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Investigation of Chemical Composition, Antioxidant, Anticholinesterase and Anti-urease activities of *Euphorbia helioscopia*

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Abstract: In this study, chemical composition, antioxidant, anticholinesterase and anti-urease activities of the essential oil, hexane, acetone, methanol and water extracts of *Euphorbia helioscopia* were investigated. The chemical composition of the essential oil was analyzed by GC and GC/MS and *β*-cubebene (19.3 %), palmitic acid (12.2 %) and caryophyllene oxide (11.7 %) were identified as major compounds. The antioxidant activity of essential oil and extracts was performed by several methods such as β-carotene-linoleic acid, DPPH$^{\bullet}$, ABTS$^{\bullet+}$ radical scavenging, CUPRAC and metal chelating assays. The water extract showed higher antioxidant activity than BHA and α-tocopherol in β-carotene-linoleic acid, DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and CUPRAC assays with IC_{50}: 1.08±0.39, 15.57±0.48, 1.89±0.33 and 12.50±0.11 µg/mL values, respectively. Also, the anticholinesterase and anti-urease activities were tested against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and urease enzymes, spectrophotometrically. The acetone extract (81.23±0.58 %) showed very close BChE inhibitory activity to galantamine. The hexane extract (96.97±0.36 %) of *E. helioscopia* showed higher anti-urease activity than thiourea (96.93±0.17 %) whereas the essential oil (91.37±0.26 %) indicated very close anti-urease activity to the standard.

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1. INTRODUCTION

Euphorbiaceae family is distributed in all parts of the world except the Antarctic continent with over 5000 taxa. *Euphorbia* species, the most well-known of this family, is represented by approximately 2150 taxa in the world, while there are 109 taxa in our country. Euphorbia species is known as 'Sütleğen' in Turkey and these species is characterized by milk latex tissues. In the folk medicine, *Euphorbia* species are used in the treatment of various diseases such as a migraine, gonorrhea, skin diseases, intestinal parasites and warts cures [1]. Until this time, it is seen that secondary metabolites such as terpenes, flavonoids, tannins, steroids, alkaloids, and lipids are isolated from *Euphorbia* species [2-5]. It has also been reported that

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Euphorbia species have bioactive properties such as anti-inflammatory, antiarthritic, antiviral, antitussives, antitumor, anti-allergy, anti-asthma and antioxidant [1, 6-7].

Alzheimer's disease (AD) is a neurodegenerative disorder with clinical features such as memory loss, speech impairment and visuospatial defect [8]. Lack of acetylcholine, tau protein hyperphosphorylation, β-amyloid (Aβ) aggregation, oxidative stress, and neuroinflammation are promising approaches used in the treatment of Alzheimer's disease [9]. Reactive oxygen species (ROS), the major source of oxidative stress, cause oxidation of proteins and lipids in the brain causing Alzheimer's disease (AD) [10]. The most important approach to the treatment of AD is the inhibition of the acetylcholinesterase enzyme which leads to the breakdown of acetylcholine. Today, many drugs derived from synthetic and natural sources are used in the treatment of AD. Natural drugs are more preferred because of the harmful and toxic effects of synthetic drugs [11]. For this reason, researchers are increasingly interested in researching new sources of natural medicines. Herbal medicines have given promising results among the natural sources due to the existence of volatile oils and secondary metabolites.

When the literature studies are examined, it is seen that researchers related to the essential oil composition and the extracts obtained from plants and their biological activities have gained more importance in recent years. According to our knowledge, there have been a limited number of reports about phytochemical contents and bioactivities of *E. helioscopia* in the literature. Therefore, in this study, we aimed to evaluate chemical composition, antioxidant, anticholinesterase and anti-urease activities of the essential oil and the hexane, acetone, methanol and water extracts of *E. helioscopia* with the total phenolic and flavonoid contents in details.

2. METHOD

2.1. Plant Material

The aerial parts of *E. helioscopia* were collected from Yusufeli, Artvin, Turkey in August, 2016. The voucher specimen has been deposited at the herbarium of Natural Products Laboratory of Muğla Sıtkı Koçman University.

2.2. Instruments and Chemicals

Bioactivity measurements were carried out on a 96-well microplate reader, SpectraMax 340PC^{384} (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). Chemical composition of the essential oils was 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′-dithiobis(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.

2.3. Isolation of the Essential Oil

The essential oil of dried aerial parts of *E. helioscopia* was extracted by hydrodistillation in a Clevenger type apparatus for 4 h. The oil was dried over anhydrous sodium sulphate and stored under + 4°C until analyzed.

2.4. Analysis of the Essential Oil

2.4.1. Gas chromatography (GC)

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 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.

2.4.2. 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 and whenever possible, by co-injection with authentic compounds [12].

2.5. Extraction

The aerial parts of *E. helioscopia* were extracted separately with different solvents according to their increasing polarity: hexane, acetone, methanol at room temperature for 24 h and four times. Solvents were evaporated on a rotary evaporator to obtain hexane, acetone and methanol extracts. The remaining plant part was allowed to stand for one day with water at 80 °C. The water extract was obtained by lyophilisation using a freeze-drier. All extracts were stored at +4°C until analysis.

2.6. Antioxidant Activity

The total antioxidant activity of the essential oil and extracts was evaluated using β-carotene-linoleic acid test system as previously reported in the literature [13]. Radical scavenging activities were measured by DPPH free and ABTS cation radical scavenging assays [13]. Reducing powers were determined using CUPRAC assays [13]. Metal chelating activity on ferrous ions was determined using the method described in the literature [14].

2.7. Total Phenolic and Flavonoid Content

The phenolic content of extracts was stated as microgram of pyrocatechol equivalents (PEs) [15]. The phenolic contents were calculated according to the following equation that was obtained from standard pyrocatechol graph:

$$\text{Absorbance} = 0.0078\ [\text{pyrocatechol } (\mu g)] + 0.0623\ (r^2, 0.9992)$$

Measurement of flavonoid content of the extracts was based on the aluminum nitrate method and results were expressed as microgram of quercetin equivalents [16]. The flavonoid contents were calculated according to following equation that was obtained from the standard quercetin graph:

Absorbance=0.0412[quercetin (μg)] – 0.0662 (r^2, 0.9998)

2.8. Enzyme Inhibitory Activity

Anticholinesterase and anti-urease activities were measured using Ellman *et al.* [17] and Weatherburn [18] respectively.

2.9. Statistical Analysis

All data on antioxidant, anticholinesterase and anti-urease tests were the average of three parallel sample measurements. Data were recorded as mean ± S.E.M. Significant differences between means were determined by student's test, p values <0.05 were regarded as significant.

3. RESULTS AND DISCUSSION

3.1. Essential Oil Composition

GC-FID and GC/MS techniques were used to determine the chemical composition of the essential oil of *E. helioscopia*. The chemical composition of the essential oil, retention indices and relative percentage (%) of compounds are shown in Table 1. Thirty compounds, representing about 99.9 % of the essential oil of *E. helioscopia* were identified. *β*-cubebene (19.3 %), palmitic acid (12.2 %) and caryophyllene oxide (11.7 %) were identified as major compounds, respectively. Sesquiterpene hydrocarbons (34.8 %) were the most abundant compounds in the essential oil.

There is only one study about the essential oil composition of *E. helioscopia*. In that report, the chemical composition of the essential oil of *E. helioscopia* collected from Greece, was studied by Fokialakis *et al.* [19] and phytol (21.2 %), *β*-caryophyllene (10.0 %) and docosanoic acid methyl ester (8.1 %) were found as major compounds. Season, the geographical location and date of collection cause these differences in the chemical composition of the essential oil [20].

Table 1. Chemical composition of the essential oil of *E. helioscopia*

No	RI[a]	Compounds	Percentage %[b]	Identification methods[c]
1	1144	*Cis-β*-terpineol	0.4	Co-GC, MS, RI
2	1159	Terpinene-4-ol	0.3	Co-GC, MS, RI
3	1178	*α*-Terpineol	0.6	Co-GC, MS, RI
4	1189	Borneol	0.9	Co-GC, MS, RI
5	1290	Thymol	0.2	Co-GC, MS, RI
6	1299	Carvacrol	0.2	Co-GC, MS, RI
7	1359	*β*-Damascenone	0.6	MS, RI
8	1381	*β*-Bourbonene	0.5	Co-GC, MS, RI
9	1394	*β*-Cubebene	19.3	MS, RI
10	1424	*β*-Caryophyllene	1.6	Co-GC, MS, RI
11	1442	*τ*-Elemene	9.3	MS, RI
12	1458	*α*-Farnesene	1.7	MS, RI
13	1478	*τ*-Muurolene	1.6	MS, RI
14	1512	*δ*-Cadinene	0.8	MS, RI

Table 1. Continues

No	RI[a]	Compounds	Percentage %[b]	Identification methods[c]
15	1538	α-Cadinol	2.5	MS, RI
16	1576	Spathulenol	9.3	Co-GC, MS, RI
17	1580	Caryophyllene oxide	11.7	Co-GC, MS, RI
18	1720	Myristic acid	1.9	Co-GC, MS, RI
19	1833	Hexahydrofarnesly acetone	5.3	MS, RI
20	1851	Pentadecanoic acid	0.4	Co-GC, MS, RI
21	1942	Phytol	6.9	MS, RI
22	2001	Palmitic acid	12.2	Co-GC, MS, RI
23	2100	Heneicosane	1.9	MS, RI
24	2108	Linolenic acid	3.6	MS, RI
25	2120	Linoleic acid	1.6	Co-GC, MS, RI
26	2140	Octadecane, 3-ethyl-5-(2-ethyl butyl)	0.3	MS, RI
27	2200	Docasane	0.2	MS, RI
28	2225	Phytol acetate	2.2	MS, RI
29	2300	Tricosane	0.7	MS, RI
30	2400	Tetracosane	1.2	MS, RI
		Oxygenated monoterpenes	2.6	
		Sesquiterpene hydrocarbons	34.8	
		Oxygenated sesquiterpenes	23.5	
		Oxygenated diterpenes	9.1	
		Others	29.9	
		Total identified (%)	99.9	
		Total number of compounds	30	

[a] Retention indices on DB–5 fused silica column. [b] Percentage concentration. [c] Identification methods: Co-I: Co-injection: based on comparison with authentic compounds; MS: based on comparison with WILEY, ADAMS and NIST 08 MS databases; RI: based on comparison of calculated with those reported in ADAMS and NIST 08.

3.2. Total Phenolic and Total Flavonoid Contents

Phenolic and flavonoid compounds are responsible for the bioactive properties of the natural compounds. Phenolic compounds are of great interest to researchers because of their beneficial effects on oxidative stress-related diseases in addition to their antioxidant. Flavonoids scavenge many oxidation molecules such as singlet oxygen and other free radicals. Also, flavonoids prevent the formation of reactive oxygen species [21,22].

The calibration curve of pyrocatechol (0.0078[pyrocatechol (µg)] + 0.0623; r^2, 0.9992) was used to determine the total phenolic content and quercetin (0.0412[quercetin (µg)] – 0.0662; r^2, 0.9998) for the total flavonoid content. Table 2 presents the total phenolic and flavonoid contents of the different extracts of *E. helioscopia*. Among the extracts, water extract has the highest phenolic (161.20±0.98 µg PEs/mg) and flavonoid (11.22±0.05 µg QEs/mg) contents, followed by the methanol extract and the acetone extract. It is well known that phenolic compounds are secondary metabolites responsible for antioxidant activity. The water extract containing the highest amount of total phenolic and flavonoid compounds showed the highest antioxidant activity in all studied tests.

Maoulainine *et al.* [23] studied the total phenolic and flavonoid contents of the methanol and ethanol extracts of *E. helioscopia* and the highest phenolic and flavonoid contents were found in the methanol extracts (51.49±0.012 mg GAE/g dry weight and 11.38±0.004 mg

QE/dry weight, respectively). Total flavonoid contents of *E. helioscopia* collected from four different regions of Chongqing were investigated by Dan *et al.* [24] and contents of total flavonoids were found in the range of 5.485-5.742 mg/g and the results obtained are consistent with the literature. Total phenolic and flavonoid contents of the hexane, acetone and water extracts of *E. helioscopia* were determined for the first time in our study.

Table 2. Total phenolic and total flavonoid contents of the extracts of *E. helioscopia*[a]

		Total phenolic contents µg PEs/mg extracts[b]	Total flavonoid contents µg QEs/mg extract[c]
E. helioscopia	**Hexane**	10.40±0.26	1.01±0.01
	Acetone	44.98±0.58	3.93±0.17
	Methanol	55.17±0.83	5.36±0.52
	Water	161.20±0.98	11.22±0.05

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

3.3. Antioxidant Activity

The essential oil and the hexane, acetone, methanol and water extracts of *E. helioscopia* were screened for their antioxidant activity by using five methods, namely β-carotene-linoleic acid, DPPH free radical scavenging, ABTS cation radical scavenging, cupric-reducing antioxidant capacity (CUPRAC) and metal chelating activity. All of the extracts and the essential oil showed antioxidant activities in a dose-dependent manner. Table 3 shows the IC_{50} values of the extracts and standard compounds (BHA, α-tocopherol, and EDTA).

Table 3. Antioxidant activity of the essential oil and extracts of *E. helioscopia* by β-Carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, CUPRAC and metal chelating assays[a]

		Antioxidant Activity				
		β-Carotene-linoleic acid assay	**$DPPH^{\bullet}$ assay**	**$ABTS^{\bullet+}$ assay**	**CUPRAC assay**	**Metal chelating assay**
		IC_{50} (µg /mL)	**IC_{50} (µg /mL)**	**IC_{50} (µg/mL)**	**$A_{0.50}$ (µg/mL)[b]**	**Inhibition (%)[c]**
E. helioscopia	**Essential oil**	113.79±0.55	2.81±0.75[c]	21.45±0.43[c]	512.11±0.72	-[e]
	Hexane extract	579.81±0.71	4.57±0.97[c]	17.45±0.68[c]	295.23±0.89	29.40±0.27
	Acetone extract	2.33±0.16	90.18±0.22	17.15±0.19	53.03±0.49	19.85±0.31
	Methanol extract	1.14±0.21	16.43±0.67	15.10±0.82	18.20±0.35	13.51±0.12
	Water extract	1.08±0.39	15.57±0.48	1.89±0.33	12.50±0.11	79.31±0.51
Standards	**α-Tocophero**	2.10±0.08	37.20±0.41	38.51±0.54	66.72±0.81	*NT*[d]
	BHA	1.34±0.04	19.80±0.36	11.82±0.09	24.40±0.69	*NT*[d]
	EDTA	*NT*[d]	*NT*[d]	*NT*[d]	*NT*[d]	94.7±0.6

[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 of 200 µg/mL concentration of the essential oil and extracts of *Euphorbia helioscopia*.
[d]: NT: not tested.
[e]:- Not active.

In all studied methods, the highest antioxidant activity was found in the water extract and followed by the methanol extract. The water extract was found to be more active than BHA and α-tocopherol which used as standards in β-carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and

CUPRAC assays with IC_{50}: 1.08±0.39, 15.57±0.48, 1.89±0.33 and 12.50±0.11 µg/mL values, respectively. The methanol extract exhibited higher antioxidant activity than BHA and α-tocopherol in β-carotene-linoleic acid, DPPH$^{\bullet}$ and CUPRAC assays with IC_{50}: 1.14±0.21, 16.43±0.67 and 18.20±0.35 µg/mL, respectively. Also, in ABTS$^{\bullet+}$ assay, the methanol extract (IC_{50}: 15.10±0.82 µg/mL) showed higher antioxidant activity than α-tocopherol (IC_{50}: 38.51±0.54 µg/mL).

In previous studies, antioxidant activity of the dichloromethane and methanol extracts of *E. helioscopia* were evaluated by thin layer chromatography (TLC) autographic assay method, using DPPH$^{\bullet}$ as spray reagent. At 100 µg concentration, when methanol extract appeared as a yellow spot against purple background, dichloromethane extract did not respond to DPPH$^{\bullet}$ [1]. In a report of Rauf *et al.* [25], DPPH$^{\bullet}$ scavenging effects of various extracts of *E. helioscopia* were determined and activity was decreased in order of methanol>ethyl acetate>ethanol>chloroform>hexane. In another study, antioxidant activity of aqueous, ethanol, petroleum ether, chloroform and methanol extracts of leaves, stem parts, and also latex of *E. helioscopia* were evaluated by using DPPH, TAC, FRAP, FTC and BCL assays. The highest antioxidant activity was found in latex and followed by the methanol extract of leaves in all the methods. The other extracts also showed significant antioxidant activity [26]. Antioxidant properties of the methanol and ethanol extracts of *E. helioscopia* leaves, flowers and stem were examined by using the DPPH$^{\bullet}$ scavenging assay by Maoulainine *et al.* [23]. The highest radical scavenging effect was observed in flowers methanol extract with IC_{50} value of 26.66±0.000 µg/mL. The results obtained in our study are similar to these findings.

When literature findings are examined, it is seen that DPPH$^{\bullet}$ scavenging assay was generally used for testing to antioxidant properties of *E. helioscopia*. In our study, antioxidant activities of the essential oil and the hexane, acetone, methanol and water extracts of *E. helioscopia* were evaluated by using β-carotene-linoleic acid, ABTS cation radical scavenging, cupric-reducing antioxidant capacity and metal chelating activity assays for the first time.

3.4. Anticholinesterase Activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the essential oil and the extracts of *E. helioscopia* compared with that of galantamine were given in Table 4. The inhibitory activity of the extracts was divided into three separate classes; potent (>50 %), moderate (30-50 %), inactive or low (<30 %) activity [27]. According to this classification, when the hexane extract (47.49±0.19 %) of *E. helioscopia* showed moderate inhibitory activity against AChE, all other extracts showed low inhibitory activity against AChE. All extracts except methanol and water extracts of *E. helioscopia* were found to be potent inhibitors against BChE. Moreover, the acetone extract (81.23±0.58 %) of *E. helioscopia* exhibited very close BChE inhibitory activity to galantamine (82.23±0.67 %).

In previous studies, anticholinesterase activities of different *Euphorbia* species were investigated. Pisano *et al*. [28] evaluated cholinesterase inhibitory activities of aqueous and alcoholic extracts from leaves, stems, and flowers of *E. characias* and ethanol leaves extract showed fifty times more activity than galantamine. Cholinesterase inhibitory activities of the methanolic extracts of *E. denticulata* (flowers, leaf, stem, and mix of aerial parts) were studied by Zengin *et al.* [29] and the extracts were reported as medium-low activator for AChE and BChE. In a different study of *Euphorbia* species, *E. hebecarpa* is tested for AChE inhibitory activity and was inactive [30]. Cholinesterase inhibitory activities of the essential oil and hexane, acetone, methanol and water extracts of *E. helioscopia* were studied for the first time in this report.

Table 4. Anticholinesterase and anti-urease inhibitory activities of the essential oil and the extracts of *E. helioscopia*[a]

		Anticholinesterase Activity		Anti-urease Activity
		AChE assay	BChE assay	
E. helioscopia	**Essential oil**	18.68±0.16	50.85±0.67	91.37±0.26
	Hexane extract	47.49±0.19	54.32±0.92	96.97±0.36
	Acetone extract	8.74±0.14	81.23±0.58	54.17±0.92
	Methanol extract	6.97±0.07	20.88±0.15	70.94±0.32
	Water extract	0.67±0.02	9.87±0.31	19.32±0.18
Standards	**Galantamine**	80.41±0.98	82.23±0.67	*NT*[b]
	Thiourea	*NT*[b]	*NT*[b]	96.93±0.17

[a] : Inhibition % of 200 µg/mL concentration of the essential oil and the extracts of *E. helioscopia*
[b] : NT: not tested.

3.5. Anti-urease Activity

Since urease inhibitors can be used as potential drugs in the treatment of ulcer diseases, the discovery of new inhibitors is gaining in importance. Table 4 shows anti-urease activity by inhibition (%) at 200 µg/mL concentration. The hexane extract (96.97±0.36 %) of *E. helioscopia* showed higher anti-urease activity than thiourea (96.93±0.17 %) used as a standard. In addition, the essential oil (91.37±0.26 %) exhibited very close anti-urease activity to the standard.

Natural compounds isolated from *Euphorbia* species exhibiting anti-urease activity are reported in the literature. Ahmad *et al.* [31,32] and Lodhi *et al.* [33] isolated five diterpenes esters from *E. decipiens* inhibited urease enzyme and the first natural urease inhibitor was discovered by Ahmad *et al.* [31]. In a different study, the methanolic bark fraction of *E. umbellata* was reported to exhibit 78.6 % inhibition at 1024 µg/mL concentration [34]. In our study, the essential oil and all the extracts except the water extract were highly active, and these results are similar to previous studies on other *Euphorbia* species.

4. CONCLUSION

In this report, antioxidant, anticholinesterase and anti-urease activities of the essential oil and various extracts of *E. helioscopia* were determined with the total phenolic and flavonoid contents. Also, chemical composition of the essential oil was analyzed by GC and GC/MS and totally 30 compounds were determined. The water extract with the highest amount of total phenolic and flavonoid contents exhibited the highest antioxidant activities in all assays. Furthermore, the acetone extract exhibited very close BChE inhibitory activity to galantamine. In anti-urease activity test, when the hexane extract showed higher activity than thiourea, the essential oil exhibited very close activity to the standard. Consequently, *E. helioscopia* could become a source of potential bioactive compounds in pharmaceutical and food industries with strong enzyme inhibitory and antioxidant activities. However, further studies are needed for discovering of new natural bioactive compounds from these species.

Conflict of interest

The authors declare that they have no conflict of interest.

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