ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 10:2 (2016) 195-206

records of natural products

Antioxidant Potential and Inhibition of Key Enzymes Linked to Alzheimer's Diseases and Diabetes Mellitus by Monoterpene-Rich Essential Oil from *Sideritis galatica* Bornm. Endemic to Turkey

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(Received July 15, 2014; Revised November 18, 2014; Accepted November 24, 2014)

Abstract: The present study was designated to (1) characterize the essential oil from *S. galatica* (SGEOs) and (2) evaluate its antioxidant and enzyme inhibitory activities. Antioxidant capacity was tested different methods including free radical scavenging (DPPH, ABTS and NO), reducing power (FRAP and CUPRAC), metal chelating and phosphomolybdenum. Inhibitory activities were analyzed on acetylcholiesterase, butrylcholinesterase, α-amylase and α-glucosidase. SGEOs were chemically analyzed and identified by gas chromatography and gas chromatography/mass spectrophotometry. 23 components, representing 98.1% of SGEOs were identified. Monoterpene hydrocarbons (74.1%), especially α- (23.0%) and β-pinene (32.2%), were the main constituents in SGEOs. The main sesquiterpene hydrocarbons were β-caryophyllene (16.9%), germacrene-D (1.2%) and caryophyllene oxide (1.2%), respectively. Generally, SGEOs has shown moderate free radical, reducing power, metal chelating and enzyme inhibitory activities. These activities related to chemical profile in SGEOs. Our findings supported that the possible utility of SGEOs is a source of natural agents for food or pharmaceutical industries.

Keywords: *Sideritis galatica*; Antioxidants; Monoterpenes; Cholinesterase; Anti-diabetic.

1. Introduction

The genus *Siderits* is widespread particularly in the Mediterranean area and represented by more than 150 species. In Turkey, the genus includes 46 species with high endemism ratio (>80%) [1]. The high endemism ratio and wide distribution show that Turkey is one of the gene centre of this genus. Members of *Sideritis* are a group of plants known as "mountain tea" in Anatolia. (Local name: dağ çayı or yayla çayı). Aerial parts of a number of *Sideritis* species in different countries including Turkey are used as anti-inflammatory, anti-ulcer, anti-microbial, and anti-spasmodics [2-4]. For example, *Sideritis* species are widely used to prepare traditional

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 08/01/2015 EISSN:1307-6167

teas in Anatolia. The tea is widely used to treatment gastrointestinal disorders (stomache ache and flatulence etc.) and common colds (fever, flu and bronchitis etc) [5]. The uses of its species in traditional medicine could be causes of the high number of studies on members of the genus *Sideritis.* In these studies, many active constituents were identified in extracts or essential oil of *Sideritis* species such as terpenes, phenolics, coumarins and lignans. At this point, Table 1 shows some studied *Sideritis* extracts and essential oils with main components.

Sideritis galatica Bornm., which is endemic to Turkey, densely hair perennial shrub growing to height of 65 cm and widely distributed in Central Anatolia Region of Turkey (especially Ankara). At our previous study, the phenolic composition, antioxidant and enzyme inhibition activities of different solvent extracts (petroleum ether, ethyl acetate, methanol and water) from *S. galatica* were evaluated [6]. To our best knowledge, no work has been carried out on the chemical composition as well as antioxidant and enzyme inhibitory activities with respect to *S. galatica* essential oil (SGEOs). The aims of this work were to (1) determine chemical profile, (2) evaluate antioxidant potentials, (3) investigate inhibitory (on cholinesterase, α-amylase and α-glucosidase) activities of SGEOs, and (4) understand the usefulness of SGEOs as a source of natural agents in food and pharmacological industries.

Table 1. Main components in extracts and essential oils from *Sideritis* species reported.

Species	**Essential oil/ Extracts**	**Main Components**	**References**
S. albiflora	Methanol	Carvacrol, Rosmarinic acid	[7]
S. angustifolia	Essential oil	*α*-Pinene, *β*-Bisabolol	[8]
S. arguta	Methanol	Ferulic acid, Chlorogenic acid	[9]
S. argyrea	Essential oil	*α*-Pinene, *β*-Pinene	[8]
S. armeniaca	Essential oil	*α*-Pinene, *β*-Pinene	[10]
S. brevidens	Acetone	*p*-Hydroxybenzoic acid, *p*-Coumaric acid	[11]
S. caesarea	Essential oil	*β*-Caryophyllene, Caryophyllene oxide	[12]
S. chamaedryfolia	Essential oil	*β*-Caryophyllene, Caryophyllene oxide	[8]
S. congesta	Ethyl acetate	Ferulic acid, *p*-Coumaric acid	[9]
S. erythyrantha var. *cedretorum*	Essential oil	*α*-Pinene, *α*- Bisabolol	[13]
S. erythrantha var. *erythrantha*	Essential oil	*α*-Pinene, *β*-Caryophyllene	[13]
S. flavovirens	Essential oil	Fenchyl acetate, Fenchone	[8]
S. foetens	Essential oil	Thymol, *p*-Cymene	[8]
S. galatica	Methanol	Benzoic acid, Chlorogenic acid	[6]
S. huber-morathii	Methanol	Flavonoids, Hyroxycinnamates	[14]
S. ibanyezii	Essential oil	Fenchyl acetate, Sabinene	[15]
S. italica	Essential oil	β-Cubebene, Kaur-15-ene	[16]
S. lanata L.	Essential oil	Hexadecanoic acid, Spathulenol	[17]
S. leptoclada	Methanol	Caffeic acid, Rosmarinic acid	[7]
S. montana L. subsp. *montana*	Essential oil	Germacrene D, Bicyclogermacrene	[17]
S. montana L. subsp. *remota*	Essential oil	Germacrene D, Bicyclogermacrene	[17]
S. mugronensis	Essential oil	δ-Cadinene, 1,8-Cineole	[18]
S. niveotomentosa	Acetone	*p*-Hydroxybenzoic acid, *p*-Coumaric acid	[11]
S. ozturkii	Essential oil	*α*-Pinene, *β*-Pinene	[19]
S. phlomoides	Methanol	Flavonoids, Hyroxycinnamates	[14]
S. taurica	Essential oil	*β*-Pinene, *α*- Bisabolol	[10]
S. tmolea	Essential oil	*β*-Caryophyllene, *α*-Cadinol	[20]
S. vuralii	Essential oil	*β*-Pinene, 1,8-Cineole	[12]

2. Materials and Methods

2.1. Plant material

Sideritis galatica Bornm. was collected from Çubuk, Ankara-Turkey on 13 July 2013. Taxonomic identification of the plant material was confirmed by the senior taxonomist Dr.

Olcay Ceylan, in Department of Biology, Mugla Sıtkı Kocman University. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Mugla Sıtkı Kocman University, Mugla-Turkey (Voucher No: MUH1466).

2.2. Isolation and analysis of the essential oil

The air-dried and ground plant material (500 g) was submitted to water-distillation for 5 h using a British-type Clevenger apparatus (ILDAM Ltd., Ankara-Turkey). The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at +4°C until tested and analyzed.

The essential oil sample was analyzed by GC-FID and GC/MS techniques. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system coupled to an Agilent 7890A GC (Agilent Technologies Inc., Santa Clara, CA). HP-Innowax FSC column (60 m x 0.25mm, 0.25µm film thickness) was used with helium (purity 99.99%) as a carrier gas (1.2 mL/min). The 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. The split ratio was used at 40:1. The injector temperature was at 250°C mass spectra were recorded at 70 eV. Mass range was from 35 to 450 *m/z*. GC-FID analysis was carried out by simultaneous auto-injection using Agilent 7693A series autosampler; 1µL of essential oil diluted with *n*-hexane (10%, v/v) was injected into GC/MS system.

The GC analysis was carried out using an Agilent 7890A GC system. In order to obtain the same elution order with GC/MS, simultaneous triplicate injections were done by using the same column and same operational conditions. The FID temperature was 300°C.

The identification of constituents was achieved on the basis of retention index determined by co-injection with reference to a homologous series of n-alkanes (C_8-C_{30}), under same experimental conditions. Further identification was carried out by comparison of their mass spectra with those from NIST 05 and Wiley 8th version and home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values [21].

2.3. Total antioxidant activity by phosphomolybdenum method

The total antioxidant activity of the sample was evaluated by phosphomolybdenum method according to Berk et al. (2011) [22] with slight modification. Sample solution (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample absorbance was read at 695 nm after a 90 min incubation at 95 °C. The total antioxidant capacity was expressed as equivalents of trolox as determined by the equation obtained from the standard trolox graph.

2.4. Reducing power

2.4.1. Cupric ion reducing (CUPRAC) method

The cupric ion reducing activity (CUPRAC) was determined according to the method of Apak et al (2006) [23]. Sample solution (0.5 mL) was added to premixed reaction mixture containing $CuCl_2$ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM) and NH_4Ac buffer (1 mL, 1 M, pH 7.0). Similarly, a blank was prepared by adding sample solution (0.5 mL) to premixed reaction mixture (3 mL) without $CuCl_2$. Then, the sample and blank absorbances were read at 450 nm after a 30 min incubation at room temperature. The absorbance of the blank was subtracted from that of the sample. The EC_{50} value (the effective concentration at which the absorbance was 0.5) was calculated for sample and trolox.

2.4.2. Ferric reducing antioxidant power (FRAP) method

The FRAP assay was carried out as described by Aktumsek et al. (2013) [24] with slight modification. Sample solution (0.1 mL) was added to premixed FRAP reagent (2 mL) containing acetate buffer (0.3 M, pH 3.6), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in a ratio of 10:1:1 (v/v/v). Then, the sample absorbance was read at 593 nm after a 30 min incubation at room temperature. FRAP activity was expressed as equivalents of trolox according to the equation obtained from the standard trolox graph. The results were evaluated by EC_{50} values.

2.5. Metal chelating activity on ferrous ions

The metal chelating activity on ferrous ions was determined by the method described by Aktumsek et al. (2013) [24]. Briefly, sample solution (2 mL) was added to $FeCl_2$ solution (0.05 mL, 2 mM). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Similarly, a blank was prepared by adding sample solution (2 mL) to $FeCl_2$ solution (0.05 mL, 2 mM) and water (0.2 mL) without ferrozine. Then, the sample and blank absorbances were read at 562 nm after 10 min incubation at room temperature. The absorbance of the blank was subtracted from that of the sample. The metal chelating activity was expressed as equivalents of EDTA according to the equation obtained from the standard EDTA graph.

2.6. Radical scavenging activity

2.6.1. Free radical scavenging activity (DPPH)

The effect of the sample on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was estimated according to Sarikurkcu (2011) [25]. Sample solution (1 mL) was added to a 4 ml of a 0.004% methanol solution of DPPH. The sample absorbance was read at 517 nm after a 30 min incubation at room temperature in dark. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I\% = 100x\ (A_{Control} - A_{Sample})/\ A_{Control}$$

where $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{Sample} is the absorbance of the test compound. Trolox was used as a control. 50% of free radical inhibition (IC_{50}) of samples was calculated. The lower the IC_{50} value indicates high antioxidant capacity.

2.6.2. ABTS (2,2 Azino-bis (3-ethylbenzothiazloine-6-sulfonic acid)) radical cation scavenging activity

The scavenging activity aganist ABTS cation radical was measured according to the method of Re et al. (1999) [26] with slight modification. Briefly, $ABTS^{\cdot+}$ radical cation was produced directly by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12-16 in dark at the room temperature. Prior to beginning the assay, ABTS solution was diluted with methanol to an absorbance of 0.700±0.02 at 734 nm. Sample solution (1 mL) was added to ABTS solution (2 mL) and mixed. The sample absorbance was read at 734 nm after a 30 min incubation at room temperature. The results were reported as IC_{50}.

2.6.3. Nitric oxide (˙NO) radical scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generated nitric oxide, which can be measured by the Griess reaction [26]. Sample solution (0.5 mL) was mixed with sodium nitroprusside (0.5 mL, 5 mM) in phosphate buffer (0.2 M, pH 7.4) and incubated for 150 min at room temperature. Similarly, a blank was prepared by adding sample solution (0.5 mL) to phosphate buffer (0.5 mL). Diluted Griess reagent (1 mL, 1:1) was added to the incubated sample and allowed to stand for 30 min. The sample and blank absorbances were read at 548 nm. The absorbance of the blank was subtracted from that of the sample and the results were reported as IC_{50}.

2.7. Enzyme inhibitory activity

2.7.1. Cholinesterase inhibition

Cholinesterase (ChE) inhibitory activity was measured using Ellman's method, as previously reported [28]. Sample solution (50 µL) was mixed with DTNB (125 µL) and AChE (or BuChE) solution (25 µL) in Tris-HCl buffer (pH 8.0) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of acetylthiocholine iodide (ATCI) or butyrylthiocholine chloride (BTCl) (25 µL). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (AChE or BChE) solution. The sample and blank absorbances were read at 405 nm after a 10 min incubation at 25 °C. The absorbance of the blank was subtracted from that of the sample and the results were reported as IC_{50}.

2.7.2. α-Amylase inhibition

α-Amylase inhibitory activity was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method [28]. Sample solution (25 µL) was mixed with α-amylase solution (50 µL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well microplate and incubated for 10 min at 37 °C. After pre-incubation, the reaction was initiated with the addition of starch solution (50 µL, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α-amylase) solution. The reaction mixture was incubated 10 min at 37 °C. The reaction was then stopped with the addition of HCl (25 µL, 1 M). This was followed by addition of the iodine-potassium iodide solution (100 µL). The sample and blank absorbances were read at 630 nm. The absorbance of the blank was subtracted from that of the sample and the results were reported as IC_{50}.

2.7.3. α-Glucosidase inhibition

α-Glucosidase inhibitory activity was performed by the previous method [28]. Sample solution (50 µL) was mixed with glutathione (50 µL), α-glucosidase solution (50 µL) in phosphate buffer (pH 6.8) and PNPG (50 µL) in a 96-well microplate and incubated for 15 min at 37 °C. Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α-glucosidase) solution. The reaction was then stopped with the addition of sodium carbonate (50 µL, 0.2 M). The sample and blank absorbances were read at 400 nm. The absorbance of the blank was subtracted from that of the sample and the results were reported as IC_{50}.

3. Results and Discussion

3.1. Essential oil composition

The chemical composition of *S. galatica* essential oil (SGEOs) was investigated using GC-MS technique. The yield of the essential oil was 0.13%. This value is comparable to the values reported in the literature for other *Sideritis* species such as *S. sipylea* (0.40%, reported by Aligiannnis et al., (2001) [2], *S. angustifolia* (0.90%, reported Ruiz-Navajas et al., (2012) [29] and *S. italica* (0.02%, reported by Giuliani et al., (2011) [30]. Twenty three components were

identified, representing the 98.1% of the oil (Table 2). Monoterpene hydrocarbons were dominant group (74.1%) in the SGEOs, with β-pinene being the principal component (32.2%) followed by α-pinene (23.0%) and (*Z)*-β-ocimene (9.5%), respectively. Sesquiterpenes were presented in a high percentage (21.6%), with β-caryophyllene (16.9%), germacrene D (1.2%) and caryophyllene oxide (1.2%) being the main compounds. This is the first comprehensive report to analyze the chemical composition of SGEOs. However, the chemical composition of the essential oils obtained from several *Sideritis* species was widely investigated. According to our knowledge, The *Sideritis* species can be classified into several groups (monoterpe-rich, sesquiterpene-rich etc.) depending on the main constituents of chemical composition. In this direction, the composition of SGEOs from our study falls into the monoterpene-rich group, with α and β-pinene as the main components. Similar to our findings, monoterpene-rich group contained in several *Sideritis* species with high levels of α- and β-pinene. For example, α-and β-pinene were identified as predominant compounds in *S. scardica* populations by Trendafilova et al. (2013) [31]. Aboutabl et al. (2009) [32] reported that monoterpene hydrocarbons were mainly β-pinene (17.7 %) and α-pinene (12.8%) in the chemical composition of *S. taurica.* Likewise, in a previous study on essential oil composition of *S. clandestina* subsp. *peloponnesiaca* the oil was rich monoterpenes, with α and β-pinene being the major components (46.6%). In the oil of *S. sipylea, S. raeseri* subsp. *attica* and *S. clandestina* subsp. *clandestina,* grown in Greece, α- and β-pinene were identified as major compounds, reaching percentage of 43.96%, 42.84% and 27.42%, respectively [2]. However, several *Sideritis* species were characterized with high level of sesquiterpene hydrocarbons, such as bicylogermacrene, germacrene D and α-cadinol [20, 33, 34]. The differences of the level of main chemical components (monoterpene, sesquiterpene or diterpenes) are responsible for the different biological activities shown *Sideritis* species. Moreover, the variability may be considerable as chemotaxonomic markers for *Sideritis* genus [35].

Table 2. Chemical composition of the essential oil of aerial parts of *Sideritis galatica.*

No	RI[a]	Components	(%)
1	1028	α-Pinene	23.0
2	1115	β-Pinene	32.2
3	1128	Sabinene	1.5
4	1156	δ-3-Carene	0.6
5	1173	α-Phellandrene	0.5
6	1191	Heptanal	0.4
7	1203	Limonene	2.9
8	1215	β-Phellandrene	1.4
9	1228	*(E)*-2-Hexenal	0.1
10	1242	*(Z)*-β-Ocimene	9.5
11	1259	*(E)*-β-Ocimene	1.4
12	1276	*p*-Cymene	0.6
13	1289	Terpinolene	0.5
14	1452	1-Octen-3-ol	0.3
15	1504	α-Copaene	0.2
16	1618	β-Caryophyllene	16.9
17	1673	*(Z)*-β-Farnesene	0.6
18	1691	α-Humulene	0.4
19	1732	Germacrene D	1.2
20	1757	Bicyclogermacrene	0.8
21	1776	δ-Cadinene	0.3
22	2018	Caryophyllene oxide	1.2
23	2663	Benzyl benzoate	1.6
		Monoterpene hydrocarbons	**74.1**
		Sesquiterpene hydrocarbons	**21.6**
		Others	**2.4**
		Total identified	**98.1**

[a] Retention index relative to *n*-alkanes on HP-innowax capillary column.

3.2. Free radical scavenging activities

Free radical scavenging activity of SGEOs was investigated by three different test systems, namely, the DPPH, ABTS and NO assays. These activities are expressed as IC_{50}. A low IC_{50} value indicates an active free radical scavenging ability. IC_{50} value of trolox was also determined in parallel experiments. All data are presented in Table 3. When DPPH, ABTS and NO react with antioxidants, which can donate hydrogen and are reduced; the changes in colour were measured with wavelength which showed maximum absorbance.

According to the results obtained, SGEOs had lower free radical scavenging activity on DPPH (IC_{50}:16.447 mg/mL) and ABTS (IC_{50}:8.518 mg/ml) than trolox (IC_{50}: 0.058 mg/mL for DPPH and IC_{50}: 0.197 mg/ml for ABTS). On the other hand, nitric oxide scavenging activity of SGEOs (IC_{50}:0.899 mg/ml) was stronger than trolox (IC_{50}:1.119 mg/ml). The results might be explained by presence of monoterpene hydrocarbons (α and β-pinene). The results show a strong similarity with these reports. For example, Marin et al. (2008) [36] reported that some monoterpene hydrocarbons such as β-pinene and α-pinene were found not possess strong scavenging activity on free radicals. In accordance with our results, essential oils obtained by hydrodistillation of some *Sideritis* and Lamiaceae species showed lower free radical scavenging activity, which contained high level of monoterpene hydrocarbons [34-41]. However, the chemical transformation of pinenes is also known in mammals. For example, the most common chemical evolution of α-pinene is their hydroxylation to verbenol and also myrtenol and myrtenic acid. Likewise, pinocarveol and α-terpinol are known as the transformation products of β-pinene in mammals. These products in both α and β-pinene transformation exhibit strong biological activities (anti-oxidant and anti-inflamatory etc.) [42]. At this point, SGEOs are considered as a valuable source of monoterpene hydrocarbons, especially pinenes.

3.3. Phosphomolybdate and metal chelating assays

Phosphomolybdate method is based on the reduction of Mo (IV) to Mo (V) by the antioxidants and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The trolox equivalent of SGEOS was 2.55 mmol/g oil in this assay. Apparently, SGEOs exhibited lower efficiency with 2.5 mmol TEs/g oil in this assay (Table 3). Like other antioxidant assays, monoterpene hydrocarbons are known to have low activity in phosphomolybdate assay. Therefore, this low antioxidant activity might be attributed to the presence of monoterpene hydrocarbons.

Although iron and copper are essential metals in the human health, they contained unpaired electrons that enable them to participate in oxidizing reactions. Therefore, metal chelating activity is known as an important antioxidant mechanism. The method is based on Fe^{2+} ions by ferrozine, which results in quantitative formation of Fe^{+2} ions complex. The metal chelating activity of SGEOs was assessed as equivalents of EDTAEs (mg EDTAEs/g oil). The chelating activity was recorded as 29.09 mgEDTAEs/g oil (Table 3). Interestingly, the main component of SGEOS, that is, monoterpene hydrocarbons are not able to form complex with Fe^{2+}. Thus, the components showed no metal chelating activity. This case was confirmed with other monoterpene-rich oils from other members of the family Lamiaceae [43, 44]. These results suggest that the chelating activity obtained could be attributed to the possible synergistic interactions in components of its chemical composition.

3.4. Reducing power

Reducing power or ability is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant compounds. Thus, the FRAP and CUPRAC assays were used to estimate the reducing power of SGEOs. These assays were evaluated by using EC_{50} (the effective concentration at which the absorbance was 0.5). As shown in Table 3, SGEOs demonstrated poor ferric (EC_{50}: 2.066 mg/mL) and cupric reducing capacity (EC_{50}:

1.068 mg/mL). The trolox concentration required to reduce the ferric and cupric ion was lower than SGEOs, indicating a better activity. Similar to our results, monoterpene- rich essential oils such as *Eucalyptus camaldulensis, Vitex agnus-castus* displayed little reducing power in FRAP and CUPRAC assays [45,46].

3.5. Enzyme inhibitory activities

It is well known that Alzheimer's diseases (AD) and diabetes mellitus (DM) are the commonest public health problems. A modern therapeutic approach to management of these diseases is related to the inhibition of key enzymes. For example, acetylcholinesterase (AChE) hydrolyses the ester bond in acetylcholine, which is known as a neurotransmitter. Inhibition of this enzyme gives rise to an increase in neurotransmitter concentration, which positively affects AD [47]. Again, α-amylase and α-glucosidase are known as key enzymes in starch metabolism. The inhibition of these enzymes delays the increase of blood glucose level in DM patients [48]. With this perspective, the inhibitors are synthetically developed to treat diseases above stated. However, it was reported that these compounds may have side effects (gastrointestinal disturbance and cytotoxicity etc.) there is an increasing interest in finding natural inhibitors from plant materials to replace synthetic [49-51]. In this direction, intensive research for utilization natural enzyme inhibitors that may serve as potential candidates in treatment of AD and DM, are carried out.

The anti-diabetic activity of SGEOs was investigated by the inhibition of α-amylase and α-glucosidase. The neuroprotective activity of SGEOs was also investigated by the inhibition of acetylcholinesterase and butrylcholinesterase. Galathamine and acarbose were used as standard inhibitors for neuroprotective and anti-diabetic activity, respectively. The enzyme inhibitory activities were measured with spectrophotometric methods and the results (IC_{50} (mg/mL)) were illustrated in Table 4. The cholinesterase inhibitory activity of SGEOs was very low when compared to galanthamine. Acetylcholinesterase (IC_{50}: 0.618 mg/mL) and butrylcholinesterase (IC_{50}: 0.632 mg/mL) inhibition ability of SGEOs appear to be close. The lower cholinestrase inhibitory activity of SGEOs may be originated from monoterpenes. These results reveal that monoterpenes (especially, α- and β-pinene) in SGEOS have very weak cholinesterase inhibitory activities. In this direction, this is a critical point as elucidation of the component(s) responsible for the activity of any essential oil is important. Our results are in agreement with previous studies that monoterpene- rich oils are showed low inhibitory potential on cholinesterase [52, 53].

SGEOs exhibited a marked inhibitory activity on α-glucosidase with an IC_{50} of 0.632 mg/mL, while α-amylase inhibitory activity (IC_{50}: 0.899 mg/mL) of the sample was lower than acarbose ((IC_{50}: 0.548 mg/mL). As a result of this case, it should be suggested that α-glucosidase inhibitory activity of SGEOs may be related with monoterpene hydrocarbons contents. The results were consistent with the previous findings [52, 54]. However, monoterpene-rich oils from several plant species exhibited very low inhibitory activities on α-glucosidase. This case can be explained with complex nature of volatile components or synergic and antagonistic actions. Recently, many studies are supported by the results above stated [55, 56].

Table 3. Total antioxidant capacity, metal chelating, radical scavenging activity (IC_{50}: mg/mL) and reducing power (EC_{50}: mg/mL) of trolox and SGEOs

Sample	Radical scavenging activity (IC_{50}, mg/mL)			Reducing power (EC_{50}, mg/mL)		Metal chelating (mmol EDTAEs/g oil)	Total antioxidant (mgTEs/g oil)
	DPPH	ABTS	Nitric oxide	CUPRAC	FRAP		
Essential oil	16.447±0.216[a]	8.518±0.160	0.899±0.024	1.068±0.043	2.066±0.051	29.09±0.10[b]	2.55±0.02[c]
Trolox	0.058±0.002	0.197±0.004	1.119±0.016	0.074±0.002	0.051±0.001	nt	nt

[a] Values expressed are means ± S.D. of three parallel measurements; nt: no tested
[b]TEs, trolox equivalents
[c]EDTAEs, disodium edetate equivalents

Table 4. Enzyme inhibitory activity (IC_{50}: mg/mL) of standards and the essential oil from *Sideritis galatica*

Sample	Neuroprotective activity		Anti-diabetic activity	
	Acetylcholinesterase	Butyrylcholinesterase	α-Amylase	α-Glucosidase
Essential oil	0.618±0.080[a]	0.632±0.160	0.899±0.056	0.632±0.073
Galanthamine	0.003±0.002	0.018±0.0013	nt	nt
Acarbose	nt	nt	0.548±0.017	2.062±0.045

[a] Values expressed are means ± S.D. of three parallel measurements; nt: no tested

4. Conclusion

This is the first report describing the chemical composition, antioxidant and enzyme inhibitory potentials of SGEOs. α-pinene (23.0%), β-pinene (32.2%) and β-caryophyllene (16.9%) were identified as the major components in SGEOs. From the results, SGEOs was called as monoterpene-rich oil (>70%). Antioxidant and enzyme inhibitory activities were measured by spectrophotometric methods. SGEOs exhibited a moderate activity in these methods. This result can be explained by lower antioxidant and enzyme inhibitory activity of monoterpene hydrocarbons (α and β-pinene). In recent times, there has been a growing awareness of the possible health risks associated with synthetic additives and drugs. This realization has stimulated an increase in research into natural agents, particularly from medicinal plants, in search of alternative source. At this point, this investigation suggests that SGEOs can be considered as a source of natural agents for development new natural products such as food additives and drugs.

References

[1] P. H. Davis, J. Cullen and M. J. Elgar Coode, eds. (1988). Flora of Turkey and the east Aegean islands. Vol. 10. Edinburgh University Press, Edinburg, UK, p 203.

[2] N. Aligiannis, E. Kalpoutzakis, I. B. Chinou and S. Mitakou (2001). Composition and antimicrobial activity of the essential oils of five taxa of *Sideritis* from Grecee, *J. Agric. Food Chem.* **49**, 811-815

[3] E. Kupeli, P F. Sahin, I. Calis, E. Yesilada and N. Ezer (2007) Phenolic compounds of *Sideritis ozturkii* and their in vivo anti-inflammatory and anticiceptive activites, *J. Ethnopharmacol*. **112**, 356-360.

[4] M. Charami, D. Lazari, A. Karioti, H. Skaltsa, D. Hadjipavlou-Litina and C. Souleles (2008). Antioxidant and anti-inflammatory activities of *Sideritis perfoliata* subsp. *perfoliata* (Lamiaceae), *Phytother. Res.* **22**, 450-454.

[5] T. Baytop (1999). Therapy with medicinal plants in Turkey (Past and Present). Nobel Tıp Kitapevi, Istanbul, p 193.

[6] G. Zengin, C. Sarikurkcu, A. Aktumsek and R. Ceylan (2014). *Sideritis galatica* Bornm.: A source of multifunctional agents for the management of oxidative damage, Alzheimer's and diabetes mellitus, *J. Funct. Foods* **11**, 538-547

[7] T. Askun, G. Tumen, F. Satil and M. Ates (2009). Characterization of the phenolic composition and antimicrobial activities of Turkish medicinal plants, *Pharm. Biol*. **47**, 563–571

[8] C. Mateo, J. Sanz and J. Calderón (1984). The essential oils of some eastern Spain *Sideritis*, *Phytochemistry* **23**, 319-322.

[9] N. Erkan, H. Cetin and E Ayranci (2011). Antioxidant activities of *Sideritis congesta* Davis et Huber-Morath and *Sideritis arguta* Boiss et Heldr: Identification of free flavonoids and cinnamic acid derivatives, *Food Res. Int.* **44**, 297-303.

[10] N. Kirimer, N. Tabanca, T. Özek, K. H. C. Baser, G. Tümen and H. Duman (2003). Composition of essential oils from five endemic *Sideritis* species, *J. Essent. Oil Res.* **15**, 221-225.

[11] S. Carikci, T. Kılıç, A. Azizoğlu and G. Topçu (2012). Chemical Constituents of Two Endemic *Sideritis* Species from Turkey with Antioxidant Activity, *Rec. Nat. Prod.* **6**, 101-109.

[12] N. Kirimer, N. Tabanca, G. Tümen, H. Duman and K. H. C. Başer (1999). Composition of the essential oils of four endemic *Sideritis* species from Turkey, *Flavour Fragr. J.* **14**, 421-425.

[13] N. Tabanca, N. Kirimer and K. H. C. Baser (2001). The composition of essential oils from two varieties of *Sideritis erythrantha* var. *erythrantha* and var. *cedretorum*, *Turk. J. Chem.* **25**, 201-208.

[14] Z. Tunalier, M. Kosar, N. Ozturk, K. H. C. Baser, H. Duman and N. Kirimer (2004). Antioxidant properties and phenolic composition of *Sideritis* species, *Chem. Nat. Compd+*. **40**, 206-210.

[15] M. Kardali, A. Velasco-Negueruela and M. J. Pérez Alonso (2000). Essential oil constituents of *Sideritis ibanyezii* Pau, *Bot. Complutensis.* **24**, 101-106.

[16] A. Basile, F. Senatore, R. Gargano, S. Sorbo, M. Del Pezzo, A. Lavitola, A. Ritieni, M. Bruno, D. Spatuzzi, D. Rigano and M. L.Vuotto (2006). Antibacterial and antioxidant activities in *Sideritis italica* (Miller) Greuter et Burdet essential oils, *J. Ethnopharmacol.* **107**, 240-248.

[17] N. Kirimer, N. Tabanca, T. Özek, G. Tümen and K. H. C. Baser (2000). Essential oils of annual *Sideritis* species growing in Turkey, *Pharmaceut. Biol.* **38**, 106-111

[18] A. Jimenez, S. Manez and A. Villar (1990). Seasonal changes in the volatiles of *Sideritis mugronensis*, *Sci. Pharm.* **58**, 399-402.

[19] N. Kirimer, N. Tabanca, B. Demirci, K. H. C. Baser, H. Duman and Z. Aytac (2001). The essential oil of a new *Sideritis* species: *Sideritis ozturkii* Aytac and Aksoy, *Chem. Nat. Compd+*. **37**, 234-237.

[20] M. Ozcan, J. C. Chalchat and A. Akgül (2001). Essential oil composition of Turkish mountain tea (*Sideritis* spp.), *Food Chem.* **75**, 459-463.

[21] R. P. Adams (2001). Identification of essential oils components by gas chromatography /quadrupole mass spectroscopy, Allured Publishing Corporation, Illinois, USA.

[22] S. Berk, B. Tepe, S. Arslan and C. Sarikurkcu (2011). Screening of the antioxidant, antimicrobial and DNA damage protection potentials of the aqueous extract of *Asplenium ceterach* DC, *Afr. J. Biotechnol*. **10**, 8902-8908.

[23] R. Apak, K. Guclu, M. Ozyurek, S. E. Karademir and E. Ercag (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas, *Int. J. Food Sci. Nutr*. **57**, 292-304.

[24] A. Aktumsek, G. Zengin, G. O. Guler, Y. S. Cakmak and A. Duran (2013). Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species, *Food Chem. Toxicol.* **55**, 290-296.

[25] C. Sarikurkcu (2011). Antioxidant activities of solvent extracts from endemic *Cyclamen mirabile* Hildebr. tubers and leaves, *Afr. J. Biotechnol*. **10**, 831-839.

[26] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Bio. Med.* 26, 1231–1237.

[27] A. Srivastava and T. Shivanandappa (2011). Antioxidant and cytoprotective properties of 2-(hydroxymethyl)-3-methoxybenzaldehyde, *Food Chem.* **128**, 458–464.

[28] G. Zengin, C. Sarikurkcu, A. Aktumsek, R. Ceylan and O. Ceylan (2014). A comprehensive study on phytochemical characterization of *Haplophyllum myrtifolium* Boiss. endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer, skin diseases and type II diabetes, *Ind. Crops Prod.* **53**, 244-251.

[29] Y. Ruiz-Navajas, M. Viuda-Martos, J. A. Perez-Alvarez, E. Sendra and J. Fernandez-Lopez (2012). Chemical characterization and antibacterial activity of two aromatic herbs (*Santolina chamaecyparissus* and *Sideritis angustifolia*) widely used in the folk medicine, *J. Food Saf.* **32**, 426-434.

[30] C. Giuliani, L. M. Bini, F. Papa, G. Cristalli, G. Sagratini, S. Vittori, D. Lucarini and F. Maggi (2011). Glandular Trichomes and Essential oil composition of endemic *Sideritis italic* (Mill) Greuter et Burdet from Central Italy, *Chem. Biodivers.* **8**, 2179-2194.

[31] A. B. Trendafilova, M. N. Todoravo, L. N. Evsatieva and D. V. Antonova (2013). Variability in the Essential-Oil compostion of *Sideritis scardica* Grieseb. from Native Bulgarian Populations, *Chem. Biodivers.* **10**, 484-492.

[32] E. A. Aboutabl, K. M. Meselh and A. M. El-Azzouny (2009). Composition and antiwormal activity of essential oil from *Sideritis taurica* Stephan ex Wild Grown in Egypt, *J. Essent. Oil Res.* **21**, 94-96.

[33] M N. Todorova, R C. Christov and L N. Evstatieva (2000). Essential oil composition of three *Sideritis* species from Bulgaria, *J. Essent. Oil Res.* **12**, 418-420.

[34] J. Pala-Paul, M. J. Perez-Alonso, A. Velasco-Neguerula, M. T. Ballesteros and J. Sanz (2006). Essential oil composition of *Sideritis hirsute* L. From Guadalajara Province, Spain, *Flavour Fragr. J.* **21**, 410-415

[35] A. Ertas, M. Ozturk, M. Boga and G. Topcu (2009). Antioxidant and anticholinesterases activity evaluation of ent-kaurane diterpenoids from *Sideritis arguta* (perpendicular), *J. Nat. Prod.* **72**, 500–502.

[36] R. Marin, M. A. Apel, R. P. Limberger, M. C. B. Raseira, J. F. M.Pereira and J. A. S. Zuanazzi (2008). Volatile components and antioxidant activity from some *Myrtaceous* fruits cultivated in Southern Brazil, *Lat. Am. J. Pharm.* **27**, 172-178.

[37] M Kelen and B. Tepe (2008). Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora, *Bioresour. Technol.* **99**, 4096-4104.

[38] W. Wang, N. Wu, Y. G. Zu and Y. J. Fu (2008). Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components, *Food Chem*. **108**, 1019-1022.

[39] A. H. Ebrahimabadi, A. Mazoochi, F. J. Kashi, Z. Djafari-Bidgoli and H. Batooli (2010). Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss. from Iran, *Food Chem. Toxicol.* **48**, 1371-1376.

[40] E. O. Kose, I. G. Deniz, C. Sarikurkcu, O. Aktas and M. Yavuz (2010). Chemical composition, antimicrobial and antioxidant activities of the essential oils of *Sideritis erythrantha* Boiss. and Heldr. (var. *erythrantha* and var. *cedretorum* P.H. Davis) endemic in Turkey, *Food Chem. Toxicol.* **48**, 2960-2965.

[41] A. Delazar, M. R. Delnavazi, N. Yassa, S. Parkhideh, N. Delazar, L. Nahar and S. D. Sarker (2012). Essential oil composition and isolation of freeradical-scavenging phenolic glycosides from the aerial parts of *Ajuga chamaepitys* growing in Iran, *Rev. Bras. Farmacogn.* **22**, 399-305.

[42] B. Mercier, J. Prost and M. Prost (2009). The essential oil of turpentine and its major volatile fraction (α and β-pinenes): A review, *Int. J. Occup. Environ. Health*. **22**, 331-342.

[43] S. Bounatirou, S. Smiti, M. G. Miguel, L. Faleiro, M. N. Rejeb, M. Neffati, M. M. Costa, A. C. Figueiredo, J. G. Barroso and L. G. Pedro (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link, *Food Chem.* **105**, 146-155.

[44] N. Zouri, I. Ayadi, N. Fakhfakh, A. Rebai and S. Zouri (2012). Variation of chemical composition of essential oils in wild populations of *Thymus algeriensis* Boiss. et Reut., a North African endemic Species, *Lipids Health Dis*. **11**, 28-39.

[45] C. Sarikurkcu, K. Arisoy, B. Tepe, A. Cakir, G. Abali and E. Mete (2009). Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnus castus* L. fruits from Turkey, *Food Chem. Toxicol*. **47**, 2479-2483.

[46] M. Kiendrebeogo, A. Y. Coulibaly, R. C. H. Nebie, B. Zeba, C. E. Lamien, A. Lamien-Meda and O. G. Nacoulma (2011). Antiacetylcholinesterase and antioxidant actvitiy of essential oils from six medicinal plants from Burkina Faso, *Rev. Bras. Farmacogn.* **21**, 63-69.

[47] L. M. Brierer, V. Haroutunian, S. Gabriel, P. J. Knott, L. S. Carlin, D. P. Purohit, D. P. Perl, J. Schmeidler, P. Kanof and K. L. Davis (1995). Neurochemicl correlates of dementia severity in Alzheimer disease: relative importance of cholinergiv deficits, *J. Neurochem*. **64**, 749-760.

[48] A. J. Krentz and C. J. Bailey (2005). Oral diabetic agents: current role in type 2 diabetes mellitus, *Drugs* **65**, 385-411.

[49] A. Kariotia, A. Protopappa, N. Megoulas and H. Skaltsa (2007). Identification of tyrosinase inhibitors from *Marrubium velutinum* and *Marrubium cylleneum*, *Bioorgan. Med. Chem.* **15**, 2708-2714.

[50] S. H. Lee, S. A. Sancheti, M. R. Bafna, S. S. Sancheti and S. Y. Seo (2011). Acetylcholinesterase inhibitory and antioxidant properties of *Rhododendron yedoense* var. *poukhanense* bark, *J. Med. Plants Res.* **5**, 248-254.

[51] S. Saha and R. Verna (2012). Inhibitory potential of traditional herbs on α-amylase activity, *Pharm. Biol*. **50**, 326-331.

[52] R. M. Loizzo, R. Tundis, F. Conforti, A. M. Saab, G. A. Statti and F. Menichini (2007). Comparative chemical composition, antioxidant and hypoglycemic activities of *Juniperus oxycedrus* sp. *oxycedrus* L. berry and wood oils from Lebanon, *Food Chem.* **105**, 572-578.

[53] O. Ustun, F. S. Senol, M. Kurkcuoglu, I. E. Orhan, M. Kartal and K. H. C. Baser (2012). Investigation on chemical composition, anticholinesterase, and antioxidant activities of extracts and essential oils of Turkish *Pinus* species and pycogenol, *Ind. Crop. Prod*. **38**, 115-123

[54] G. Oboh, A. O. Ademosun, V. O. Odubanjo and I. A. Akinbola (2013). Antioxidative properties and inhibiton of key enzymes relevant to type-2 Diabetes and hypertension by essential oils from Black Pepper, *Adv. Pharmacol. Sci*. 926047, 1-6.

[55] S. Savelev, E. Okello, N. S. L. Perry, R. M. Wilkins and E. K. Pery (2003). Synergistic and antagonistis interactions of anticholiensterase terpenoids in *Salvia lavandulaefolia* essential oil, *Pharmacol. Biochem. Be*. **75**, 661-668.

[56] J. Mastelic, I. Jerkovic and I. Blazevic (2008). Comparative study on the antioxidant and biological activities of carvacrol, tyhmol and eugenol derivates, *J. Agric. Food Chem.* **56**, 3989-3996.