended in

DMEM, were transferred to six-well plates with a density of 5×10^5 cells per well. After 24 h of incubation in six-well plates, the cells were used for the assays presented below.

4.8. Cell Viability Assay

The investigate how **SH1-SH12** affect the viability of selected cell lines, the MTT assay was performed following the modified method introduced by Carmichael et al. [82]. Cells were seeded and cultured in 6-well plates, as described in Section 4.7. Then, the cells were incubated with increasing concentrations of **SH1-SH12** (up to 1000 µg/mL) in duplicate. After incubation with MTT, the solution was aspirated and DMSO was added to dissolve formazan crystals. The absorbance (read at 570 nm) in each well was referred to the untreated control cells (taken as 100%) and expressed as a percent of the mean control value, according to the method described in the previous study by the research team [83].

4.9. DNA Biosynthesis Assay

The extract and fractions selected in the preliminary cytotoxicity assay (**SH1**, **SH4**, **SH11**) were assessed in the DNA biosynthesis assay where the amount of [^{3}H]-thymidine incorporated into the DNA of cells is measured as described in the previous study [84]. The mean radioactivity of untreated control wells was considered 100%. Values observed in the tested wells were expressed as a percent of the mean control value.

4.10. Flow Cytometry Evaluation of Annexin V Binding

To assess the ability of **SH1**, **SH4**, **SH11**, and cisplatin (cisPt) to induce apoptosis in MCF-7 cells, a flow cytometric assay with Annexin V-FITC Apoptosis Detection Kit II was performed, according to the producer's protocol. In brief, after 24 h of incubation with various concentrations of the tested extracts and reference drug, the cells were transferred from 6-well plates to test tubes and suspended in a binding buffer. Annexin V-FITC and PI (propidium iodide) (5 µL, each) were added to each sample and the mixtures were subsequently incubated at room temperature for 15 min. The analysis was performed using a flow cytometer and FACSDiva software.

4.11. Analysis of Protein Expression Using Western Blot Technique

Cell lysate samples (**SH1** and **SH4**: 800 µg/mL, **SH11** 400 µg/mL, cisPt 100 µM) containing 30 µg of protein each were subjected to SDS-PAGE. The electrophoresis was run at 100 V for 1.5 h.

The protein transfer to nitrocellulose membranes was done in the electrophoresis unit (1 h at 20 mA). After the transfer, nitrocellulose was washed with 5% non-fat dairy milk in TBS-T (TRIS-buffered saline with Tween 20 (20 mM TRIS-HCl buffer, pH 7.6, with 150 mM NaCl and 0.05% Tween 20)) for 1 h. Subsequently, overnight incubation of membranes with monoclonal antibodies against BCL-2, Bax, ATG5, LC3B, and p-FAK in TBS-T took place. Then, secondary alkaline phosphatase-conjugated antibodies against rabbit immunoglobulin (1:1000) diluted in TBS-T were added to each nitrocellulose membrane and 1 h of incubation with gentle shaking took place. After the incubation, the nitrocellulose membranes were washed with TBS-T four times and exposed to Sigmafast BCIP/NBT in the darkness. Images of the nitrocellulose membranes were subsequently captured and analyzed.

4.12. Analysis of Protein Concentration Using ELISA Technique

The evaluation of the protein concentrations (p-Akt, p-ERK 1/2, IL-8, IL-10, TNF-α) in MCF-7 cell lysates was done using high-sensitivity assay kits. Cell lysates were obtained and stored as described previously [85]. Untreated cells acted as the control. All tests were performed according to the producer's protocols, on microplates precoated with specific antibodies, provided with the kits.

4.13. Statistical Analysis

Data from three replicates are summarized as mean ± standard deviation (SD). Statistical analysis was done in GraphPad Prism Version 6.0 (GraphPad Software, Inc., San Diego, CA, USA). The one-way ANOVA with Bonferroni multiple comparison test was performed to calculate the differences between the results obtained in the control and tested cells, in addition to linear regression parameters confirming their statistical significance. Calculations for regression parameters were made using MS Excel 2019. Statistically significant differences were defined as $p < 0.05$.

5. Conclusions

The results obtained throughout this study demonstrate that *S. hispanica* seeds, particularly the oil, are a source of multiple natural products, including saturated and unsaturated fatty acids, and phytosterols. **SH12** might be a product of special interest in the future. The procedure of oil cold pressing is uncomplicated and this product exhibits no cytotoxicity toward cells in vitro. However, extracts and fractions obtained from *S. hispanica* seeds contain multiple bioactive compounds such as polyphenols including quinic and cinnamic acid derivatives, and apigenin, but also fatty acids and organic polyols. In the biological assays, **SH1**, **SH4,** and **SH11** exhibited cytotoxic activity in the MCF-7 human mammary carcinoma cell line via the inhibition of the PI3K/Akt and ERK 1/2 cell signaling pathways. **SH1**, **SH4**, and **SH11** were also observed to alter the expression of proteins related to both apoptosis and autophagy. Their inhibitory activity on IL-8 expression may lead to the suppression of angiogenesis and tumor metastasis. Nevertheless, an in-depth investigation of the activity of the extracts, in addition to their constituents, is required. So far, the results obtained in this study might suggest that *S. hispanica* seeds are a promising source of bioactive compounds that could potentially find use in breast cancer therapy.

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Abbreviations

3:5-dCQa	3:5-di-caffeoylquinic acid
3-CQa	3-caffeoylquinic acid
4,5-dCQa	4,5-di-caffeoylquinic acid
4-CQa	4-caffeoylquinic acid
5-CQa	5-caffeoylquinic acid

A	Apigenin
ACN	Acetonitrile
Akt	Protein kinase B
ATG5	Autophagy-related protein 5
BAK	BCL-2 homologous antagonist
Bax	BCL-2-like protein 4
BCL-2	B-cell lymphoma 2
BCL-xL	B-cell lymphoma extra-large
CA	Caffeic acid
cisPt	Cisplatin
CLA	Conjugated linoleic acid
DMEM	Dulbecco's modification of Eagle medium
EDTA	Ethylenediaminetetraacetic acid
EI	Electron ionization
ERK	Extracellular signal-regulated kinase
FA	Formic acid
FAK	Focal adhesion kinase
FBS	Fetal bovine serum
GC-MS	Gas chromatography-mass spectrometry
IC_{25}	Quarter-maximal inhibitory concentration
IC_{50}	Half-maximal inhibitory concentration
IL-8	Interleukin 8
LA	Linoleic acid
LC-PDA-MS	Liquid chromatography-photodiode array-mass spectrometry
LC3B	Light chain 3B
LOD	Limit of detection
LOQ	Limit of quantification
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NF-κB	Nuclear factor kappa B
OA	Oleic acid
PA	Palmitic acid
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PI	Propidium iodide
PI3K	Phosphoinositide 3-kinase
QA	Quinic acid
RI	Retention index
SDS	Sodium dodecyl sulfate
TBS-T	TRIS-buffered saline with Tween 20
TCA	Trichloroacetic acid
TIC	Total ion current
TMS	Trimethylsilyl
TNF-α	Tumor necrosis factor-alpha
TRIS	Tris(hydroxymethyl)aminomethane
UFA	Unsaturated fatty acids
UPW	Ultra-pure water

References

1. Duran, A.; Hamzaoğlu, E. A New Species of *Scorzonera* L. (*Asteraceae*) from South Anatolia, Turkey. *Biologia* **2004**, *59*, 47–50.
2. Zaika, M.A.; Kilian, N.; Jones, K.; Krinitsina, A.A.; Nilova, M.V.; Speranskaya, A.S.; Sukhorukov, A.P. *Scorzonera* sensu lato (*Asteraceae*, *Cichorieae*)—Taxonomic reassessment in the light of new molecular phylogenetic and carpological analyses. *PhytoKeys* **2020**, *137*, 1–85. [CrossRef] [PubMed]
3. Wang, Y.; Edrada-Ebel, R.; Tsevegsuren, N.; Sendker, J.; Braun, M.; Wray, V.; Lin, W.; Proksch, P. Dihydrostilbene derivatives from the Mongolian medicinal plant *Scorzonera radiata*. *J. Nat. Prod.* **2009**, *72*, 671–675. [CrossRef] [PubMed]
4. Buranov, A.U.; Elmuradov, B.J. Extraction and characterization of latex and natural rubber from rubber-bearing plants. *J. Agric. Food Chem.* **2010**, *58*, 734–743. [CrossRef]

5. Bahadır Acıkara, Ö.; Citoğlu Gülçin, S.; Dall'Acqua, S.; Özbek, H.; Cvačka, J.; Žemlička, M.; Šmejkal, K. Bioassay-guided isolation of the antinociceptive compounds motiol and β-sitosterol from Scorzonera latifolia root extract. *Pharmazie* **2014**, *69*, 711–714. [CrossRef]
6. Karakaya, S.; Polat, A.; Aksakal, Ö.; Sümbüllü, Y.Z.; İncekara, Ü. Ethnobotanical study of medicinal plants in Aziziye district (Erzurum, Turkey). *Turk. J. Pharm. Sci.* **2020**, *17*, 211–220. [CrossRef]
7. Yaldiz, G.; Koca Çalişkan, U.; Aka, C. In vitro screening of natural drug potentials for mass production. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2017**, *45*, 292–300. [CrossRef]
8. Tsevegsuren, N.; Edrada, R.A.; Lin, W.; Ebel, R.; Torre, C.; Ortlepp, S.; Wray, V.; Proksch, P. Biologically active natural products from Mongolian medicinal plants *Scorzonera divaricata* and *Scorzonera pseudodivaricata*. *J. Nat. Prod.* **2007**, *70*, 962–967. [CrossRef]
9. Lendzion, K.; Gornowicz, A.; Bielawski, K.; Bielawska, A. phytochemical composition and biological activities of *Scorzonera* species. *Int. J. Mol. Sci.* **2021**, *22*, 5128. [CrossRef]
10. Wu, Q.-X.; Su, Y.-B.; Zhu, Y. Triterpenes and steroids from the roots of *Scorzonera austriaca*. *Fitoterapia* **2011**, *82*, 493–496. [CrossRef]
11. Yang, Y.-J.; Yao, J.; Jin, X.-J.; Shi, Z.-N.; Shen, T.-F.; Fang, J.-G.; Yao, X.-J.; Zhu, Y. Sesquiterpenoids and tirucallane triterpenoids from the roots of *Scorzonera divaricata*. *Phytochemistry* **2016**, *124*, 86–98. [CrossRef] [PubMed]
12. Bahadır-Acıkara, Ö.; Özbilgin, S.; Saltan-İşcan, G.; Dall'Acqua, S.; Rjašková, V.; Özgökçe, F.; Suchý, V.; Šmejkal, K. Phytochemical analysis of *Podospermum* and *Scorzonera n*-hexane extracts and the HPLC quantitation of triterpenes. *Molecules* **2018**, *23*, 1813. [CrossRef] [PubMed]
13. Bahadır Acıkara, Ö.; Akkol, E.K.; Süntar, I.; Ergene, B.; Saltan-Çitoğlu, G.; Çoban, T. Assessment of anti-inflammatory and free radical scavenger activities of selected *Scorzonera* species and determination of active components. *Int. J. Pharmacogn. Phytochem. Res.* **2014**, *6*, 492–498.
14. Donia, A.E.R.M. Phytochemical and pharmacological studies on *Scorzonera alexandrina* Boiss. *J. Saudi Chem. Soc.* **2016**, *20*, S433–S439. [CrossRef]
15. Akkol, E.K.; Acikara, O.B.; Süntar, I.; Citolu, G.S.; Kele, H.; Ergene, B. Enhancement of wound healing by topical application of *Scorzonera* species: Determination of the constituents by HPLC with new validated reverse phase method. *J. Ethnopharmacol.* **2011**, *137*, 1018–1027. [CrossRef]
16. Akkol, E.K.; Šmejkal, K.; Kurtul, E.; Ilhan, M.; Güragac, F.T.; Çitoğlu, G.S.; Acıkara, Ö.B.; Cvačka, J.; Buděšínský, M. Inhibitory activity of *Scorzonera latifolia* and its components on enzymes connected with healing process. *J. Ethnopharmacol.* **2019**, *245*, 112168. [CrossRef]
17. Granica, S.; Lohwasser, U.; Jöhrer, K.; Zidorn, C. Qualitative and quantitative analyses of secondary metabolites in aerial and subaerial of *Scorzonera hispanica* L. (black salsify). *Food Chem.* **2015**, *173*, 321–331. [CrossRef]
18. Zidorn, C.; Ellmerer-Müller, E.P.; Stuppner, H. Sesquiterpenoids from *Scorzonera hispanica* L. *Pharmazie* **2000**, *55*, 550–551.
19. Granica, S.; Zidorn, C. Phenolic compounds from aerial parts as chemosystematic markers in the *Scorzonerinae* (Asteraceae). *Biochem. Syst. Ecol.* **2015**, *58*, 102–113. [CrossRef]
20. Petkova, N. Characterization of inulin from black salsify (*Scorzonera hispanica* L.) for food and pharmaceutical purposes. *Asian J. Pharm. Clin. Res.* **2018**, *11*, 221–225. [CrossRef]
21. Park, B.Y.; Oh, S.R.; Ahn, K.S.; Kwon, O.K.; Lee, H.K. (-)-Syringaresinol inhibits proliferation of human promyelocytic HL-60 leukemia cells via G1 arrest and apoptosis. *Int. Immunopharmacol.* **2008**, *8*, 967–973. [CrossRef] [PubMed]
22. Jeong, Y.H.; Chung, S.Y.; Han, A.R.; Sung, M.K.; Jang, D.S.; Lee, J.; Kwon, Y.; Lee, H.J.; Seo, E.K. P-glycoprotein inhibitory activity of two phenolic compounds, (-)-syringaresinol and tricin from *Sasa borealis*. *Chem. Biodivers.* **2007**, *4*, 12–16. [CrossRef] [PubMed]
23. Liu, W.; Zhang, L.; Shi, J.; Liu, Y.; Zhou, L.; Hou, K.; Qu, X.; Teng, Y. clinical significance of pAkt and pErk1/2 expression in early-stage breast cancer patients treated with anthracycline-based adjuvant chemotherapy. *Oncol Lett* **2015**, *9*, 1707–1714. [CrossRef] [PubMed]
24. Han, Q.; Xiao, F.; Ma, L.; Zhou, J.; Wang, L.; Cheng, H.; Zhu, J.; Yao, F.; Lyu, J.; Du, L. DDR1 promotes migration and invasion of breast cancer by modulating the Src-FAK signaling. *Neoplasma* **2022**, 220316N289. [CrossRef] [PubMed]
25. Sulzmaier, F.J.; Jean, C.; Schlaepfer, D.D. FAK in cancer: Mechanistic findings and clinical applications. *Nat. Rev. Cancer* **2014**, *14*, 598–610. [CrossRef]
26. Lindsay, J.; Esposti, M.D.; Gilmore, A.P. Bcl-2 Proteins and Mitochondria—Specificity in membrane targeting for death. *Biochim. Biophys. Acta (BBA) Mol. Cell Res.* **2011**, *1813*, 532–539. [CrossRef]
27. Marquez, R.T.; Xu, L. Bcl-2:Beclin 1 Complex: Multiple, Mechanisms Regulating Autophagy/Apoptosis Toggle Switch. *Am. J. Cancer Res.* **2012**, *2*, 214.
28. Buzun, K.; Gornowicz, A.; Lesyk, R.; Bielawski, K.; Bielawska, A. Autophagy Modulators in Cancer Therapy. *Int. J. Mol. Sci.* **2021**, *22*, 5804. [CrossRef]
29. Waugh, D.J.J.; Wilson, C. The Interleukin-8 pathway in Cancer. *Clin. Cancer Res.* **2008**, *14*, 6735–6741. [CrossRef]
30. Cruceriu, D.; Baldasici, O.; Balacescu, O.; Berindan-Neagoe, I. The Dual Role of Tumor Necrosis Factor-Alpha (TNF-α) in Breast Cancer: Molecular Insights and Therapeutic Approaches. *Cell. Oncol.* **2020**, *43*, 1–8. [CrossRef]
31. Dennis, K.L.; Blatner, N.R.; Gounari, F.; Khazaie, K. Current Status of IL-10 and Regulatory T-Cells in Cancer. *Curr. Opin. Oncol.* **2013**, *25*, 637. [CrossRef] [PubMed]
32. Chang, C.M.; Lam, H.Y.P.; Hsu, H.J.; Jiang, S.J. Interleukin-10: A double-edged sword in breast cancer. *Tzu Chi Med. J.* **2021**, *33*, 203–211. [CrossRef] [PubMed]

33. Gornowicz, A.; Kałuża, Z.; Bielawska, A.; Gabryel-Porowska, H.; Czarnomysy, R.; Bielawski, K. Cytotoxic efficacy of a novel dinuclear platinum(II) complex used with anti-MUC1 in human breast cancer cells. *Mol. Cell. Biochem.* **2014**, *392*, 161–174. [CrossRef] [PubMed]
34. Lopes, C.M.; Dourado, A.; Oliveira, R. Phytotherapy and nutritional supplements on breast cancer. *Biomed. Res. Int.* **2017**, *2017*, 7207983. [CrossRef] [PubMed]
35. Puskulluoglu, M.; Uchańska, B.; Tomaszewski, K.A.; Zygulska, A.L.; Zielińska, P.; Grela-Wojewoda, A. Use of complementary and alternative medicine among Polish cancer patients. *Nowotwory. J. Oncol.* **2021**, *71*, 274–281. [CrossRef]
36. Drozdoff, L.; Klein, E.; Kiechle, M.; Paepke, D. Use of biologically-based complementary medicine in breast and gynecological cancer patients during systemic therapy. *BMC Complement. Altern. Med.* **2018**, *18*, 259. [CrossRef]
37. Leggett, S.; Koczwara, B.; Miller, M. The impact of complementary and alternative medicines on cancer symptoms, treatment side effects, quality of life, and survival in women with breast cancer—A systematic review. *Nutr. Cancer* **2015**, *67*, 373–391. [CrossRef]
38. McGrowder, D.A.; Miller, F.G.; Nwokocha, C.R.; Anderson, M.S.; Wilson-Clarke, C.; Vaz, K.; Anderson-Jackson, L.; Brown, J. medicinal herbs used in traditional management of breast cancer: Mechanisms of action. *Medicines* **2020**, *7*, 47. [CrossRef]
39. Gharby, S.; Harhar, H.; Bouzoubaa, Z.; Asdadi, A.; El Yadini, A.; Charrouf, Z. Chemical characterization and oxidative stability of seeds and oil of sesame grown in Morocco. *J. Saudi Soc. Agric. Sci.* **2017**, *16*, 105–111. [CrossRef]
40. Xu, H.; Li, Y.; Han, B.; Li, Z.; Wang, B.; Jiang, P.; Zhang, J.; Ma, W.; Zhou, D.; Li, X.; et al. Anti-breast-cancer activity exerted by β-sitosterol- d -glucoside from sweet potato via upregulation of microRNA-10a and via the PI3K-Akt signaling pathway. *J. Agric. Food Chem.* **2018**, *66*, 9704–9718. [CrossRef]
41. Zhao, M.; Zhong, Q.; Tian, M.; Han, R.; Ren, Y. Comparative transcriptome analysis reveals differentially expressed genes associated with the development of Jerusalem artichoke tuber (*Helianthus tuberosus* L.). *Ind. Crops Prod.* **2020**, *151*, 112455. [CrossRef]
42. Yilmaz, E.; Güneşer, B.A. Cold pressed versus solvent extracted lemon (*Citrus limon* L.) seed oils: Yield and properties. *J. Food Sci. Technol.* **2017**, *54*, 1891–1900. [CrossRef]
43. Konopka, I.; Roszkowska, B.; Czaplicki, S.; Tańska, M. Optimization of pumpkin oil recovery by using aqueous enzymatic extraction and comparison of the quality of the obtained oil with the quality of cold-pressed oil. *Food Technol. Biotechnol.* **2016**, *54*, 413–420. [CrossRef] [PubMed]
44. Tantivejkul, K.; Vucenik, I.; Eiseman, J.; Shamsuddin, A.K.M. Inositol hexaphosphate (IP6) enhances the anti-proliferative effects of adriamycin and tamoxifen in breast cancer. *Breast Cancer Res. Treat.* **2003**, *79*, 301–312. [CrossRef] [PubMed]
45. Teng, Y.N.; Wang, C.C.N.; Liao, W.C.; Lan, Y.H.; Hung, C.C. caffeic acid attenuates multi-drug resistance in cancer cells by inhibiting efflux function of human P-glycoprotein. *Molecules* **2020**, *25*, 247. [CrossRef]
46. Rosendahl, A.H.; Perks, C.M.; Zeng, L.; Markkula, A.; Simonsson, M.; Rose, C.; Ingvar, C.; Holly, J.M.P.; Jernström, H. Caffeine and caffeic acid inhibit growth and modify estrogen receptor and insulin-like growth factor I receptor levels in human breast cancer. *Clin. Cancer Res.* **2015**, *21*, 1877–1887. [CrossRef] [PubMed]
47. Ghasemzadeh, A.; Talei, D.; Jaafar, H.Z.E.; Juraimi, A.S.; Mohamed, M.T.M.; Puteh, A.; Halim, M.R.A. Plant-growth regulators alter phytochemical constituents and pharmaceutical quality in Sweet potato (*Ipomoea batatas* L.). *BMC Complement. Altern. Med.* **2016**, *16*, 152. [CrossRef]
48. Elansary, H.O.; Szopa, A.; Kubica, P.; Ekiert, H.; Al-Mana, F.A.; El-Shafei, A.A. Polyphenols of *Frangula alnus* and *Peganum harmala* leaves and associated biological activities. *Plants* **2020**, *9*, 1086. [CrossRef]
49. Shendge, A.K.; Chaudhuri, D.; Basu, T.; Mandal, N. A natural flavonoid, apigenin isolated from *Clerodendrum viscosum* leaves, induces G2/M phase cell cycle arrest and apoptosis in MCF-7 cells through the regulation of p53 and caspase-cascade pathway. *Clin. Transl. Oncol.* **2021**, *23*, 718–730. [CrossRef]
50. Hostanska, K.; Nisslein, T.; Freudenstein, J.; Reichling, J.; Saller, R. evaluation of cell death caused by triterpene glycosides and phenolic substances from *Cimicifuga racemosa* extract in human MCF-7 breast cancer cells. *Biol. Pharm. Bull.* **2004**, *27*, 1970–1975. [CrossRef]
51. Whyte, J.; Bergin, O.; Bianchi, A.; McNally, S.; Martin, F. Key signalling nodes in mammary gland development and cancer. Mitogen-activated protein kinase signalling in experimental models of breast cancer progression and in mammary gland development. *Breast Cancer Res.* **2009**, *11*, 209. [CrossRef] [PubMed]
52. Seo, J.H.; Moon, H.S.; Kim, I.Y.; Guo, D.D.; Lee, H.G.; Choi, Y.J.; Cho, C.S. PEGylated conjugated linoleic acid stimulation of apoptosis via a p53-mediated signaling pathway in MCF-7 breast cancer cells. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 621–626. [CrossRef] [PubMed]
53. Dan, H.C.; Cooper, M.J.; Cogswell, P.C.; Duncan, J.A.; Ting, J.P.Y.; Baldwin, A.S. Akt-dependent regulation of NF-κB is controlled by mTOR and Raptor in association with IKK. *Genes Dev.* **2008**, *22*, 1490–1500. [CrossRef] [PubMed]
54. Kello, M.; Takac, P.; Kubatka, P.; Kuruc, T.; Petrova, K.; Mojzis, J. Oxidative stress-induced DNA damage and apoptosis in clove buds-treated MCF-7 cells. *Biomolecules* **2020**, *10*, 139. [CrossRef] [PubMed]
55. Chun, J.; Han, L.; Xu, M.Y.; Wang, B.; Cheng, M.S.; Kim, Y.S. The induction of apoptosis by a newly synthesized diosgenyl saponin through the suppression of estrogen receptor-α in MCF-7 human breast cancer cells. *Arch. Pharm. Res.* **2014**, *37*, 1477–1486. [CrossRef] [PubMed]
56. Luo, M.; Guan, J.L. Focal Adhesion Kinase: A prominent determinant in breast cancer initiation, progression and metastasis. *Cancer Lett* **2010**, *289*, 127–139. [CrossRef]

57. Kello, M.; Kulikova, L.; Vaskova, J.; Nagyova, A.; Mojzis, J. Fruit Peel Polyphenolic Extract-Induced Apoptosis in Human Breast Cancer Cells Is Associated with ROS Production and Modulation of p38MAPK/Erk1/2 and the Akt Signaling Pathway. *Nutr. Cancer* **2017**, *69*, 920–931. [CrossRef]
58. Yu, C.H.; Chu, S.C.; Yang, S.F.; Hsieh, Y.S.; Lee, C.Y.; Chen, P.N. Induction of apoptotic but not autophagic cell death by *Cinnamomum cassia* extracts on human oral cancer cells. *J. Cell. Physiol.* **2019**, *234*, 5289–5303. [CrossRef]
59. Marie Hardwick, J.; Soane, L. Multiple functions of BCL-2 family proteins. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a008722. [CrossRef]
60. Edlich, F. BCL-2 proteins and apoptosis: Recent insights and unknowns. *Biochem. Biophys. Res. Commun.* **2018**, *500*, 26–34. [CrossRef]
61. Irshad, M.; Jafar Mehdi, S.; Al-Fatlawi, A.A.; Zafaryab, M.; Ali, A.; Ahmad, I.; Singh, M.; Moshahid Rizvi, M.A. Phytochemical composition of *Cassia fistula* fruit extracts and its anticancer activity against human cancer cell lines. *J. Biol. Act. Prod. Nat.* **2014**, *4*, 158–170. [CrossRef]
62. Ruwizhi, N.; Aderibigbe, B.A. Cinnamic acid derivatives and their biological efficacy. *Int. J. Mol. Sci.* **2020**, *21*, 5712. [CrossRef] [PubMed]
63. Yousefi, S.; Perozzo, R.; Schmid, I.; Ziemiecki, A.; Schaffner, T.; Scapozza, L.; Brunner, T.; Simon, H.U. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. *Nat. Cell Biol.* **2006**, *8*, 1124–1132. [CrossRef] [PubMed]
64. Koukourakis, M.I.; Kalamida, D.; Giatromanolaki, A.; Zois, C.E.; Sivridis, E.; Pouliliou, S.; Mitrakas, A.; Gatter, K.C.; Harris, A.L. Autophagosome proteins LC3A, LC3B and LC3C have distinct subcellular distribution kinetics and expression in cancer cell lines. *PLoS ONE* **2015**, *10*, e0137675. [CrossRef] [PubMed]
65. Weng, J.R.; Yen, M.H.; Lin, W.Y. Cytotoxic constituents from *Celastrus paniculatus* induce apoptosis and autophagy in breast cancer cells. *Phytochemistry* **2013**, *94*, 211–219. [CrossRef]
66. Tokgun, O.; Tokgun, P.E.; Turel, S.; Inal, B.; Inci, K.; Tan, S.; Can Alvur, O. *Bryonia multiflora* extract induces autophagy via regulating long non-coding RNAs in breast cancer cells. *Nutr. Cancer* **2021**, *73*, 1792–1803. [CrossRef]
67. Esquivel-Velázquez, M.; Ostoa-Saloma, P.; Palacios-Arreola, M.I.; Nava-Castro, K.E.; Castro, J.I.; Morales-Montor, J. The role of cytokines in breast cancer development and progression. *J. Interf. Cytokine Res.* **2015**, *35*, 1–16. [CrossRef]
68. Santos, A.F.; Santos Mota, N.S.R.; Schiefer, E.M.; da Cunha, R.S.; Junkert, A.M.; Stinghen, A.E.M.; Pontarolo, R.; Crisma, A.R.; Weffort-Santos, A.M.; Pedrosa, R.C.; et al. The toxicity of *Aspidosperma subincanum* to MCF7 cells is related to modulation of oxidative status and proinflammatory pathways. *J. Ethnopharmacol.* **2021**, *281*, 114512. [CrossRef]
69. Kim, S.J.; Pham, T.H.; Bak, Y.; Ryu, H.W.; Oh, S.R.; Yoon, D.Y. 7-Methoxy-luteolin-8-C-β-6-deoxy-xylo-pyranos-3-uloside exactly (mLU8C-PU) isolated from *Arthraxon hispidus* inhibits migratory and invasive responses mediated via downregulation of MMP-9 and IL-8 expression in MCF-7 breast cancer cells. *Environ. Toxicol.* **2018**, *33*, 1143–1152. [CrossRef]
70. Bahadır Acikara, Ö.; Hošek, J.; Babula, P.; Cvačka, J.; Budešínský, M.; Dračinský, M.; Saltan İşcan, G.; Kadlecová, D.; Ballová, L.; Šmejkal, K. Turkish *Scorzonera* species extracts attenuate cytokine secretion via inhibition of NF-κB activation, showing anti-inflammatory effect in vitro. *Molecules* **2016**, *21*, 43. [CrossRef]
71. Costantini, S.; Rusolo, F.; De Vito, V.; Moccia, S.; Picariello, G.; Capone, F.; Guerriero, E.; Castello, G.; Volpe, M.G. Potential anti-inflammatory effects of the hydrophilic fraction of pomegranate (*Punica granatum* L.) seed oil on breast cancer cell lines. *Molecules* **2014**, *19*, 8644–8660. [CrossRef] [PubMed]
72. Nazaruk, J.; Jakoniuk, P. Flavonoid composition and antimicrobial activity of *Cirsium rivulare* (Jacq.) All. flowers. *J. Ethnopharmacol.* **2005**, *102*, 208–212. [CrossRef] [PubMed]
73. Juszczak, A.M.; Czarnomysy, R.; Strawa, J.W.; Končić, M.Z.; Bielawski, K.; Tomczyk, M. In vitro anticancer potential of *Jasione montana* and its main components against human amelanotic melanoma cells. *Int. J. Mol. Sci.* **2021**, *22*, 3345. [CrossRef] [PubMed]
74. Isidorov, V.A.; Smolewska, M.; Purzyåska-Pugacewicz, A.; Tyszkiewicz, Z. Chemical composition of volatile and extractive compounds of pine and spruce leaf litter in the initial stages of decomposition. *Biogeosciences* **2010**, *7*, 2785–2794. [CrossRef]
75. National Institute of Standard and Technology. *NIST Chemistry WebBook*; National Institute of Standard and Technology: Gaithersburg, MD, USA, 2013.
76. Isidorov, V.A.; Kotowska, U.; Vinogorova, V.T. GC identification of organic compounds based on partition coefficients of their TMS derivatives in a hexane-acetonitrile system and retention indices. *Anal. Sci.* **2005**, *21*, 1483–1489. [CrossRef]
77. Isidorov, V.A.; Szczepaniak, L. Gas chromatographic retention indices of biologically and environmentally important organic compounds on capillary columns with low-polar stationary phases. *J. Chromatogr. A* **2009**, *1216*, 8998–9007. [CrossRef]
78. Isidorov, V.A.; Stocki, M.; Vetchinikova, L. Inheritance of specific secondary volatile metabolites in buds of white birch *Betula pendula* and *Betula pubescens* hybrids. *Trees Struct. Funct.* **2019**, *33*, 1329–1344. [CrossRef]
79. Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, 043101. [CrossRef]
80. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing Corporation: Carol Streams, IL, USA, 2007; ISBN 1932633219.
81. ICH. In Proceedings of the 6th International Conference on Harmonization. Geneva, Switzerland, 1 November 2005.
82. Carmichael, J.; DeGraff, W.G.; Gazdgar, A.F.; Minna, J.D.; Mitchel, J.B. evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936–942.

83. Gornowicz, A.; Szymanowski, W.; Bielawski, K.; Kałuża, Z.; Michalak, O.; Bielawska, A. Mucin 1 as a molecular target of a novel diisoquinoline derivative combined with anti-MUC1 antibody in AGS gastric cancer cells. *Molecules* **2021**, *26*, 6504. [CrossRef]
84. Gornowicz, A.; Szymanowski, W.; Czarnomysy, R.; Bielawski, K.; Bielawska, A. Anti-HER2 monoclonal antibodies intensify the susceptibility of human gastric cancer cells to etoposide by promoting apoptosis, but not autophagy. *PLoS ONE* **2021**, *16*, e0255585. [CrossRef] [PubMed]
85. Buzun, K.; Gornowicz, A.; Lesyk, R.; Kryshchyshyn-Dylevych, A.; Gzella, A.; Czarnomysy, R.; Latacz, G.; Olejarz-Maciej, A.; Handzlik, J.; Bielawski, K.; et al. 2-{5-[(Z,2Z)-2-Chloro-3-(4-nitrophenyl)-2-propenylidene]-4-oxo-2-thioxothiazolidin-3-yl}-3-methylbutanoic acid as a potential anti-breast cancer molecule. *Int. J. Mol. Sci.* **2022**, *23*, 4091. [CrossRef] [PubMed]

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SUPPLEMENTARY MATERIAL

LC-PDA-MS and GC-MS Analysis of *Scorzonera hispanica* Seeds and Their Effects on Human Breast Cancer Cell Lines

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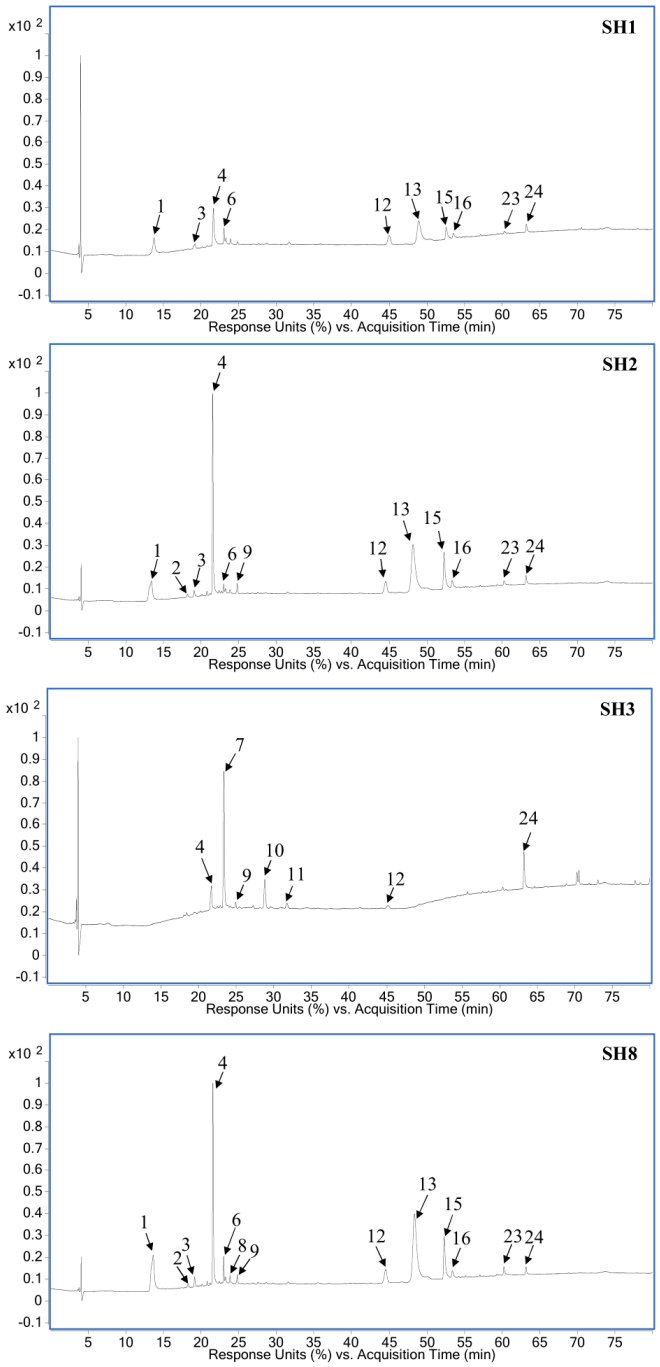

Figure S1. UV–VIS chromatogram of SH1-3 and SH8 extracts obtained by HPLC-PDA-MS (280 nm).

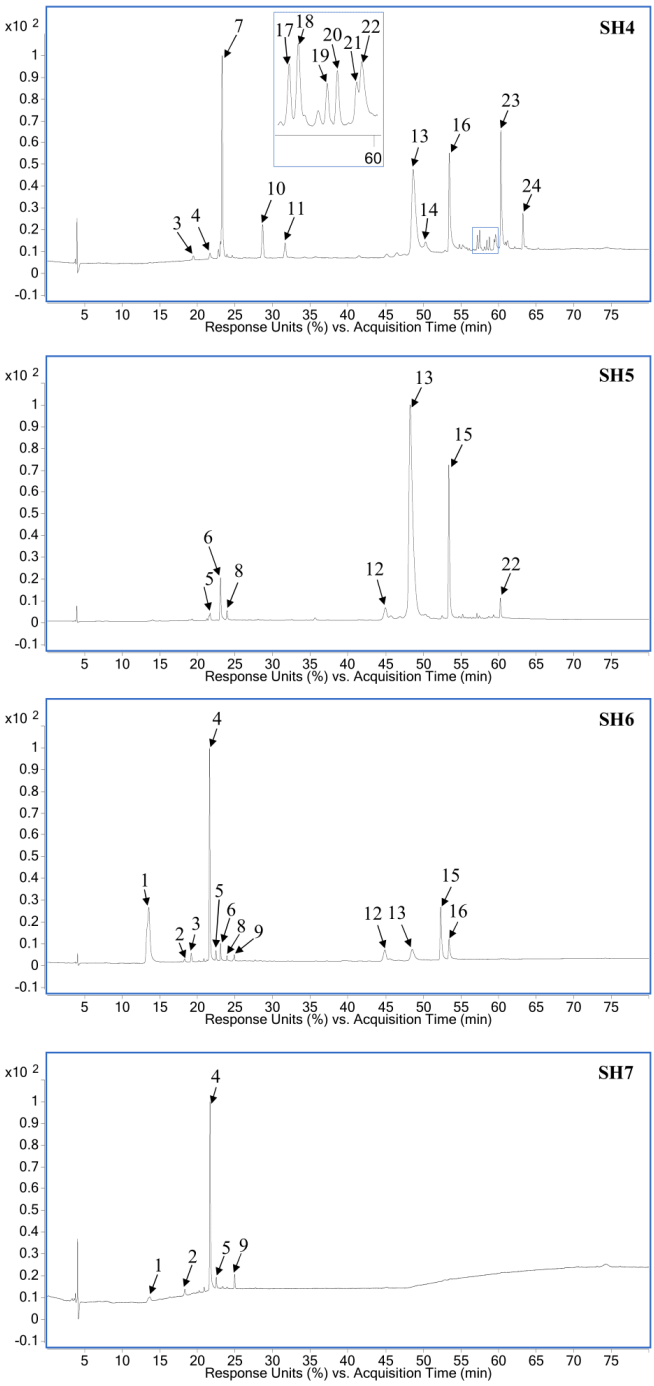

Figure S2. UV–VIS chromatogram of SH4-6 fractions and SH7 residue obtained by HPLC-PDA-MS (280 nm).

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1. *Lendzion K, Gornowicz A, Bielawski K, Bielawska A.: Phytochemical Composition and Biological Activities of Scorzonera Species. International Journal of Molecular Sciences 2021, vol. 22(10), ID: 5128, 42 strony. DOI: 10.3390/ijms22105128*

wchodzącej w skład mojej rozprawy doktorskiej polegał na współtworzeniu koncepcji pracy, zebraniu i analizie literatury dotyczącej składu chemicznego i aktywności biologicznej gatunków z rodzaju *Scorzonera*, stworzeniu schematu pracy, przygotowaniu tabel, rycin i manuskryptu, co określam jako **85%** udziału w przygotowaniu wyżej wymienionej publikacji.

2. *Lendzion K, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of Scorzonera hispanica Seeds and Their Effects on Human Breast Cancer Cell Lines. International Journal of Molecular Sciences 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584*

wchodzącej w skład mojej rozprawy doktorskiej polegał na współtworzeniu koncepcji pracy i hipotez badawczych, przeprowadzeniu badań biologicznych na hodowli komórkowej *in vitro*, analizie i interpretacji wyników uzyskanych w toku pracy, a także ich analizie statystycznej, przygotowaniu rycin i dyskusji, współtworzeniu manuskryptu, funkcji autora korespondencyjnego, co określam jako **55%** udziału w przygotowaniu wyżej wymienionej publikacji.

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wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na współudziale w tworzeniu koncepcji pracy oraz nadzorze merytorycznym nad publikacją.

2. *Lendzion K, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of Scorzonera hispanica Seeds and Their Effects on Human Breast Cancer Cell Lines. International Journal of Molecular Sciences 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584*

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Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionych prac przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

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2. *Lendzion K, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of Scorzonera hispanica Seeds and Their Effects on Human Breast Cancer Cell Lines. International Journal of Molecular Sciences 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584*

wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na współtworzeniu koncepcji pracy, udziale w analizie danych z oznaczeń techniką ELISA, pozyskaniu środków na prowadzenie badań oraz ocenie i edycji manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionych prac przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Gornowicz Agnieszka
.....
Podpis (czytelny)

Białystok, 20 października 2022 r.

Prof. dr hab. Krzysztof Bielawski
Imiona i nazwisko współautora

Zakład Syntezy i Technologii Środków Leczniczych
Uniwersytet Medyczny w Białymstoku
Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Lendzion K, Gornowicz A, Bielawski K, Bielawska A.: Phytochemical Composition and Biological Activities of Scorzonera Species. International Journal of Molecular Sciences 2021, vol. 22(10), ID: 5128, 42 strony. DOI: 10.3390/ijms22105128*

wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na współudziale w tworzeniu koncepcji pracy, oraz nadzorze merytorycznym nad tekstem publikacji.

2. *Lendzion K, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of Scorzonera hispanica Seeds and Their Effects on Human Breast Cancer Cell Lines. International Journal of Molecular Sciences 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584*

wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na współtworzeniu koncepcji pracy oraz korekcie merytorycznej tekstu przygotowanego do druku.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionych prac przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Krzysztof Bielawski
.....
Podpis (czytelny)

Białystok, 20 października 2022 r.

Dr hab. Michał Tomczyk
Imiona i nazwisko współautora

Zakład Farmakognozji
Uniwersytet Medyczny w Białymstoku
Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Lendzion K, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of Scorzonera hispanica Seeds and Their Effects on Human Breast Cancer Cell Lines. International Journal of Molecular Sciences 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584*

wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na współtworzeniu koncepcji pracy, nadzorze nad otrzymaniem ekstraktów i frakcji, a także nad wykonaniem i interpretacją wyników ich analizy metodą LC-PDA-MS, oraz współtworzeniu manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

.....
Podpis (czytelny)

Białystok, 24 października 2022 r.

Prof. dr hab. Wojciech Miltyk
Imiona i nazwisko współautora

Zakład Analizy i Bioanalizy Leków
Uniwersytet Medyczny w Białymstoku
Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Lendzion K, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of Scorzonera hispanica Seeds and Their Effects on Human Breast Cancer Cell Lines. International Journal of Molecular Sciences 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584*

wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na ocenie merytorycznej i edycji manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

KIEROWNIK
Zakładu Analizy i Bioanalizy Leków
prof. dr hab. n. farm. Wojciech Miltyk
Podpis (czytelny)

Białystok, 20 października 2022 r.

Dr hab. Robert Czarnomysy
Imiona i nazwisko współautora

Zakład Syntezy i Technologii Środków Leczniczych
Uniwersytet Medyczny w Białymstoku
Miejsce pracy/afiliacja

Oświadczenie współautora

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wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na wykonaniu oznaczeń metodą cytometrii przepływowej.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Czarnomysy Robert
.....
Podpis (czytelny)

Białystok, 20 października 2022 r.

mgr farm. Jakub Władysław Strawa
Imiona i nazwisko współautora

Zakład Farmakognozji
Uniwersytet Medyczny w Białymstoku
Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

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wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na nadzorze nad otrzymaniem ekstraktów i frakcji, wykonaniu i interpretacji wyników ich analizy metodą LC-PDA-MS, oraz współtworzeniu manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Podpis (czytelny)

W przypadku prac dwu- lub wieloautorskich zaleca się złożenie oświadczenia **przez współautora wskazujące na jego merytoryczny (a NIE procentowy) wkład w powstanie pracy [np. twórca hipotezy badawczej, pomysłodawca badań, wykonanie specyficznych badań (np. przeprowadzenie konkretnych doświadczeń, opracowanie i zebranie danych, wykonanie zestawień statystycznych itp.), wykonanie analizy wyników, przygotowanie manuskryptu artykułu, i inne]. Określenie wkładu danego współautora powinno być na tyle precyzyjne, aby umożliwić dokładną ocenę jego udziału i roli w powstaniu każdej pracy.*

Białystok, 24 października 2022 r.

Dr n. farm. Katarzyna Bielawska
Imiona i nazwisko współautora

Zakład Analizy i Bioanalizy Leków
Uniwersytet Medyczny w Białymstoku
Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Lendzion K, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of Scorzonera hispanica Seeds and Their Effects on Human Breast Cancer Cell Lines. International Journal of Molecular Sciences 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584*

wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na wykonaniu i interpretacji wyników analizy GC-MS oraz współtworzeniu manuskryptu.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Katarzyna Bielawska
Podpis (czytelny)

Białystok, 20 października 2022 r.

Dr n. farm. Bożena Popławska
Imiona i nazwisko współautora

Zakład Biotechnologii
Uniwersytet Medyczny w Białymstoku
Miejsce pracy/afiliacja

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Lendzion K, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of Scorzonera hispanica Seeds and Their Effects on Human Breast Cancer Cell Lines. International Journal of Molecular Sciences 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584*

wchodzącej w skład rozprawy doktorskiej Pani mgr Karoliny Lendzion polegał na wykonaniu oznaczeń metodą Western blot.

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Panią mgr Karolinę Lendzion jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

B. Popławska
Podpis (czytelny)

13. Dorobek naukowy

13.1. Wykaz publikacji stanowiących rozprawę doktorską

1. **Lendzion K**, Gornowicz A, Bielawski K, Bielawska A.: Phytochemical Composition and Biological Activities of *Scorzonera* Species. *International Journal of Molecular Sciences* 2021, vol. 22(10), ID: 5128, 42 strony. DOI: 10.3390/ijms22105128 IF = **6.208**, MEiN = **140 pkt.**

2. **Lendzion K**, Gornowicz A, Strawa JW, Bielawska K, Czarnomysy R, Popławska B, Bielawski K, Tomczyk M, Miltyk W, Bielawska A.: LC-PDA-MS and GC-MS Analysis of *Scorzonera hispanica* Seeds and Their Effects on Human Breast Cancer Cell Lines. *International Journal of Molecular Sciences* 2022, 23(19), ID: 11584. 29 stron. DOI: 10.3390/ijms231911584 IF = **6.208**, MEiN = **140 pkt.**

13.2. Wykaz doniesień zjazdowych

1. **Lendzion Karolina**, Gornowicz Agnieszka, Strawa Jakub Władysław, Tomczyk Michał, Bielawski Krzysztof, Bielawska Anna. *Evaluation of cytotoxic activity of Scorzonera hispanica seed extracts against MDA-MB-231 breast cancer cell line.* X Konwersatorium Chemii Medycznej. Lublin, 3-5.09.2021.
2. **Lendzion Karolina**, Gornowicz Agnieszka, Tomczyk Michał, Strawa Jakub, Roszczenko Piotr, Bielawska Anna. *Assessment of cytotoxic activities of Scorzonera hispanica seed extracts in MFC-7 breast cancer cell line.* 18th Hellenic Symposium on Medicinal Chemistry, on-line, 25-27.02.2021.
3. Bielawska Anna, Gornowicz Agnieszka, **Lendzion Karolina**, Popławska Bożena, Bielawski Krzysztof. *Ocena wpływu komórek macierzystych Citrus aurantium na proliferację fibroblastów skóry ludzkiej.* IV Sympozjum Szkoła Chemii Medycznej, Wrocław, Poland, 25-27.09.2019.

13.3. Wykaz innych aktywności naukowych

Aktywność dydaktyczna:

1. Prowadzenie zajęć dydaktycznych dla studentek II roku kierunku Kosmetologia II° - przedmioty: Przemysłowa produkcja kosmetyków, Nanotechnologia w kosmetologii, Biotechnologia kosmetyków w roku akademickim 2019/2020, 2020/2021, 2021/2022, 2022/2023.

Inne aktywności:

1. Prowadzenie warsztatu pt. *Technologia przyszłości w kosmetologii i pielęgnacji - komórki macierzyste* w ramach XVIII Podlaskiego Festiwalu Nauki i Sztuki (11.05.2022).