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RESEARCH ARTICLE

Chemical composition, antioxidant, anticholinesterase and anti-urease activities of *Sideritis pisidica* Boiss. & Heldr. endemic to Turkey

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ABSTRACT

The objective of this study was to investigate the chemical composition, antioxidant, anticholinesterase and anti-urease activities of essential oil, hexane, acetone and methanol extracts of *Sideritis pisidica* Boiss. & Heldr. The essential oil and fatty acid composition were analyzed by GC and GC/MS. δ-Cadinene (19.5 %), T-cadinol (16.7 %) and β-cubebene (10.4 %) were identified as the main compounds of the essential oil whereas the most abundant compounds of fatty acids were found as linolenic (42.7 %), palmitic (31.3 %) and linoleic (13.4%) acids. The methanol extract demonstrated the highest antioxidant activity in all tests, except for metal chelating assay. The hexane extract was found to be significantly active in metal chelating (IC_{50}: 22.97±1.36 μg/mL), acetylcholinesterase (62.54±0.88 %) and urease inhibitory (78.93±0.17 %) assays. These results show that the *Sideritis pisidica* could be used as the potential source of natural products in the food and pharmaceutical areas.

Key words: *Sideritis pisidica*; essential oil; fatty acid; antioxidant activity; anticholinesterase activity; anti-urease activity

1. Introduction

The *Sideritis* genus, a member of the Lamiaceae family, has more than 150 species which are distributed in tropical and mild regions of the Northern Hemisphere. The highest biodiversity of these species is found in the Mediterranean area especially Greece and Turkey [1-3]. *Sideritis* species are known as "mountain tea" in Anatolia and consumed as tea, flavoring agents and for medicinal purposes in various regions. *Sideritis pisidica* Boiss. & Heldr. is called çay çalbası [4] and eldiven çayı [5] by local people. Up to now, several biological activities such as antioxidant, anticonvulsant, antispasmodic, anti-inflammatory, anti-rheumatic, anti-ulcer, antimicrobial, cytotoxic, and analgesic have been reported from *Sideritis* species [6-9].

In earlier studies, phenolic acids, steroids, diterpenoids, fatty acids, flavonoids, iridoid and phenylethanoid glycosides have been reported in *Sideritis* species [10-13]. In addition, the biological activity of extracts, essential oils and isolated pure compounds from these species were reported [10, 11, 14-18]. There are some chemical studies performed on *S. pisidica*. Up to date, the essential oil [19-22] and fatty acid [23] compositions of *S. pisidica* have been studied. Also, antioxidant, anti-acetylcholinesterase activity and phenolic composition of the aqueous and ethanolic extract of *S. pisidica* have been reported by Erdogan-Orhan *et al.* [24] and Özkan [12].

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So far, no study has been reported about antioxidant and enzyme inhibitory activities of the essential oil and the hexane, acetone and methanol extract of *S. pisidica* with the total phenolic and flavonoid contents. The aim of this study was to determine chemical composition, antioxidant, anticholinesterase and anti-urease activities of the essential oil and the hexane, acetone and methanol extract of *S. pisidica* with the total phenolic and flavonoid contents.

2. Results and Discussion

2.1. Essential oil composition

The chemical composition of the essential oil of *S. pisidica* was analyzed by GC-FID and GC/MS techniques. The chemical composition of the essential oil, relative percentage (%) and Kovats indices of compounds are given in Table 1. Thirty-one compounds, representing about 99.9 % of the essential oil of *S. pisidica* were identified. The major compound was δ-cadinene (19.5 %), followed by T-cadinol (16.7 %) and β-cubebene (10.4 %). The most abundant compounds in the essential oil were sesquiterpene hydrocarbons (56.1 %) and oxygenated sesquiterpenes (39.2 %), respectively.

In previous studies, the essential oil composition of *S. pisidica* was reported [19-22]. In these studies, α-pinene, β-pinene, myrcene, α-bisabolol, sabinene, β-caryophyllene, eugenol, thymol and camphor were identified as major compounds. When our findings were compared with the literature, the results showed some qualitative and quantitative differences between the compounds of the essential oils. The chemical composition of the essential oil of *S. pisidica* can be influenced

Table 1. Chemical composition of the essential oil of *Sideritis pisidica*

No	Compounds	RI[a]	LRI[b]	*S. pisidica* (%[c])	Identification Methods
1	Eucalyptol	1027	1026	0.2	Co-GC, MS, RI
2	*α*-Terpineol	1178	1176	0.2	Co-GC, MS, RI
3	Bornyl acetate	1270	1270	0.1	Co-GC, MS, RI
4	Thymol	1290	1293	*tr*	Co-GC, MS, RI
5	Carvacrol	1299	1306	1.5	Co-GC, MS, RI
6	*α*-Cubebene	1347	1355	0.3	MS, RI
7	*α*-Copaene	1371	1379	3.7	Co-GC, MS, RI
8	*β*-Bourbonene	1381	1386	0.7	Co-GC, MS, RI
9	*β*-Elemene	1392	1389	0.6	MS, RI
10	*β*-Cubebene	1394	1390	10.4	MS, RI
11	*α*-Gurjunene	1408	1413	1.2	MS, RI
12	*β*-Caryophyllene	1424	1421	5.3	Co-GC, MS, RI
13	*τ*-Elemene	1442	1445	0.3	MS, RI
14	*α*-Humulene	1448	1452	0.5	Co-GC, MS, RI
15	Alloaromadendrene	1460	1465	0.1	Co-GC, MS, RI
16	Germacrene D	1474	1479	*tr*	MS, RI
17	*β*-Guainene	1482	1486	2.9	MS, RI
18	*τ*-Gurjunene	1487	1492	6.9	MS, RI
19	*α*-Muurolene	1495	1496	1.0	MS, RI
20	*δ*-Cadinene	1512	1522	19.5	Co-GC, MS, RI
21	*β*-Cadinene	1530	1535	2.7	Co-GC, MS, RI
22	Spathulenol	1576	1572	8.7	Co-GC, MS, RI
23	Caryophyllene oxide	1580	1578	*tr*	Co-GC, MS, RI
24	Globulol	1586	1590	2.4	MS, RI
25	Ledol	1590	1592	tr	MS, RI
26	Viridiflorol	1596	1602	3.0	MS, RI
27	Alloaromadendrene oxide	1598	1604	0.2	MS, RI
28	Cubenol	1600	1605	1.6	MS, RI
29	*δ*-Cadinol	1608	1608	6.6	MS, RI
30	T-Cadinol	1620	1625	16.7	MS, RI
31	Hexahydrofarnesyl acetone	1833	1844	2.6	MS, RI
	Oxygenated monoterpenes			2.0	
	Sesquiterpene hydrocarbons			56.1	
	Oxygenated sesquiterpenes			39.2	
	Others			2.6	
	Total identified (%)			99.9	
	Total number of compounds			31	

[a]: Retention indices on DB–5 fused silica column, [b]: Retention indices of literature on DB-5 column (33), [c]: Percentage concentration, [Co-GC]: Co-injection with authentic compounds, [RI]: Retention Index literature comparison, [tr]: trace

by season, the geographical location and date of collection [25].

2.2. Fatty acid composition

The fatty acid composition of the aerial parts of *S. pisidica* was analyzed with GC-FID and GC/MS techniques. Table 2 shows the fatty acid composition of *S. pisidica*. Totally, nine fatty acids, ranging from $C_{14:0}$ to $C_{24:0}$ were detected. The major fatty acids were linolenic (42.7 %), palmitic (31.3 %) and linoleic acids (13.4 %). Linolenic acid known as the essential fatty acid for human metabolism was found to be the major fatty acid of *S. pisidica*. The total unsaturated fatty acid percentage was found as 62.9 % and linoleic and oleic acid ratio (L/O) was 1.97. Oleic acid (6.8 %), stearic acid (4.1 %) and behenic acid (1.6 %) were found in small quantities while other fatty acids were determined in trace amounts. Ertan *et al*. [23] previously studied the fatty acid composition of the seeds of 15 *Sideritis* species including *S. pisidica*. The fatty acid composition of leaves and flowers of Lamiaceae were mainly composed of ($C_{18:3}$) linolenic acid [23, 26].

Table 2. The fatty acid composition (%) of *S.pisidica*

Fatty acids	*Sideritis pisidica* (%)
Tetradecanoic acid ($C_{14:0}$)	*tr*[b]
Palmitic acid ($C_{16:0}$)	31.3
Linolenic acid ($C_{18:3}$)	42.7
Linoleic acid ($C_{18:2}$)	13.4
Oleic acid ($C_{18:1}$)	6.8
Stearic acid ($C_{18:0}$)	4.1
Arachidic acid ($C_{20:0}$)	*tr*
Behenic acid ($C_{22:0}$)	1.6
Tetracosanoic acid ($C_{24:0}$)	*tr*
Total saturation	37.0
Total unsaturation	62.9
Saturation/Unsaturation	0.59
L/O[a]	1.97

[a] L/O: linoleic acid-oleic acid ratio.
[b] *tr*: trace

2.3. Total phenolic and total flavonoid contents

Phenolic compounds have antioxidant properties due to their ability to act as hydrogen donor and metal chelating [27]. Flavonoids are phenolic compounds widely distributed in plants and create a significant portion of the human diet [28, 29]. The acetone extract of *S. pisidica* has the highest amount of the total phenolic content (100.24±1.06 μg PEs/mg) while the methanol extract has the highest amount of the total flavonoid content (43.75±0.20 μg QEs/mg). The total phenolic contents of extracts of *S. pisidica* were found in the following order: acetone > methanol > hexane. The total flavonoid contents of extracts of *S. pisidica* were decreased in the order of methanol > acetone > hexane. In addition, the methanol extract of *S. pisidica* with higher concentrations of flavonoid contents showed the highest activity in all antioxidant activity assays except metal chelating assay (Table 3).

Table 3. Total phenolic and total flavonoid contents of the extracts of *Sideritis pisidica* [a]

Extracts	Total phenolic contents μg PEs/mg extracts[b]	Total flavonoid contents μg QEs/mg extract[c]
Hexane	20.40±0.65	30.66±0.81
Acetone	100.24±1.06	34.48±0.33
Methanol	28.09±0.69	43.75±0.20

[a] Values expressed are means ± S.E.M. of three parallel measurements. ($p<0.05$)
[b] PEs, pyrocatechol equivalents.
[c] QEs, quercetin equivalents.

2.4. Antioxidant activity

Many studies demonstrate that antioxidants protect against the chronic disease and aging by inhibiting or reducing the oxidation processes that produce free radicals [30]. In order to determine the antioxidant activity of the essential oil and the extracts of *S. pisidica*, lipid peroxidation inhibition by β-carotene-linoleic acid, DPPH free radical scavenging, ABTS cation radical scavenging, CUPRAC, and metal chelating assays were used. The antioxidant activities of the essential oil and the extracts of *S. pisidica* compared with BHA, α-tocopherol and EDTA were given in Table 4. The essential oil and the extracts of *S. pisidica* were tested at different concentrations and IC_{50} values determined.

In β-carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and CUPRAC assays, the methanol extract showed the highest activity with IC_{50} values of 17.81±1.23, 39.07±0.37, 23.75±0.09, 55.90±0.02 μg/mL, respectively. The methanol extract showed very close radical scavenging activity to that of α-tocopherol in $DPPH^{\bullet}$ assay while it exhibited the highest activity, even higher than that of α-tocopherol in $ABTS^{\bullet+}$, and CUPRAC assays (Table 4). These results suggest that the best antioxidant activity obtained with the methanol extract could be responsible for

Table 4. Antioxidant activity of the essential oil and extracts of *Sideritis pisidica* by β-Carotene-linoleic acid, DPPH•, ABTS•+, CUPRAC and metal chelating assays [a]

		Antioxidant Activity				
		β-Carotene-linoleic acid assay	DPPH• assay	ABTS•+ assay	CUPRAC assay	Metal chelating assay
		IC_{50} (μg /mL)	IC_{50} (μg /mL)	IC_{50} (μg/mL)	$A_{0.50}$ (μg/mL)[b]	IC_{50} (μg/mL)
S. pisidica	Essential oil	>200	6.52±0.58[c]	>200	>200	*NA*[e]
	Hexane extract	89.34±0.57	>200	>200	160.08±0.09	22.97±1.36
	Acetone extract	24.44±0.48	187.51±0.25	86.20±1.13	112.12±0.11	57.98±0.70
	Methanol extract	17.81±1.23	39.07±0.37	23.75±0.09	55.90±0.02	71.93±0.88
Standards	α-Tocopherol[d]	2.10±0.08	37.20±0.41	38.51±0.54	66.72±0.81	*NT*
	BHA[d]	1.34±0.04	19.80±0.36	11.82±0.09	24.40±0.69	*NT*
	EDTA[d]	*NT*[f]	*NT*	*NT*	*NT*	3.47±0.16

[a] IC_{50} values represent the means ± SEM of three parallel measurements ($p< 0.05$).
[b] $A_{0.50}$ values represent the means ± SEM of three parallel measurements ($p< 0.05$).
[c] % inhibition at 200 μg/mL concentration of the essential oil of *Sideritis pisidica*.
[d] Reference compound. [e] *NA*: not active [f] *NT*: not tested
BHA: butylatedhydroxyl anisole; EDTA: Ethylenediaminetetraacetic acid

its high level of total flavonoid content. In metal chelating assay, the highest activity was found in the hexane extract (IC_{50}: 22.97±1.36 μg/mL) and followed by the acetone extract (IC_{50}: 57.98±0.70 μg/mL). Non-phenolic components in the hexane extracts could be responsible for the observed metal chelating activity. The essential oil exhibited no any chelating activity (Table 4).

2.5. Anticholinesterase activity

Recent studies show that compound having acted as antioxidants may be used in the treatment of neuronal diseases [31]. Table 5 shows the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the essential oil and extracts of *S. pisidica* compared with that of galantamine – that is used as a standard drug for the treatment of mild Alzheimer's disease.

The hexane extract (62.54±0.88 %) of *S. pisidica* exhibited high activity while the acetone and methanol extract were found to be inactive against AChE enzyme. The essential oil (58.37±1.03 %) was found as the most active against BChE enzyme. Also, the hexane and acetone extract of *S. pisidica* showed moderate inhibitory activity against BChE enzyme with inhibition % values at 200 μg/ mL concentration of 47.47±0.98 % and 43.43±1.10, respectively (Table 5).

2.6. Anti-urease activity

Urease has a significant role in the pathogenesis of gastric and peptic ulcers and cancer due to cause to survive *Helicobacter pylori* in the acidic environment of the stomach. Besides, urease induces infections and urolithiasis by *Proteus mirabilis* and *Yersinia enterocolitica,* infection-induced acute pyelonephritis and reactive arthritis [32]. Urease inhibitors have attracted much attention as potential drugs. Table 5 shows inhibition % values at 200 μg/mL concentration of the essential oil and the extracts of *S. pisidica* for anti-urease activity. Against urease enzyme, the hexane extract of *S. pisidica* (78.93±0.17 %) exhibited near activity to thiourea (88.76±0.22 %) used as a standard and followed by the methanol extract (66.64±1.03 %).

3. Conclusion

This is the first study to investigate the antioxidant, anticholinesterase and anti-urease activities of the essential oil and the hexane, acetone and methanol extracts of an endemic species *S. pisidica* from Turkey with the total phenolic and flavonoid contents. The methanol extract had the highest total flavonoid content and the best antioxidant activity in β-carotene-linoleic acid, DPPH•, ABTS•+, and CUPRAC assays. Also, the hexane extract was found to be significantly active in metal chelating assay, urease and acetylcholinesterase inhibitory activities. Thirty-one compounds were identified in the essential oil representing 99.9% of the total oil; the major compounds were *δ*-cadinene (19.5 %), T-cadinol (16.7 %) and β-cubebene (10.4 %). The most abundant fatty acids were found as linolenic (42.7 %), palmitic (31.3 %) and linoleic (13.4%) acids.

Table 5. Anticholinesterase and anti-urease activities of the essential oil and the extracts of *Sideritis pisidica* [a]

Sideritis species		Cholinesterase Inhibitory Activity		Urease Inhibitory Activity
		AChE assay	BChE assay	Urease assay
S. pisidica	Essential oil	11.60±1.20	58.37±1.03	7.88±0.22
	Hexane extract	62.54±0.88	47.47±0.98	78.93±0.17
	Acetone extract	*NA*[c]	43.43±1.10	43.21±0.28
	Methanol extract	*NA*	16.35±0.91	66.64±1.03
Standards	Galantamine[b]	80.41±0.98	82.23±0.67	*NT*
	Thiourea[b]	*NT*	*NT*	88.76±0.22

[a] (%) inhibition at 200 μg/mL concentration values represent the means ± S.E.M. of three parallel measurements ($p<0.05$). [b] Reference compounds. [c] *NA*: not active. [d] *NT*: not tested.

The results show that *Sideritis pisidica* could be used as a potential source of natural products in the food and pharmaceutical areas. However, further investigations are recommended including isolation of new drug candidates from this species as well as *in vitro* and *in vivo* tests to explore the activity in biological systems.

4. Materials and Methods

4.1. Plant material

The aerial part of *S. pisidica* was collected from Muğla, Turkey in 2013. The plant was identified by Dr. Hasan Yildirim at Ege University, Izmir, Turkey. The voucher specimen has been deposited at the herbarium of Ege University with voucher no EGE42371.

4.2. Instruments and chemicals

Bioactivity measurements were carried out on a 96-well microplate reader, SpectraMax 340PC384 (Molecular Devices, Silicon Valley, CA). The measurements and calculations of the activity results were evaluated by using Softmax PRO v5.2 software (Molecular Devices, Silicon Valley, CA). Analyses of chemical composition of the essential oils and fatty acids were performed using GC (Shimadzu GC-17 AAF, V3, 230V series gas chromatography, Japan) and GC/MS (Varian Saturn 2100T, USA).

Pyrocatechol, quercetin, *n*-hexane, methanol, ethanol, ferrous chloride, copper (II) chloride and ethylenediaminetetraacetic acid (EDTA) were purchased from E. Merck (Darmstadt, Germany). Butylatedhydroxyl anisole (BHA), α-tocopherol, β-carotene, polyoxyethylene sorbitan monopalmitate (Tween-40), linoleic acid, Folin–Ciocalteu's reagent (FCR), neocuproine, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2′-azino *bis* (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (Ferene), acetylcholinesterase (AChE) from electric eel (Type-VI-S, EC 3.1.1.7, 425.84 U/mg, Sigma, St. Louis, MO), butyrylcholinesterase (BChE) from horse serum (EC 3.1.1.8, 11.4 U/mg, Sigma, St. Louis, MO),), urease [Type-III from Jack Beans, EC 232-656-0, 20990 U/g solid], 5,5′-dithio*bis*(2-nitrobenzoic) acid (DTNB), galantamine, thiourea, acetylthiocholine iodide, and butyrylthiocholine chloride were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical grade.

4.3. Extraction

The aerial part of *S. pisidica* (430 g) was extracted separately with 2.5 L hexane, acetone and methanol, respectively at room temperature for 24 h and four times. Solvents were removed with a rotary evaporator. All extracts were stored at +4°C until analysis.

4.4. Isolation of the essential oil

The essential oil of dried aerial parts of *S. pisidica* was obtained by hydrodistillation in a Clevenger type apparatus for 4 h. The oil was dried over anhydrous sodium sulphate and stored under + 4°C until analysis.

4.5. Analysis of the essential oil

4.5.1. Gas chromatography (GC-FID)

A Flame Ionization Detector (FID) and a DB-5 fused silica capillary non-polar column (30 m×0.25 id., film thickness

0.25 µm) were used for GC analyses. The injector temperature and detector temperature were adjusted 250 °C and 270 °C, respectively. Carrier gas was He at a flow rate of 1.4 mL/min. Sample size was 1.0 µL with a split ratio of 20:1. The initial oven temperature was held at 60 °C for 5 min, then increased up to 240 °C with 4 °C/min increments and held at this temperature for 10 min. The percentage composition of the essential oil was determined with GC solution computer program.

4.5.2. Gas chromatography /mass spectrometry (GC/MS)

An Ion trap MS spectrometer and a DB-5 MS fused silica non-polar capillary column (30 m×0.25 mm ID, film thickness 0.25 µm) were used for the GC/MS analyses. Carrier gas was helium at a flow rate of 1.4 mL/min. The oven temperature was held at 60°C for 5 min, then increased up to 240 °C with 4 °C/min increments and held at this temperature for 10 min. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. Ion source temperature was 200°C. The injection volume was 0.2 µL with a split ratio of 1:20. EI–MS measurements were taken at 70 eV ionization energy. Mass range was from m/z 28 to 650 amu. Scan time 0.5 s with 0.1 inter scan delays. Identification of components of the essential oils was based on GC retention indices and computer matching with the Wiley, NIST-2005 and TRLIB Library as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [33] and whenever possible, by co-injection with authentic compounds.

4.6. Analysis of fatty acids

The n-hexane extract was dissolved in 0.5 M NaOH (2 mL) in a 25 mL flask. After the flask was heated in a water bath (50°C), then 2 mL BF_3:MeOH was added. The mixture was boiled for 2 minutes, and then the mixture was left until it cooled down, and then the volume was completed to 25 mL with saturated NaCl solution. Esters were extracted with n-hexane; thus, the organic layer was separated. The hexane layer was washed with a potassium bicarbonate solution (4 mL, 2 %) and dried with anhydrous Na_2SO_4 and filtered. The organic solvent was removed under reduced pressure by a rotary evaporator to give methyl esters of fatty acid. Derivatization of methyl esters of fatty acids was carried out according to our previous method [34]. Qualitative and quantitative analysis of the fatty acid esters were performed by GC and GC/MSD as reported previously [35].

4.7. Total phenolic and flavonoid contents

The phenolic content in the extracts were expressed as microgram of pyrocatechol equivalents (PEs), determined with FCR according to the method of Slinkard & Singleton [36] as described in the literature. The phenolic contents were calculated according to the following equation that was obtained from standard pyrocatechol graph:

Absorbance=0.017[pyrocatechol (µg)] + 0.0025 (r^2, 0:9996)

Measurement of flavonoid content of the extracts was based on the aluminum nitrate method [37], and results were expressed as microgram of quercetin equivalents. The flavonoid contents were calculated according to following equation that was obtained from the standard quercetin graph:

Absorbance=0.019[quercetin (µg)] – 0.035 (r^2, 0:9985)

4.8. Antioxidant activity

Total antioxidant activity by β-carotene-linoleic acid test [38], DPPH$^{\bullet}$ free radical scavenging activity [38], $ABTS^{\bullet+}$ cation radical scavenging activity [38], CUPRAC antioxidant activity [38], Metal chelating activity [38] were determined according to our reported procedures with slight modifications [39]. The sample concentration providing 50 % inhibition activity (IC_{50} µg/mL) was calculated from the graph of antioxidant activity percentages (Inhibition %) against sample concentrations (µg/mL). The sample concentration having 0.50 absorbance ($A_{0.5}$) was calculated from the plot of CUPRAC absorbance against sample concentration.

4.9. Anticholinesterase activity

Acetylcholinesterase and butyrylcholinesterase enzymes inhibitory activities were measured the spectrophotometric method developed by Ellman *et al.* [40] with slight modifications [38]. Galantamine was used as reference compound. The results were given as inhibition percentage (%) of the enzyme at 200 µg/mL concentration of the extracts and essential oil.

4.10. Anti-urease activity

Anti-urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn (1967) [41]. Briefly, solution for the reaction was comprised of urease enzyme (Jack bean source 25 µL), 50 µL of urea (100 mM) and 100 mM sodium phosphate buffer

(pH 8.2). The reaction solution along with test samples (10 µL, 1mM) was incubated at 30˚C for 15 minutes in 96-well plate. Simply 45µL of 1% (w/v) phenol reagent, alkali reagent (70 µL) and sodium nitroprusside 0.005% (w/v) was added to each well. After 50 min of incubation, increase in absorbance was measured with the of micro plate reader at 630 nm. Thiourea was used as reference compound. The results were given as inhibition percentage (%) of the enzyme at 200 µg/mL concentration of the extracts and essential oil.

4.11. Statistical analysis

All data on the antioxidant, anticholinesterase and anti-urease activity tests were the averages of three parallel sample measurements. The data were recorded as the mean ± S.E.M. Significant differences between the means were determined by student's *t* test, and *p* values <0.05 were regarded as significant.

Authorship statement

Author contributions: Concept – E.D., G.TÇ., M.E.D.; Design – E.D., G.TÇ., M.E.D.; Supervision – G.TÇ., M.E.D.; Resource – G.TÇ., M.E.D.; Materials – H.Y., M.E.D.; Data Collection and/or Processing – E.D., G.TÇ., H.Y.; Analysis and/or Interpretation - E.D., G.TÇ.; Literature Search – E.D., G.TÇ., M.E.D.; Writing – E.D., G.TÇ.; Critical Reviews – E.D., G.TÇ., M.E.D.

Conflict of interest statement

The authors declared no conflict of interest in the manuscript.

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