NPC Natural Product Communications

2014
Vol. 9
No. 0
1 - 2

Dual Excitatory and Smooth Muscle-relaxing Effect of *Sideritis montana* L. Extract on Guinea-pig Ileum

Barbara Tóth[a], Loránd Bartho[b], Andrea Vasas[a], Zsolt Sándor[b], Nikoletta Jedlinszki[a], Gyula Pinke[c] and Judit Hohmann[a,*]

[a]*Department of Pharmacognosy, University of Szeged, H-6720 Szeged, Eötvös u. 6., Hungary*

[b]*Department of Pharmacology and Pharmacotherapy, University of Pécs, H-7624 Pécs, Szigeti u. 12., Hungary*

[c]*Department of Botany, Faculty of Agricultural and Food Sciences, University of West Hungary, H-9200 Mosonmagyaróvár, Vár u. 2., Hungary*

hohmann@pharm.u-szeged.hu.

Received: October 16th, 2014; Accepted: XX, 2014

The neuronal and smooth muscle effects of methanol extract prepared from the air-dried flowering aerial parts of *Sideritis montana* L. (SEM) was tested *in vitro* on guinea-pig ileum. The chemical composition of the investigated extract was analysed by HPLC-MS, and chrysoeriol, chlorogenic acid and caffeic acid were detected as main constituents. The isolated organ assay showed that *S. montana* extract caused an immediate contraction and a more slowly developing inhibitory response in the ileum. The SME-induced contractions were strongly inhibited by the acetylcholine muscarinic receptor antagonist atropine (0.5 μM), but not by the Na^+ channel blocker tetrodotoxin (TTX; 0.5 μM) or the histamine H_1 receptor antagonist chloropyramine (0.5 μM). Selective desensitization of capsaicin-sensitive neurons by the sensory neuron stimulant and blocker capsaicin did not influence the contractile effect of SME. As to the spasmolytic effect, SME inhibited the effects of electrical field stimulation, exogenous acetylcholine, or histamine. These smooth muscle-relaxing effects were reversible by repeated renewals of the bathing solution in 40 min.

Keywords: *Sideritis montana*, Lamiaceae, phenolic compounds, spasmolytic activity, muscarinic receptors.

Sideritis montana L. belongs to the family Lamiaceae, genus *Sideritis*. The genus *Sideritis* comprises more than 150 perennial and annual species, most of them grow in the Mediterranean area [1], but some species are also found in Germany and Western China [2].

Sideritis species are commonly known as ”mountain tea”. The decoctions or the infusions of aerial parts are traditionally used in Mediterranean folk medicine against different diseases, infections and injuries, because of their anti-inflammatory [3], analgesic [4], antitussive, spasmolytic [5], antimicrobial [6], anticonvulsant [7], antiulcerative [8], and wound-healing [2] effects. Different extracts and fractions, and isolated compounds, such as diterpenoids [9], phenylpropanoid glycosides [10] and flavonoids [11] were investigated to validate their use in folk medicine. These studies have shown anti-inflammatory, antioxidant, antiulcerative, analgesic and antimicrobial activities [2,12,13]. Some diterpenoids isolated from *Sideritis* ssp, and their semisynthetic derivatives showed anti-HIV activity [2]. Different extracts possessed antiproliferative, selective estrogen modulatory and antiherpetical activity [2].

The typical chemical constituents of the *Sideritis* genus are flavonoids [14,15], iridoids [16], coumarins, lignanes [17], phenylpropanoid glycosides [18], and sterols [19]. Almost every species of the *Sideritis* genus contain diterpenes [20], flavonoids [21], and essential oil [22,23], and with regard to the literature data, these constituents are responsible for the main pharmacological activities.

The aim of this study was to evaluate the effect of *S. montana* methanolic extract (SME) on the motor responses of the ileum of the guinea-pig and to determine the possible mechanisms encountered. The chemical composition of the investigated plant extract was characterized by HPLC-MS.

A methanol extract from the air-dried aerial parts of *S. montana* was prepared. The HPLC-MS analysis of the extract revealed the presence of phenolic compounds, the flavonoid chrysoeriol and phenolic acids, chlorogenic acid and caffeic acid (Figure 1). These compounds were detected previously from some *Sideritis* species [24], but chlorogenic acid and caffeic acid were identified for the first time from *S. montana*. Previously only the components of the essential oil and seeds of this plant were reported besides chrysoeriol [25].

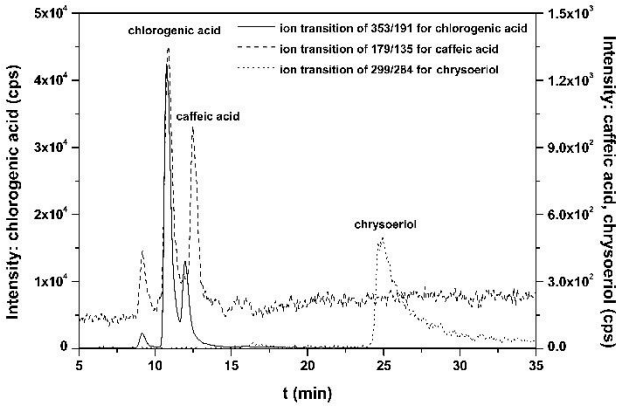

Figure 1: MRM spectra of the *S. montana* methanol extract. For clarity only one ion transition was illustrated for all components (353→191 for chlorogenic acid, 179→135 for caffeic acid and 299→256 for chrysoeriol).

The pharmacological investigation of *S. montana* methanolic extract (SME) showed that it caused an immediate contraction and a more

slowly developing inhibitory response in the ileum. Contraction in response to 2 mg/mL of SME was not reproducible upon repeated administration (n=4) and anyway this amount yielded a too high DMSO concentration in the organ bath, therefore a standard concentration of 1 mg/mL was used, whose action proved to be reproducible with contact times of 3 min and a washout period of 40 min between the two administrations. For assessing concentration-effect relationship, lower concentrations were also tested (Table 1). At none of the corresponding volumes of administration did the solvent (DMSO) cause a contraction exceeding 5% of the maximal spasm.

Table 1: Contractile effects of different concentrations of *S. montana* extract (SME) on the guinea-pig small intestine.

Sample	% ± SD	n
SME 0.33 mg/mL	18.1 ± 8.82	6
SME 0.66 mg/mL	27.6 ± 11.94	11
SME 1.00 mg/mL, 1st administration	28.6 ± 5.03	7
SME 1.00 mg/mL, 2nd administration	31.8 ± 6.88	7

1 mg/mL in two successive administrations were performed (each for 3 min), separated by a 40 min washout period. Contractions are expressed as % of the maximal longitudinal spasm in response to histamine (10 μM).

Pharmacological analysis has shown that SME-induced contractions were strongly inhibited by the acetylcholine muscarinic receptor antagonist atropine (0.5 μM; Table 2), but not by the Na^+ channel blocker (nerve conduction inhibitor) tetrodotoxin (TTX; 0.5 μM) or the histamine H_1 receptor antagonist chloropyramine (0.3 μM) (Table 2). Likewise, the sensory neuron stimulant and blocker capsaicin (1 μM, administered for 50 min) failed to inhibit the effect of SME. Capsaicin caused an immediate excitation, then a more delayed inhibition [26], but at the time of SME administration it had no effect on either basal tone or the electrically-evoked cholinergic contractions, while the preparation was totally unresponsive to capsaicin itself (tested in separate experiments, n=5).

Table 2: Effects of drug pretreatments on the SME-induced contractile response. (Expression of the results, see Table 1.)

Sample	% ± SD*	n
SME (1 mg/mL) before atropine	30.3 ±5.39	6
SME (1 mg/mL) in the presence of atropine	6.7 ± 5.63	
SME (1 mg/mL) before tetrodotoxin (TTX)	30.1 ± 9.17	5
SME (1 mg/mL) in the presence of TTX	31.1 ± 3.80	
SME (1 mg/mL) before chloropyramine	34.3 ± 2.68	5
SME (1 mg/mL) in the presence of chloropyramine	30.8 ± 5.59	
SME (1 mg/mL) before capsaicin	30.9 ± 4.25	5
SME (1 mg/mL) in the presence of capsaicin	32.4 ± 9.17	

*p < 0.05 or less. Concentrations of drugs were 0.5 μM for atropine and tetrodotoxin (contact time, 20 min), 0.3 μM for chloropyramine (contact time, 20 min) and 1 μM for capsaicin (administered for 50 min, with rinsing and readministering capsaicin at min 15 and 30). Before the first administration of SME the solvents of the respective drug was administered (0.5 μL/mL ethanol for capsaicin and 1 μl/mL isotonic saline for the rest of the drugs).

SME (0.33−1 mg/mL or 0.1−1 mg/mL, administered for 20 min) concentration-dependently inhibited the effects of electrical field stimulation, exogenous acetylcholine, or histamine (Tables 3−5). These effects were reversible by repeated renewals of the bathing solution in 40 min (data not shown).

Table 3: Effects of different concentrations of SME (contact time 20 min) on the cholinergic twitch response evoked by single shocks of electrical field stimulation. (Expression of the results, see Table 1.)

Sample	% ± SD*	n
Twitch response before	52.0 ± 9.31	6
In the presence of SME (0.33 mg/mL)	43.5 ± 13.72	
Twitch response before	58.2 ± 17.54	8
In the presence of SME (0.66 mg/mL)	46.0 ± 11.88	
Twitch response before	52.4 ± 20.91	13
In the presence of SME (1.00 mg/mL)	30.8 ± 17.31	

*p < 0.05 or less. Responses before SME administration were evoked while the respective amount of DMSO was present in the organ bath. (DMSO usually caused slight enhancement of the twitch response.) SME was administered for 10 min first, then removed by rinsing and immediately readministered. Electrical stimulation was stopped for the first 10 min of SME administration. In this way, the initial contractile response to SME did not interfere with the effect of field stimulation.

Table 4: Effects of different concentrations of SME (contact time, 20 min) on the acetylcholine (ACh, 0.1 μM) -evoked contractile response. (Expression of the results, see Table 1.)

Sample	% ± SD*	n
Effect of ACh before	72.1 ± 11.40	5
In the presence of SME (0.10 mg/mL)	66.9 ± 12.30	
Effect of ACh before	75.0 ± 8.82	6
In the presence of SME (0.33 mg/mL)	47.1 ± 15.92	
Effect of ACh before	71.6 ± 11.27	6
In the presence of SME (0.66 mg/mL)	41.2 ± 7.59	
Effect of ACh before	66.8 ± 14.82	7
In the presence of SME (1.00 mg/mL)	15.9 ± 3.17	

*p < 0.05 or less. Responses before SME administration were evoked while the respective amount of DMSO was present in the organ bath. SME was administered for 10 min first, then removed by rinsing and immediately readministered for another 10 min.

Table 5: Effects of different concentrations of SME on the histamine (0.3 μM) -evoked contractile response. (Expression of the results, see Table 1.)

Sample	% ± SD*	n
Effect of histamine before	60.7 ± 12.98	6
In the presence of SME (0.33 mg/mL)	59.5 ± 18.13	
Effect of histamine before	60.2 ± 17.46	7
In the presence of SME (0.66 mg/mL)	45.8 ± 10.58	
Effect of histamine before	64.0 ± 7.10	6
In the presence of SME (1.00 mg/mL)	34.3 ± 16.90	

*p < 0.05 or less. Responses before SME administration were evoked while the respective amount of DMSO was present in the organ bath. SME was administered for 10 min first, then removed by rinsing and immediately readministered for another 10 min.

These results indicate that the effect of SME on the small intestine exerts two kinds of effect: concentration-dependent contraction and relaxation of the smooth muscle, quicker and slower in development, respectively.

The SME-induced contraction seems not to involve nerve axonal conduction (spike generation through voltage-dependent Na^+ channels), as shown by the lack of effect of TTX. Also histamine-like contractile activity was not involved, since the histamine H_1 receptor antagonist chloropyramine did not inhibit the contractile effect of SME. The concentrations of TTX and chloropyramine used are sufficient to fully block the effect of electrical field stimulation of nerves and of histamine (0.3 μM), respectively, as shown by earlier experiments (n > 50 and n=15, respectively) [26].

The inhibitory effect of the muscarinic acetylcholine receptor antagonist atropine on the SME-induced contraction indicates the involvement of such receptors in this response. We basically see two possibilities for the interpretation of this result. First, SME itself may possess acetylcholine-like activity, i.e., it stimulates muscarinic receptors directly. Second, endogenous acetylcholine is released from the preparation by SME. Within the gut wall, nerve endings of neuronal plexus neurons are the most probable source of this biogenic amine. In this case acetylcholine is most probably released by a mechanism independent of Na^+ channels, i.e. spike generation, as shown by the lack of effect of TTX. It is known that nerve endings (unlike axons) are mostly resistant to the effect of TTX.

The sensory stimulant capsaicin causes cholinergic (atropine-sensitive) contraction in the guinea-pig small intestine. Although this response is strongly attenuated by TTX [26] and therefore only partly mimics that of SME, we tested the effect of capsaicin desensitization on the SME-induced contraction. Capsaicin exposure is able to selectively block the effect of capsaicin itself by rendering capsaicin-sensitive nerve endings functionally impaired [26]. A long exposure of the preparations was used to a moderate

concentration of capsaicin. At the end of this incubation (without removing capsaicin from the organ bath) the preparations were insensitive to capsaicin, whereas the effect of electrical field stimulation was unimpaired, as shown by preliminary experiments [26]. This procedure failed to inhibit the excitatory action of SME, indicating no involvement of capsaicin-sensitive nerves.

Upon longer incubation SME showed inhibitory effects on contractions evoked by electrical field stimulation of cholinergic nerves, exogenous ACh, or histamine. Although concentration-inhibition curves of SME against these contractile influences were not fully superposable, the differences in the inhibitory effects make less than one order of magnitude; consequently, we think that they all are manifestations of a non-selective smooth muscle-relaxant effect of SME.

The spasmolytic effect of chrysoeriol was previously detected on isolated rat jejunum. This compound at 30 μg/mL concentration antagonised non-competitively the concentration-response curve induced by $CaCl_2$ (E_{max} = 70.0%) [27]. Another investigation resulted that the spasmolytic effect of chrysoeriol mediated possibly through K^+ channel opening action [28]. Moreover, the spasmolytic activity of aqueous extract of *Lippia integrifolia* was also demonstrated and confirmed that the high caffeoyl quinic acid (one of them is chlorogenic acid) content (0.10 %) could be responsible for this effect [29]. The detected compounds could be, at least partly, responsible for the spasmolytic effect of *S. montana* methanol extract which can evolve through different pharmacological mechanisms.

Taken together, these data indicate an acetylcholine-like excitatory and a non-selective smooth muscle relaxant effect of *S. montana* extract.

Experimental

Plant material: *Sideritis montana* L. was collected near Öskü (Hungary), in July 2013. Botanical identification was carried out by one of the authors, Gyula Pinke (Department of Botany, Faculty of Agricultural and Food Sciences, University of West Hungary). A voucher specimen (No. 822) has been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Preparation of the extract: The extract was prepared from 50 g air-dried, powdered plant material with 3 × 500 mL MeOH with the use of an ultrasonic bath for 3 × 15 min. After filtration, the solutions were combined and evaporated to dryness (5.9 g) under reduced pressure.

HPLC-MS apparatus and measurement conditions: Chromatographic separation of *S. montana* methanol extract was performed using a Shimadzu liquid chromatographic system [2 pumps (LC-20AD); diode array detector (SPD-M20A)]; Autosampler (SIL-20A); Controller (CBM-20A); Degasser (DGU-20A3); Column thermostat (CTO-20AC) equipped with a LiChrospher 100 RP-18e (4 mm × 250 mm, 5 μm) column. Mobile phase A was 0.1% formic acid in acetonitrile and mobile phase B was 0.1% formic acid in water. Gradient elution was applied (0 min: 95% mobile phase B; 12 min: 70% mobile phase B; 40 min: 40% mobile phase B; 50 min: 0% mobile phase B; 51 min: 95% mobile phase B; 61 min: 95% mobile phase B).The flow rate was 600 μL/min. The HPLC was coupled to an API 2000 MS/MS with an electrospray (ESI) interface. The source temperature was 300 °C. The measurements were carried out in negative ionization mode and the qualification was accomplished by using multiple reaction monitoring (MRM) with transitions of m/z 179→135 and 179→107 for caffeic acid, m/z 353→191 and 353→179 for chlorogenic acid and m/z 299→284 and 299→256 for chrysoeriol. Data aquisition and evaluation were performed using Analyst 1.5.1 software.

Animals and assay arrangement: All experiments have been approved by the Regional Committee for Animal Research (Baranya County, Hungary), approval number, BA02/2000-1/2012. Guinea-pigs (short-haired, coloured, 420-600 g) of either sex were killed by a blow to the occiput and bled out. Preterminal ileal segments were removed, gently flushed with Krebs-Henseleit solution and placed into a Petri dish. Longitudinally-oriented preparations of approximately 3 cm length were placed into 5 mL organ baths containing Krebs-Henseleit solution of 37 °C, bubbled with a mixture of 95% O_2 and 5% CO_2. Movements were recorded isotonically, by means of lever transducers and bridge amplifiers (Hugo Sachs−Harvard Apparatus, March−Hugstetten, Germany), under a constant tension, which was 6 mN. Experiments started after an equilibration period of 30 min. Histamine (10 μM) was then administered for 1.5 min for evoking maximal longitudinal spasm. After another 30 min of washout submaximal gut contractions were evoked by electrical field stimulation [30] or by administering acetylcholine (0.1 μM for 1 min) or histamine (0.3 μM for 1 min). Cholinergic "twitch" responses of approximately half-maximal size were evoked by electrical field stimulation (near-maximal voltage of 15 V/cm, 0.1 ms pulse width, single electrical shocks at 0.05 Hz), applied by means of a high-performance stimulator (Experimetria, Budapest, Hungary), through a pair of platinum wire electrodes, placed at the top and the bottom of the organ bath. The experiments with histamine were performed while the muscarinic acetylcholine receptor antagonist atropine (0.5 μM) was present in the organ bath.

Drugs and solutions: Composition of the bathing solution was as follows NaCl 119 mM, $NaHCO_3$ 25 mM, KCl 2.5 mM, $MgSO_4$ 1.5 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, glucose 11 mM. Drugs used were acetylcholine chloride, atropine sulfate, capsaicin, histamine hydrochloride, tetrodotoxin (Sigma), chloropyramine (Egis Pharmaceutical Company, Budapest). Capsaicin (2 mM) was dissolved in ethanol; the rest of drugs was dissolved and diluted in isotonic saline. *S. montana* extract was dissolved in DMSO, at a concentration of 0.33 g/mL; higher concentrations could not be prepared.

Statistical analysis: Data are indicated as mean ± SD. For comparing responses before and after drug treatment, Wilcoxon's signed rank test was used. A value of $p < 0.05$ or smaller was taken as significant.

Acknowledgments - This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11/1-2012-0001 'National Excellence Program', as well as by TÁMOP-4.2.2.B-10/1-2010-0029. This paper was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

References

[1] Barber JC, Francisco-Ortega J, Santos-Guerra A, Turner KG, Hansen RK. (**2002**) Origin of Macaronesian *Sideritis* L. (Lamioideae, Lamiaceae) inferred from nuclear and chloroplast sequence datasets. *Molecular Phylogenetics and Evolution, **23***, 293–306.

[2] Gonzalez-Burgos E, Carretero ME, Gomez-Serranillos MP. (**2011**) *Sideritis* spp.: Uses, chemical composition and pharmacological activities – A review. *Journal of Ethnopharmacology*, ***135***, 209–225.

[3] Charami MT, Lazari D, Karioti A, Skaltsa H, Hadjipavlou-Litina D, Souleles C. (**2008**) Antioxidant and antiinflammatory activities of *Sideritis perfoliata* subsp. *perfoliata* (Lamiaceae). *Phytotherapy Research*, ***22***, 450–454.

[4] Küpeli E, Pınar Sahin F, Calıs I, Yesilada E, Ezer N. (**2007**) Phenolic compounds of *Sideritis ozturkii* and their *in vivo* anti-inflammatory and antinociceptive activities. *Journal of Ethnopharmacology*, ***112***, 356–360.

[5] Brankovic S, Kitic D, Radenkovic M, Veljkovic S, Jankovic T, Savikin K, Zdunic G. (**2011**) Spasmolytic activity of the ethanol extract of *Sideritis raeseri* spp. *raeseri* Boiss. & Heldr. on the isolated rat ileum contractions. *Journal of Medicinal Food*, ***14***, 495–498.

[6] Iscan G, Kirimer N, Kurkcuoglu M, Baser KHC. (**2005**) Composition and antimicrobial activity of the essential oils of two endemic species from Turkey: *Sideritis cilicica* and *Sideritis bilgerana*. *Chemistry of Natural Compounds*, ***41***, 679-682.

[7] Aboutabl EA, Nassar MI, Elsakhawy FM, Maklad YA, Osman AF, El-Khrisy EAM. (**2002**) Phytochemical and pharmacological studies on *Sideritis taurica* Stephan ex Wild. *Journal of Ethnopharmacology*, ***82***, 177–184.

[8] Gurbüz I, Ozkan AM, Yesilada E, Kutsal O. (**2005**) Anti-ulcerogenic activity of some plants used in folk medicine of Pinarbasi (Kayseri, Turkey). *Journal of Ethnopharmacology*, ***101***, 313–318.

[9] González-Burgos E., Carretero ME, Gómez-Serranillos MP. (**2013**) Nrf2-dependent neuroprotective activity of diterpenoids isolated from *Sideritis* spp. *Journal of Ethnopharmacology*, ***147***, 645–652.

[10] Venditti A, Bianco A, Maggi F, Nicoletti M. (**2013**) Polar constituents composition of endemic *Sideritis italica* (Mill.) Greuter et Burter from Central Italy. *Natural Product Research*, ***27***, 1408–1412.

[11] Akcoş Y, Ezer N, Çalış İ, Demirdamar R, Tel BC. (**1999**) Polyphenolic compounds of *Sideritis lycia* and their anti-inflammatory activity. *Pharmaceutical Biology*, ***37***, 118–122.

[12] Petreska J, Stefova M, Ferreres F, Moreno DA, Tomás-Barberán FA, Stefkov G, Kulevanova S, Gil-Izquierdo A. (**2011**) Dietary burden of phenolics per serving of "Mountain tea" (Sideritis) from Macedonia and correlation to antioxidant activity. *Natural Product Communications*, ***6***, 1305-1314.

[13] Karapandzova M, Qazimi B, Stefkov G, Baceva K, Stafilov T, Panovska TK, Kulevanova S. (2013) Chemical characterization, mineral content and radical scavenging activity of *Sideritis scardica* and *S. raeseri* from R. Macedonia and R. Albania. *Natural Product Communications*, ***8***, 639-644.

[14] Palomino OM, Gomez-Serranillos P, Carretero E, Villar A. (**1996**) High-performance liquid chromatography of flavonoids from *Sideritis* species. *Journal of Chromatography A*, ***731***, 103–108.

[15] Alipieva K, Petreska J, Gil-Izquierdo A, Stefova M, Evstatieva L, Bankova V. (**2010**) Influence of the extraction method on the yield of flavonoids and phenolics from Sideritis spp. (Pirin Mountain tea). *Natural Product Communications*, ***5***, 51-54.

[16] Kirmizibekmez H, Ariburnu El, Masullo M, Festa M, Capasso A, Yesilada E, Piacente S. (**2012**) Iridoid, phenylethanoid and flavonoid glycosides from *Sideritis trojana*. *Fitoterapia*, ***83***, 130–136.

[17] Gonzalez AG, Fraga BM, Hernandez MG, Luis JG, Larruga F. (**1979**) Comparative phytochemistry of the genus *Sideritis* from the Canary Islands. *Biochemical Systematics and Ecology*, ***7***, 115–120.

[18] Kassi E, Paliogianni A, Dontas I, Aligiannis N, Halabalaki M, Papoutsi Z, Skaltsounis AL, Moutsatsou P. (**2011**) Effects of *Sideritis euboea* (Lamiaceae) aqueous extract on IL-6, OPG and RANKL secretion by osteoblasts. *Natural Product Communications*, ***6***, 1689-1696.

[19] Navarro A, De Las Heras B, Villar A. Anti-Inflammatory and immunomodulating properties of a sterol fraction from *Sideritis foetens*. (**2001**) *Biological and Pharmaceutical Bulletin*, ***24***, 470–473.

[20] Fraga BM, Hernández MG, Fernández C, Santana JMH. (**2009**) A chemotaxonomic study of nine Canarian *Sideritis* species. *Phytochemistry*, ***70***, 1038–1048.

[21] Alipieva KI, Kostadinova EP, Evstatieva LN, Stefova M, Bankova VS. (**2009**) An iridoid and a flavonoid from *Sideritis lanata* L. *Fitoterapia*, ***80***, 51–53.

[22] Kirimer N, Baser KHC, Demirci B, Duman H. (**2004**) Essential oils of *Sideritis* species of Turkey belonging to the section Empedoclia. *Chemistry of Natural Compounds*, ***40***, 19-23.

[23] Özkan G, Krüger H, Schulz H, Özcan M. (**2005**) Essential oil composition of three *Sideritis* species used as herbal teas in Turkey. *Journal of Essential Oil-Bearing Plants*, ***8***, 173–177.

[24] (a) Tomás-Barberán FA, Rejdali M, Harborne JB, Heywood VH. (1**988**) External and vacuolar flavonoids from Ibero-North African *Sideritis* species. A chemosystematic approach. *Phytochemistry*, ***27***, 165-170.; (b) Armata M, Gabrieli C, Termentzi A, Zervou M, Kokkalou E. (**2008**) Constituents of *Sideritis syriaca* ssp. *syriaca* (Lamiaceae) and their antioxidante activity. *Food Chemistry*, ***111***, 179–186.; (c) Ríos JL, Máñez S, Payá M, Alcaraz MJ. (**1992**) Antioxidant activity of flavonoids from *Sideritis javalambrensis*. *Phytochemistry*, ***31***, 1947–1950.

[25] (a) Kirimer N, Tabanca N, Özek T, Tumen G, Baser KHC. (**2000**) Essential oils of annual *Sideritis* species growing in Turkey. *Pharmaceutical Biology*, ***38***, 106–111.; (b) Emre I, Kursat M, Yilmaz O, Erecevit P. **(2011)** Some biological compounds, radical scavenging capacities and antimicrobial activities in the seeds of *Nepeta italica* L. and *Sideritis montana* L. subsp. *montana* from Turkey. *Grasas y Aceites*, ***62***, 68–75.

[26] Bartho L, Benko R, Patacchini R, Pethö G, Holzer-Petsche U, Holzer P, Lázár Zs, Undi S, Illényi L, Antal A, Horváth ÖP. (**2004**) Effects of capsaicin on visceral smooth muscle: a valuable tool for sensory neurotransmitter identification. *European Journal of Pharmacology*, ***500***, 143-157.

[27] Gorzalczany S, Moscatelli V, Acevedo C, Ferraro G. (**2013**) Spasmolytic activity of *Artemisia copa* aqueous extract and isolated compounds. *Natural Product Research*, ***27***, 1007-1011.

[28] Gilani AH, Khan A, Ghayur MN, Ali SF, Herzig JW. (**2006**) Antispasmodic effects of Rooibos tea (*Aspalathus lineraris*) is mediated predominantly through K^+-channel activation. *Basic & Clinical Pharmacology & Toxicology*, ***99***, 365-373.

[29] Gorzalczany S, Sülsen V, Redko F, Vescina C, Muschietti L, Martino V, Acevedo C. (**2008**) Choleretic and antispasmodic effects of *Lippia integrifolia* aqueous extract. *Revista Brasileira de Farmácia*, ***18***, 16-20.

[30] Paton WDM, Vizi ES. (**1969**) The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *British Journal of Pharmacology*, ***35***, 10-20.