molecules

MDPI

Article

A Comprehensive Phytochemical Analysis of *Sideritis scardica* Infusion Using Orbitrap UHPLC-HRMS

Dimitrina Zheleva-Dimitrova [1,*,†], Yulian Voynikov [2,†], Reneta Gevrenova [1] and Vessela Balabanova [1]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, 1000 Sofia, Bulgaria; rgevrenova@pharmfac.mu-sofia.bg (R.G.); vbalabanova@pharmfac.mu-sofia.bg (V.B.)
[2] Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, 1000 Sofia, Bulgaria; y_voynikov@pharmfac.mu-sofia.bg
* Correspondence: dzheleva@pharmfac.mu-sofia.bg
† These authors contributed equally to this work.

Abstract: *Sideritis scardica* Griseb, also known as "mountain tea" and "Olympus tea" (Lamiaceae family) is an endemic plant from the mountainous regions of the Balkan Peninsula. In this study, we focused on an in-depth phytochemical analysis of *S. scardica* infusion using ultra-high-performance liquid chromatography hyphenated with high-resolution mass spectrometry (UHPLC–HRMS). Quantitative determination of the main secondary metabolites was carried out by UHPLC–HRMS analyses using the external standard method. The results revealed more than 100 metabolites, including five sugar acids and saccharides, 21 carboxylic, hydroxybenzoic, hydroxycinnamic acids, and derivatives, 15 acylquinic acids, 10 phenylpropanoid glycosides, four iridoid glycosides, 28 flavonoids, seven fatty acids, and four organosulfur compounds. Furthermore, a dereplication and fragmentation patterns of five caffeic acids oligomers and four acylhexaric acids was performed for the first time in *S. scardica*. Regarding the quantitative analysis, the phenylethanoid verbascoside (**53**) (151.54 ± 10.86 mg/g lyophilized infusion, li), the glycosides of isoscutellarein (**78**) (151.70 ± 14.78 mg/g li), methylisoscutelarein (**82**) (107.4 ± 9.07 mg/g li), and hypolaetin (**79**) (78.33 ± 3.29 mg/g li), as well as caffeic acid (**20**) (87.25 ± 6.54 mg/g li), were found to be the major compounds in *S. scardica* infusion. The performed state-of-the-art phytochemical analysis of *S. scardica* provides additional knowledge for the chemical constituents and usage of this valuable medicinal plant.

Keywords: *Sideritis scardica* Griseb; UHPLC–HRMS; phytochemical analysis

Citation: Zheleva-Dimitrova, D.; Voynikov, Y.; Gevrenova, R.; Balabanova, V. A Comprehensive Phytochemical Analysis of *Sideritis scardica* Infusion Using Orbitrap UHPLC-HRMS. *Molecules* **2024**, *29*, 204. https://doi.org/10.3390/molecules29010204

Academic Editor: Hyun-Ock Pae

Received: 2 December 2023
Revised: 22 December 2023
Accepted: 27 December 2023
Published: 29 December 2023

1. Introduction

Sideritis scardica Griseb (Lamiaceae family) is an endemic plant of the mountainous regions of the Balkan Peninsula [1,2]. It is often referred to as "mountain tea", "ironwort", "Olympus tea", and "Pirin tea" [3]. Mountain tea is a perennial herbaceous plant with a well-developed root system, the stem is 15–40 cm and woody at the base, the leaves are opposite with gray hairs, the flowers are clustered in a dense spike, the middle bracts are 12–20 mm long, i.e., longer than the flowers, the corolla is lemon yellow with glandules, and the calyx is tubular-campanulate [2,3]. Usually, *Sideritis* plants are applied in traditional medicine, mostly as an aromatic herbal tea [4–7]. The tea is made from the aerial parts of the plant by infusion or decoction [8]. Historically, *S. scardica* has been used to treat inflammation, common colds, asthma, bronchitis, and gastrointestinal disorders. It is supposed to relieve pain, including rheumatic pain, as well as reducing stress and anxiety. The plant name comes from the Greek word "sideros", meaning "iron", as it was used in ancient times to heal wounds from iron weapons [4]. Regular consumption of mountain tea by rats has been shown to lead to weight loss and prevent insulin resistance by lowering blood glucose and triglyceride levels and increasing liver glycogen content [8]. Additionally, antioxidant properties and positive effects on memory and cognitive abilities have also

been observed [7,9,10]. *Sideritis* species have also been used topically on the skin and as an antiseptic solution to sooth the pain of tooth extraction [3].

The traditional medicinal usage of the species is based on the phytochemical constituents, including phenolic acids (chlorogenic acid, 3-caffeoylquinic acid, feruloylquinic acid, and others), flavonoids and their derivatives (hypolaetin, isoscutellarein, and others), phenylethanoid glycosides (lavandulifolioside, verbascoside, echinacoside, allysonoside, and others), and terpenoids (mostly iridoid glycosides) [6,8,11,12]. These chemical compounds have been explored in phytochemical studies, operating with various extraction techniques such as hydrodistillation and solvent and supercritical extractions [8,12–15]. Precisely, the most abundant secondary metabolites of *S. scardica* water extracts (i.e., when making infusion or decoction) are flavonoids, hydoxycinnamic acid derivatives, and phenylethanoid glycosides [7,14,16,17]. Identification of closely related species of the genus *Sideritis* is based on the dominant 5-caffeoylquinic acid, lavandulifolioside, verbascoside, isoscutellarein 7-O-allosyl(1→2)glucoside, hypolaetin 7-O-[6‴O-acetyl]-allosyl(1→2)glucoside, isoscutellarein 7-O-[6‴-O-acetyl]-allosyl(1→2) glucoside, 3′-O-methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside, and 4′-O-methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl-(1→2)-[6″-O-acetyl]-glucoside. Thus, these compounds have been used as chemotaxonomical markers [6].

Based on the literature available on *Sideritis scardica*, there is no detailed metabolite profiling of the species, something which seems important in light of its health benefits. An in-depth UHPLC–HRMS analysis of the main metabolites of *S. scardica*, together with quantitative determination, was conducted. More than 100 secondary metabolites were identified/tentatively elucidated in a lyophilized infusion of mountain tea. The performed phytochemical analysis of *S. scardica* will provide additional knowledge of the chemical constituents and usage of this valuable medicinal plant for the future.

2. Results and Discussion

2.1. Metabolite Profiling of S. scardica Lyophilized Infusion

Herein, an in-depth UHPLC–HRMS analysis of *S. scardica* infusion was conducted by allowing the dereplication/annotation of 103 metabolites, including five sugar acids and saccharides, 21 carboxylic, hydroxybenzoic, hydroxycinnamic acids, and derivatives, five caffeic acids oligomers, 15 acylquinic acids, four acylhexaric acids, 10 phenylpropanoid glycosides, four iridoid glycosides, 28 flavonoids, seven fatty acids, and four organosulfur compounds (Table 1). This study allowed the identification of caffeic acids oligomers, acylquinic, acylhexaric acids, and flavonoids not previously reported in the taxon. The total ion chromatogram (TIC) in negative ion mode of the studied extract is presented in Figure 1.

2.1.1. Sugar Acids and Saccharides

Compound **1** ($[M-H]^-$ at m/z 165.041) gave fragment ions at m/z 147.02 $[M-H-H_2O]^-$, 129.018 $[M-H-2H_2O]^-$, 111.01 $[M-H-3H_2O]^-$, and 101.023 $[M-H-3H_2O-CO]^-$, as well as ions corresponding to the loss of 60 Da $[M-H-C_2H_4O_2]^-$ and 90 Da $[M-H-C_3H_6O_3]^-$, respectively. Thus, compound **1** was annotated as xylonic acid. Analogously, **4** was related to pentose with a base peak at m/z 75.00 ($C_2H_2O_3$). Moreover, **2** (with an additional CH_2 group compared to **4**) and **3** (with additional CH_2O) were identified as hexose and gluconic acid, respectively (Table 1). The identity of asystoside (**5**) was suggested by the transitions 583.261→421.209→289.166→161.445, resulting from the losses of hexosyl (162.053 Da), pentosyl (132.043 Da), and oct-1-en-3-ol units ($C_8H_{16}O$, 128.122 Da) (Table 1). All above-mentioned compounds were previously identified in the species [12].

2.1.2. Carboxylic, Hydroxybenzoic, Hydroxycinnamic Acids and Their Derivatives

Eight hydroxybenzoic acids (**9**, **12**, **15**, **16**, **18**, **19**, **21**, and **24**), four hydroxycinnamic acids (**17**, **20**, **25**, and **26**) and their glycosides (**10**, **11**, **13**, **14**, **22**, and **23**), together with quinic (**6**), oxaloglutaric (**7**), and citric acid (**8**), were identified based on the comparison

with reference standards and literature data in the assayed extract (Table 1) [12,17]. Compound **8** ([M−H]$^-$ at m/z 191.018) showed fragment ions at m/z 173.008 [M−H−H_2O]$^-$, 147.028 [M−H−CO_2]$^-$, a base peak at m/z 111.007 [M−H−CO_2−2H_2O]$^-$, and was related to citric acid [18]. A key step in the dereplication of phenolic acid glycosides was the neutral losses of 162.05, 132.04, and 308.11 Da, corresponding to hexose, pentose, and rutinose, respectively, together with the base peaks of the respective phenolic acid deprotonated molecule. Thus, pentosylhexosides of hydroxybenzoic acid (**10**) and vanillic acid (**11**), dihexoside of caffeic acid (**14**), and hexoside of coumaric acid (**22**) were annotated. Compounds **13** ([M−H]$^-$ at m/z 487.146) and **23** ([M−H]$^-$ at m/z 501.161) gave base peaks at m/z 179.034 [caffeic acid−H]$^-$ and 193.050 [ferulic acid−H]$^-$, corresponding to the loss of rutinose ([M−H−rutinose]$^-$), and were identified as caffeic acid *O*-rutinoside and ferulic acid *O*-rutinoside, respectively (Table 1, Figure 2). Compounds **10**, **11**, **12**, **14**, **15**, **16**, **17**, **18**, **19**, **22**, **23**, **24**, and **25** are reported for the first time in *S. scardica*.

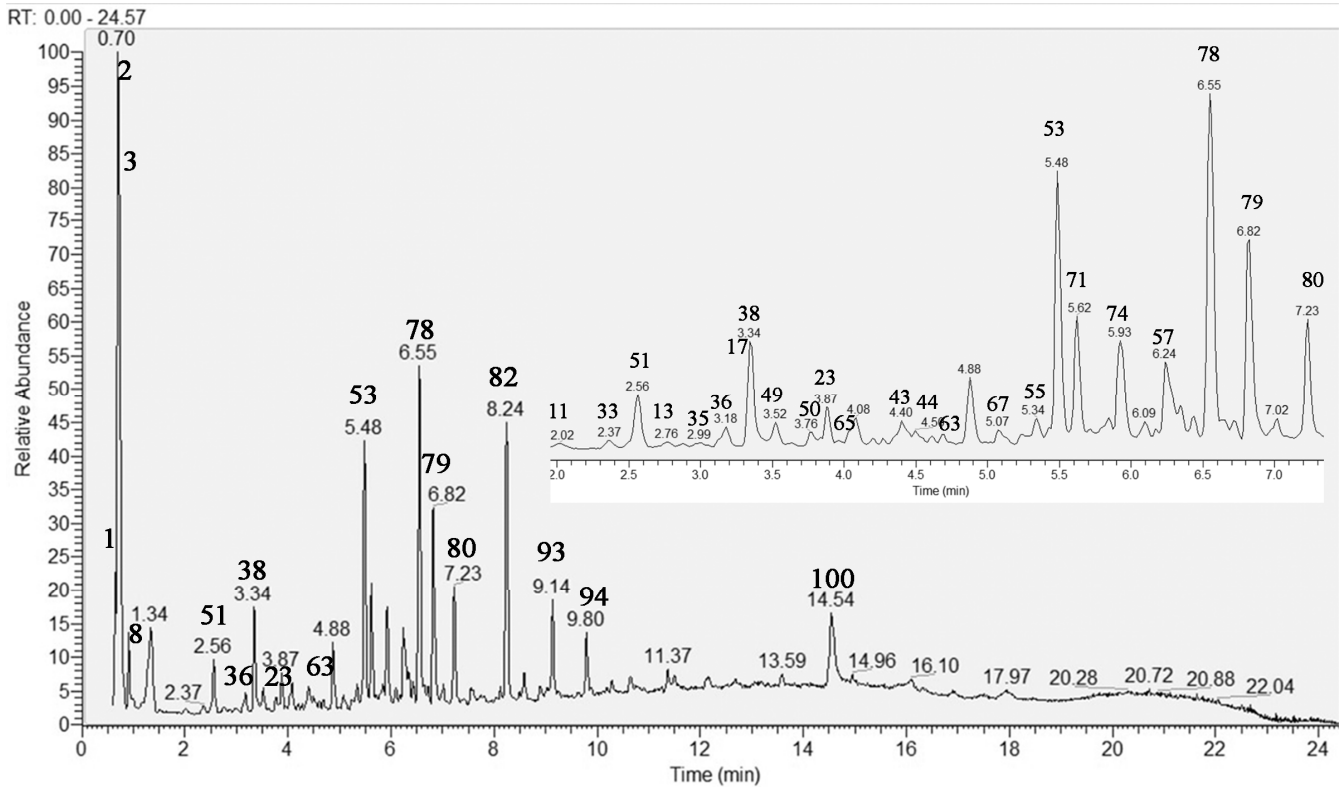


Figure 1. Total ion chromatogram (TIC) in negative ion mode of *Sideritis scardica* extract; the same chromatogram 2–7 min. For compound numbering see Table 1.

2.1.3. Caffeic Acids Oligomers

Caffeic acid oligomers consist of ester-bonded monomers such as danshensu, caffeic acid, and others and are present in Lamiaceae species [19–21]. Based on the accurate masses, MS/MS data, and literature data, a dimer rosmarinic acid (**29**), two trimers (**27** and **30**), and two tetramers (**28** and **31**) were dereplicated in the studied *S. scardica* extract. The fragmentation pattern and retention time of rosmarinic acid (**29**) were compared with reference standard. Key points in the caffeic acid oligomers annotation were the indicative fragment ions derived from the cleavage of *a* and *b* ester bonds with loss of danshensu [M−H−198.05]$^-$, danshensoyl [M−H−180.04]$^-$, and caffeoyl residue [M−H−162.03]$^-$, respectively [19]. Compound **30** ([M−H]$^-$ at m/z 491.099) afforded a base peak at m/z 311.056, corresponding to the easier loss of danshensu, due to the dibenzooxepin structure, restraining the cleavage of the *a* bond. Based on a comparison with literature data, **30** was tentatively identified as isosalvianolic acid C [19] (Table 1, Figure 3). With re-

spect to **27**, an abundant fragment ion at m/z 493.114 $[M-H-CO_2]^-$ and a base peak at m/z 339.059 $[M-H-198.05]^-$ were indicative of the presence of CO_2 group attached to the benzofuran ring and danshensu residue. This allowed us to deduce the structure of lithospermic acid [19,20]. The fragmentation pathway of **28** included prominent ions at m/z 673.157 $[M-H-CO_2]^-$, 537.105 $[M-H-180.04]^-$, 519.095 $[M-H-198.05]^-$, 493.115 $[M-H-180.04-CO_2]^-$, 339.051 $[M-H-2 \times 198.05]^-$, and a base peak at m/z 321.041 $[M-H-198.05-180.04-CO_2]^-$, indicating, consequently, losses of two danshensu residues and carboxyl groups. Moreover, diagnostic ions at m/z 537.093, corresponding to deprotonated lythospermic acid, as well as the lack of loss of caffeoyl residue, suggested a terminal danshensu residue linked to lithospermic acid. Thus, compound **28** was dereplicated as salvianolic acid B (Table 1, Figure 3). Similarly, **31** was related to didehydrosalvianolic acid B (Table 1, Figure 3) [19]. Compounds 27, **28**, 30, and **31** are reported for the first time in *S. scardica*.

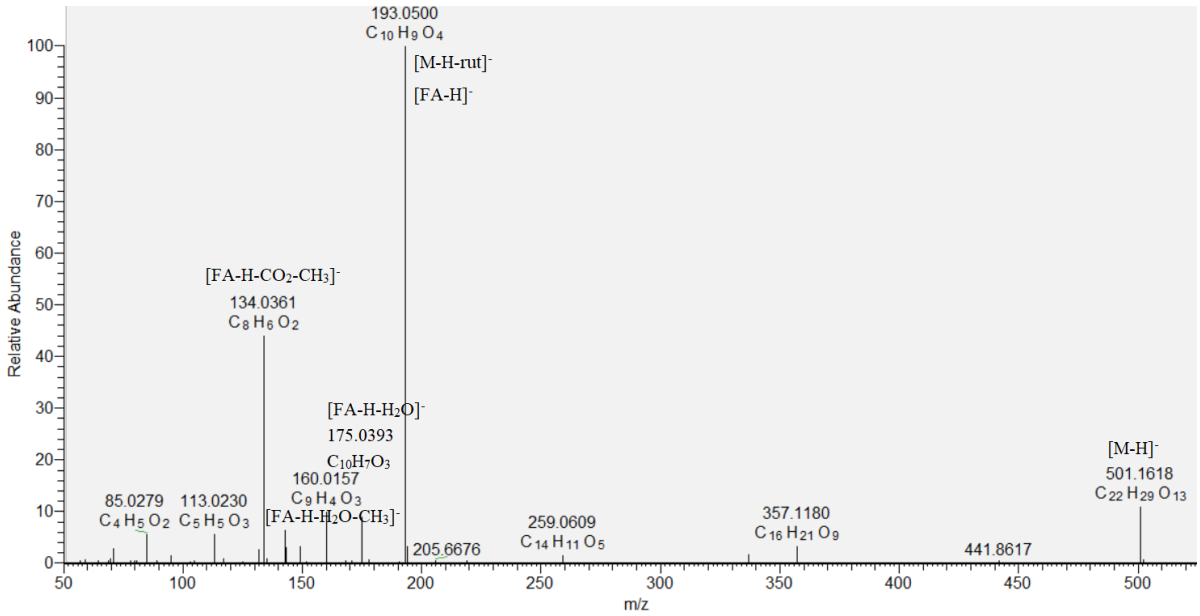


Figure 2. MS/MS spectrum of ferulic acid *O*-rutinoside (**23**).

2.1.4. Acylquinic Acid

Overall, three caffeoylquinic (**33**, **36**, and **38**), four *p*-coumaroylquinic (**35**, **41**, **42**, and **44**), two syringoylquinic (**37** and **40**), three feruloylquinic (**39**, **43**, and **45**), together with two hexosides (**32** and **34**), and a syringoyl–caffeoylquinic acid (**46**) were dereplicated or annotated in the studied extract (Table 1). The acylquinic acids (AQAs) annotation was based on the fragment ions and their relative abundances corresponding to each subclass AQAs [22–24]. Three isobars shared the same deprotonated molecule $[M-H]^-$ at m/z 353.086. Compound **36** was identified as chlorogenic acid (5-caffeylquinic acid) due to the base peak at m/z 191.055 $[\text{quinic acid}-H]^-$. The positional isomer neochlorogenic acid (3-caffeylquinic acid) (**33**) was apparent by the higher relative abundances of the fragment ions at m/z 179.033 (61.2%) and 135.043 (48.7%) than those of **36**. Compounds **33** and **36** were unambiguously identified by comparison with reference standards. In the MS/MS spectrum of **38**, **40**, and **42**, a base peak at m/z 173.044 $[\text{quinic acid}-H-H_2O]^-$ was detected, indicating caffeoyl, syringoyl, and *p*-coumaroyl residues at position 4 of the quinic acid. Thus, **38**, **40**, and **42** were annotated as 4-caffeylquinic, 4-syringoyl, and 4-*p*-coumaroyl acids, respectively [24]. The compounds 3-*p*-coumaroylquinic acid (3-*p*-CoQA) (**35**) and 3-feruloylquinic acid (3-FQA) (**39**) were identified from the base peaks at m/z 163.039 $[p\text{-CoA}-H]^-$ and 193.050 $[FA-H]^-$ (Table 1). Compounds **37**, **41**, and **43** showed a base peak at m/z 191.055 and fragment ions at m/z 197.045 $[\text{syringic acid}-H]^-$, 163.038

[*p*-CoA−H]$^-$, and 193.050 [FA−H]$^-$, respectively, and were identified as 5-syringoylquinic, 5-*p*-coumaroylquinic, and 5-feruloylquinic (FQA) acids [22–24] (Table 1).

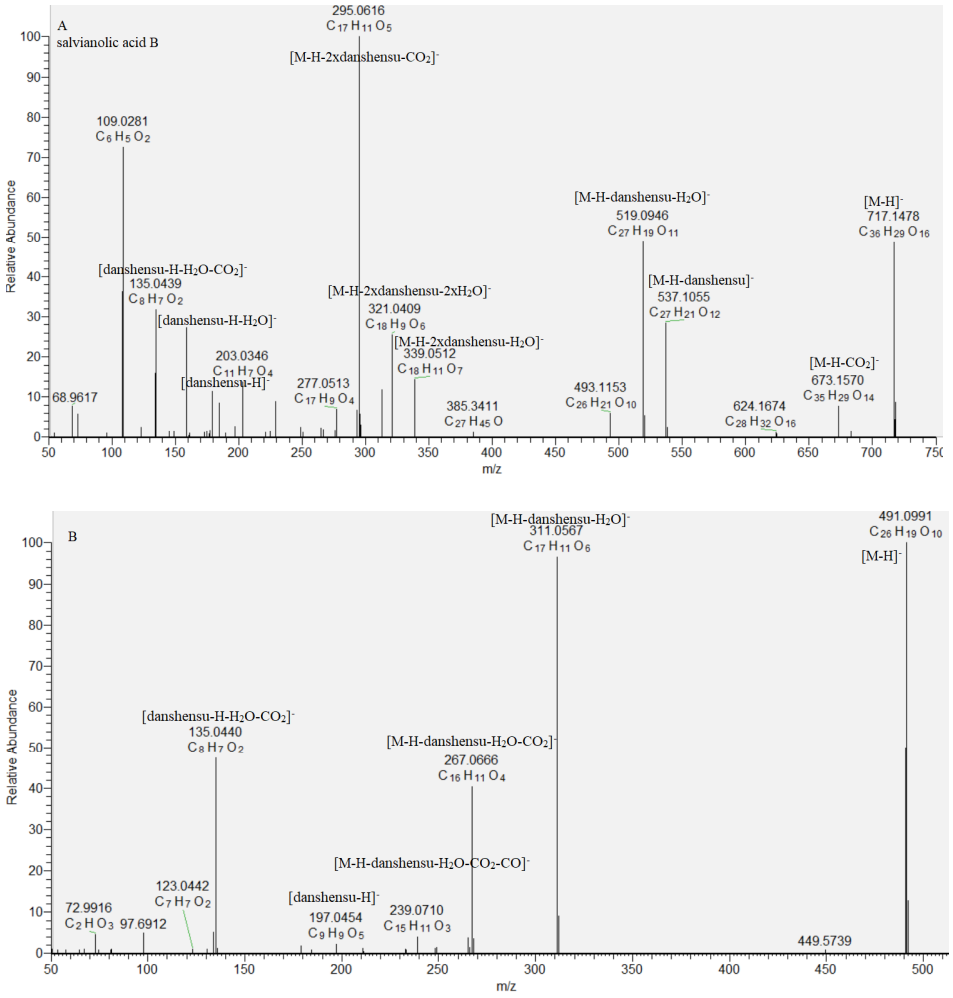


Figure 3. MS/MS spectrum of (**A**) salvianolic acid B (**28**) and (**B**) isosalvianolic acid C (**30**).

With respect to compound **46**, the base peak at 197.045 [syringic acid-H]$^-$, together with a diagnostic fragment ion at *m*/*z* 335.077 [CQA−H−H_2O]$^-$ indicated syringoyl–caffeoylquinic acid. Additionally, two hexosides of neochlorogenic (**32**) and chlorogenic acid (**34**) were also dereplicated (Table 1).

2.1.5. Acylhexaric Acids

Key steps in the acylhexaric acids annotation were the subsequent losses of one hydroxydihydrocaffeoyl (**47**, **48**) and two (**49**, **50**) hydroxydihydrocaffeoyl and syringoyl residues (Table 1, Figure 4). Thus, the base peak in the MS/MS spectra was consistent with [hexaric acid (HA)−H]$^-$ at *m*/*z* 209.030 ($C_6H_9O_8$) supported by the series of indicative ions at *m*/*z* 191.019 [HA-H-H_2O]$^-$, 147.029 [HA−H−H_2O−CO_2]$^-$, 129.018 [HA−H−2H_2O−CO_2]$^-$, 111.007 [HA−H−3H_2O−CO_2]$^-$, and 85.028 [HA−H−2H_2O−2CO_2]$^-$ (Table 1) [25]. Compounds **49** and **50** shared the same [M−H]$^-$ at *m*/*z* 569.116. They formed the prominent fragment ions at *m*/*z* 389.073 [M−H−180.04]$^-$, 371.063 [M−H−198.05]$^-$, and 209.030 [M−2 × 180.04]$^-$, resulting from the concomitant loss of hydroxydihydrocaffeoyl and syringoyl residues. Syringoyl moiety was suggested by the fragment ions at *m*/*z* 197.045 [syringic acid (SA)−H]$^-$, 182.021 [(SA−H)−CH_3]•$^-$ and 153.055 [(SA−H)−CO_2]$^-$. Compounds **49** and **50** were identified as isomeric hydroxydihydrocaffeoyl–syringoyl–hexaric acids (Table 1, Figure 4). Acylhexaric acids are reported for the first time in *S. scardica*.

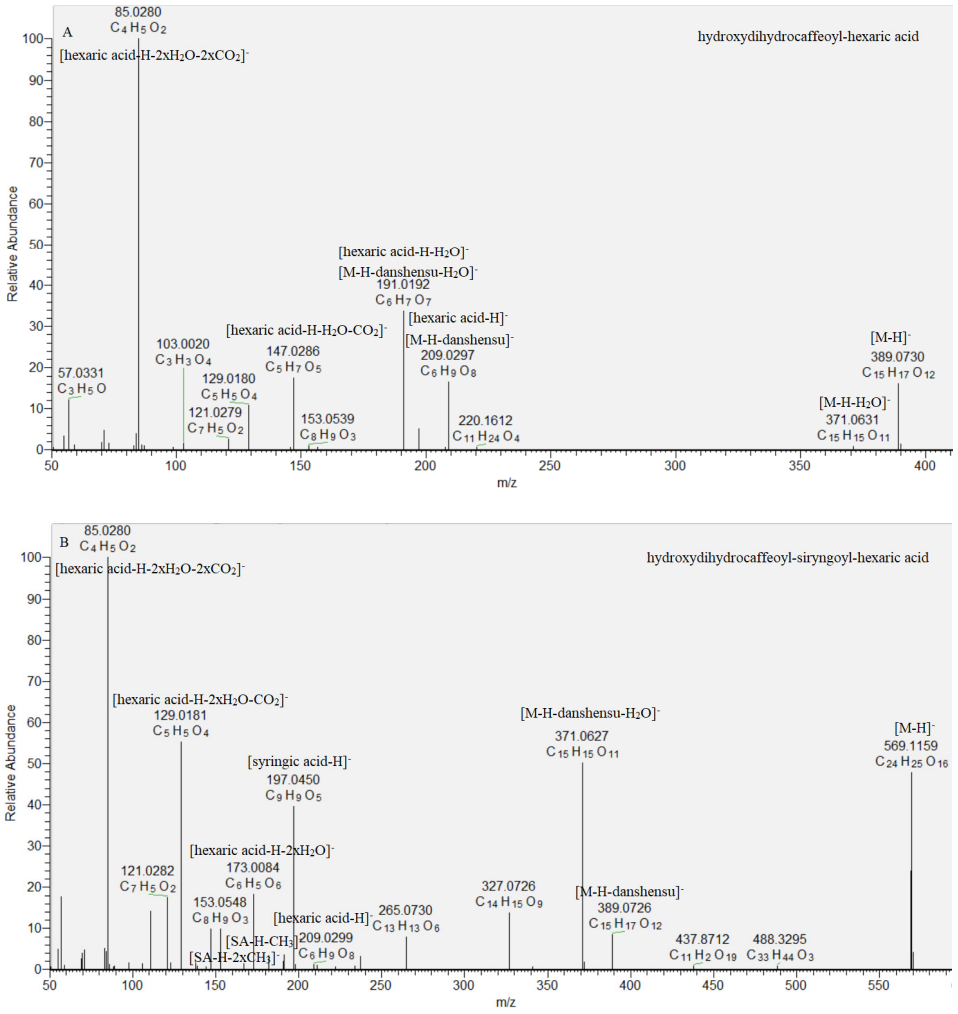


Figure 4. MS/MS spectrum of (**A**) hydroxydihydrocaffeoyl–hexaric acid (**47**) and (**B**) hydroxydihydrocaffeoyl–siryngoyl–hexaric acid (**49**).

2.1.6. Phenylethanoid Glycosides

A class of secondary metabolites distinctive for *Sideritis* species were phenylethanoid glycosides [12]. The typical fragmentation pattern revealed the loss of 162.05, 146.05, 179.03, 18.01 Da—corresponding to glucosyl and rhamnosyl moieties—deprotonated caffeic acid, and H_2O, respectively. Detailed discussion on the MS/MS fragmentation has been previously provided [12]. Based on a comparison with literature data, 10 phenylethanoid glycosides were dereplicated in the studied *S. scardica* extract (Table 1).

2.1.7. Iridoid Glycosides

The characteristic loss of hexose (−162.05 Da) and 7-(hydroxymethyl)-4,5-dihydrocyclopentapyran-4,5-diol (−182.06 Da, $C_9H_{10}O_4$) indicated the presence of iridoid glycosides [12]. Compound **61** with deprotonated molecules at m/z 523.166 was dereplicated as melittoside [12]. Fragment ions at m/z 163.039, 179.034, and 193.050 corresponding to the deprotonated coumaric, caffeic, and ferulic acids, led to the identification of **62**, **63**, and **64** as *p*-coumaroylmelittoside, caffeoylmelitoside, and feruloylmelitosside, respectively. Compounds **63** and **64** were found for the first time in *S. scardica* (Table 1). Previously, **63** has been isolated from *S. lanata* [26], while **64** has been found in *S. trojana* [27].

2.1.8. Flavonoids

Flavonoids are the dominant secondary metabolites in *Sideritis* species [12]. The main flavonoids in the studied species were *O*-glycosides of the flavones isoscutellarein

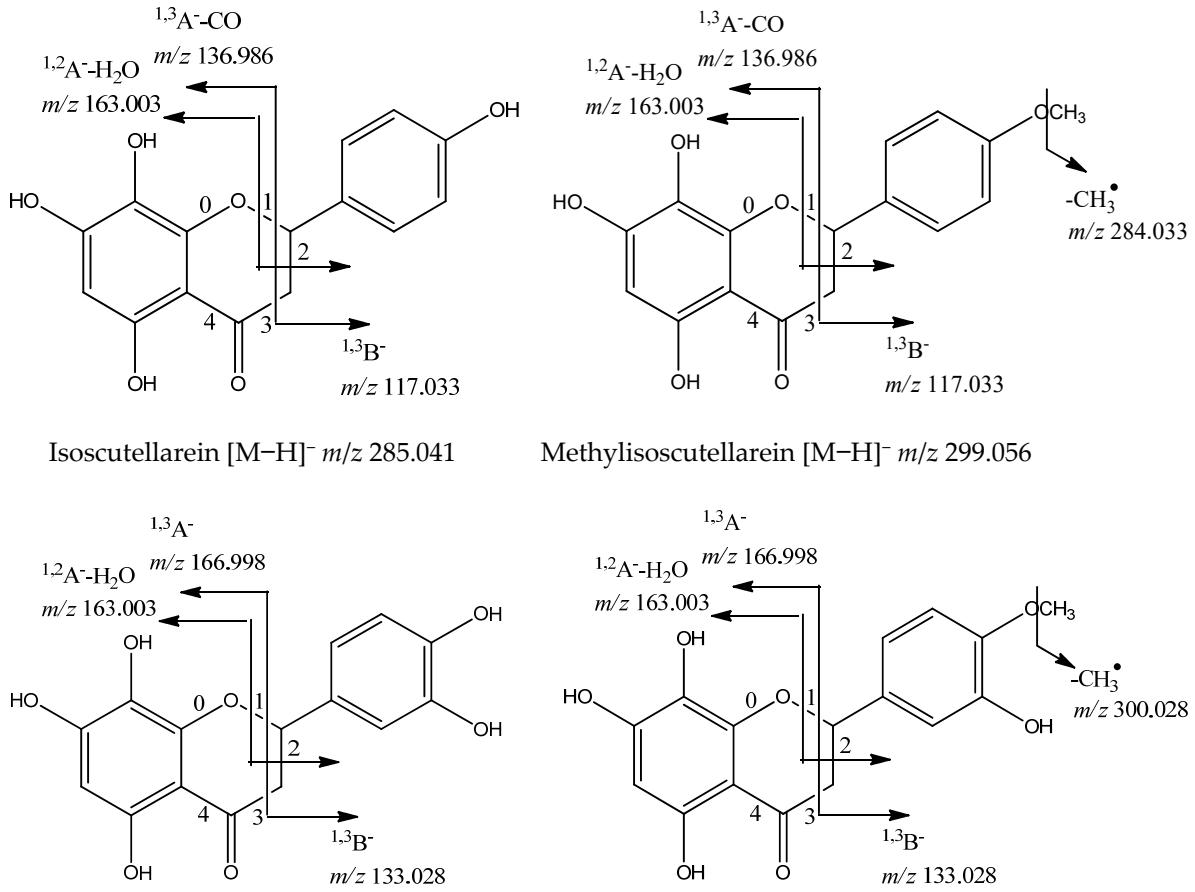
([Agl−H]$^-$ at *m*/*z* 285.041), methylisoscutellarein ([Agl−H]$^-$ at *m*/*z* 299.056), hypolaetin ([Agl−H]$^-$ at *m*/*z* 301.036), methylhypolaetin ([Agl−H]$^-$ at *m*/*z* 315.052), and apigenin ([Agl−H]$^-$ at *m*/*z* 285.041). The MS/MS fragmentation patterns of the glycosides have been described previously [12] and are based on the loss of 162.054, 324.106, and 42.016 Da, corresponding to O-hexose/dihexose/acetyl group, respectively. The occurrence of a 180 Da loss [M−H-hex-H_2O]$^-$ was indicative of 1→2 glycosylation between two sugars [16]. Significant fragments in the flavone aglycone annotation were a series of neutral losses of CO (−28 Da), CO_2 (−44 Da), CH_2O (−30 Da), and H_2O (−18 Da), supported by the retro-Diels–Alder (rDA) cleavages $^{0,4}A^-$, $^{1,2}A^-$, $^{1,3}A^-$, $^{1,2}B^-$, and $^{1,3}B^-$ [22,23]. In general, five glycosides of isoscutellarein (IS) (**70**, **71**, **72**, **78**, **83**), three of methylisoscutellarein (MIS) (**80**, **82**, **88**), two of hypolaetin (HL) (**67**, **73**), four of methylhypolaetin (MHL) (74, **76**, **79**, and **84**), five of apigenin (Api) (**65**, **69**, **75**, **77**, **86**), and one of luteolin (Lu) (**66**) were dereplicated in the *S. scardica* extract (Table 1). Apigenin and luteolin were evidenced by the rDA ions at *m*/*z* 151.002 ($^{1,3}A^-$), 107.012 ($^{0,4}A^-$), 117.033 ($^{1,3}B^-$) (Api), and 133.028 ($^{1,3}B^-$) (Lu). The aglycones isoscutellarein and hypolaetin were deduced from the rDA cleavages $^{1,2}A^-$-H_2O at *m*/*z* 163.003, $^{1,3}A^-$-CO at *m*/*z* 136.986, as well as $^{1,3}B^-$ at *m*/*z* 117.033 (IS) and $^{1,3}B$- at *m*/*z* 133.028 (HL). Their methoxylated derivatives revealed a fragment ion [Agl−H−CH_3]•$^-$ at *m*/*z* 284.033 (MIS) and 300.028 (MHL). Compound **70** ([M−H]$^-$ at *m*/*z* 579.136) gave a base peak at *m*/*z* 285.041 [M−H−pent-hex]$^-$. Thus, **70** was related to isoscutellarein *O*-pentosylhexoside. Illustrations of the fragmentation pathways of glycosides of the four abovementioned flavones aglycons are presented in Figure 5. The flavanone naringenin [M−H]$^-$ at *m*/*z* 271.061 (**85**) was identified based on the RDA fragments at *m*/*z* 151.002 ($^{1,3}A^-$), 107.012 ($^{0,4}A^-$), and 119.049 ($^{1,3}B^-$). In addition, its dihexoside (**68**) and coumaroylhexoside (**87**) were annotated based on the neutral loss of two hexoses and coumaroylhexose, respectively. Compounds **68**, **70**, **85**, and **87** were annotated for the first time.

2.1.9. Fatty Acids and Organosulfur Compounds

A saturated (**98**), two monounsaturated (**94** and **97**), and four polyunsaturated (**93**, **95**, **96**, and **99**) fatty acids were tentatively identified in *S. scardica* extract. Among them, the main fatty acids were trihydroxyoctadecadienoic acid (**93**) and trihydroxyoctadecenoic acid (**94**), previously reported for the species [12]. The dereplication and fragmentation pathway of dihydroxy fatty acids have been previously described [28]. In addition, four organosulfur compounds (**100**–**103**) were dereplicated based on fragment ions at *m*/*z* 96.959 [HO_4S]$^-$ and 79.956 [O_3S]$^-$. These compounds have been previously described for *S. scardica* [12].

2.2. Quantitative Determination

The quantitative determination of the main compounds in the profile of *S. scardica* lyophilized infusion was based on a common approach, where the HPLC analysis of the analytes was performed with a mobile phase composed of formic acid acetonitrile and water [22]. The content of the assayed compounds is revealed in Table 1. The main compounds in the tested lyophilized infusion were isoscutellarein-7-*O*-hexosyl-(1→2)-[6″-O-acetyl]-hexoside (**78**) and verbascoside (**53**), followed by 4′-*O*-methylisoscutellarein-7-*O*-[6‴-*O*-acetyl]hexosyl-(1→2)hexoside (**82**). Other dominant phenolic compounds include caffeic acid (**20**), 4′-methylhypolaetin-7-*O*-acetyl–hexosyl–hexoside (**79**), and isoscutellarein 7-*O*-hexosyl (1→2)-hexoside (**71**) (Table 1). Moreover, the data reveled moderate quantity of the phenylethanoid glycosides leucoseptoside A (**57**) and martynoside (**60**), as well as iridoid glycoside melittoside (**61**) (Table 1). The content of phenylethanoid glycosides ranged from 0.74 mg/g (**54**) to 151.54 mg/g lyophilized infusion (li) (**53**). With respect to caffeic acid oligomers, their quantities were found to range from 0.19 ± 0.033 mg/g li (**31**) to 6.07 ± 0.46 mg/g li (**29**), while caffeoylhexaric acids ranged from 0.81 ± 0.075 (**48**) to 3.53 ± 0.237 (**50**) (Table 1). However, this is the first attempt to quantify the secondary metabolites of the above classes in *Sideritis* species.



Isoscutellarein [M−H]$^-$ *m/z* 285.041 Methylisoscutellarein [M−H]$^-$ *m/z* 299.056

Hypolaetin [M−H]$^-$ at *m/z* 301.036 Methylhypolaetin [M−H]$^-$ at *m/z* 315.052

Figure 5. Fragmentation pathways for flavones aglycons caused by cleavage of C-ring bonds in negative ion mode.

4-Caffeoylquinic acid (**38**) was found to be the dominant acylquinic acid (7.65 ± 0.96 mg/g li), followed by chlorogenic (**36**) and 5-feruloylquinic acid (**43**). Earlier quantitative research on the genus *Sideritis* showed that 5-caffeoylquinic acid has been found in all studied species as the most abundant hydroxycinnamic acid. In addition, the dominant phenolic compounds were the isoscutellarein derivatives isoscutellarein 7-*O*-[6‴-*O*-acetyl]-allosyl-(1→2)glucoside and 4′-*O*-methylisoscutellarein 7-*O*-allosyl-(1→2)-[6″-O-acetyl]-glucoside. Recently, eight compounds were detected in different *Sideritis* species: 5-caffeoylquinic acid, lavandulifolioside, verbascoside, isoscutellarein 7-O-allosyl(1→2)glucoside, hypolaetin 7-O-[6″-O-acetyl]-allosyl(1→2)glucoside, isoscutellarein 7-O-[6″-O-acetyl]-allosyl(1→2) glucoside, 3′-O-methylhypolaetin 7-O-[6″-O-acetyl]-allosyl(1→2)glucoside, 4′-O-methylhypolaetin, and 7-O-[6″-O-acetyl]-allosyl-(1→2)-[6″-O-acetyl]-glucoside). They represent 50% to 80% of the total phenolic content in *S. scardica*, *S. raeseri*, *S. syriaca*, and *S. Taurica*, and up to 90% in *S. lanata* [6]. The most abundant compounds present in the analyzed *Siderits* samples belonged to the group of phenylethanoid glycosides. The content of phenylethanoid glycosides ranged from 1.22 mg/g dry herb for *S. lanata* to 108.3 mg/g dry herb for *S. scardica* from Rhodopi Mountain, Bulgaria. The contribution of phenylethanoid glycosides to total phenolic content was around 50% for all samples, except for *S. lanata* where it accounted only for around 7% [6]. Eleven acetylated glycosides of isoscutellarein, hypolaetin, methylhypolaetin and methylisoscutellarein were previously isolated from 80% EtOH extract [30]. The differences between previous studies and our results can be ascribed to the different extraction methods and solvents.

Table 1. UHPLC–HRMS metabolite profiling of *Sideritis scardica* infusion with content (mg/g lyophilized infusion) of compounds assayed.

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass $[M-H]^-$	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
				Sugar acids and saccharides				
1.	Xylonic acid	$C_5H_{10}O_6$	165.0405	165.0395 (60.5), 147.0288 (8.8), 129.0181 (12.3), 111.0073 (0.3), 105.0179 (33.1), 101.0228 (2.7), 87.0073 (43.1), 75.0072 (100)	0.68	−6.006	2	-
2.	Hexose	$C_6H_{12}O_6$	179.0561	179.0541 (68.6), 161.0444 (11.1), 143.0338 (10.1), 125.0231 (1.53), 99.0437 (1.71), 81.0331 (4.8), 75.0072 (100)	0.70	−4.975	2	-
3.	Gluconic acid	$C_6H_{12}O_7$	195.0510	195.0503 (96.1), 177.0396 (18.6), 159.0288 (14.7), 147.0287 (15.5), 141.0184 (5.9), 129.0180 (48.3), 111.0073 (5.3), 105.0179 (332.8), 75.0072 (100)	0.72	−3.619	2	-
4.	Pentose	$C_5H_{10}O_5$	149.0456	149.0444 (21.6), 131.0334 (5.2), 101.0224 (0.8), 89.0229 (17.8), 75.0072 (100)	0.74	−7.358	2	-
5.	Asystoside	$C_{25}H_{44}O_{15}$	583.2607	583.2618 (100), 421.2090 (4.9), 289.1662 (15.4), 161.0445 (15.8), 101.0230 (21.3), 71.0123 (30.6)	6.34	1.880	2	-
				Carboxylic acids				
6.	Quinic acid	$C_7H_{12}O_6$	191.0561	191.0553 (100), 173.0446 (1.8), 155.0340 (0.2), 137.022 (0.2), 127.0387 (3.3), 99.0438 (0.6), 93.0331 (5.6), 85.0279 (18.2), 71.0123 (1.6), 59.0123 (1.37)	0.69	−4.194	2	-
7.	Oxaloglutaric acid	$C_7H_8O_7$	203.0197	203.0191 (100), 159.0292 (2.3), 141.0181 (27.8), 115.0022 (11.7), 97.0279 (97.1), 95.0123 (14.2), 79.0174 (11.2), 72.9915 (7.1), 71.0123 (50.6), 69.0330 (66.2)	0.88	−2.984	2	-
8.	Citric acid	$C_6H_8O_7$	191.0197	191.0191 (2.6), 173.0084 (1.43), 154.9979 (0.7), 147.0286 (0.4), 129.0181 (6.1), 111.0074 (100), 101.0231 (0.7), 87.0073 (43.8), 85.0280 (27.0)	0.90	−3.119	2	-

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
Hydroxybenzoic, hydroxycinnamic acids, and their derivatives								
9.	Gallic acid	$C_7H_6O_5$	169.0143	169.0133 (37.5), 125.0231 (100), 97.0280 (3.8), 69.0330 (4.9)	1.13	−5.660	1	3.45 ± 0.51
10.	Hydroxybenzoic acid *O*-pentosylhexoside [a]	$C_{18}H_{24}O_{12}$	431.1194	431.1200 (69.3), 299.0776 (2.3), 137.0232 (100), 93.0331 (73.0)	1.78	1.231	2	1.32 ± 0.13
11.	Vanillic acid *O*-pentosylhexoside [a]	$C_{19}H_{26}O_{13}$	461.1301	461.1310 (76.2), 329.0879 (1.3), 167.0340 (100), 152.0104 (52.4), 123.0438 (11.3), 108.0203 (27.4)	1.99	2.052	2	2.27 ± 0.35
12.	Protocatechuic acid [a]	$C_7H_6O_4$	153.0193	153.0182 (15.9), 123.0439 (0.1), 109.0281 (100), 81.0330 (1.4), 65.0380 (0.38)	2.01	−7.397	1	7.98 ± 0.54
13.	Caffeic acid *O*-rutinoside (swertiamacroside)	$C_{21}H_{28}O_{13}$	487.1457	487.1465 (28.9), 179.0342 (100), 161.0234 (14.4), 135.0439 (45.5), 113.0232 (2.9)	2.80	1.572	2	4.68 ± 0.45
14.	Caffeic acid *O*-dihexoside [a]	$C_{21}H_{28}O_{14}$	503.1406	503.1414 (34.7), 341.0878 (42.9), 179.0341 (100), 135.0438 (77.1)	2.82	1.513	2	-
15.	2,3-Dihydroxybenzoic acid [a]	$C_7H_6O_4$	153.0193	153.0182 (51.9), 123.0074 (26.7), 108.0203 (100), 95.0124 (32.1), 85.0280 (33.1)	2.95	−7.267	2	-
16.	*p*-Hydroxybenzoic acid [a]	$C_7H_6O_3$	137.0244	137.0231 (35.2), 108.0200 (3.5), 93.0331 (100)	2.97	−9.614	1	-
17.	Dihydrocaffeic acid [a]	$C_9H_{10}O_4$	181.0506	181.0499 (52.1), 163.0377 (0.4), 137.0596 (100), 135.0439 (19.6), 123.0436 (58.0), 121.0282 (24.9), 119.0489 (15.1), 109.0281 (27.6), 93.0332 (2.5), 59.0124 (86.3)	3.33	−4.154	2	-
18.	2,4-Dihydroxybenzoic acid [a]	$C_7H_6O_4$	153.0193	153.0182 (76.8), 135.0075 (29.7), 123.0439 (0.26), 109.0281 (100), 108.0201 (0.2), 91.0174 (5.6), 81.0333 (0.3), 65.0381 (14.5)	3.47	−7397	1	4.77 ± 0.74
19.	*p*-Hydroxyphenyl acetic acid [a]	$C_8H_8O_3$	151.0400	151.0389 (100), 136.0156 (20.1), 123.0075 (4.6), 109.0283 (11.5), 107.0489 (2.3)	3.49	−7.133	1	-
20.	Caffeic acid	$C_9H_8O_4$	179.0350	179.0341 (21.3), 135.0439 (100), 117.0335 (0.7), 107.0487 (1.35), 91.0537 (0.5)	3.54	−4.759	1	87.25 ± 6.54
21.	Gentisic acid	$C_7H_6O_4$	153.0193	153.0182 (45.9), 109.0281 (100), 91.0175 (1.1), 108.0203 (8.7), 81.0330 (1.9), 65.0382 (0.1)	3.65	−7.397	1	0.42 ± 0.05
22.	Ferulic acid *O*-rutinoside [a,b]	$C_{22}H_{30}O_{13}$	501.1614	501.1618 (10.2), 193.0500 (100), 175.0393 (8.4), 160.0157 (9.7), 134.0361 (43.4), 113.0230 (5.5)	3.78	3.125	2	-

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
23.	*p*-Coumaric acid *O*-hexoside [a]	$C_{15}H_{18}O_8$	325.0928	163.0391 (100), 145.0288 (4.14), 119.0488 (35.7)	3.81	3.197	2	-
24.	Syringic acid [a]	$C_9H_{10}O_5$	197.0455	197.0449 (40.0), 182.0213 (48.9), 153.0547 (100), 138.0311 (11.5), 123.0075 (26.7), 121.0281 (85.6), 106.0046 (9.67), 95.0123 (8.1), 89.0018 (14.97)	4.34	−3.231	2	1.69 ± 0.26
25.	*O*-coumaric acid [a]	$C_9H_8O_3$	163.0401	163.0390 (9.7), 135.0075 (0.2), 119.0489 (100)	4.55	−6.363	1	-
26.	Ferulic acid	$C_{10}H_{10}O_4$	193.0506	193.0499 (100), 178.0260 (14.1), 165.0545 (13.5), 149.0600 (21.4), 134.0358 (12.3), 123.0438 (92.0), 79.0538 (4.1)	5.17	−3.637	1	-
	Caffeic acid oligomers							
27.	Lithospermic acid [a]	$C_{27}H_{22}O_{12}$	537.1038	537.1035 (7.1), 493.1142 (30.4), 339.0515 (100), 313.0726 (8.8), 295.0613 (23.6), 267.0671 (10.9), 179.0345 (12.4), 135.0440 (49.3)	4.97	−0.743	2	0.51 ± 0.05
28.	Salvianolic acid B [a]	$C_{36}H_{30}O_{16}$	717.1460	717.1478 (49.4), 673.1570 (7.7), 537.1055 (28.0), 519.0946 (47.9), 493.1153 (5.9), 339.0512 (13.0), 321.0409 (26.0), 313.0717 (11.5), 295.0616 (100), 277.0513 (6.7), 229.0141 (8.7), 203.0346 (13.9), 197.0447 (2.5), 179.0340 (11.3), 161.0237 (1.1), 135.0439 (32.2), 109.0281 (71.9)	6.06	2.401	2	1.42 ± 0.01
29.	Rosmarinic acid	$C_{18}H_{16}O_8$	359.0772	359.0782 (14.1), 197.0449 (26.0), 179.0342 (11.3), 161.0233 (100), 135.0439 (14.64), 133.0282 (19.8), 109.0275 (0.4)	6.33	2.56	1	6.07 ± 0.46
30.	Isosalvianolic acid C [a]	$C_{26}H_{20}O_{10}$	491.0983	491.0991 (100), 311.0567 (98.4), 267.0666 (39.3), 265.0508 (3.9), 249.0559 (1.5), 197.0454 (2.2), 179.0339 (1.8), 135.0440 (48.7)	7.29	1.466	2	0.42 ± 0.03
31.	Didehydrosalvianolic acid B [a]	$C_{36}H_{28}O_{16}$	715.1305	715.1324 (52.4), 535.0894 (20.2), 517.0786 (5.4), 337.0357 (8.1), 319.0241 (12.4), 311.0575 (7.6), 293.0461 (100), 265.0503 (7.1), 197.0446 (8.9), 135.0438 (10.5), 109.0279 (5.5)	7.76	2.786	2	0.19 ± 0.03

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
				Acylquinic acids				
32.	(Neo)chlorogenic acid *O*-hexoside [a]	$C_{22}H_{28}O_{14}$	515.1406	515.1414 (52.2), 353.0883 (5.6), 191.0553 (100), 179.0351 (3.4), 135.0441 (4.0), 93.0332 (11.0)	2.13	1.536	2	-
33.	Neochlorogenic acid	$C_{16}H_{18}O_9$	353.0877	353.0883 (40.7), 191.0555 (100), 179.0341 (61.2), 173.0451 (3.1), 161.0235 (3.5), 135.0439 (48.7), 93.0331 (4.5)	2.36	0.575	1	-
34.	Chlorogenic acid *O*-hexoside [a]	$C_{22}H_{28}O_{14}$	515.1406	515.1414 (100), 323.0767(51.9), 191.0554 (94.8), 179.0327 (4.5), 161.0238 (33.5), 135.0434 (6.4), 111.0435 (4.0)	2.85	2.487	1	-
35.	3-*p*-coumaroylquinic acid [a]	$C_{16}H_{18}O_8$	337.0928	337.0932 (6.9), 191.0555 (6.9), 173.0449 (3.5), 163.0390 (100), 135.0437 (0.5), 119.0488 (26.5), 111.0438 (0.7), 93.0332 (0.8)	2.99	0.918	2	-
36.	Chlorogenic acid	$C_{16}H_{18}O_9$	353.0877	353.0879 (4.6), 191.0554 (100), 179.0380 (0.9), 161.0235 (1.9), 111.0437 (0.8), 93.0331 (2.7), 85.0280 (7.5)	3.17	0.325	1	5.22 ± 0.21
37.	5-Syringoylquinic acid [a]	$C_{16}H_{20}O_{10}$	371.0983	371.0987 (36.1), 197.0448 (4.9), 191.0554 (100), 173.0443 (14.6), 153.0538 (2.3), 121.0279 (9.4), 111.0435 (2.8), 93.0331 (31.6), 85.0279 (3.7)	3.30	0.862	3	-
38.	4-Caffeoylquinic acid [a]	$C_{16}H_{18}O_9$	353.0877	353.0883 (32.7), 191.0555 (39.50), 179.0342 (75.3), 173.0447 (100), 135.0439 (52.1), 111.0436 (3.3), 93.0331 (21.3)	3.36	0.515	2	7.65 ± 0.96
39.	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	367.1034	367.1036 (19.5), 193.0500 (100), 173.0447 (4.7), 137.0226 (3.4), 134.0361 (56.7)	3.42	0.339	2	0.63 ± 0.04
40.	4-Syringoylquinic acid [a]	$C_{16}H_{20}O_{10}$	371.0983	371.0978 (9.8), 197.0456 (11.5), 191.0554 (100), 173.0443 (14.6), 153.0538 (2.3), 121.0283 (4.9), 111.0435 (2.8), 93.0331 (14.5)	3.43	−1.509	3	-
41.	5-*p*-Coumaroylquinic acid [a]	$C_{16}H_{18}O_8$	337.0928	337.0940 (8.9), 191.0554 (100), 173.0446 (6.6), 163.0390 (5.31), 119.0488 (4.8), 111.0437 (2.0), 93.0331 (16.7)	3.96	3.172	2	1.85 ± 0.03
42.	4-*p*-Coumaroylquinic acid [a]	$C_{16}H_{18}O_8$	337.0928	337.0941 (8.6), 191.0555 (2.7), 173.0445 (100), 163.0390 (18.5), 119.0488 (9.0), 111.0436 (3.1), 93.0330 (22.2)	4.02	3.558	2	1.82 ± 0.05

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
43.	5-Feruloylquinic acid	$C_{17}H_{20}O_9$	367.1034	367.1038 (23.7), 193.0500 (16.9), 191.0554 (100), 173.0446 (81.4), 155.0340 (3.8), 134.0361 (19.2), 111.0438 (4.9), 93.0331 (39.7)	4.38	0.912	2	5.13 ± 0.08
44.	1-*p*-Coumaroylquinic acid [a]	$C_{16}H_{18}O_8$	337.0928	337.0932 (7.2), 191.0554 (100), 173.0445 (100), 135.0447 (2.3), 163.0393 (0.5), 119.0487 (1.1), 111.0437 (0.5), 93.0331 (5.3)	4.60	1.007	2	0.56 ± 0.04
45.	1-Feruloylquinic acid	$C_{17}H_{20}O_9$	367.1034	367.1039 (10.2), 191.0554 (100), 179.0340 (0.5), 173.0448 (2.3), 161.0239 (0.3), 134.0360 (3.1), 111.0440 (1.41), 93.0331 (5.15)	4.90	−2.996	2	1.12 ± 0.16
46.	Syringoyl–caffeoylquinic acid [a]	$C_{25}H_{26}O_{13}$	533.1300	533.1305 (14.5), 335.0777 (44.6), 291.0875 (20.7), 197.0450 (100), 153.0546 (12.6), 137.0232 (38.4), 123.0073 (17.0), 111.0439 (10.4), 93.0331 (49.0)	6.90	0.799	2	0.81 ± 0.08
				Acylhexaric acids				
47.	Hydroxydihydrocaffeoyl–hexaric acid [a,b]	$C_{15}H_{18}O_{12}$	389.0725	389.0730 (16.8), 371.0631 (0.8), 209.0297 (16.8), 191.0192 (34.2), 153.0539 (1.0), 147.0286 (17.9), 129.0180 (10.8), 85.0280 (100)	1.65	1.236	3	1.12 ± 0.16
48.	Hydroxydihydrocaffeoyl–hexaric acid isomer [a,b]	$C_{15}H_{18}O_{12}$	389.0725	389.0728 (16.8), 371.0621 (2.7), 209.0294 (17.2), 197.0442 (3.7), 191.0191 (45.3), 173.0087 (3.1), 147.0286 (20.6), 129.0180 (13.5), 111.0079 (4.5), 85.0280 (100)	2.60	0.773	3	0.81 ± 0.08
49.	Hydroxydihydrocaffeoyl–siryngoyl–hexaric acid [a,b]	$C_{24}H_{26}O_{16}$	569.1148	569.1159 (49.1), 389.0726 (8.8), 371.0627 (52.7), 327.0726 (13.8), 209.0299 (1.4), 197.0450 (39.1), 191.0186 (3.7), 182.0211 (3.3), 173.0084 (18.4), 166.9975 (1.6), 147.0285 (10.0), 138.0309 (1.7), 129.0181 (54.5), 123.0072 (1.6), 121.0282 (17.5), 111.0073 (14.3), 97.6908 (1.7), 85.0280 (100)	3.53	1.937	3	1.77 ± 0.15
50.	Hydroxydihydrocaffeoyl–siryngoyl–hexaric acid isomer [a,b]	$C_{24}H_{26}O_{16}$	569.1148	569.1158 (43.9), 389.0731 (7.6), 371.0625 (42.8), 327.0728 (12.2), 209.0295 (1.13), 197.0450 (40.8), 191.0186 (3.8), 182.0214 (4.3), 173.0084 (15.4), 166.9978 (1.0), 153.0548 (10.2), 147.0288 (8.0), 138.0315 (2.2), 129.0181 (59.1), 123.0074 (3.6), 121.0281 (16.5), 111.0073 (12.5), 85.0280 (100)	3.77	2.148	3	3.53 ± 0.237

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
				Phenylethanoid glycosides				
51.	Decaffeoyl aceteoside/verbasoside	$C_{20}H_{30}O_{12}$	461.1664	461.1673 (100), 315.1085 (5.2), 297.0984 (2.6), 135.0439 (29.4), 113.0230 (46.0), 85.0280 (20.6), 71. 0123 (20.7)	2.57	0.881	2	35.09 ± 2.46
52.	Hydroxyverbascoside	$C_{29}H_{36}O_{16}$	639.1931	639.1946 (86.8), 621.1832 (5.3), 459.1533 (1.9), 179.0342 (36.0), 161.0232 (5.3), 135.0440 (22.3), 133.0283 (37.6), 113.0231 (8.6)	4.49	2.381	2	13.45 ± 0.47
53.	Verbascoside	$C_{29}H_{36}O_{15}$	623.1987	623.1996 (60.3), 461.1674 (9.3), 315.1078 (1.80), 179.0340 (2.4), 161.0234 (100), 135.0440 (8.7), 133.0283 (23.9)	5.48	2.305	2	151.54 ± 10.86
54.	Echinacoside	$C_{35}H_{46}O_{20}$	785.2509	785.2529 (72.5), 623.2175 (7.7), 461.1666 (6.3), 179.0349 (3.6), 161.0234 (100), 135.0440 (24.9), 133.0282 (47.0)	5.23	2.424	2	0.74 ± 0.005
55.	Forsythoside B/samioside/ lavandulifolioside	$C_{34}H_{44}O_{19}$	755.2403	755.2424 (80.5), 593.2080 (8.2), 461.1675 (8.7), 267.1614 (1.5), 179.0341 (6.8), 161.0234 (100), 135.0439 (23.5), 133.0282 (45.8), 113.0231 (9.4)	5.38	2.606	2	6.57 ± 0.103
56.	Alyssonoside	$C_{35}H_{46}O_{19}$	769.2560	769.2578 (100), 593.2076 (8.5), 461.1658 (8.9), 193.05 (20.9), 175.0393 (44.1), 161.0236 (10.2), 160.0156 (44.4), 135.0442 (15.4), 134.0362 (20.9), 132.0206 (14.9), 123.0439 (7.4), 113.023 (15.1), 85.0281 (9.3), 71.0124 (10.0)	6.07	22.207	2	2.16 ± 0.14
57.	Leucoseptoside A	$C_{30}H_{38}O_{15}$	637.2138	637.2154 (100), 461.1669 (16.2), 315.1091 (3.8), 193.0501 (13.00), 175.0392 (67.8), 161.0233 (109), 160.0155 (60.1), 113.0230 (18.9)	6.25	2.505	2	22.80 ± 0.82
58.	Leontoside B/stachyoside D	$C_{36}H_{48}O_{19}$	783.2716	783.2734 (100), 193.0501 (34.7), 175.0392 (92.4), 167.0700 (1.5), 160.0156 (73.8), 132.0205 (26.8)	6.97	2.142	2	0.90 ± 0.05
59.	Acetylverbascoside	$C_{31}H_{38}O_{16}$	665.2087	665.2103 (65.0), 503.1778 (3.3), 461.1676 (3.1), 161.0234 (100), 179.0339 (2.9), 135.0440 (10.4), 133.0283 (38.4), 113.0229 (1.9)	6.99	2.348	2	0.90 ± 0.01
60.	Martynoside	$C_{31}H_{40}O_{15}$	651.2294	651.2317 (95.8), 475.1836 (1.2), 329.1252 (1.1), 193.0500 (13.6), 175.0392 (100), 160.0156 (69.1), 132.0204 (25.1), 113.0230 (14.0)	7.21	3.450	2	11.35 ± 0.75

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
				Iridoid glycosides				
61.	Melittoside	$C_{21}H_{32}O_{15}$	523.1668	523.1676 (10.9), 361.1139 (4.5), 343.1040 (5.4), 325.0919 (1.3), 313.9523 (0.4), 283.0820 (0.5), 253.0722 (1.3), 223.0613 (1.7), 205.0506 (1.6), 179.0553 (100), 161.0447 (16.8), 119.0337 (34.9), 101.0230 (30.8), 89.0229 (80.9)	1.33	1.446	2	13.22 ± 1.36
62.	*p*-Coumaroylmelittoside	$C_{30}H_{38}O_{17}$	669.2035	669.2049 (100), 489.1423 (5.4), 325.0932 (28.3), 307.0823 (5.0), 265.0729 (3.2), 235.0605 (3.4), 205.0499 (16.9), 163.0390 (71.9), 145.0283 (84.4), 119.0488 (46.4), 93.0330 (4.9), 89.0230 (6.4)	3.98	1.909	2	3.71 ± 0.06
63.	Caffeoylmelittoside [a,b]	$C_{30}H_{38}O_{18}$	685.1974	685.1998 (100), 649.1036 (1.3), 523.1473 (1.9), 187.0396 (1.1), 181.0498 (24.9), 179.0342 (61.7), 163.0391 (39.9), 161.0235 (12.7), 135.0440 (78.4), 93.0331 (2.4), 89.0227 (2.3)	4.79	1.799	2	0.70 ± 0.01
64.	Feruloylmelittoside [a,b]	$C_{31}H_{40}O_{18}$	699.2142	699.2161 (75.9), 519.1519 (8.4), 357.0992 (54.0), 193.0500 (100), 163.0389 (30.6), 135.0435 (16.10), 134.0361 (70.0)	5.56	2.778	2	-
				Flavonoids				
65.	Apigenin 6,8-*C*-hexosyl hexoside	$C_{27}H_{30}O_{15}$	593.1522	593.1522 (100), 503.1212 (4.8), 473.1094 (14.5), 413.0891 (2.0), 395.0770 (0.8), 383.0776 (18.0), 353.0672 (30.1), 325.0729 (2.8), 297.0770 (10.7), 161.0235 (2.2), 117.0333 (3.3)	4.05	1.630	2	1.59 ± 0.18
66.	Luteolin 7-*O*-dihexoside	$C_{27}H_{30}O_{16}$	609.1461	609.1473 (100), 447.0949 (4.7), 285.0408 (61.9), 284.0331 (18.1), 256.0380 (1.0), 151.0025 (3.8), 133.0281 (3.4), 107.0121 (2.1)	4.94	2.023	2	0.58 ± 0.01
67.	Hypolaetin 7-*O*-hexosyl (1→2)-hexoside	$C_{27}H_{30}O_{17}$	625.1410	625.1422 (100), 463.0883 (6.6), 445.0775 (6.5), 301.0356 (88.8), 300.0279 (30.4), 283.0244 (0.7), 255.0292 (3.6), 227.0350 (2.3), 166.9975 (3.3), 163.0029 (1.1), 137.0232 (4.0), 133.0280 (7.5)	5.08	1.900	1	5.30 ± 0.09
68.	Naringenin 7-*O*-dihexoside	$C_{27}H_{32}O_{15}$	595.1668	595.1658 (100), 475.1172 (4.6), 355.0674 (5.6), 271.0619 (54.8), 270.0215 (1.8), 269.0457 (38.2), 151.0027 (43.4), 119.0488 (21.5), 107.0123 (18.3)	5.45	−1.703	2	-

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
69.	Apigenin 7-*O*-allosyl (1→2) glucoside	$C_{27}H_{30}O_{15}$	593.1512	593.1522 (64.7), 431.0986 (3.0), 269.0459 (100), 225.0546 (0.7), 151.0027 (1.4), 161.0232 (1.7), 117.0333 (3.3), 107.0125 (1.8)	5.50	1.731	2	1.93 ± 0.04
70.	Isoscutellarein 7-*O*-pentosyl–hexoside [a,b]	$C_{26}H_{28}O_{15}$	579.1356	579.1366 (38.9), 461.0086 (0.4), 285.0408 (100), 257.0451 (1.7), 241.0506 (1.6), 229.0497 (1.5), 213.0554 (5.1), 187.0393 (4.4), 136.9867 (0.9), 117.0330 (0.5)	5.51	1.825	2	1.01 ± 0.05
71.	Isoscutellarein 7-*O*-hexosyl (1→2)-hexoside	$C_{27}H_{30}O_{16}$	609.1461	609.1472 (87.2), 447.0912 (0.4), 429.0833(6.8), 285.0409 (100), 284.0328 (9.5), 255.0303 (2.2), 167.0496 (0.5), 163.0028 (2.1), 136.9868 (1.7), 117.0333 (1.8)	5.62	1.826	2	47.47 ± 0.95
72.	Isoscutellarein 7-*O*-hexoside	$C_{21}H_{20}O_{11}$	447.0933	447.0941 (26.4), 285.0408 (100), 229.0509 (1.0), 136.9868 (2.3), 117.0332 (1.1)	5.81	1.801	2	0.58 ± 0.01
73.	Hypolaetin 7-*O*-acetylhexosiyl–hexoside	$C_{29}H_{32}O_{18}$	667.1516	625.1406 (3.2), 463.0898 (3.2), 445.079 (6.6), 301.0357 (76.0), 300.0278 (30.8), 283.0285 (1.1), 255.0298 (1.4), 227.0347 (1.0), 166.9973 (1.9), 163.0020 (1.0), 137.0232 (4.0), 133.0284 (8.3), 109.0282 (1.0)	5.93	3.152	2	19.17 ± 1.07
74.	Methylhypolaetin 7-*O*-dihexoside	$C_{28}H_{32}O_{17}$	639.1567	639.1579 (75.3), 315.0516 (100), 300.0279 (40.7), 271.0264 (1.2), 243.0296 (2.5), 165.9901 (0.7), 136.9871 (6.2), 117.1944 (0.5), 133.0283 (2.5)	5.96	1.920	2	17.33 ± 0.25
75.	Apigenin 7-*O*-glucoside	$C_{21}H_{20}O_{10}$	431.0984	431.0988 (100), 269.0453 (24.9), 268.0381 (54.3), 211.0395 (1.6), 151.0025 (3.2), 117.0330 (1.8), 170.0124 (2.0)	6.06	1.044	1	0.46 ± 0.02
76.	Methylhypolaetin 7-*O*-hexoside	$C_{22}H_{22}O_{12}$	477.1039	477.1045 (30.5), 315.0515 (100), 300.0278 (32.0), 227.0350 (1.6), 136.9870 (6.2)	6.13	1.406	2	0.26 ± 0.01
77.	Apigenin 7-*O*-[6‴-*O*-acetyl]-hexosyl(1→2)-hexoside	$C_{29}H_{32}O_{16}$	635.1618	635.1629 (60.6), 593.1563 (1.1), 431.0981 (2.3), 269.0458 (100), 225.0560 (12.4), 151.0024 (1.7), 117.0332 (5.1), 107.0126 (2.6)	6.44	1.798	2	2.18 ± 0.003
78.	Isoscutellarein 7-*O*-hexosyl-(1→2)-[6″-*O*-acetyl]-hexoside	$C_{29}H_{32}O_{17}$	651.1567	651.1581 (69.3), 429.0831 (8.9), 285.0408 (100), 255.0285 (1.0), 239.0344 (1.1), 163.0026 (1.5), 136.9863 (0.8), 117.0334 (1.1)	6.58	2.161	2	151.70 ± 14.79

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
79.	4′-Methylhypolaetin 7-*O*-acetyl–hexosyl–hexoside	$C_{30}H_{34}O_{18}$	681.1672	681.1688 (89.6), 639.1533 (1.2), 357.0594 (0.9), 315.0516 (100), 300.0279 (44.2), 271.0248 (1.6), 243.0291 (1.4), 136.9868 (7.9), 133.0283 (4.1)	6.83	2.236	2	78.33 ± 3.29
80.	4′-Methylisoscutellarein 7-*O*-dihexoside	$C_{28}H_{32}O_{16}$	623.1618	623.1630 (100), 461.1117 (0.6), 299.0565 (83.6), 284.0330 (39.2), 255.0299 (3.0), 117.0330 (0.7)	7.23	1.929	2	24.20 ± 0.98
81.	Tremasperin	$C_{30}H_{34}O_{16}$	649.1774	649.1786 (5.4), 607.1672 (2.6), 283.0616 (100), 268.0381 (55.5), 284.0649 (5.8), 240.0431 (2.2), 151.0024 (0.5)	8.21	1.913	2	0.55 ± 0.04
82.	4′-*O*-methylisoscutellarein 7-*O*-[6‴-*O*-acetyl]hexosyl-(1→2)hexoside	$C_{30}H_{34}O_{17}$	665.1723	665.1740 (84.8), 299.0565 (100), 284.0330 (30.6), 255.0293 (2.5), 240.0429 (2.5), 227.0343 (2.5), 163.0025 (1.1), 136.9867 (9.2), 117.0338 (1.9)	8.24	2.447	2	107.44 ± 9.07
83.	Isoscutellarein 7-*O*-acetylhexosyl-*O*-acetylhexoside	$C_{31}H_{34}O_{18}$	693.1672	693.1663 (85.8), 471.0903 (7.4), 285.0407 (100), 213.0551 (6.0), 163.0022 (4.5), 136.9864 (1.9), 117.0331 (3.4)	8.26	−1.323	2	-
84.	Methylhypolaetin 7-*O*-acetylhexosyl-*O*-acetylhexoside	$C_{32}H_{36}O_{19}$	723.1778	723.1794 (89.7), 315.0515 (100), 300.0280 (44.9), 271.0255 (1.1), 243.0298 (1.4), 199.0390 (4.6), 136.9866 (9.5), 133.0284 (5.9)	8.47	2.182	2	0.31 ± 0.02
85.	Naringenin	$C_{15}H_{12}O_5$	271.0612	271.0615 (100), 227.0701 (0.7), 165.0180 (2.5), 151.0025 (65.7), 125.0228 (1.3), 119.0489 (52.3), 107.0124 (15.5), 93.0331 (11.9)	8.60	1.193	2	-
86.	Apigenin 7-*O*-p-coumaroyl-*O*-hexoside	$C_{30}H_{26}O_{12}$	577.1352	577.1360 (100), 431.0990 (13.4), 413.0890 (7.9), 269.0459 (77.0), 145.0283 (83.4), 163.0391 (4.4), 117.0332 (38.3), 107.0121 (1.1), 151.0026 (2.1)	9.06	1.457	2	0.75 ± 0.01
87.	Naringenin 7-*O*-coumaroylhexoside [a]	$C_{30}H_{28}O_{12}$	579.1508	579.1514 (100), 415.1033 (3.9), 307.0829 (12.0), 271.0616 (79.4), 151.0026 (40.9), 163.0391 (15.7), 145.0283 (57.4), 119.0489 (40.9), 107.0125 (15.2), 117.0332 (21.9)	9.15	0.985	2	1.09 ± 0.09
88.	4′-Methylisoscutellarein 7-*O*-(6‴-acetyl)-hexosyl(1→2)-[6′-*O*-acetyl]hexoside	$C_{32}H_{36}O_{18}$	707.1829	707.1844 (12.1), 299.0565 (100), 284.0330 (31.9), 300.0598 (6.8), 298.0496 (8.2), 255.0292 (4.7), 240.0424 (3.2), 227.0341 (1.7), 163.0023 (3.5), 136.9867 (10.7), 117.0332 (1.5)	9.89	2.125	2	0.09 ± 0.003
89.	Pectolinarigenin	$C_{17}H_{14}O_6$	313.0718	313.0721 (100), 298.0485 (53.9), 283.0251 (52.0), 269.0468 (2.4), 255.0302 (14.4), 227.0342 (2.17), 211.0386 (1.3), 183.0446 (2.1), 178.9918 (3.0), 163.0031 (11.8), 135.0075 (2.8), 117.0331 (13.1)	10.36	0.922	1	3.11 ± 0.50

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass [M−H]⁻	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
90.	Eupatilin	$C_{18}H_{16}O_7$	343.0823	343.0826 (85.2), 328.0594 (100), 313.0360 (54.6), 298.0125 (13.3), 285.0412 (2.0), 270.0174 (42.6), 257.0095 (2.6), 133.0282 (3.7), 123.0439 (4.6)	11.05	0.915	2	0.47 ± 0.07
91.	8-Methoxycirsilineol	$C_{18}H_{16}O_7$	343.0823	343.0826 (100), 328.0594 (48.7), 313.0360 (71.8), 299.0952 (0.7), 298.0124 (209), 270.0175 (10.6), 242.0220 (4.2), 161.0233 (0.8), 117.0333 (8.9)	11.24	0.828	2	14.15 ± 2.30
92.	Genkwanin	$C_{16}H_{12}O_5$	283.0611	283.0615 (100), 268.0379 (67.4), 240.0428 (6.2), 239.0352 (1.8), 178.9915 (1.2), 151.0025 (4.1), 107.0125 (3.3)	11.42	1.036	2	-
				Fatty acids				
93.	Trihydroxyoctadecadienoic acid	$C_{18}H_{32}O_5$	327.2177	327.2166 (100), 309.2069 (0.8), 291.1971 (3.5), 229.1443 (12.5), 211.1334 (16.2), 183.1383 (1.6), 171.1015 (6.0), 85.0280 (2.5), 57.0329 (0.9)	9.15	0.986	2	-
94.	Trihydroxyoctadecenoic acid	$C_{18}H_{34}O_5$	329.2334	329.2338 (100), 311.2232 (1.4), 293.2119 (0.4), 229.1442 (17.2), 211.1335 (23.2), 183.1381 (2.7), 171.1020 (4.4), 127.1115 (1.6)	9.80	1.466	2	-
95.	Dihydroxyoctadecatrienoic acid	$C_{18}H_{30}O_4$	309.2071	309.2076 (100), 291.1972 (54.3), 247.2075 (1.0), 185.1179 (5.4), 137.0959 (17.9), 97.0645 (4.1)	10.90	1.641	2	-
96.	Dihydroxyoctadecadienoic acid	$C_{18}H_{32}O_4$	311.2228	311.2233 (100), 293.2132 (7.2), 275.2029 (6.4), 201.1128 (61.0), 183.1387 (1.6), 171.1015 (11.2), 127.1114 (4.3)12.62	12.62	1.662	2	-
97.	Dihydroxyoctadecenoic acid	$C_{18}H_{34}O_4$	313.2384	313.2389 (100), 295.2266 (5.8), 277.2166 (4.9), 201.1126 (42.8), 171.1012 (5.11), 127.1116 (4.8), 125.0960 (3.3)	13.75	1.460	2	-
98.	Dihydroxyoctadecanoic acid	$C_{18}H_{36}O_4$	315.2541	315.2544 (100), 297.2450 (4.3), 287.2241 (4.3), 171.1380 (0.6), 141.1272 (3.2), 127.1116 (0.6), 89.0230 (0.5)	14.87	1.101	2	-
99.	Hydroxylinoleic acid	$C_{18}H_{32}O_3$	295.2279	295.2282 (100), 277.2175 (14.6), 195.1384 (17.5), 113.0960 (1.1)	15.98	1.056	2	-

Table 1. *Cont.*

No	Identified/Tentatively Annotated Compound	Molecular Formula	Exact Mass $[M-H]^-$	Fragmentation Pattern in (-) ESI-MS/MS	tR (min)	Δ ppm	Level of Confidence [29]	Content [mg/g li] Mean ± SD
				Organosulfur compounds				
100.	Dodecyl sulfate	$C_{12}H_{26}O_4S$	265.1479	265.1482 (100), 96.9586 (66.4), 79.9558 (1.6)	14.55	1.081	2	-
101.	Lauryl ether sulfate	$C_{14}H_{30}O_5S$	309.1741	309.1746 (100), 122.9746 (1.9), 104.9527 (0.2), 96.9586 (54.3), 79.9558 (6.6)	16.10	1.624	2	-
102.	4-Dodecylbenenesulfonic acid	$C_{18}H_{30}O_3S$	325.1842	325.1846 (100), 216.0095 (0.2), 183.0113 (46.5), 197.0272 (0.8), 184.0147 (1.9)	17.44	0.957	2	-
103.	Myristyl sulfate	$C_{14}H_{30}O_4S$	293.1792	293.1796 (100), 96.9586 (73.6), 79.9558 (2.2)	17.89	1.455	2	-

[a]—reported for the first time in the studied species; [b]—undescribed in the literature; level of confidence: 1—compound identified by comparison with reference standard; 2—putatively annotated compound; 3—putatively characterized compound classes.

2.3. Study Strength, Limitation and Future Direction

The study strength is that the presented extraction method of infusion is similar to the approach used in traditional medicine to process *S. scardica* tea. Therefore, this provides an insight into the phytochemical composition of common tea used in the traditional medicine and in-home remedies. A notable contribution of this study is the first-time dereplication and fragmentation patterns of five caffeic acids oligomers and four acylhexaric acids in *S. scardica*, expanding the current understanding of its chemical profile. The quantitative analysis identified major compounds in *S. scardica* infusion, with phenylethanoid verbascoside, glycosides of isoscutellarein, methylisoscutelarein, hypolaetin, and caffeic acid standing out as significant constituents. The reported concentrations add quantitative depth to the qualitative richness of the chemical composition. However, there are some limitations to the proposed method. In the quantitative assessment, a semi-quantitation was conducted, multiple detected substances were quantified based on a standard with a similar, yet different, chemical structure, as detailed above. Hence, a variation in the ionization between a standard and analytes may be a limitation. Future quantification based on the individual isolated secondary metabolites is recommended. In addition, isolation and accurate identification of the newly annotated caffeic acid oligomers and caffeoylhexaric acids will strengthen the validity of the present work.

3. Materials and Methods

3.1. Chemicals

Acetonitrile (hypergrade for LC–MS), formic acid (for LC–MS), and methanol (analytical grade) were purchased from Chromasolv (Sofia, Bulgaria). The reference standards used for compound identification were obtained from Extrasynthese (Genay, France) for protocatechuic, gentisic acids, and apigenin. Chlorogenic, caffeic, rosmarinic, cichoric acid, pectolinarigenin, and scutellarein were supplied from Phytolab (Vesten-bergsgreuth, Bavaria, Germany).

3.2. Plant Material

S. scardica seedlings were bought from a certified greenhouse "Mursalski-biogroup" (Bulgaria) and subsequently bred on alluvial soil with sunny exposure in an herbal garden (Rayanovtsi village, Vidin region) in Bulgaria at 349 m a.s.l. (43.7023° N 22.5206° E). The plant was identified by one of the authors (D.Z.) according to Assenov (1989) [31]. The plant material (aerial parts of 4-year-old plants) was collected during the flowering stage in July 2022 and dried for one week in the shade at room temperature. Then it was comminuted with a grinder (Rohnson, R-942, 220–240 V, 50/60 Hz, 200 W, Prague, Czech Republic) and stored in a dry and cool place until further analysis. The fresh/dried mass ratio is 4:1.

3.3. Sample Extraction

Air-dried aerial parts (100 g) were infused twice with boiled water (1:20 w/v) and extracted for 15 min at room temperature. The herbal infusion was lyophilized (lyophilizer Biobase BK-FD10P, BIOBASE, Jinan, China) to yield crude extracts of 12.5 g.

3.4. UHPLC–HRMS Dereplication/Annotation

The UHPLC–HRMS analyses were performed, as described previously [22], using a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) equipped with a heated electrospray ionization (HESI-II) probe (Thermo Scientific). The equipment was operated in negative ion mode within the m/z range from 130 to 2000 at a resolution of 70,000. Other instrument parameters for full MS mode were set as follows: automatic gain control (AGC) target 3×10^6, maximum injection time (IT) 100 ms, number of scan ranges 1. For the DD-MS^2 mode, the instrument parameters were as follows: microscans 1, resolution 17,500, AGC target 1×10^5, maximum IT 50 ms, MSX count 1, Top5, isolation window 2.0 m/z, stepped normalized collision energy (NCE) 10, 20, 60 eV. The chromatographic separation was achieved on a reversed phase column

Kromasil EternityXT C18 (1.8 µm, 2.1 × 100 mm) at 40 °C. The UHPLC analyses were run with a mobile phase containing 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The run time was 33 min. The gradient elution program was used as follows: 0–1 min, 0–5% B; 1–20 min, 5–30% B; 20–25 min, 30–50% B; 25–30 min, 50–70% B; 30–33 min, 70–95%; 33–34 min 95–5%B. The equilibration time was 4 min. The injection volume and the flow rate were set to 1 µL and 300 µL/min, respectively. Data acquisition was performed using Xcalibur 4.2 (Thermo Scientific, Waltham, MA, USA) instrument control/data handling software.

3.5. UHPLC–HRMS Quantification

The UHPLC–HRMS quantification was conducted using the external standard method. Standard calibrations of protocatechuic (**12**), gentisic (**21**), caffeic (**20**), rosmarinic (**27**), chlorogenic (**36**), cichoric acids, apigenin, and scutellarein were established at five data points covering the concentration range of each analyte according to the level expected in the plant samples. Working solutions containing 0.05, 0.025, 0.012, 0.006, and 0.003 mg/mL of the assayed analytes were prepared from a stock solution in methanol containing 0.1 mg/mL. Based on the similar structure, the quantity of **9**, **10**, **14**, and **21** was determined based on the calibration curve of gentisic acid; **11**, **12**, **18**, and **24** as protocatechuic acid; **13**, **14**, **20**, **51**–**56**, and **63** as caffeic acid; **27**–**31** as rosmarinic acid; **36**, **38**, **39**, **41**–**44** as chlorogenic acid; **65**, **66**, **69**, **75**, **77**, **86**–**91** as apigenin; while **70**–**74**, **76**, **78**–**84**, and **88** as scutellarein. Regression equations were as follows: gentisic acid y = 1,977,866,070x + 2460.9184 (R^2 = 0.9951) protocatechuic acid y = 1,416,556,589.51x + 1581.8936 (R^2 = 0.9965); caffeic acid y = 1,133,573,161x − 410.2916 (R^2 = 0.9928); rosmarinic acid y = 2,190,130,610x + 22,248.9144 (R^2 = 0.9950); chlorogenic acid y = 4,602,799,047.3258x − 921.0750 (R^2 = 0.9926); cichoric acid y = 4,589,524,443.3458x + 85.8499 (R^2 = 0.9999); apigenin y = 4,594,027,463.2779x + 21,088.0103 (R^2 = 0.9983); scutellarein y = 1,976,006,256x − 41,744.9522 (R^2 = 0.9420). The peak areas were calculated by integrating the Area Under the Curve (AUC) of the full-scan intensity scans for the corresponding molecular ion. These scans were also filtered for the presence of the characteristic base peak. MZmine 2.53 software was applied to the UHPLC–HRMS raw files of the studied *S. scardica* lyophilized infusion to obtain the peak area in the quantitative analysis. Results are expressed as mg/g lyophilized infusion.

4. Conclusions

In conclusion, an in-depth phytochemical analysis of *S. scardica* infusion using UHPLC–HRMS was performed. More than 100 metabolites, including sugar acids and saccharides, carboxylic, hydroxybenzoic, hydroxycinnamic, acylquinic and acylhexaric acids, caffeic acids oligomers, phenylpropanoid and iridoid glycosides, flavonoids, fatty acids, and organosulfur compounds were dereplicated/annotated. In addition, 62 metabolites of *S. scardica* were quantified. The presented extraction method of infusion is similar to the approach used in traditional medicine to process *S. scardica* tea. Therefore, the performed state-of-the-art phytochemical analysis of *S. scardica* provide additional knowledge with respect to the chemical constituents of this valuable medicinal plant.

Author Contributions: Conceptualization, D.Z.-D. and R.G.; methodology, D.Z.-D. and Y.V.; software, D.Z.-D. and Y.V.; validation, D.Z.-D.; formal analysis, D.Z.-D.; investigation, D.Z.-D. and V.B.; resources, D.Z.-D.; data curation, D.Z.-D.; writing—original draft preparation, D.Z.-D.; writing—review and editing, D.Z.-D., V.B. and R.G.; visualization, D.Z.-D.; supervision, R.G.; project administration, D.Z.-D.; funding acquisition, D.Z.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0004-C01.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors are grateful to Stephan Mladenov, Faculty of Medicine, Medical University Sofia, for the supply of *S. scardica* plant material.

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

References

1. Petrova, A.; Vladimirov, V. Balkan Endemics in the Bulgarian Flora. *Phytol. Balcan.* **2010**, *16*, 293–311.
2. Assenov, I.; Ganchev, G. Morphological Characteristics of *Sideritis scardica* Griseb. *Pharmacia* **1978**, *2*, 29–32.
3. Evstatieva, L. Sideritis scardica Griseb. In *Red Data Book of the Republic of Bulgaria*; Peev, D., Ed.; Joint Edition of the Bulgarian Academy of Sciences & Ministry of Environment and Water of Bulgaria, Digital Edition; Bulgarian Academy of Sciences: Sofia, Bulgaria, 2011; Volume 1, Available online: http://e-ecodb.bas.bg/rdb/en/ (accessed on 1 December 2023).
4. González-Burgos, E.; Carretero, M.E.; Gómez-Serranillos, M.P. *Sideritis* spp.: Uses, Chemical Composition and Pharmacological Activities—A Review. *J. Ethnopharmacol.* **2011**, *135*, 209–225. [CrossRef] [PubMed]
5. Yaneva, I.; Balabanski, V. History of the Uses of Pirin Mountain Tea (*Sideritis scardica* Griseb.) in Bulgaria. *Bulg. J. Public Health* **2013**, *5*, 48–57.
6. Stanoeva, J.P.; Stefova, M.; Stefkov, G.; Kulevanova, S.; Alipieva, K.; Bankova, V.; Aneva, I.; Evstatieva, L.N. Chemotaxonomic Contribution to the *Sideritis* species Dilemma on the Balkans. *Biochem. Syst. Ecol.* **2015**, *61*, 477–487. [CrossRef]
7. Irakli, M.; Tsifodimou, K.; Sarrou, E.; Chatzopoulou, P. Optimization Infusions Conditions for Improving Phenolic Content and Antioxidant Activity in *Sideritis scardica* Tea Using Response Surface Methodology. *J. Appl. Res. Med. Aromat. Plants* **2018**, *8*, 67–74. [CrossRef]
8. Todorova, M.; Trendafilova, A. *Sideritis scardica* Griseb.; An Endemic Species of Balkan Peninsula: Traditional Uses, Cultivation, Chemical Composition, Biological Activity. *J. Ethnopharmacol.* **2014**, *152*, 256–265. [CrossRef]
9. Jeremic, I.; Petricevic, S.; Tadic, V.; Petrovic, D.; Tosic, J.; Stanojevic, Z.; Petronijevic, M.; Vidicevic, S.; Trajkovic, V.; Isakovic, A. Effects of *Sideritis scardica* Extract on Glucose Tolerance, Triglyceride Levels and Markers of Oxidative Stress in Ovariectomized Rats. *Planta Med.* **2019**, *85*, 465–472. [CrossRef]
10. Wightman, E.; Jackson, P.A.; Khan, J.; Forster, J.; Heiner, F.; Feistel, B.; Suarez, C.G.; Pischel, I.; Kennedy, D.O. The Acute and Chronic Cognitive and Cerebral Blood Flow Effects of a *Sideritis scardica* (Greek Mountain Tea) Extract: A Double Blind, Randomized, Placebo Controlled, Parallel Groups Study in Healthy Humans. *Nutrients* **2018**, *10*, 955. [CrossRef]
11. Żyżelewicz, D.; Kulbat-Warycha, K.; Oracz, J.; Żyżelewicz, K. Polyphenols and Other Bioactive Compounds of *Sideritis* Plants and Their Potential Biological Activity. *Molecules* **2020**, *25*, 3763. [CrossRef]
12. Mróz, M.; Malinowska-Pańczyk, E.; Bartoszek, A.; Kusznierewicz, B. Comparative Study on Assisted Solvent Extraction Techniques for the Extraction of Biologically Active Compounds from *Sideritis raeseri* and *Sideritis scardica*. *Molecules* **2023**, *28*, 4207. [CrossRef] [PubMed]
13. Kostadinova, E.; Nikolova, D.; Alipieva, K.; Stefova, M.; Stefkov, G.; Evstatieva, L.; Matevski, V.; Bankova, V. Chemical Constituents of the Essential Oils of *Sideritis scardica* Griseb. and *Sideritis raeseri* Boiss and Heldr. from Bulgaria and Macedonia. *Nat. Prod. Res.* **2007**, *21*, 819–823. [CrossRef] [PubMed]
14. Petreska Stanoeva, J.; Stefova, M. Assay of Urinary Excretion of Polyphenols after Ingestion of a Cup of Mountain Tea (*Sideritis scardica*) Measured by HPLC-DAD-ESI-MS/MS. *J. Agric. Food Chem.* **2013**, *61*, 10488–10497. [CrossRef] [PubMed]
15. Tadić, V.M.; Jeremic, I.; Dobric, S.; Isakovic, A.; Markovic, I.; Trajkovic, V.; Bojovic, D.; Arsic, I. Anti-inflammatory, Gastroprotective, and Cytotoxic Effects of *Sideritis scardica* extracts. *Planta Med.* **2012**, *78*, 415–427. [CrossRef] [PubMed]
16. Petreska, J.; Stefova, M.; Ferreres, F.; Moreno, D.A.; Tomás-Barberán, F.A.; Stefkov, G.; Kulevanova, S.; Gil-Izquierdo, A. Potential Bioactive Phenolics of Macedonian *Sideritis* Species Used for Medicinal "Mountain Tea". *Food Chem.* **2011**, *125*, 13–20. [CrossRef]
17. Alipieva, K.; Petreska, J.; Gil-Izquierdo, Á.; Stefova, M.; Evstatieva, L.; Bankova, V. Influence of the Extraction Method on the Yield of Flavonoids and Phenolics from *Sideritis* spp. (Pirin Mountain Tea). *Nat. Prod. Commun.* **2010**, *130*, 51–54. [CrossRef]
18. Al Kadhi, O.; Melchini, A.; Mithen, R.; Saha, S. Development of a LC-MS/MS Method for The Simultaneous Detection of Tricarboxylic Acid Cycle Intermediates in A Range of Biological Matrices. *J. Anal. Methods Chem.* **2017**, *2017*, 5391832. [CrossRef]
19. Liu, A.H.; Guo, H.; Ye, M.; Lin, Y.H.; Sun, J.H.; Xu, M.; Guo, D.A. Detection, Characterization and Identification of Phenolic Acids in Danshen Using High-Performance Liquid Chromatography with Diode Array Detection and Electrospray Ionization Mass Spectrometry. *J. Chromatogr. A* **2007**, *1161*, 170–182. [CrossRef]
20. Zheleva-Dimitrova, D.; Simeonova, R.; Gevrenova, R.; Savov, Y.; Balabanova, V.; Nasar-Eddin, G.; Bardarov, K.; Danchev, N. In vivo Toxicity Assessment of *Clinopodium vulgare* L. Water Extract Characterized by UHPLC-HRMS. *Food Chem. Toxicol.* **2019**, *134*, 110841. [CrossRef]
21. Zhu, L.; Ma, S.; Li, K.; Xiong, P.; Qin, S.; Cai, W. Systematic Screening of Chemical Constituents in the Traditional Chinese Medicine Arnebiae Radix by UHPLC-Q-Exactive Orbitrap Mass Spectrometry. *Molecules* **2022**, *27*, 2631. [CrossRef]
22. Gevrenova, R.; Zengin, G.; Sinan, K.I.; Zheleva-Dimitrova, D.; Balabanova, V.; Kolmayer, M.; Voynikov, Y.; Joubert, O. An In-Depth Study of Metabolite Profile and Biological Potential of *Tanacetum balsamita* L. (Costmary). *Plants* **2023**, *12*, 22. [CrossRef] [PubMed]
23. Gevrenova, R.; Zheleva-Dimitrova, D.; Balabanova, V.; Voynikov, Y.; Sinan, K.I.; Mahomoodally, M.F.; Zengin, G. Integrated phytochemistry, bio-functional potential and multivariate analysis of *Tanacetum macrophyllum* (Waldst. & Kit.) Sch.Bip. and *Telekia speciosa* (Schreb.) Baumg. (Asteraceae). *Ind. Crops Prod.* **2020**, *155*, 112817.
24. Clifford, M.N.; Knight, S.; Kuhnert, N. Discriminating between the six isomers of dicaffeoylquinic acid by LC-MS n. *J. Agric. Food Chem.* **2005**, *53*, 3821–3832. [CrossRef] [PubMed]

25. Kleczek, N.; Malarz, J.; Gierlikowska, B.; Kiss, A.; Stojakowska, A. Constituents of *Xerolekia speciosissima* (L.) Anderb. (Inuleae), and Anti-Inflammatory Activity of 7,10-Diisobutyryloxy-8,9-epoxythymyl Isobutyrate. *Molecules* **2020**, *25*, 4913. [CrossRef] [PubMed]
26. Alipieva, K.; Kostadinova, E.; Evstatieva, L.; Stefova, M.; Bankova, V. An iridoid and a flavonoid from *Sideritis lanata* L. *Fitoterapia* **2009**, *80*, 51–53. [CrossRef]
27. Kirmizibekmez, H.; Ariburnu, E.; Masullo, M.; Festa, M.; Capasso, A.; Yesilada, E.; Piacente, S. Iridoid, phenylethanoid and flavonoid glycosides from *Sideritis trojana*. *Fitoterapia* **2012**, *83*, 130–136. [CrossRef]
28. Sinan, K.I.; Zengin, G.; Zheleva-Dimitrova, D.; Etienne, O.K.; Mahomoodally, M.F.; Bouyahya, A.; Lobine, D.; Chiavaroli, A.; Ferrante, C.; Menghini, L.; et al. Qualitative Phytochemical Fingerprint and Network Pharmacology Investigation of *Achyranthes aspera* Linn. Extracts. *Molecules* **2020**, *25*, 1973. [CrossRef]
29. Sumner, L.W.; Amberg, A.; Barrett, D.; Beale, M.H.; Beger, R.; Daykin, C.A.; Fan, T.W.-M.; Fiehn, O.; Goodacre, R.; Griffin, J.L. Proposed Minimum Reporting Standards for Chemical Analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* **2007**, *3*, 211–221. [CrossRef]
30. Moussavi, N.; Azizullah, H.; Malterud, K.; Inngjerdingen, K. Immunomodulating polyphenols from *Sideritis scardica*. *J. Funct. Food* **2022**, *96*, 105197. [CrossRef]
31. Assenov, I. *Sideritis* L. In *Flora of PR Bulgaria*; Velchev, V., Ed.; Bulgarian Academy of Sciences Publishing House: Sofia, Bulgaria, 1989; Volume 9, pp. 369–374.