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Phytochemical Investigation of Endemic *Sideritis cypria* Post

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Abstract: Non-polar and polar compounds were isolated from the leaves and chemical composition of the essential oil from aerial parts of *Sideritis cypria* endemic in Northern Cyprus was also determined. The structures of all the isolated compounds were elucidated by means of spectroscopic (UV, NMR: 1D and 2D-NMR: ^{1}H, ^{13}C, COSY, HSQC, HMBC) methods and comparison with literature data. The essential oil composition of the plant material was analysed by GC-FID and GC-MS, simultaneously. To the best of our knowledge, secondary metabolites of this endemic species were determined for the first time. As a result, two mixtures of four *ent*-kaurane diterpenes; sidol (**1**) and isosidol (**2**), linearol (**3**) and isolinearol (4); four phenylethanoid glycosides; verbascoside (**5**), lavandulifolioside (**6**), leonoside A (**7**), leucoseptoside A (**8**); four flavone glycosides, apigenin-7-*O*-glucopyranoside (**9**), isoscutellarein-7-*O*-[6'''-*O*-acetyl-allopyranosyl-(1→2)-glucopyranoside] (**10**) and a mixture of apigenin-7-*O*-(4''-*O*-*p*-coumaroyl)-glucopyranoside (**11**) and apigenin-7-O-(3''-*O*-*p*-coumaroyl)-glucopyranoside (**12**) were elucidated. 7-*O*-acetyl-8-*epi*-loganic acid (**13**), an iridoid glycoside, was reported herein for the first time for genus *Sideritis*. In addition, major compounds of the essential oil were determined as α-pinene (14.73±0.15%), β-pinene (16.60±0.20%), β-phellandrene (17.83±0.23%) and *epi*-cubebol (7.70±0.20%), respectively.

Keywords: *Sideritis*; phytochemistry; *ent*-kaurane diterpenes; iridoids; flavonoids; essential oil.

1. Introduction

Lamiaceae family was said to comprise 8 subfamilies before, then this number increased to 10, however, in recent years 2 more subfamilies have been added by the transfer of two new genera from the family Verbenaceae [1-3]. Two of these subfamilies, Lamioideae and Nepetoideae, are wider than the others. In general, Nepetoideae contains mostly essential oil-rich genera while Lamioideae, in which genus *Sideritis* is also included mostly rich in secondary metabolites such as iridoids,

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phenylethanoid glycosides, diterpenes etc. [4,5]. Essential oil compositions of *Sideritis* spp. have been extensively investigated [6]. Moreover, the compositions of essential oils of *Sideritis* spp. belonging to the section *Empedoclia* have also been investigated [7,8].
Genus *Sideritis* of the Lamiaceae family comprises over 150 species, 44 of which grow in Turkey, one of the two main gene centers with Spain with a high rate of endemism (79.5%) [6,7,9]. In Cyprus, this genus is represented by 3 species: *S. curvidens* Stapf, *S. perfoliata* L. and an endemic species *S. cypria* Post [10].

Phytochemical constituents of several *Sideritis* spp. have been published previously. In general, flavonoid and phenylpropanoid glycosides [11-15], diterpenoid compounds with different carbon skeletons (kaurane, labdane, pimarane, etc.) are the major compounds [16]. Diterpenoids reported from this genus are mostly *ent*-kaurane type [17-20]. Iridoid glycosides [21,22] and coumarins [23] are rarely reported from this genus.

The studies performed on the pharmacological effects of *Sideritis* spp. are attributed to the following major compounds; diterpenes, flavonoids and essential oil, respectively [6,22,24-32].

Sideritis cypria named as ‘‘*Dağçayı* or *Adaçayı*” is used traditionally as herbal tea in order to treat stomach problems, headache, sore throat and common cold. It is also consumed as a tonic and diaphoretic in Cyprus [33,34].

The goal of this work was to characterize the phytochemical content, which comprises of chemical composition characterization of the essential oil and also isolation and structure elucidation of both non-polar and polar compounds, of *Sideritis cypria* endemic in Northern Cyprus.

2. Materials and Methods

2.1. Plant Material

Aerial parts of *Sideritis cypria* were collected from the heights of Pentadactylos (*Beşparmak*) Mountains during the flowering phase in June 2016. Voucher specimens are kept at the Herbarium of the Near East University, Turkish Republic of Northern Cyprus (NEUN) as NEUN 6903.

2.2. Phytochemical Studies

2.2.1. Isolation of Essential Oil

100 g of the air dried aerial parts of *Sideritis cypria* were distilled with 1 L distilled water for 3 hours using a Clevenger-type apparatus by hydrodistillation. The resulting essential oil was stored at 4°C until analysis. The oil yield was calculated as v/w on dry basis. The essential oil yield was 0.49%.

2.2.2. Analysis of Essential Oil

2.2.2.1. GC-MS Analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

2.2.2.2. GC-FID Analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection

was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of the essential oil components were carried out by comparison of their relative retention times with those of certified standards or by comparison of their linear retention index (LRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder3 Library) [35,36] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [37,38] was used for the identification.

2.2.3. Chemicals, Reagents and Devices

All chemicals were purchased from Sigma-Aldrich®. For evaporation under reduced pressure Buchi® rotavapor (R-210) which attached with heating bath (B-491), vacuum pump (V-700) and vacuum controller (V-850) was used. For lyophilization of samples, Christ® Alpha 1-4 LD plus was used. For medium-pressure liquid chromatographic (MPLC) separations, Buchi Sepacore® Chromatography systems was used with Buchi borosilicate 3.3 column (36 mm x 230 mm) packed with LiChroprep RP-18 (Merck, Darmstadt). For reversed phase Vacuum Liquid Chromatography (RP-VLC), LiChroprep RP-18 (Merck, Darmstadt) was used for stationary phase. Silica gel 60 (0.063–0.200 mm; Merck, Darmstadt) and Sephadex™ LH-20 (GE Healthcare, Sweden) were utilized for open column chromatography (CC) studies. TLC analyses were carried out on silica gel 60 F_{254} precoated plates (Merck, Darmstadt). 1% Vanillin in MeOH and 5% H_2SO_4 in EtOH were used successively as reagents. For NMR spectroscopy experiments measurements were performed on a Bruker DRX 500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively. Negative and positive mode HRLCMS-MS measurements were recorded on an UPLC-Quadrupole Orbitrap instrument.

2.2.4. Preparation of Crude Extracts

Powdered, air-dried leaves of *Sideritis cypria* (200 g) were macerated twice with acetone (2.5 L) at room temperature for one day. The combined acetone extracts were concentrated under reduced pressure to yield 6.21% crude extract.

Powdered, air-dried leaves of *S. cypria* (147 g) were macerated twice with 3 L of ethanol:water (80:20) at room temperature for one day. After the EtOH extract was concentrated using rotary evaporator until a water suspension was obtained and made up to 500 mL with distilled water, it was partitioned with DCM (dichloromethane), EtOAc (ethyl acetate) and *n*-BuOH (*n*-butanol), respectively. EtOAc extract was adsorbed onto silica gel for dry column application and *n*-BuOH extract was concentrated under reduced pressure to dryness at 40°C.

2.2.5. Isolation of Non-polar Compounds

Acetone extract (absorbed in 50 g silica gel) was subjected to silica gel vacuum liquid chromatography (200 g) and eluted with petroleum ether, cyclohexane, a stepwise gradient of cyclohexane in acetone (10%-50%, 200 mL each) yielding 7 subfractions A-G. Fr. E (3.68 g) was applied dry with 7 g silica gel on 100 g silica gel eluting DCM:Acetone (9:1 to 7:3; 1,4 L in total) and DCM:Acetone:MeOH (4:3:3; 400 mL in total) to obtain Fr. E1-15. For purification of the compounds **1-4**, Fr. E12 (362.8 mg) was fractionated by gel chromatography (Sephadex LH-20) eluting Cyclohexane:DCM:MeOH (7:4:1) yielded compounds **1** and **2** as a mixture (61.9 mg) and also compounds **3** and **4** mixture (31.2 mg).

2.2.6. Isolation of Polar Compounds

n-BuOH extract was submitted to reverse phase vacuum liquid chromatography (100 g) and eluted with H_2O followed by increasing concentrations of MeOH in H_2O (between 10%and 90%) to give 11 major fractions Fr. A-K.

Fr. B (2.62 g) was chromatographed over RP_MPLC (Reverse phase medium pressure liquid chromatography) with a stepwise gradient of MeOH in H_2O (up to 60%) to obtain Fr. B1-12. For purifiying compound **5** (52 mg) and **6** (105 mg), Fr. B11 was fractionated by gel chromatography (Sephadex LH-20) and eluted with 100% MeOH, gave 3 subfractions B11a-B11c and then Fr. B11b was submitted to normal phase column chromatography (NP_CC) eluted with DCM:MeOH:H_2O (80:20:2 to 70:30:3). In addition, Fr. B8 was purified with MPLC using a mixture of H_2O (A) and MeOH:isopropanol (3:1; B) with an increasing amount of B to give compound **13**.

Fr. F (823.3 mg) was fractionated with increasing concentrations of MeOH in H_2O (up to 80%) by MPLC in order to yield 9 subfractions (F1-F9). Fr. F8 was obtained as pure compound **10**. Fr. F5 was chromatographed over silica gel (35 g, elution: DCM:MeOH:H_2O - 80:20:2 to 70:30:3) to give F5a-F5f. At this point, Fr. F5e gave compound **7** and Fr. F5a was purified by gel chromatography (Sephadex LH-20) with 50% MeOH elution to obtain compound **8**.

Fr. J (645 mg) was separated by gel chromatography (Sephadex LH-20) eluted with 100% MeOH and totally 6 subfractions (Fr. J1-J6) was obtained. Fr. J5 (266 mg) was purified by silica gel (Solvent system: DCM:MeOH:H_2O - 90:10:1 to 80:20:2) to obtain compound **11** and **12** (40.3 mg) as a mixture.

EtOAc extract (absorbed into 8 g silica gel) was chromatographed over silica gel (150 g) EtOAc:MeOH:H_2O (100:10:2.5 to 100:20:15) elution to obtain major fractions A-J grouped by TLC. For obtaining compound **9** (Subfraction D8 - 17,2 mg), Fr. D was purified by gel chromatography (Sephadex LH-20) with 100% MeOH.

*Sidol (**1**) / isosidol (**2**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., $CDCl_3$): *see* Supporting information; Positive-ion HRMS: *m/z* 385.2342 $[M+Na]^+$ (calc. for $C_{22}H_{34}O_4$, Mol. wt. 362.25), *m/z* 725.4982 $[2M+H]^+$, 747.4799 $[2M+Na]^+$.

*Linearol (**3**) / isolinearol (**4**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., $CDCl_3$): *see* Supporting information; Positive-ion HRMS: *m/z* 385.2340 $[M+Na]^+$ (calc. for $C_{22}H_{34}O_4$, Mol. wt. 362.25), *m/z* 725.4973 $[2M+H]^+$, 747.4791 $[2M+Na]^+$.

*Verbascoside (**5**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., CD_3OD): *see* Supporting information; Positive-ion HRMS: *m/z* 647.1943 $[M+Na]^+$ (calc. for $C_{29}H_{36}O_{15}$, Mol. wt. 624.21), *m/z* 1271.4001 $[2M+Na]^+$; Negative-ion HRMS: *m/z* 623.1976 $[M-H]^-$.

*Lavandulifolioside (**6**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., CD_3OD): *see* Supporting information.

*Leonoside A (**7**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., CD_3OD): *see* Supporting information; Positive-ion HRMS: *m/z* 793.2516 $[M+Na]^+$ (calc. for $C_{35}H_{46}O_{19}$, Mol. wt. 770.26); Negative-ion HRMS: *m/z* 769.2548 $[M-H]^-$.

*Leucoseptoside A (**8**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., CD_3OD): *see* Supporting information; Positive-ion HRMS: *m/z* 661.2097 $[M+Na]^+$ (calc. for $C_{30}H_{38}O_{15}$, Mol. wt. 638.22), *m/z* 1299.4313 $[2M+Na]^+$; Negative-ion HRMS: *m/z* 637.2133 $[M-H]^-$, 1275.4325 $[2M-H]^-$.

*Apigenin-7-O-glucopyranoside (**9**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., DMSO-d_6): *see* Supporting information; Positive-ion HRMS: *m/z* 433.1126 $[M+H]^+$ (calc. for $C_{21}H_{20}O_{10}$, Mol. wt. 432.11); Negative-ion HRMS: *m/z* 431.0968 $[M-H]^-$.

*Isoscutellarein-7-O-[6'''-O-acetyl-allopyranosyl-(1→2)-glucopyranoside] (**10**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., DMSO-d_6): *see* Supporting information; Positive-ion HRMS: *m/z* 653.1708 $[M+H]^+$ (calc. for $C_{29}H_{32}O_{17}$, Mol. wt. 652.16); Negative-ion HRMS: *m/z* 651.1559 $[M-H]^-$.

*Apigenin-7-O-(4''-O-p-coumaroyl)-glucopyranoside (11) / Apigenin-7-O-(3''-O-p-coumaroyl)-glucopyranoside (**12**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., DMSO-d_6): *see* Supporting information; Positive-ion HRMS: *m/z* 579.1484 $[M+H]^+$ (calc. for $C_{30}H_{26}O_{12}$, Mol. wt. 578.14); Negative-ion HRMS: *m/z* 577.1399 $[M-H]^-$.

*7-O-acetyl-8-epi-loganic acid (**13**):* ^{1}H and ^{13}C-NMR (500 MHz and 125 MHz, resp., CD_3OD): *see* Supporting information; Negative-ion HRMS: *m/z* 417.1398 $[M-H]^-$ (calc. for $C_{18}H_{26}O_{11}$, Mol. wt. 418.15).

3. Results and Discussion

The extracts of *Sideritis cypria* leaves were subjected to different column chromatography techniques, to afford four known diterpenes (**1-4**) from acetone extract and also various types of nine polar compounds (**5-13**) from EtOH extract (Figure 1). Each of the isolated compounds was elucidated by comparison of their spectroscopic data with published values in literature.

The diterpenes isolated in the present study were identified as sidol (**1**) and linearol (**3**) *ent*-kaur-16-ene derivatives, together with isosidol (**2**), and isolinearol (**4**) *ent*-kaur-15-ene derivatives. The structures of each isomer were established by the help of 2D-NMR (COSY, HSQC, HMBC) experiments and HRMS spectrometric data. These compounds were first reported from *Sideritis leucantha* and *S. linearifolia* [39]. The structures of phenylethanoid glycosides isolated from *S. cypria* were determined as verbascoside (**5**) [40], lavandulifolioside (**6**) [41], leonoside A (**7**) [42] and leucoseptoside A (**8**) [43], respectively. Apigenin 7-*O*-glucopyranoside (**9**) [44] and isoscutellarein 7-*O*-[6'''-*O*-acetyl-allopyranosyl-(1→2)-glucopyranoside] (**10**) [45] were isolated as pure compounds. However, two *p*-coumaric acid ester derivatives of flavone glycoside of apigenin were isolated as a mixture, **11** and **12**. The structures of the compounds **11** and **12** were elucidated using 1D-NMR (^{1}H and ^{13}C) and 2D-NMR (COSY, HSQC, HMBC) experiments and HRMS spectrometric data.. Based on spectral evidences, their structures have been determined as apigenin 7-*O*-(4''-*O*-*p*-coumaroyl)-glucopyranoside (**11**; major) and apigenin 7-*O*-(3''-*O*-*p*-coumaroyl)-glucopyranoside (**12**; minor) [46,47]. Moreover, 7-*O*-acetyl-8-*epi*-loganic acid (**13**)**,** one known iridoid glycoside [48,49], was isolated which was reported herein for the first time for genus *Sideritis*.

Mediterranean *Sideritis* spp. have been divided into four main diterpene-containing group in terms of chemotaxonomy [16]. Our results suggest that *S. cypria* could also be a part of *ent*-kaurene type tetracyclic diterpenes-rich group. In addition, it is suggested that *ent*-kaur-15-ene derivatives (**2** and **4**), in Turkish samples, are less frequent than *ent*-kaur-16-ene derivatives (**1** and **3**) in the same literature [16].

Iridoids and phenylethanoid glycosides are considered as important taxonomic markers for the family Lamiaceae. According to the studies performed on the genus *Sideritis*, it can be concluded that the molecular diversity is not very rich at the level of species studied. So far, few iridoids have been reported from *Sideritis* spp. These iridoid glycosides were 10-*O*-(*E*)-*p*-coumaroylmelittoside isolated from *S. lanata* [50], ajugoside from *S. perfoliata* L. subsp. *perfoliata* [51], ajugol from *S. scardica* [52], melittoside, 5-allosyloxy-aucubin from *S. italica* [4] and harpagide, 8-*O*-acetylharpagide, 8-*epi*-loganic acid from *S. montana* L. subsp. *montana*. In addition, 8-*epi*-loganic acid was reported for the first time from the genus *Sideritis* [53], however, in the present study, 7-*O*-acetyl derivate of 8-*epi*-loganic acid was reported for the first time for the genus *Sideritis*. On the other hand, phenylethanoid glycosides isolated from *Sideritis* spp. were di- and tri- glycosidic derivatives. Common diglycosidic forms were verbascoside (=acteoside) [14], leucosceptoside A and martynoside [54] while triglycosidic derivatives, such as lavandulifolioside [54], isolavandulifolioside, lamalboside, leonoside A [21], alyssonoside and echinacoside [55], were derived from verbascoside by binding the third sugar unit on the diglycosidic sugar moiety.

Flavonoid glycosides are also considered as taxonomic markers especially for some genera of Lamiaceae [6,16,56]. Up to now, flavonoid content of *Sideritis* taxa from Western Mediterranean [57,58] and Macaronesian region [59,60] were studied particularly where chemotaxonomic relations have already been revealed. The diversity of flavonoid content of different *Sideritis* spp. could be affected by geographical region. Besides, several Turkish *Sideritis* spp. were also investigated for their

flavonoid glycosides pertaining to Eastern Mediterranean region until now [12,14,15,61]. As reported, several Turkish *Sideritis* spp. comprise mono- or di- glycosidic forms of apigenin and/or scutellarein derivatives. Our results agreed with these investigations.

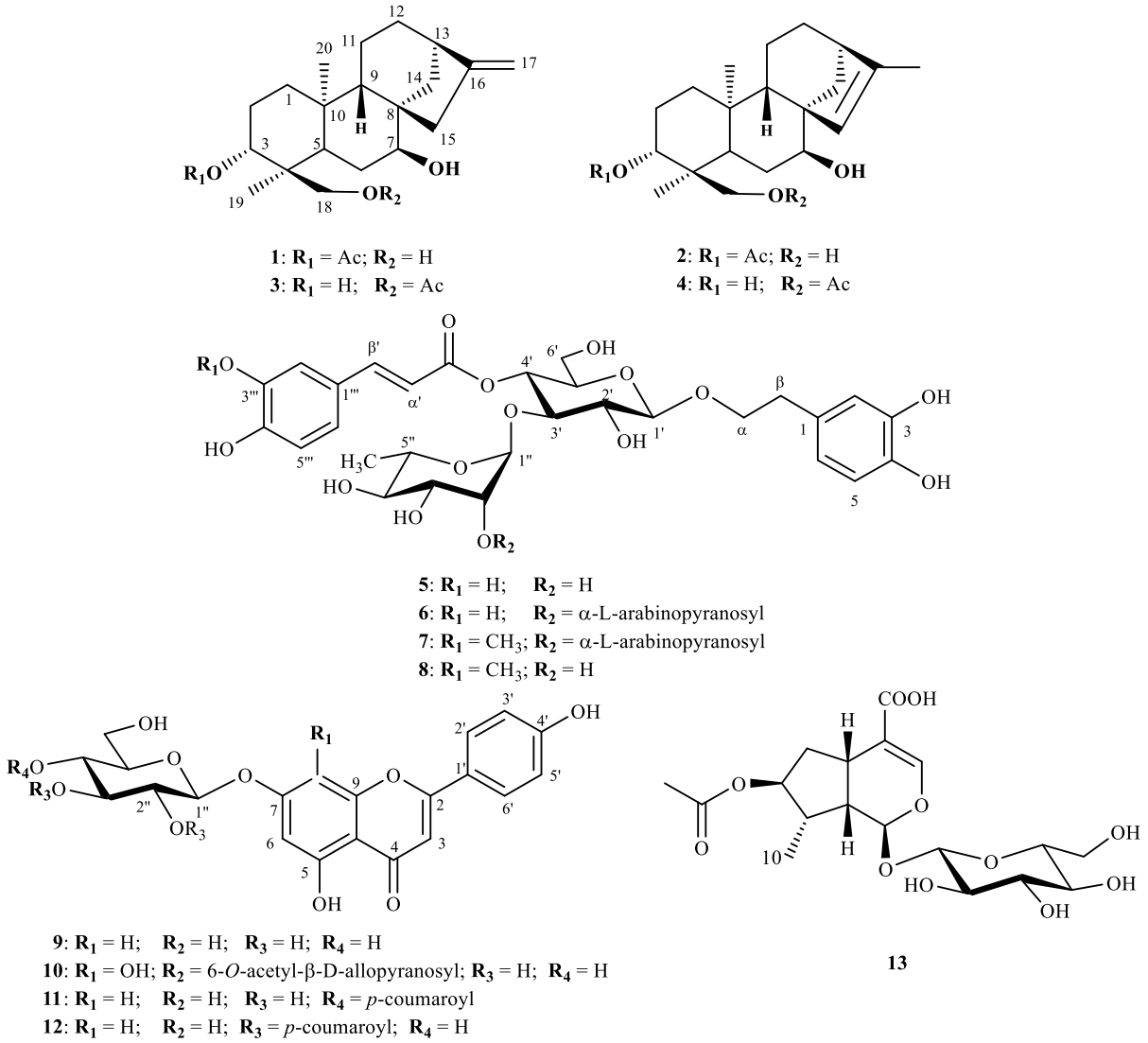

Figure 1. The chemical structures of isolated and elucidated compounds **1**−**13** from *Sideritis cypria* extracts

Regarding the isolated flavonoids, phenylethanoids and iridoids glycosides in the present study, arabinose-containing triglycosidic forms of phenylethanoids such as lavandulifolioside (**6**) and leonoside A (**7**) were also isolated from *Stachys* spp. which were found rarely both in nature and genera. In addition, Lenherr *et al.* [62] reported 8-hydroxyflavone glycosides containing acetylated allose as sugar moiety for the first time for *Stachys* genus from *Stachys recta.* Flavonoids isolated in the present study compound **9**, **10** and **12**, were also isolated from *Stachys lanata.* Conspicuously isolated compound **10,** a diglycosidic 8-hydroxyflavone containing an acetylated allose which is shown before as an important taxonomic marker for both Lamiaceae and related families and tribe *Stachydeae* includes *Sideritis*, *Stachys* and other close relationship genera, is also a supporting evidence for chemotaxonomical approach [5,16,56,63,64]. In addition flavonoid *p*-coumaroylglucosides, such as isolated compounds **11** and **12** in the present study, are also reported as chemotaxanomic markers by Tomás-Barberán *et al.* [56]. In the same study, it is suggested that existence of both type of compounds is an evidence of closer relationship for subtribes *Marrubiae* and *Lamiae* [56]. Even though iridoids are rare for these genera, 7-*O*-acetyl-8-*epi*-loganic acid (**13**) was

also isolated before from *Stachys spinosa* [41,45,47,49,65]. The idea of chemotaxonomicaly close relationship of these two genera, *Sideritis* and *Stachys*, is supported with our findings [4,21].

Hydrodistilled essential oil was analyzed simultaneously by GC-FID and GC-MS and 37 compounds were identified representing 99.43±0.15% of total essential oil. α-pinene (14.73±0.15%), β-pinene (16.60±0.20%) and β-phellandrene (17.83±0.23%) and *epi*-cubebol (7.70±0.20%) were determined as main compounds (Table 1).

Table 1. The Essential oil composition of aerial parts of *Sideritis cypria.*

Compound	**LRI** [a]	**LRI**[b]	**Relative percentage amounts** %
α-Pinene	1032	1025[e]	14.73±0.15[c]
α-Thujene	1035	1026[f]	1.27±0.06
β-Pinene	1118	1117[e]	16.60±0.20
Sabinene	1132	1122[f]	3.93±0.06
Myrcene	1174	1160[f]	0.50±0
α-Phellandrene	1176	1168[e]	1.53±0.06
α-Terpinene	1188	1177[f]	0.33±0.06
Limonene	1203	1212[g]	4.73±0.06
β-Phellandrene	1218	1209[f]	17.83±0.23
γ-Terpinene	1255	1245[f]	0.60±0
p-Cymene	1280	1282[g]	1.37±0.06
Terpinolene	1290	1285[e]	0.20±0
α-Cubebene	1466	1480[g]	0.40±0
α-Copaene	1497	1488[g]	0.47±0.11
β-Cubebene	1549	1541[f]	t[d]
Pinocarvone	1586	1575[f]	0.27±0.06
Terpinen-4-ol	1611	1601[f]	1.43±0.06
β-Caryophyllene	1612	1608[g]	2.57±0.15
Cadina-3,5-diene	1639		0.40±0
trans-Pinocarveol	1670	1661[f]	0.47±0.11
epi-Zonarene	1677		0.40±0
α-Humulene	1687	1666[f]	0.57±0.06
Cryptone	1690	1674[f]	2.37±0
α-Terpineol	1706	1694[f]	1.13±0.06
Bicyclosesquiphellandrene	1722		0.53±0.06
δ-Cadinene	1773	1755[f]	3.33±0.06
Cadina-1,4-diene (=*Cubenene*)	1799		0.33±0.06
Cuminaldehyde	1802	1784[f]	1.37±0.06
Myrtenol	1804	1790[f]	0.33±0.06
epi-Cubebol	1900	1900[f]	7.70±0.20
Cubebol	1957	1918[f]	3.40±0.10
Caryophyllene oxide	2008	1986[f]	2.67±0.06
Cubenol	2080	2047[f]	1.30±0.10
1-*epi*-Cubenol	2088	2088[f]	2.20±0.10
Cumin alcohol	2113	2058[h]	0.40±0
T-Cadinol	2187	2187[1]	0.90±0.10
14-Hydroxy-β-caryophyllene	2357		0.87±0.25
Total			99.43±0.15

[a]LRI: Linear retention indices calculated against *n*-alkanes on the HP Innowax column;
[b]LRI from literature (68[e], 69[f], 70[g], 71[f], 72[i]); [c]mean % calculated from Flame Ionization Detector (FID) data ± SD (n=3); [d]t: Trace (< 0.1 %)

The chemical content of *Sideritis cypria* essential oil was previously reported for post-flowering season [25]. When compared with the results of the present work, essential oil yield was found to be the same, however, some of the major compounds and their percentage amounts varied. In addition *trans*-piperitol, which was one of the major components in the previous work was not found at all in the current material. Kırımer *et al.* [8] divided essential oils of 50 taxa of genus *Sideritis*

growing in Turkey into 7 main groups. According to this data, our results can be put into the group of oils which composed of pinene and *epi*-cubebol as major compounds. In addition, oil yields of the members of this group were approximately 0.5% on average (between 0.01 and 0.85%) of all examined samples which also agreed with our finding. As Todorova and Trendafilova [66] reviewed, main constituents of essential oil compositions of *S. scardica*, which is an endemic species in Balkan peninsula, collected from different locations varied. Greece samples showed the highest monoterpene hydrocarbon rate while FYROM samples determined as oxygenated sesquiterpene rich. Diterpene compounds found to be trace amounts in the samples collected from Turkey. Moreover, Çarıkçı *et al.* [67] determined the essential oil compositions of five *Sideritis* species (*S. phrygia, S. pisidica, S. brevibracteata, S. bilgerana, S. hispida*) as sesquiterpene rich group. However Kırımer *et al.* [8] reported the major compounds of *S. pisidica* and *S. bilgerana* as monoterpene rich group.

In conclusion, this report presents the first phytochemical investigation of *S. cypria* endemic in Northern Cyprus. As illustrated by isolated molecules and elucidated structures various kinds of molecules such as diterpenes, phenylethanoids, flavonoids and an iridoid glycoside some of which are important taxanomical markers, and also composition of the essential oil, these results could be helpful for chemotaxanomical classification of genus *Sideritis*.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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