Food Chemistry 111 (2008) 179–186

Contents lists available at ScienceDirect

Food Chemistry

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

Constituents of *Sideritis syriaca.* ssp. *syriaca* (Lamiaceae) and their antioxidant activity

M. Armata [a], C. Gabrieli [a], A. Termentzi [a], M. Zervou [b], E. Kokkalou [a,*]

[a] *Laboratory of Pharmacognosy, Department of Pharmacognosy–Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, 54 124 Thessaloniki, Greece*
[b] *Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, Vas. Konstantinou 48, 11635 Athens, Greece*

ARTICLE INFO

Article history:
Received 21 November 2007
Received in revised form 12 December 2007
Accepted 19 March 2008

Keywords:
Sideritis syriaca
Flavonoids
Phenylpropanoid derivatives
Antioxidant activity

ABSTRACT

The aerial parts of *Sideritis syriaca* ssp. *syriaca* (Lamiaceae) were extracted, after defatting, with diethyl ether, ethyl acetate and *n*-butanol. The antioxidant activities of the extracts were evaluated through in vitro model systems, such as 1,1-diphenyl-2-picryl hydrazyl (DPPH) and Co(II) EDTA-induced luminol chemiluminescence. In both model systems the ethyl acetate extract was the most effective. Phytochemical analysis of ethyl acetate extract showed the presence of two new isomeric compounds (**1** and **1′**), identified as 1-rhamnosyl, 1-coumaroyl, dihydrocaffeoyl, protocatechuic tetraester of quinic acid, as well as chlorogenic acid (**2**), apigenin 7-O-glucoside (**3**), apigenin (**4**), 4′-O-methylisoscutellarein 7-O-[6‴-O-acetyl-β-D-allopyranosyl-(1 → 2)-β-D-glucopyranoside] (**5**), isoscutellarein 7-O-[6‴-O-acetyl-β-D-allopyranosyl-(1 → 2)-β-D-glucopyranoside] (**6**), 4′-O-methylisoscutellarein 7-O[β-D-allopyranosyl-(1 → 2)-β-D-glucopyranoside] (**7**) and 4′-O-methylisoscutellarein 7-O-[β-D-allopyranosyl-(1 → 2)-6″-O-acetyl-β-D-glucopyranoside] (**8**). The above compounds were identified by spectroscopic methods.

1. Introduction

Sideritis syriaca ssp. *syriaca* is endemic to the mountains of Crete (Greece) (Aligiannis et al., 2001), and is used to prepare herbal medicines and traditional teas known as 'mountain teas'.

A limited number of reports concerning the antioxidant activities of extracts or isolated compounds of *Sideritis* species have appeared in the literature (Akcos, Eser, Calis, Demirdamar, & Tel, 1999; Basile et al., 2006; Gabrieli, Kefalas, & Kokkalou, 2005; Nakiboglu, Orturk Urek, Ayar Kayali, & Tarhan, 2007; Rios, Manez, Paya, & Alcaraz, 1992; Tepe, Sokmen, Akpulat, Yumrutas, & Sokmen, 2006; Sagdic, Aksoy, Okzan, Ekici, & Albayrak, 2008). There is only one report for *Sideritis syriaca*, which states that the methanolic extracts of its aerial parts were equal antioxidants to rosmarinic acid, when antioxidant power was measured with the DPPH·test (Koleva et al., 2003). Recent research work showed moderate antioxidant capacity of *n*-BuOH extract of *Sideritis raeseri* (Gabrieli et al., 2005).

This paper is the first detailed phytochemical analysis of *S. syriaca* polar extracts, with regard to their antioxidant capacity, evaluated not only by the DPPH·, but also with the more sensitive luminol chemiluminescence test.

2. Material and methods

2.1. Plant material

Sideritis syriaca ssp. *syriaca* (Lamiaceae) was collected from Agios Mamas, near Chania (Crete) during the flowering stage, and authenticated by Dr. C. Furnaraki, Department of Systematic Botany, Agronomical Institute of MAICH, Crete, Greece (Herbarium voucher specimen, 8264).

2.2. Phytochemical study

2.2.1. General procedures, chemicals and standards

All solvents and chemicals used were of analytical or HPLC grade and obtained from Merck (Darmstadt, Germany). Sephadex LH-20 was obtained from Pharmacia (Uppsala, Sweden) and Polyamide CC-6 from Macherey-Nagel (Düren, Germany).

Acid hydrolysis was performed by refluxing a small quantity of the compound with 5 ml of 2 M HCl:methanol (1:1) for 2 h, evaporating the mixture to dryness under reduced pressure, dissolving the residue in 2 ml of water and extracting the solution with ethyl acetate. The aqueous phase was chromatographed on paper (Whatman 1 MM) with aqueous phenol saturated solution. Allose and glucose were identified by comparison with standards (Chari, Grayer-Barkmeijer, Harborne, & Osterdahl, 1981). Enzymatic hydrolysis was performed with acetyl esterase (Sigma–Aldrich, St. Louis, MO). The flavonoids were suspended in 0.5 ml Pi buffer

* Corresponding author. Tel.: +30 2310 997634; fax: +30 2310 997662.
E-mail address: kokkalou@pharm.auth.gr (E. Kokkalou).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.03.061

(0.1 M, pH 6.4; Sigma–Aldrich), and 0.1 ml of the commercial suspension of enzyme (Sigma) were added. The mixture was incubated at 30 °C for 24 h, extracted with *n*-BuOH, concentrated to dryness and redissolved in MeOH for HPLC analyses. The LC–DAD–MS (ESI+) set up consisted of a Finnigan Mass Spectra System P4000 pump coupled with a UV 6000 LP diode array detector and a Finnigan AQA (Thermoquest) spectrometer (Thermo Finnigan, San Jose, CA). The separation was performed on a 125 mm × 2 mm Superspher 100-4 RP18 column (Macherey-Nagel, Düren, Germany; 4 μm particle size) at a flow rate of 0.33 ml/min and monitored at 365, 340 and 290 nm. The column was kept at 40 °C and the following gradient was applied, using solvents (A), AcOH (2.5%) in water, and (B), MeOH: 90% A and 10% B at 0 min, 90% A and 10% B at 2 min, 0% A and 100% B at 42 min. The MS spectra were obtained in ESI+ mode at a capillary voltage of 4.90 kV, source voltage 45 V, probe temperature 450 °C, RF lens 0.3 V, ion energy 1.0 eV, detector voltage 650 V, scan range from *m*/*z* 130 to *m*/*z* 1046 with acquisitions at 0.6 scans/s and AQA max polarities at 12 and 70 eV, simultaneously. The NMR spectra were run on a Bruker AC spectrometer (300 MHz) and a Bruker Advance (500 MHz), and the UV–vis spectra on a Hitachi U-2000 spectrophotometer.

2.2.2. Extraction and isolation

The air-dried aerial parts of *S. syriaca* (390 g) were coarsely powdered (1400–1600 μm) and exhaustively extracted (Soxhlet) with petroleum ether (bp 50–70 °C), dichloromethane and methanol. The methanolic extract (16.1% w/w in dry plant) was concentrated to dryness under reduced pressure and the residue (62.86 g) was redissolved in boiling water and filtered. The filtrate was extracted with diethyl ether (5 × 200 ml), ethyl acetate (5 × 200 ml) and *n*-butanol (5 × 200) successively. The diethyl ether and butanol extracts were concentrated to dryness and gave 1.50 g (0.38% in dry plant) and 6.7 g (1.7% in dry plant) of residue. The ethyl acetate extract was concentrated to half of its volume and gave a precipitate **(A)** which after filtration gave 3.27 g of a yellow residue. **A** was chromatographed on a polyamide CC-6 column (40 cm × 3.5 cm) with a water–methanol gradient to yield 6 fractions. Compound **1** (6 mg) and 0.8 mg of compound **2** (100% H_2O) were isolated after purification with Sephadex LH-20 (methanol), from fractions 1 and 2. The residue from 80% (H_2O–MeOH) (fraction 3), was subjected to a second polyamide column (16.5 cm × 3.5 cm) and eluted with a H_2O–MeOH gradient, to yield 40.5 mg of compound **3**. MPLC of fraction 5, (50% MeOH), was rechromatographed on silica gel (9385) (17 cm × 2.5 cm) with a dichloromethane → methanol gradient and yielded 15.2 mg of compound **4,** eluted with 80% dichloromethane. Finally fraction 6 (70–100% MeOH) was chromatographed on a silica gel column (9385) (19 × 1.5 cm) with dichloromethane → methanol gradient and yielded 1 mg of compound **5,** purified with a Sephadex LH-20 column (MeOH), and a second sub-fraction (1.5 mg), which was analysed (due to the minor quantity) with LC–DAD–MS (ESI+). Compounds **6**, **7**, **8** were identified by the same chromatographic conditions as previous, using (A), AcOH (2.5%) in water, and (B), MeOH: 90% A and 10% B at 0 min, 90% A and 10% B at 1 min, 0% A and 100% B at 16 min. The MS spectra were obtained in ESI (+) mode under the same conditions as previously, with the exception of the probe temperature (450 C) and at maximum polarities at 12 and 50 eV simultaneously. From the precipitate **A** after isolation have been identified the following constituents: Compound **1**: 1-rhamnosyl, 1-coumaroyl, dihydrocaffeoyl, protocatechuic tetraester of quinic acid. Spot appearance: blue (UV), light blue F1 (UV/NH_3); TLC (cellulose): R_f = 0.84 (AcOH 15%): UV (λ_{max}, MeOH, nm): 285, 324. LC–DAD–MS (ESI+) analysis:[12 eV] 807 [M+Na] (45%), 802 [M+H_20] (50%), 785 [M+1], 507 [quinic acid + coumaric acid + rhamnose], 339 [quinic acid + coumaric acid], [70 eV] 807 [M + Na] (100%), 785 [M + 1], 507 [quinic acid + coumaric acid + rhamnose], 339 [quinic acid + coumaric acid], 177 [protocatechuic acid + Na]. ^{1}H NMR (500 MHz, CD_3OD) δ: 7.68 (1H, d, *J* = 15.5 Hz, H = 7′), 7.23 (1H, s, H = 2‴), 7.19 (1H, dd, *J* = 8 and 1.5 Hz, H = 6‴), 7.11 (2 H, d, *J* = 7.5 Hz, H = 2′, H = 6′), 7.10 (1H, d, *J* = 8 Hz, H = 5‴), 6.84 (1H, d, *J* = 8.5 Hz, H = 5″), 6.82 (1H, d, *J* = 1.5 Hz, H = 2″), 6.77 (1H, dd, *J* = 8.5 and 1.5 Hz, H = 6″), 6.72 (2 H, d, *J* = 7.5 Hz, H = 3′, H = 5′), 6.40 (1H, d, *J* = 15.5 Hz, H = 8′), 5.51 (1H, m, H = 5), 5.40 (1H, m, H = 3), 5.36 (1H, d, *J* = 2 Hz, H = 1‴″), 3.00–4.00 (sugar protons), 3.88 (1H, m, H = 4), 2.82 (1H, m, H = 7″), 2.36 (1H, m, H = 8″), 2.34 (1H, m, H = 2ax), 2.33 (2 H, m, H = 2eq, 6eq), 1.20 (3H, d, *J* = 6 Hz, CH_3), 1.59 (1H, m, H = 6ax).

Compound **2**: chlorogenic acid. Spot appearance: blue (UV), light blue F1 (UV/NH_3); R_f = 0.74 (AcOH 15%); UV (λ_{max}, MeOH, nm): 250 sh, 290, 329. LC–DAD–MS (ESI+) analysis: [20 eV] 355 [M+1], 163 [caffeic acid], [80 eV] 377 [M+Na+1], 355 [M+1], 163 [caffeic acid]. ^{1}H NMR (500 MHz, CD_3OD) δ: 7.59 (1H, d, *J* = 15.5 Hz, H = 7′), 7.03 (1H, s, H = 2′), 6.91 (1H, d, *J* = 8.5 Hz, H = 6′), 6.70 (1H, d, *J* = 8.5 Hz, H = 5i), 6.21 (1H, d, *J* = 15.5 Hz, H = 8'), 4.95 (1H, m, H = 5), 3.96 (1H, dd, *J* = 4.5 and 3.3 Hz, H = 3), 3.66 (1H, m, H = 4), 2.38 (1H, dd, *J* = 14.5 and 3.3 Hz, H = 2eq), 2.35 (1H, dd, *J* = 3.3 and 14.5 Hz, H = 2ax), 2.33 (1H, dd, *J* = 12.5 and 3.3 Hz, H = 6eq), 1.63 (1H, m, H = 6ax).

Compounds **3** and **4** were identified as apigenin 7-O-glucoside and apigenin, respectively, by UV, ^{1}H NMR (CD_3OD, 300 MHz) and MS (Gabrieli & Kokkalou, 1990).

Compound **5**: 4′-methylisoscutellarein 7-O-[6‴-O-acetyl-β-D-allopyranosyl-(1 → 2)-β-D-glucopyranoside]. Spot appearance: deep purple (UV), deep purple F1 (UV/NH_3); UV (λ_{max}, MeOH, nm): 220, 279, 306. LC–MS analysis: 667 [M+1], 601 [M–acetyl+1], 462 [M–acetyl–glu+1], 301 [aglycone+1]. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.09 (2H, d, *J* = 85, H = 2′ and H = 6′], 7.13 (2H, d, *J* = 8.5 Hz, H = 3′ and H = 5′], 6.91 (1H, s, H = 3), 6.68 (1H, s, H = 6), 5.06 (1H, d, *J* = 6.7, H = 1′), 4.91 (1H, d, *J* = 6.7, H = 1″), 3.90 (3H, s, H = OCH_3), 3.00–4.00: sugar protons, 1.90 (3H, s, $COCH_3$).

Compounds identified only by LC–DAD–MS (ESI+) (Gabrieli et al., 2005).

Compound **6**: isoscutellarein 7-O-[6‴-O-acetyl-β-D-allopyranosyl-(1 → 2)-β-D-glucopyranoside] UV (λ_{max}, MeOH, nm): 240, 278, 306 328. LC–MS analysis: 675 [M+Na], 653 [M+1], 287 [aglycone+1].

Compound **7**: 4′-methylisoscutellarein 7-O-[β-D-allopyranosyl-(1 → 2)-β-D-glucopyranoside] UV (λ_{max}, MeOH, nm): 240, 278, 306 328. LC–MS analysis: 647 [M+Na], 625 [M+1], 463 [M–sugar+1], 301 [aglycone+1].

Compound **8**: isoscutellarein 7-O-[β-D-allopyranosyl-(1 → 2)-6″-O-acetyl-β-D-glucopyranoside] UV (λ_{max}, MeOH, nm): 240, 278, 306 328. LC–MS analysis:689.

[M+Na], 667 [M+1], 504 [M–glucose+1], 301 [M–glucose–glucose–CH_3CO+1], 301 [aglycone+1].

2.3. Antioxidant activity

2.3.1. Chemicals and reagents

The solvents used for the present work were purchased from Merck (Darmstadt, Germany) and Panreac (Barcelona, Spain). Sodium carbonate, $CoCl_2 \cdot 6H_20$ and Stabilised perhydrol 30% H_2O_2, were purchased from Merck; DPPH (1,1-diphenyl-2-picrylhydrazyl 90%), EDTA, luminol (3-aminophthalhydrazide) and boric acid from Sigma (St. Louis, MO).

2.3.2. Evaluation of antioxidant activity using the DPPH· and the Co(II)/EDTA-induced luminol chemiluminescence (CL) methods

The antioxidant activity of all extracts was first determined using the DPPH· test, according to Termentzi, Kefalas, and Kokkalou (2006). Different concentrations of all extracts were prepared. An

aliquot of 25 μl of diluted sample was added to 975 μl DPPH· solution (2×10^{-5} M) and the mixture vortexed. The decrease in the absorbance was determined at 515 nm when the reaction reached a plateau, using an HP 8452A diode array spectrophotometer, with 10 mm quartz cuvettes. For the samples diluted with methanol, methanol was used to zero the spectrophotometer. For those not diluted with methanol, the apparatus was zeroed with methanol (975 μl) and dimethylsulphoxide (DMSO 25 μl). The absorbance of the DPPH· radical without any sample was measured at 515 nm. The DPPH· concentration in the reaction medium was calculated from the calibration curve, as determined by linear regression:

A_{515} nm = 0.0248 × [DPPH· (μg/ml)] + 0.0138 (r^2 = 0.9968).

For each sample concentration tested, the percentage of DPPH· remaining in the steady state; was calculated in the following way: Percentage of remaining DPPH = [DPPH·] t_T [DPPH·] t_o, where T is the time necessary to reach the steady state. The antioxidant capacity of each sample was expressed as the amount of sample necessary to decrease the initial DPPH· concentration by 50% (EC_{50}). The antiradical efficiency (AE) was calculated as follows:

$AE = 1/EC_{50}$.

The antioxidant activity was also determined using the Co(II)/EDTA-induced luminol chemiluminescence method, according to Termentzi et al. (2006). The chemiluminescence measurements were carried out on a Model 6200 Fluorimeter, (Jenway, Dunmow, United Kingdom), keeping the lamp off and using only the photomultiplier of the apparatus. At least three different dilutions of the extracts were prepared. One millilitre of borate buffer (0.05 M, adjusted to pH 9 with 1 M NaOH), containing 1 mg/ml EDTA and 0.2 mg/ml of $CoCl_2 \cdot 6H_2O$, was added to 100 μl of luminol solution (5.6×10^{-4} M) in borate buffer (0.05 M, adjusted to pH 9 with 1 M NaOH) in a test tube, and the mixture vortexed for 15 s. Then, 25 μl of H_2O_2 aqueous solution (4.5×10^{-3} M) were deposited on the bottom of a 10 × 10 mm glass cuvette, using precision pipettes. The luminol–buffer mixture was rapidly added to the cuvette, using a Pasteur pipette, and carefully mixed for 15 s, in order to initiate the chemiluminescence reaction. When the reaction reached a plateau, the chemiluminescence (CL) intensity (Io) was recorded. Immediately afterwards, 25 μl of the sample were added and the instantaneous decrease of the light emission was recorded (I). The ratio Io/I was calculated. This ratio vs. μg extract/ml was plotted for three prepared dilutions of each extract and a linear regression was established, in order to calculate IC_{50}. IC_{50} is the amount of sample needed to decrease, by 50%, the CL intensity. The antiradical efficiency ($AE = 1/IC_{50}$) was also calculated.

Three reference antioxidants (quercetin, Trolox and ascorbic acid) were used as standards for the evaluation of the antioxidant power of our extracts. Their EC_{50}, IC_{50} and AE values are present in the form of diagrams in Figs. 1 and 2. The correlation between the two methods is given in Fig. 3.

3. Results and discussion

3.1. Structure elucidation

The structure of compound **1** was elucidated on the basis of spectral data (UV, ^{1}H NMR, LC–MS) as a quinic acid esterified with rhamnose, and protocatechuic, dihydrocaffeoyl and coumaroyl acids. The UV spectrum of **1** in methanol, as well as its R_f value, are evidence that compound **1** is a quinic acid derivative. The ^{1}H NMR spectrum exhibited signals for a coumaric, a dihydrocaffeic and a protocatechuic acid, as well as a rhamnosyl moiety. The quinic acid was esterified at positions C-1, C-3 and C-5, as the signals for the protons C-3 and C-5 shifted downfield, δ_H = 5.51 (H-5) and 5.36 (H-3) ppm, compared to free quinic acid (Merfort, 1992). The

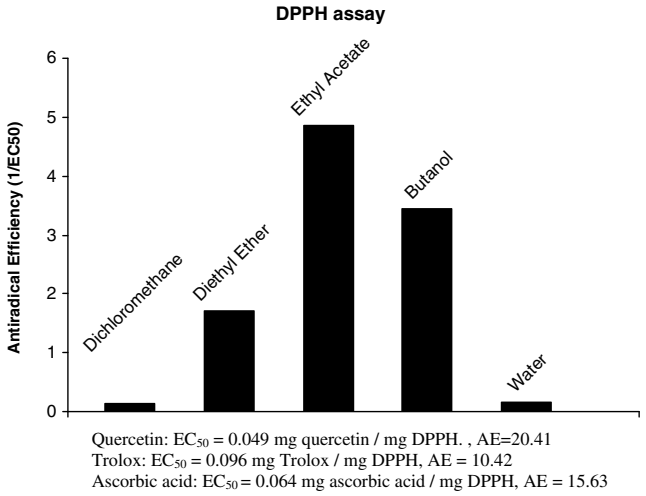

Fig. 1. Andiradical efficiencies (AE) of all fractions using the DPPH· test.

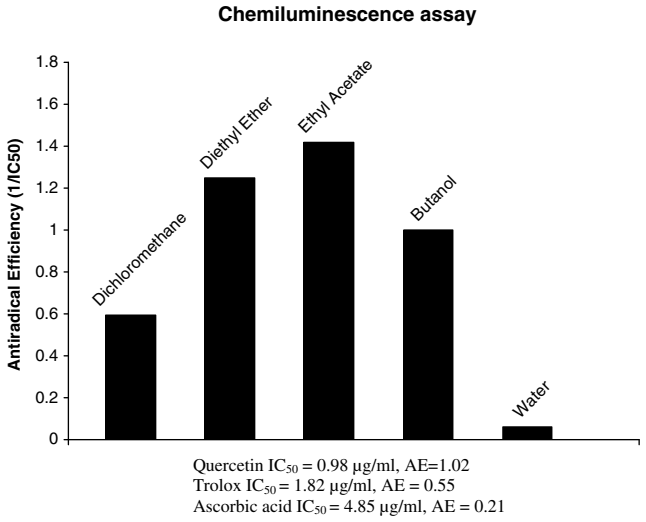

Fig. 2. Andiradical efficiencies (AE) of all fractions using the chemiluminescence test.

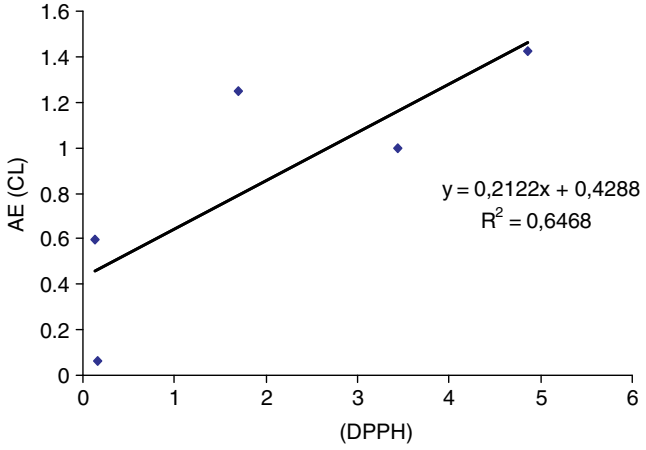

Fig. 3. Correlation between DPPH· and chemiluminescence results for antiradical efficiencies).

signals of H-5 (equatorial), H-4 (axial) and H-3 (axial) of the quinic acid moiety were assigned according to their multiplicity and their spin–spin coupling constants. The anomeric proton signal of rhamnose observed downfield at δ_H = 5.30 ppm was associated with an esteric linkage between the sugar and the free carboxyl group of quinic acid. The LC–DAD–MS (ESI+) showed that there is a main peak at 17.82 min (compound **1**) together with another at 18.47 min (compound **1′**) low intensity (Fig. 4). The UV channels were set at 310 and 290 nm, respectively. The MS for compound **1** showed an [M+1] ion at *m/z* 785 [12 eV] with very low intensity, an M+Na ion at *m/z* 807 (45%) and an M+H_2O ion at *m/z* 802 (50%). The fragment at *m/z* 339 is the base peak, corresponding to the loss of a dihydrocaffeic and a protocatechuic acid moiety. The coumaroyl moiety was not eliminated and it is obvious that coumaric acid is attached at C-1 of quinic acid, as also observed with 1,3-di-O-caffeoylquinic acid (Merfort, 1992). The other acids, protocatechuic and dihydrocaffeic, are attached to C-3 and C-5 of quinic acid, or *vice versa* and so revealed two isomers, corresponding to the peaks at 17.82 and 18.74 min. The second isomer appeared only during the LC–DAD–MS analysis, and the only difference from the first isomer was in the relative intensity of MS fragments, such as M+Na (65%), M+H_2O (50%) at 12 eV and M+Na (100%), M+H_2O (10%) at 70 eV. Figs. 5 and 6 illustrate the UV absorptions and the MS spectra of compounds **1** and **1′**, respectively. A fragmentation mechanism of these compounds is shown in Fig. 7.

The structures of compounds **2, 3, 4** and **5** were established by standard chemical and spectroscopic evidence and by comparison with literature (Ansari, Barron, Abdalla, Saleh, & Quere, 1991; Lenherr, Lahloub, & Sticher, 1984; Mabry, Markham, & Tomas, 1970; Rios et al., 1992), while compounds **6, 7** and **8** were elucidated by means of LC–DAD–MS (ESI+) analysis. Constituent **5** was hydrolysed with acid to give glucose and allose. Allose has a similar R_f value to glucose in BAW (butanol:acetic acid:water) and other chromatographic systems but separates clearly in an aqueous phenol saturated solution (allose R_f: 0.43, glucose R_f: 0.32). The presence of an acetyl group in the molecule is clearly evident from the ^{1}H NMR signal at 1.90 ppm. This was also apparent when treatment with acetyl esterase gave a new glycoside with lower R_f values in BAW and 15% HOAc (Chari et al., 1981). Evidence for the β-configuration of both sugars was drawn from the coupling constants of 7.2 Hz (H-1″) and 6.9 Hz (H-1‴) of the two anomeric proton doublets. An aliquot (0.1 mg) of the mixture containing **6, 7** and **8** was hydrolysed with acid, as above, and the same sugars were identified. Treatment with acetyl esterase afforded a mixture of glycosides, which after LC–DAD–MS analysis afforded two peaks instead of three corresponding to compounds **6** and **7** as was expected after deacetylation of compound **8**.

Radical-scavenging activity, expressed as EC_{50}, ranged from 0.21 to 6.09 mg antioxidant/mg DPPH$^{\cdot}$, and the respective antioxidant capacities are illustrated in Fig. 1. According to the DPPH as-

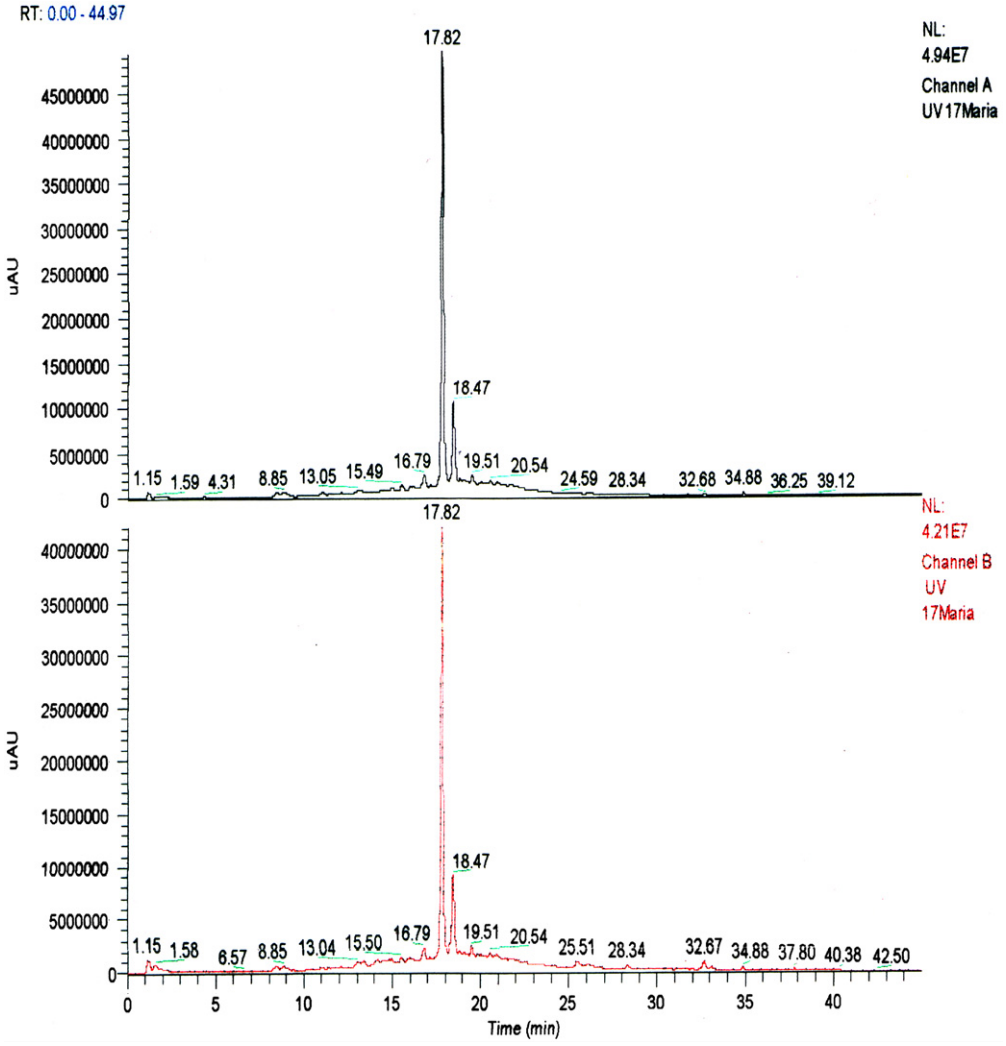

Fig. 4. HPLC chromatogram of compounds **1** and **1′** at 310 and 290 nm, respectively.

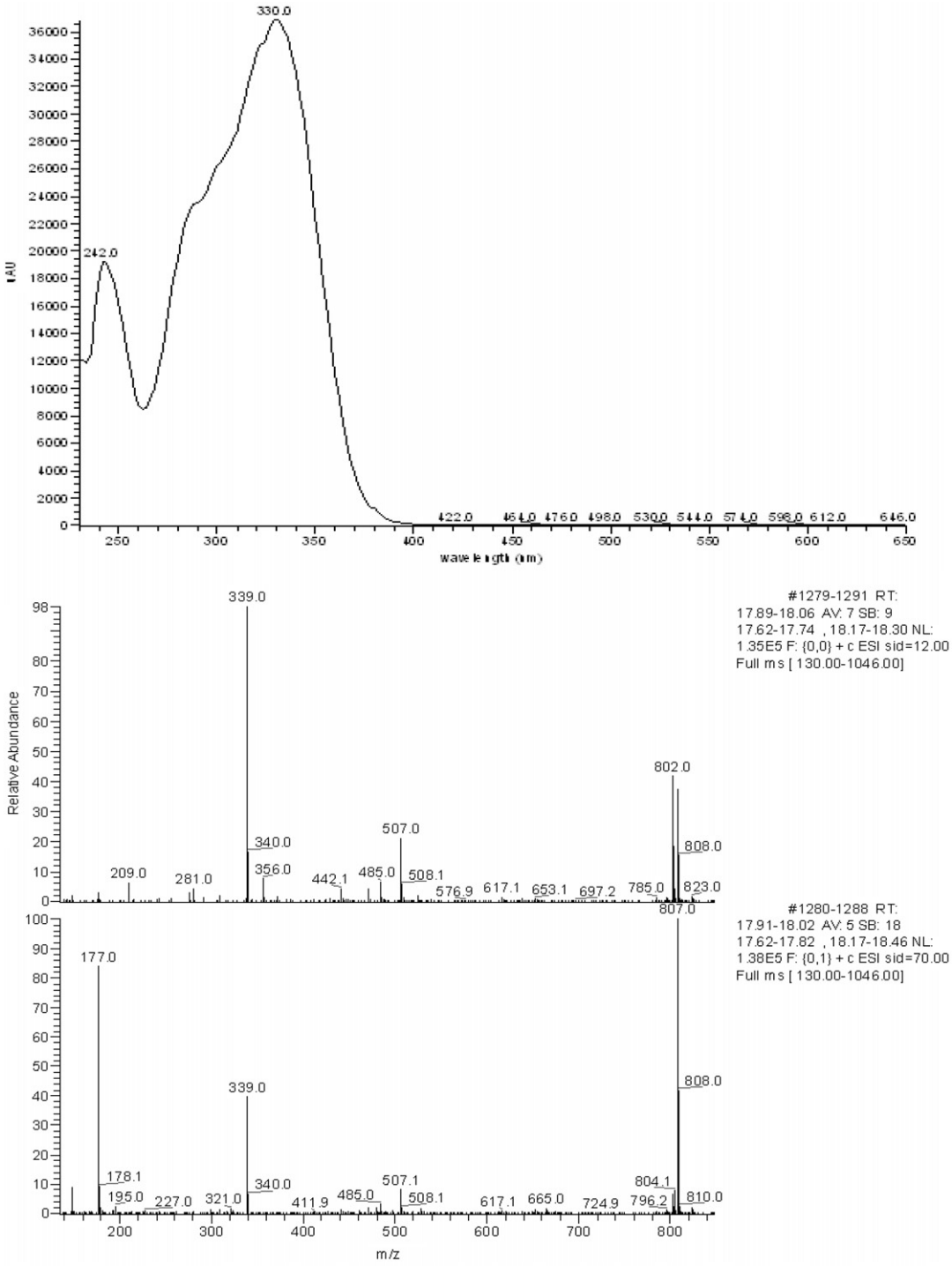

Fig. 5. UV and MS spectra of compound **1**.

say, the ethyl acetate fraction was by far the best antioxidant sample, followed by the butanolic fraction. The diethyl ether fraction came next, while dichloromethane and water fractions were the weakest antioxidants amongst the samples. Compared to the three standards used, all samples studied had weaker antioxidant power. However, the ethyl acetate fraction approached the antioxidant capacity of Trolox.

When measured by the chemiluminescence assay, IC_{50} ranged from 0.71 to 16.3 μg antioxidant/ml and the respective antioxidant capacities are illustrated in Fig. 2. The ethyl acetate fraction was, according to this method too, the strongest antioxidant among all samples, followed by the diethyl ether fraction. Butanol and dichloromethane fractions came next and the weakest was again the aqueous extract. However, according to the CL test, all extracts had stronger antioxidant power than the three standards used. The ethyl acetate fraction was a 1.5 times stronger antioxidant than quercetin, 3 times stronger than Trolox and more than 7 times stronger than ascorbic acid.

3.2. Comparison between the two methods of radical-scavenging activity

A direct correlation between the two methods of radical-scavenging activity (EC_{50} for DPPH· test vs. IC_{50} for CL test), was

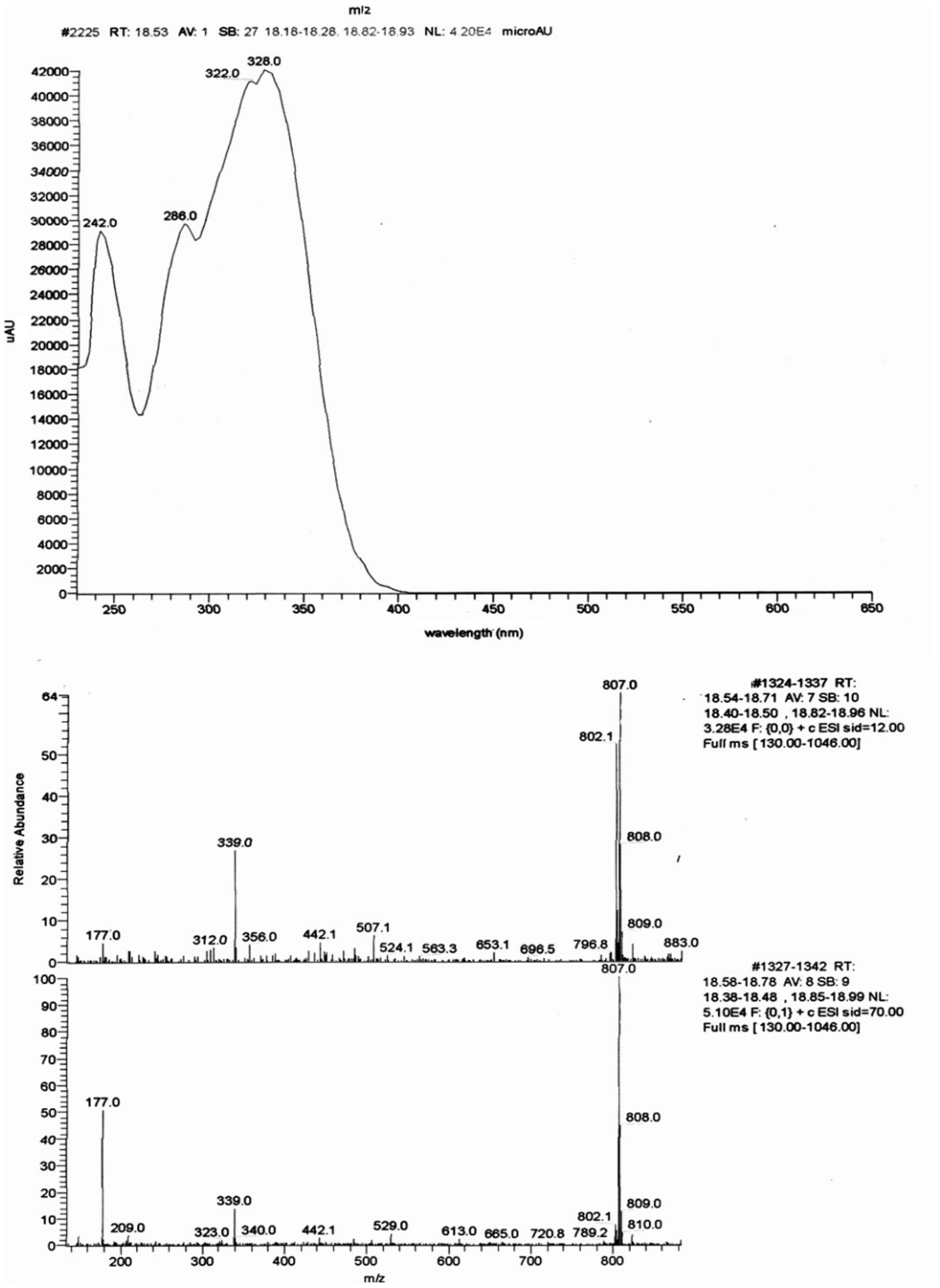

Fig. 6. UV and MS spectra of compound **1′**.

demonstrated by linear regression analysis (Fig. 3). The two methods showed a rather low correlation coefficient (0.6468). This was somehow expected, since the two methods have large differences in the measurement of the antioxidant power (Termentzi et al., 2006). That may also indicate a selectiveness of the antioxidant activity of the studied compounds.

3.3. Antioxidant capacity in correlation to the phenolic content

The antioxidant capacity of the ethyl acetate extract of *S. syriaca* aerial parts could be attributed to the phenolic content. Despite the fact that the extract was not effective when measured with the DPPH test, it seemed to possess potent antioxidant capacity when measured with the chemiluminescence test (1.4 times stronger than quercetin, 2 times stronger than trolox and 7 times stronger than ascorbic acid). This showed that the substances are capable of scavenging reactive oxygen species (O_2^- and OH**.**) and act like hydrogen donors. Apigenin, and apigenin and isoscutellarein glycosides are antioxidant agents, due to the acidic 4′-hydroxyl group (Rice-Evans, Miller, & Paganga, 1996). Furthermore, the presence of hydroxycinnamic acids enriched the antioxidant capacity. The –CH=CH–CO– group ensures great hydrogen-donating ability and

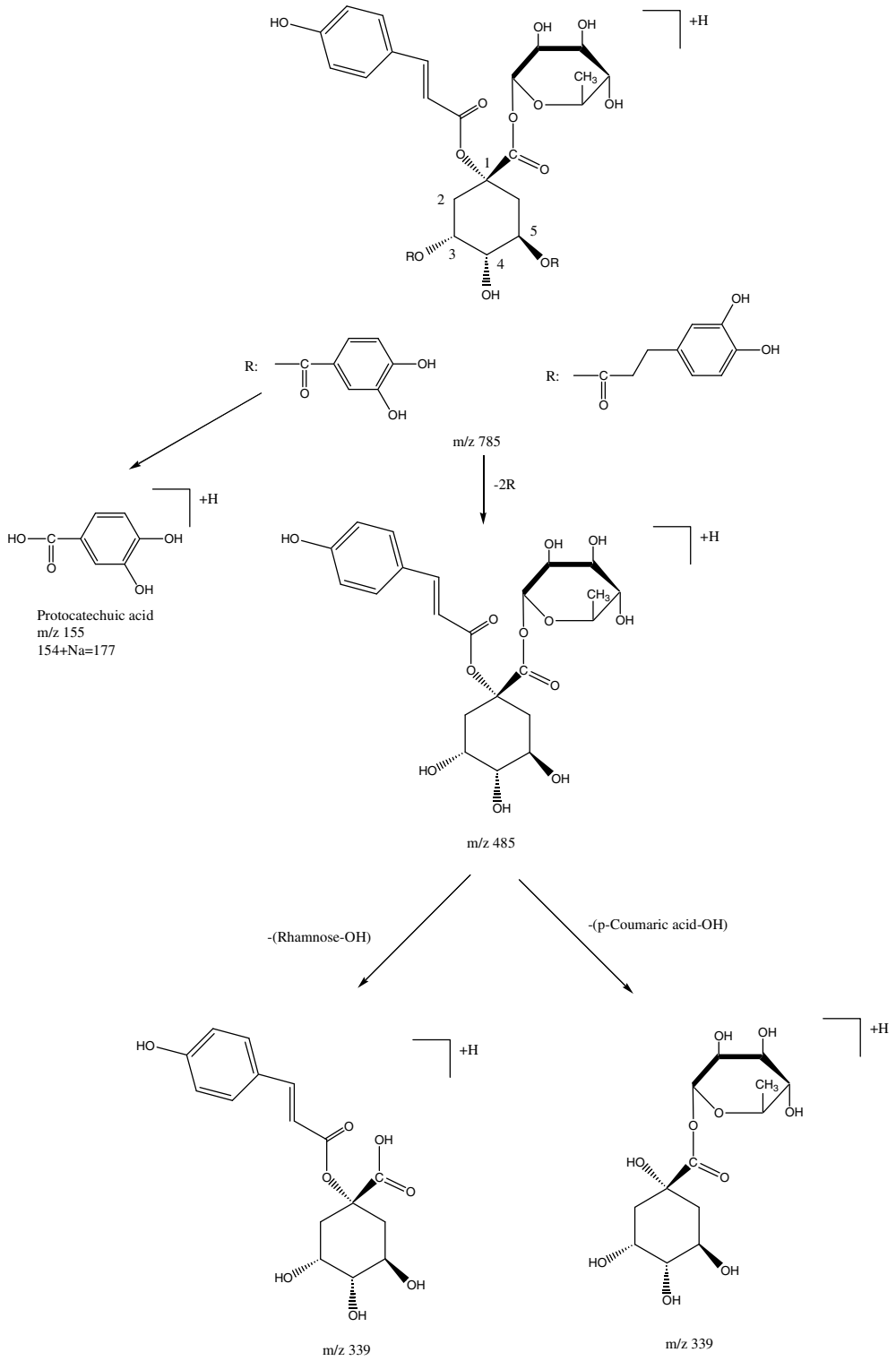

Fig. 7. MS fragmentation patterns of compounds **1** and **1′**.

thus enforces the antioxidant capacity (Cuvelier, Richard, & Bercet, 1992). 4′-Methylisoscutellarein glycosides are not expected to be potent antioxidants, due to methylation of the 4′-hydroxyl group.

4. Conclusions

In spite of the fact that the hexane extract obtained from the leaves of *S. syriaca* showed the absence of tocopherols (antioxidant factors) (Demo, Petrakis, Kefalas, & Boskou, 1998), we examined other extracts of aerial parts of this plant, which were found to possess potent antioxidant activity. We preferred to examine the most polar extracts of aerial parts and not the leaves only, since the water extracts of all these plant parts are widely used as a decoction. The results showed that, contrary to *S. raeseri*, which possesses moderate antioxidant power according to previous research work (Gabrieli et al., 2005), *S. syriaca* aerial parts could

act as antioxidants, due to their phenolic content. Phytochemical analysis suggests that this capacity could be attributed to the presence of apigenin and phenylpropanoids, substances that were absent from the *S. raeseri* extracts.

Results suggest that the aqueous beverage of *S. syriaca* aerial parts could be beneficial for health due to its antioxidant capacity. In order to enforce this conclusion, the research is continued by testing other polar extracts of constituents of this plant.

Acknowledgement

The authors are grateful to the Agronomical Institute of MAICH (Dr. P. Kefalas) for recording the LC–DAD–MS.

References

Akcos, Y., Eser, N., Calis, I., Demirdamar, R., & Tel, B. C. (1999). *Pharmaceutical Biology, 37*, 118–122.

Aligiannis, N., Kalpoutzakis, E., Chinou, I. B., Mitakou, S., Gikas, E., & Tsarbopoulos, A. (2001). Composition and antimicrobial activity of the essential oils of five taxa of *Sideritis* from Greece. *Journal of Agricultural and Food Chemistry, 49*, 811–815.

Ansari, M. A., Barron, D., Abdalla, M. F., Saleh, N. A. M., & Quere, J. L. (1991). Flavonoid constituents of *Stachys aegyptiaca*. *Phytochemistry, 30*, 1169–1173.

Basile, A., Senatore, F., Gargano, R., Sorbo, S., Del Pezzo, M., Lavitola, A., et al. (2006). Antibacterial and antioxidant activities in *Sideritis italica* (Miller) Greuter et Burdet essential oils. *Journal of Ethnopharmacology, 107*, 240–248.

Chari, V. M., Grayer-Barkmeijer, R. J., Harborne, J. B., & Osterdahl, B. G. (1981). An acylated allose-containing 8-hydroxyflavone glycosides from *Eronica filiformis*. *Phytochemistry, 20*, 1977–1979.

Cuvelier, M. E., Richard, H., & Bercet, C. (1992). Comparison of the antioxidant activity of some acid-phenols: Structure–activity relationships. *Bioscience, Biotechnology and Biochemistry, 56*, 324–325.

Demo, A., Petrakis, C., Kefalas, P., & Boskou, D. (1998). Nutrient antioxidants in some herbs and Mediterranean plant leaves. *Food Research International, 31*(5), 351–354.

Gabrieli, C., & Kokkalou, E. (1990). A glucosylated acylflavone from *Sideritis raeseri*. *Phytochemistry, 29*(2), 681–683.

Gabrieli, C. N., Kefalas, P. G., & Kokkalou, E. L. (2005). Antioxidant activity of flavonoids from *Sideritis raeseri*. *Journal of Ethnopharmacology, 96*, 423–428.

Koleva, I. I., Linssen, J. P. H., van Beek, T. A., Evstatieva, L. N., Kortenska, V., & Handjieva, N. (2003). Antioxidant activity screening of extracts from Sideritis species (Labiatae) grown in Bulgaria. *Journal of the Science of Food and Agriculture, 83*, 809–819.

Lenherr, A., Lahloub, M. F., & Sticher, O. (1984). Three flavonoid glucosides containing acetylated allose from *Stachys recta*. *Phytochemistry, 23*, 2343–2345.

Mabry, T. J., Markham, K. R., & Tomas, M. B. (1970). *The systematic identification of flavonoids*. Berlin: Springer-Verlag.

Merfort, I. (1992). Caffeoylquinic acids from flowers of *Arnica montana* and *Arnica chamissonis*. *Phytochemistry, 31*, 2111–2113.

Nakiboglu, M., Orturk Urek, R., Ayar Kayali, H., & Tarhan, L. (2007). Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey. *Food Chemistry, 104*, 630–635.

Rice-Evans, C., Miller, N., & Paganga, G. (1996). Structure–antioxidant activity of flavonoids and phenolic acids. *Free Radical in Biology and Medicine, 30*, 933.

Rios, J. L., Manez, S., Paya, M., & Alcaraz, M. J. (1992). Antioxidant activity of flavonoids from Sideritis Javalambrensis. *Phytochemistry, 31*, 1947–1950.

Sagdic, O., Aksoy, A., Okzan, G., Ekici, L., & Albayrak, S. (2008). *Innovative Food Science and Emerging Technologies, 9*, 80–84.

Tepe, B., Sokmen, M., Akpulat, H. A., Yumrutas, O., & Sokmen, A. (2006). *Food Chemistry, 98*, 9–13.

Termentzi, A., Kefalas, P., & Kokkalou, E. (2006). Antioxidant activities of various extracts and fractions of *Sorbus domestica* fruit at different maturity stages. *Food Chemistry, 98*, 599–608.