Sigma Journal of Engineering and Natural Sciences
Web page info: https://sigma.yildiz.edu.tr
DOI: 10.14744/sigma.2024.00000

Research Article

Determination of radical scavenging activity, secondary metabolite amount, and toxicity of *Onopordum bracteatum* extracts

Aytaj GASİMOVA[1], Ramazan MAMMADOV[2,3], Birsen ATLI[1], Mehlika ALPER[1,*]

[1]*Department of Molecular Biology and Genetics, Muğla Sıtkı Koçman University, Muğla, 48000, Türkiye*
[2]*Department of Biology and Ecology, Faculty of Nature and Technology, University of Odlar Yurdu, Baku, AZ1110, Azerbaijan*
[3]*Institute of Dendrology Ministry of Science and Education of the Republic of Azerbaijan Mardakan, Baku, AZ1044, Azerbaijan*

ARTICLE INFO

Article history
Received: 20 March 2023
Revised: 04 May 2023
Accepted: 07 July 2023

Keywords:
Anthelmint; Antioxidant; Brine Shrimp; *Onopordum bracteatum*, Secondary Metabolite

ABSTRACT

In the present study, the antioxidant activities and total secondary metabolite amounts of different extracts (methanol, acetone, and water) obtained from the leaf and flower parts of *Onopordum bracteatum*, which was collected from Muğla (Türkiye) were determined for the first time. Antioxidant activity studies were performed with DPPH, ABTS, β-Carotene/linoleic acid, FRAP, and CUPRAC methods. Secondary metabolite assays were carried out in order to determine the total phenolic, total flavonoid, and total tannin contents of the extracts. In addition, the toxicities (Brine shrimp) of water extracts and the anthelmintic effects of methanol and water extracts were investigated. The leaf methanol extract of *O. bracteatum* with the highest antioxidant activity in all tests, except the FRAP test (DPPH: IC_{50}= 0.26 ± 0.01 mg/mL, ABTS: IC_{50}= 0.19 ± 0.003 mg/mL, β-Carotene/linoleic acid: 72.98 ± 1.31%, CUPRAC: 34.02 ± 1.87 mg TE/g extract, FRAP: 3.96 ± 0.65 mg TE/g extract) used to determine the antioxidant activity was observed to have the highest total phenolic content (5.78 ± 0.24 mg GAE/g extract). The highest total flavonoid (32.17 ± 0.82 mg QE/g extract) and total tannin contents (16.41 ± 1.30 mg CE/g extract) were determined in leaf acetone and flower acetone extracts of *O. bracteatum*, respectively. Concerning the cytotoxic properties, concentration-dependent activity was seen in *O. bracteatum* water extracts against brine shrimp. Methanol extracts of *O. bracteatum* showed better anthelmintic activity than water extracts. The results will provide basic data for studies on the pharmacological and medical use of *O. bracteatum*.

Cite this article as: Gasimova A, Mammadov R, Atlı B, Alper M. Determination of radical scavenging activity, secondary metabolite amount, and toxicity of *onopordum bracteatum* extracts. Sigma J Eng Nat Sci 2024;42(5):1612–1620.

INTRODUCTION

Secondary metabolites are chemical compounds that are not necessary for the plant's normal development but are synthesized as a by-product in metabolism [1]. Although these metabolites do not directly participate in vital activities, they are quite necessary for the adaptation of the plant to environmental conditions. These metabolites play a role in different processes in plants; they act as

***Corresponding author.**
*E-mail address: mehlikaalper@mu.edu.tr

This paper was recommended for publication in revised form by Editor-in-Chief Ahmet Selim Dalkilic

signaling compounds, attract pollinators, protect the plant against oxidative stress and ultraviolet radiation, play a role in defense against pathogens and predators, ensure healthy growth and reproduction of plants, and can also contribute to the characteristics of vegetables and fruits such as color, odor and taste and etc. Thanks to their bioactive properties, secondary metabolites can be used as nutritional supplements in addition to their frequent use in medicine and pharmacy. Secondary metabolites have been stated to have hypoglycemic, hypolipidemic, anti-inflammatory, and antioxidant effects and are protective against thrombosis and cancer [2-4].

Free radicals are short-lived, low molecular weight, highly active molecules containing one or more electrons and contain unshared electrons in their final orbitals. When they react easily with some other substances, they can form new compounds with highly toxic effects. There is a balance between antioxidants and free radicals. Oxidative stress, which occurs due to free radicals accumulating in the event of insufficient antioxidant systems, has played a role in the emergence of sicknesses such as cancer, aging, atherosclerosis, heart diseases, hypertension, joint diseases, Parkinson's disease, and Alzheimer's disease [4]. Antioxidants neutralize free radicals and prevent them from causing damage to cells [5], and serve to extend the shelf life of food. Antioxidants, which can be divided into two large groups as endogenous and exogenous antioxidants, can be obtained from synthetic and natural sources. Plants contain various substances with antioxidant activity [4,6].

Helminths are animals with bilateral symmetry, unlined muscles, and no joints. Anthelmintic drugs are generally used to control helminths that live as parasites in the body of humans and animals [7]. Although chemical warfare has an important place in practice, it has been determined that the synthetic drugs used have negative effects on the environment, natural life, and human health due to their high toxicity. For this reason, within the scope of entomological studies, the herbal-based treatment approach, which can have a highly lethal or nutritional inhibitory effect, has come to the fore as an alternative method. Many plants grown in nature are considered potential alternatives to synthetic nematicides due to the rich bioactive phytochemicals they contain [8].

Asteraceae is the second largest family of the flora of Türkiye, which has a rich biodiversity due to its location at the intersection of 3 phytogeographic regions (Europe-Siberia, Iran-Turanian, and Mediterranean region) [9-12]. *Onopordum* is a genus belonging to the Asteraceae family, and the species of this genus are mainly distributed in Western and Central Asia, and Europe, especially in countries with Mediterranean coasts, North Africa, and the Canary Islands [13,14]. *Onopordum* taxa in Türkiye are known by different names such as "at dikeni, boğa dikeni, deve dikeni, gengel" [15,16]. It is well known that members of the Asteraceae family have been used in diet and medicine for centuries. In spite of their wide variety, most family members have a similar chemical composition, for instance, all species are known to be well sources of inulin, which is a natural polysaccharide with powerful prebiotic features. They also show diuretic and wound-healing properties as well as strong antioxidant, anti-inflammatory, and antimicrobial activity [17]. Various biological features of different *Onopordum* species have been presented in the literature [18-20]. The present study aimed to determine for the first time the antioxidant activities and total secondary metabolite amounts of different extracts (methanol, acetone, and water) obtained from the leaf and flower parts of *O. bracteatum* Boiss. & Heldr., which was collected from Muğla (Türkiye). In addition, revealing the toxicities of water extracts and investigating the anthelmintic effects of methanol and water extracts are among the aims of the study.

MATERIAL AND METHODS

The experiment procedure is shown in Figure 1.

Chemicals and Reagents

The chemicals and reagents used were obtained from *Merck* /Sigma Aldrich (Germany).

Plant Materials and Plant Extraction

O. bracteatum was collected from Muğla province (37° 10' 36" N 28° 20' 58" E) on May 2021. The aerial parts of the plant, which were dried at room temperature for about a week, were separately shredded using a blender. Each 10 g dried sample was extracted twice with 100 mL solvent (methanol, acetone, or distilled water) in a water bath set at 55°C for 6 hours. The solutions from each extraction were collected and filtered, and the solvent was removed using a rotary evaporator. After the extracts were lyophilized, they were stored at -20°C for use in experiments [21].

Antioxidant Activity Assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and 2,2' azino-bis (3-ethylbenzothiazloine-6-sulfonic acid) (ABTS) scavenging cation activity assays were carried out by following the methods described by Brand-Williams et al. [22] and Re et al. [23], respectively. For DPPH assay, DPPH methanolic solution (0.004%, 4 mL) was combined with extract solutions at different concentrations (1 mL). After 30 min, the absorbance was recorded at 517 nm. For ABTS assay, 7 mM ABTS and 2.45 mm potassium persulfate solutions were mixed and stored for 12-16 h in the dark. This mixture was diluted with methanol to acquire an absorbance of 0.700 at 734 nm. Then, 0.5 mL of this mixture was mixed with 1 mL of different concentrations of extracts. After 15 min, the absorbance was recorded at 734 nm. Butylated hydroxyanisole (BHA) served as a positive control for these two assays and the results were expressed as IC_{50}

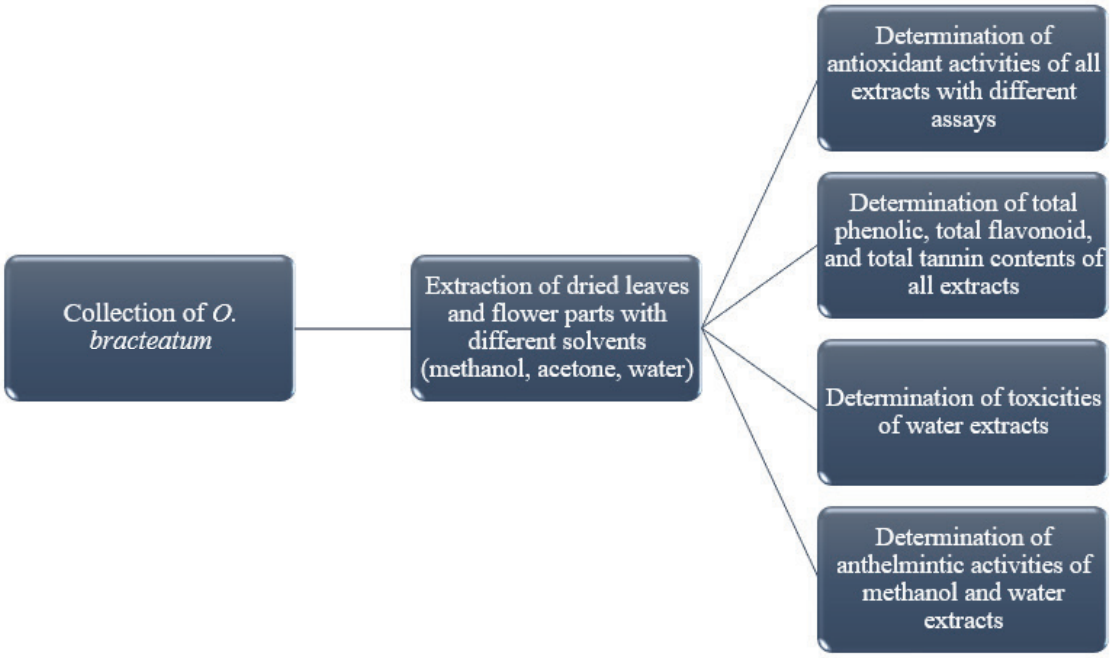

Figure 1. The experiment procedure.

(inhibitory concentration, 50%). β-Carotene/linoleic acid assay was performed according to the method expressed by Amin et al. [24]. In the β-Carotene/linoleic acid assay, BHA (1 mg/mL) was used as a positive control, and total antioxidant activity (AA) was calculated using the equation reported by Amarowicz et al. [25]. Briefly, 1 mL of extract solution was combined with 24 mL of β-carotene solution and initial absorbance at 470 nm was read. The mixture was placed at 50°C for 2 h and absorbance was measured every 30 min. The methods of Apak et al. [26] and Benzie and Strain [27] were followed to perform cupric ion-reducing antioxidant capacity (CUPRAC) and Ferric reducing antioxidant power (FRAP) assays, respectively, and the results were given as equivalents of trolox (mgTEs/g). For these assays, CUPRAC or FRAP solution was combined with the extract solutions and these mixtures were incubated 30 min. Absorbances were taken at 450 and 593 nm, respectively.

Determination of Total Secondary Metabolite Amounts

The total phenolic content of the extracts was detected as gallic acid equivalent (mg GAE/g) using Folin-Ciocalteu Reagent (FCR) [28]. Briefly, 1 mL of extract solution (1 mg/mL) was mixed with 1 mL of FCR and 46 mL of distilled water. After 3 min incubation, 3 mL of sodium carbonate solution (2%) was added to this mixture. After 2 h, the absorbance was taken at 760 nm. The total flavonoid content in the extracts was assigned as equivalent to quercetin (mg QE/g) using the aluminum colorimetric method [29]. 10 minutes after mixing 1 ml of aluminum chloride solution in methanol (2%) with the equivalent quantity of the extract solution, the measurement was made at 415 nm.The total tannin content of the extracts was given as catechin equivalent (mg CE/g) using the vanillin method [30]. In short, 0.5 mL of extract solution was combined with 1,5 mL of vanillin solution. After 15 min, the absorbance was measured at 500 nm.

Brine Shrimp Lethality Assay

Commercially purchased brine shrimp (*Artemia salina*) eggs (Sera Artemia Mix, 18g, USA) were added to 500 mL of artificial seawater at 28°C in an aquarium and they were left under the light. After 48 hours, the nauplii were collected with the help of a Pasteur pipette. Each test tube was including 10 nauplii, 4.5 mL of brine solution, and 0.5 mL of plant extracts. The tubes were maintained at 28°C for 24 h in a bright environment, and then the dead shrimps were counted. The experiment was performed for five different concentrations (0.01, 0.05, 0.1, 0.5, and 1.0 mg/mL) of water extracts of both the leaf and flower of *O. bracteatum*. There was no plant extract in the control group. % mortality was calculated for all applications [31].

Anthelmintic Activity Assay

The method of Dash et al. [32] was applied to evaluate the anthelmintic activity of the methanol and water extracts of both the leaf and flower of *O. bracteatum*. For this assay, 6 of *Tubifex tubifex* which were about 1-2 cm in size, were separately placed in a petri dish containing 20 mL solutions of each extract prepared at different concentrations (2.5, 5, 10, 20 mg/mL) by dissolving them in distilled water. A reference standard was Albendazole (20 mg/

mL). The negative control was distilled water. Petri dishes were followed and the time taken for paralysis and death of the worms was noted in minutes. The mean duration of paralysis at which movement was lost or no movement was sighted, except when the worms were vigorously swayed, was noted. The time of death of each worm was reported after it was defined that the worms did not move when swung or externally warn.

Statistical Analysis

All tests were performed in triplicate. The results are expressed as mean ± SE (Standard Error). Statistical analysis of data from the present study was carried out by using SPSS 22.0 by one-way ANOVA with posthoc Tukey's test ($P \leq 0.05$). SPSS was also used for calculating IC_{50} or LC_{50} values.

RESULTS AND DISCUSSION

Since the plants used in treating many diseases since ancient times are the source of many phytotherapeutic products, the search for new herbal medicines maintains its importance [33].

Antioxidant Activities

The radical scavenging activities of antioxidant compounds are very important in eliminating the harmful effects of free radicals on health in the medicine, food, and pharmaceutical industries [34]. To determine the antioxidant activities of methanol, acetone, and water extracts obtained from the flower and leaf parts of *O. bracteatum*, five diverse methods were used. The antioxidant activity of the extracts were shown in Table1.

According to the results of DPPH (IC_{50}= 0.26 ± 0.01 mg/mL) and ABTS (IC_{50}= 0.19 ± 0.003 mg/mL) radical scavenging assays as well as the β-Carotene/linoleic acid (72.98 ± 1.31%) and CUPRAC (34.02±1.87 mg TE/g) assays, the leaf methanol extract possesses the highest antioxidant activities among the extracts tested. However, the leaf acetone extract (4.17±0.03 mg TE/g) has high reducing power activity in FRAP assay. The extracts of *O. bracteatum* evaluated in the present study have lower DPPH radical scavenging activities than the butanol extract of *O. acanthium*, which was reported to have good antioxidant activity with an IC_{50} value of 134.4 µg/mL according to the DPPH method by Habibatni et al. [35]. In terms of the DPPH radical scavenging ability, the flower EO was found to exhibit the best antioxidant activities with an IC_{50} value of 2.66 mg/mL, among *O. arenarium* flower and stem essential oils, and its hexane extract [36].

When the antioxidant activities of ethyl acetate, methanol, and water extracts of the aerial parts of *O. caricum* were assessed, except for the ferrous ion chelating activity assay, methanol extract was reported to cause higher activity than others, in addition, the results for this extract in terms of EC_{50}/IC_{50} were reported as 1.46, 1.10, 1.30, 0.87, and 0.76 mg/mL for phosphomolybdenum, DPPH and ABTS radical scavenging, CUPRAC, and FRAP tests, respectively [37]. Differences in antioxidant activity among extracts can be due to differences in the polarity of the solvents used [38, 39]. To our knowledge, the antioxidant activity of *O. bracteatum* has not been previously reported. In the literature, there are several results about the antioxidant activities of some other species belonging to the genus *Onopordum*, for example, *O. alexandrinum* [40,41], *O. acanthium* [42-44], *O. leptolepis* [45], *O. anatolicum* and *O. heteracanthum* [46], *O. tauricum* [47].

Total Secondary Metabolite Amounts

Among the compounds that can be obtained from plants, phenolic compounds are interesting because they are natural and possess the same or even better antioxidant activities than synthetic antioxidants [48]. In the present study, the highest total phenolic, total flavonoid, and total tannin contents were determined in leaf methanol (5.78±0.24 mg GAE/g), leaf acetone (32.17±0.82 mg QE/g)

Table 1. Antioxidant activities of different extracts of *O. bracteatum*

Samples	DPPH (IC_{50}, mg/mL)	ABTS (IC_{50}, mg/mL)	β-Carotene linoleic acid (%)	CUPRAC (mgTE/g extract)*	FRAP (mgTE/g extract)*
LOM	0.26±0.01^{b}	0.19±0.003^{b}	72.98±1.31^{b}	34.02±1.87^{a}	3.96±0.65^{a,b}
LOA	0.90±0.01^{d}	0.25±0.002^{d}	61.79±6.06^{b,c}	13.18±0.37^{c}	4.17±0.03^{a}
LOW	0.96±0.01^{d}	0.37±0.01^{e}	21.59±6.04^{d}	4.74±0.01^{d}	1.18±0.07^{c}
FOM	0.66±0.01^{c}	0.22±0.004^{c}	59.18±4.88^{b,c}	17.99±0.29^{b}	1.91±0.06^{c}
FOA	1.27±0.03^{e}	0.39±0.01^{e}	57.67±8.40^{c}	14.43±0.32^{c}	1.43±0.11^{c}
FOW	0.90±0.01^{d}	0.54±0.02^{f}	21.27±0.37^{d}	6.43±0.08^{d}	3.39±0.19^{b}
BHA	0.01±0.002^{a}	0.04±0.02^{a}	90.33±0.63^{a}	nt	nt

LOM: Leaf methanol extract; LOA: Leaf acetone extract; LOW: Leaf water extract; FOM: Flower methanol extract; FOA: Flower acetone extract; FOW: Flower water extract; BHA: Butylated hydroxyanisole, nt: not tested If the lower cases in the column are the same, there is no statistical difference in Tukey's multiple range test ($P \leq 0.05$). * It was made according to the equivalence of Trolox.

Table 2. Total phenolic, total flavonoid, and total tannin amount of different extracts of *O. bracteatum*

Sample	Total Phenolic (mg GAE/g extract)*	Total Flavonoid (mg QE/g extract)**	Total Tannin (mg CE/g extract)***
LOM	5.78±0.24[a]	7.43±0.12[d]	2.96±0.32[c]
LOA	2.76±0.12[c]	32.17±0.82[a]	11.61±0.12[b]
LOW	1.81±0.07[d]	0.83±0.02[e]	0.55±0.03[d]
FOM	3.53±0.12[b]	9.62±0.10[c]	4.40±0.27[c]
FOA	3.49±0.14[b]	30.46±0.25[b]	16.41±1.30[a]
FOW	2.62±0.14[c]	1.09±0.01[e]	0.69±0.03[d]

LOM: Leaf methanol extract; LOA: Leaf acetone extract; LOW: Leaf water extract; FOM: Flower methanol extract; FOA: Flower acetone extract; FOW: Flower water extract. If the lower cases in the column are the same, there is no statistical difference in Tukey's multiple range test ($P \leq 0.05$). * It was made according to the equivalence of Gallic acid. ** It was made according to the equivalence of Quercetin.*** It was made according to the equivalence of Catechin.

and flower acetone (16.41±1.30 mg CE/g) extracts, respectively (Table 2).

In the current study, it was observed that the leaf methanol extract of *O. bracteatum* with the highest antioxidant activity in all tests used to determine the antioxidant activity, except the FRAP test had the highest total phenolic content. Accordingly, the phenolic compounds existing in the extracts may be responsible for their antioxidant properties. From this point of view, these data are com patible with the data of other studies revealing the potent relationship between total phenolic content and antioxidant activity [49-51]. To our knowledge, in the current study, the amounts of total secondary metabolites considered in *O. bracteatum* extracts tested were revealed for the first time. The butanol extract of *O. acanthium* was reported to have a significant amount of total phenolic (8.93 ± 0.133 mg GAE/100 mg dried extract) and total flavonoid contents (3.93±0.037 mg catechin/100 mg dried extract) [35]. Baştürk and Peker [46] reported that *O. anatolicum* (18.554 ±2.00 mg GAE/ kg^{-1} extract) had higher phenolic content than *O. heteracanthum* (13.015 ±5.16 mgGAE/ kg^{-1} extract). In another study, the total phenolic content of the ethanol and water extracts of *O. tauricum* flower heads were reported as 7.64±0.01 and 4.64±0.04 mg GAE/g dw, respectively, and the ethanol extract in question was found to show the highest antioxidant activity, indicating correlations between phenolic compounds and antioxidant capacity [47]. Also, there are studies in the literature for the total secondary metabolite amounts of different *Onopordum* species, for example, *O. caricum* [37], *O. acanthium* [42,52,44].

Toxic Activities

The purpose of the experiments used in the determination of acute toxicity is to determine the toxic effects of chemical substances in biological systems and to obtain data specific to the dose-response relationship. An acute toxicity test is done to determine the toxicity of drugs, cosmetics, plant extracts, cleaning agents, and different chemicals used for different purposes. To express the toxicity of a substance, that is, how toxic it is, the expression LC_{50} (lethal concentration, 50%), which is the unit of lethality according to acute toxicity, is used [53]. The brine shrimp (*Artemia salina*) lethality test is widely used in the toxicity determination of biologically active samples simply and rapidly [54]. The potential cytotoxic activities of water extracts from *O. bracteatum* on brine shrimp (*A. salina*) were shown in Table 3.

It was determined that the % mortality increased depending on the increasing concentration of the extracts. LC_{50} values of leaf water extract of *O. bracteatum* were found as 0.16±0.0009 mg/mL. The LC_{50} value was not calculated for the flower water extract due to more than 50% mortality was detected at all concentrations tested. According to Meyer et al. [55], the water extracts of both leaf and flower from *O. bracteatum* were considered toxic, due to their LC_{50} <1000 µg/mL. Habibatni et al. [35] revealed that unlike the butanol extract of *O. acanthium* with an LC_{50} value of fewer than 500 µg/mL, LC_{50} values of methanolic, chloroform, petroleum ether extract its was reported to be more than 500 µg/mL. Different biochemical contents of the plant extracts may be responsible for the differences in the results observed.

Table 3. Mean mortality rates (%) and statistical values of *O. bracteatum* extracts against *A. Salina*

Concentrations, LC_{50}, values	LOW	FOW
0.01 mg/mL	44.4±2.29[d]	63.83±2.26[d]
0.05 mg/mL	63.83±2.26[c]	72.2±2.29[c]
0.1 mg/mL	77.77±2.26[b]	80.53±2.26[b]
0.5 mg/mL	88.84±2.26[a]	94.4±2.27[a]
1.0 mg/mL	94.4± 2.27[a]	97.2±2.29[a]
LC_{50} (mg/mL)	0.16±0.0009	nd

LOW: Leaf water extract; FOW: Flower water extract; nd: not detected. If the lower cases in the column are the same, there is no statistical difference in Tukey's multiple range test ($P \leq 0.05$).

Table 4. Anthelmintic activities of *O. bracteatum* methanol and water extracts

Extracts	Concentration (mg/mL)	P (min)*	D (min)**
LOM	2,5	35	54
	5	8	11
	10	3	5
	20	2	3
FOM	2.5	27	55
	5	6	8
	10	3	5
	20	2	4
LOW	2.5	>120	>120
	5	>120	>120
	10	>120	>120
	20	36	63
FOW	2.5	>120	>120
	5	>120	>120
	10	>120	>120
	20	36	63
PC***	20	3	7
NC****	-	-	-

LOM: Leaf methanol extract; LOW: Leaf water extract; FOM: Flower methanol extract; FOW: Flower water extract.

*P: Time taken for paralysis (min). **D: Time is taken for death (min), ***PC: Positive control (Albendazole), **** Negative control (distilled water)

Anthelmintic Activities

The anthelmintic activities of methanol and water extracts of *O. bracteatum* on *T. tubifex* were presented in Table 4. According to the results, the extract causing the shortest paralysis (2 min) and death (3 min) time was found to be the leaf methanol extract at 20 mg/mL. Osukoya et al. [56] also reported that aqueous extracts of leaves of *Theobroma cacao* showed significantly lower anthelmintic activity compared to its ethanolic extracts and reference standard (albendazole). Studies on screening plants for their anthelmintic effects are ongoing [57-59].

CONCLUSION

In the present study, many different biological properties such as secondary metabolite contents, and antioxidant, cytotoxic, and anthelmintic activities of different extracts of *O. bracteatum* from Muğla were determined. Leaf methanol extract of *O. bracteatum*, which was determined to have the highest phenolic content among the extracts, was also found to be the extract with the highest antioxidant activity. Leaf acetone and flower acetone extracts of *O. bracteatum* were the extracts with the highest total flavonoid and total tannin contents, respectively. Methanol extracts of *O. bracteatum* exhibited good anthelmintic activity than its water extracts. The different activities of the tested extracts can be explained by the different polarities of the solvents used. Based on the study and literature, polar solvents can be said to have more effect than nonpolar solvents. The different biological activities of *O. bracteatum* extracts, which have been clarified, support that the species can be used as a drug source in pharmacological and medical fields. At the same time, there is no study on the *O. bracteatum* species in the literature with the methods mentioned and the data obtained from this study have the potential to guide new studies on this species, increasing the value of the study.

ACKNOWLEDGMENTS

We would like to thank Dr. Olcay CEYLAN for helping us with the collection and identification of plants. This study was produced from the master's thesis of the first author, accepted at Muğla Sıtkı Koçman University, Department of Molecular Biology and Genetics in 2022. The antioxidant potential determined via DPPH and ABTS assay and phenolic contents of methanol and acetone extracts of the leaf of *O. bracteatum* was presented as a summary at the symposium "XI МЕЖДУНАРОДНОГО СИМПОЗИУМА ФЕНОЛЬНЫЕ СОЕДИНЕНИЯ: ФУНДАМЕНТАЛЬНЫЕ И ПРИКЛАДНЫЕ АСПЕКТЫ", Moscova, 11-15 April 2022, as a preliminary study.

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

REFERENCES

[1] Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK. Secondary Metabolites of Plants and their Role: Overview. Curr Trends Biotechnol Pharm 2015;9:293–304.

[2] Chikezie PC, Ibegbulem CO, Mbagwu FN. Medicinal potentials and toxicity concerns of bioactive principles. Med Aromat Plants 2015;4:1–15. [CrossRef]
[3] Ülger TG, Ayhan NY. Functionel Effects of Plant Secondary Metabolites on Health. Acıbadem Üniv Sağlık Bilim Derg 2020;3:384–390.
[4] Mammadov R. Serbest Radikaller ve Antioksidanlar. Ankara, TR: Akademisyen Kitabevi: 2020. [CrossRef]
[5] Alper M, Özay C, Güneş H, Mammadov R. Assessment of Antioxidant and Cytotoxic Activities and Identification of Phenolic Compounds of Centaurea solstitialis and Urospermum picroides from Türkiye. Braz Arch Biol Technol 2021;64. [CrossRef]
[6] Sen S, Chakraborty R. The role of antioxidants in human health. In Oxidative stress: diagnostics, prevention, and therapy. Am Chem Soc 2011;1:1–37. [CrossRef]
[7] Jackson F. Anthelmintic resistance the state of play. Br Vet J 1993;149:123–138. [CrossRef]
[8] Tan AN. Nematisit etkili bitkiler ve bitki ekstraktları. Ege Üniv Ziraat Fak Derg 2011;48;165–173.
[9] Doğan C, Sorkun K. Pollenanalysis of honeys-from Central, Easternand Southeastern Anatolia in Türkiye. Hacettepe Bull Nat Sci Eng 1999;28:35–50.
[10] Davis PH. Flora of Türkiye and the East Aegean Islands. Edinburgh: Edinburgh Uni. Press; 1965-1985.
[11] Davis PH, Mill RR, Tan K. Flora of Türkiye and The East Aegean Islands. Edinburgh, UK: Edinburgh University Press; 1988.
[12] Güner A, Özhatay N, Ekim T, Başer KHC. Flora of Türkiye. Edinburgh, UK: Edinburgh University Press; 2000.
[13] Jeffrey C. Compositae: Introduction with key to tribes. Families and Genera of Vascular Plants, Flowering Plants, Eudicots, Asterales. Berlin, D: Springer-Verlag; 2007.
[14] Susanna A, Garcia-Jacas N. Tribe Cardueae. in: The Families and Genera of Vascular Plants, Flowering Plants. Eudicots. Asterales. Berlin and Heidelberg, D: Springer; 2007.
[15] Baytop T. Türkçe Bitki Adları Sözlüğü. Ankara, TR: Türk Dil Kurumu Yayınları; 2015.
[16] Özbek U. Onopordum L. In: Türkiye Bitkileri Listesi (Damarlı Bitkiler). İstanbul, TR: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını; 2012.
[17] Rolnik A, Olas B. The plants of the Asteraceae family as agents in the protection of human health. Int J Mol Sci 2021;22:3009. [CrossRef]
[18] El-Najjar N, Saliba N, Talhouk S, Gali-Muhtasib H. Onopordum cynarocephalum induces apoptosis and protects against 1, 2 dimethylhydrazine-induced colon cancer. Oncol Rep 2007;17:1517–1523. [CrossRef]
[19] Roselli M, Fernando RI, Guadagni F, Spila A, Alessandroni J, Palmirotta R, et al. Brachyury, a driver of the epithelial-mesenchymal transition, is overexpressed in human lung tumors: an opportunity for novel interventions against lung cancer. Clin Cancer Res 2012;18:3868–3879. [CrossRef]
[20] Abd El-Moaty HI, Wanas AS, Radwan MM, Desoukey SY. Glycosides of Onopordum alexandrinum Boiss. and its Central Nervous System (CNS) and Some Biological Activities. Int J Pharmacogn Phytochem Res 2016;8:1088–1098.
[21] Mammadov R, Ili P, Ertem Vaizogulları H, Afacan Makascı A. Antioxidant Activity and Total Phenolic Content of *Gagea fibrosa* and *Romulea ramiflora*. Iran J Chem and Chem Eng 2011;30:57–62.
[22] Brand-Williams W, Cuvelier ME, Berset C. Use of a Free Radical Method to Evaluate Antioxidant Activity. Lebensm Wiss u-Technol 1995;28:25–30. [CrossRef]
[23] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice EC. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231–1237. [CrossRef]
[24] Amin İ, Zamaliah MM, Chin WF. Total antioxidant activity and phenolic content in selected vegetables. Food Chem 2004;87:58. [CrossRef]
[25] Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem 2004;84:551–562. [CrossRef]
[26] Apak R, Guclu K, Ozyurek M, Karademir SE. Novel total antioxidant capacity ındex for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agr Food Chem 2004;52:7970–7981. [CrossRef]
[27] Benzie I, Strain J. The ferric reducing ability of plasma (FRAP) as a Measure of antioxidant power: The FRAP assay. Anal Biochem 1996;239:70–76. [CrossRef]
[28] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Meth Enzymol 1999;299:152–178. [CrossRef]
[29] Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardization of propolis extract and identification of principal constituents. J Pharm Belg 1994;49:462–468.
[30] Bekir J, Mars M, Souchard JP, & Bouajila J. Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (Punica granatum) leaves. Food Chem Toxicol 2013;55:470–475. [CrossRef]
[31] Cigdem, A., Ramazan, M., & Mahmudcon, D. Biological activities, phenolic constituents and of various extracts of cyclamen coum tubers and leaves from Türkiye. Chemistry of Plant Raw Materials 2023:255–263. [CrossRef]
[32] Dash GK, Suresh P, Sahu SK, Kar DM, Ganapaty S, Panda SB. Evaluation of Evolvulus alsinoides Linn.

for anthelmintic and antimicrobial activities. J Nat Remedies 2022;2:182–185.
[33] Singh DB, Pathak RK, Rai D. From traditional herbal medicine to rational drug discovery: strategies, challenges, and future perspectives. Rev Bras Farmacogn 2022;32:147–159. [CrossRef]
[34] Bursal E, Köksal E, Gülçin İ, Bilsel G, Gören AC. Antioxidant activity and polyphenol content of cherry stem (Cerasus avium L.) determined by LC-MS/MS. Food Res Int 2013;51:66–74. [CrossRef]
[35] Habibatni S, Zohra AF, Khalida H, Anwar S, Mansi I, Awadh Ali NA. In vitro antioxidant, Xanthine oxidase-inhibitory and in vivo Anti-inflammatory, analgesic, antipyretic activity of Onopordum acanthium. Int J Phytomed 2017;9:92–100. [CrossRef]
[36] Bouazzi S, El Mokni R, Nakbi H, Dhaouadi H, Joshi RK, Hammami S. Chemical composition and antioxidant activity of essential oils and hexane extract of *Onopordum arenarium* from Tunisia. J Chromatogr Sci 2020;58:287–293. [CrossRef]
[37] Tepe AS. Chemical composition, antioxidant and enzyme inhibitory activity of Onopordum caricum. Rec Nat Prod 2022;16:172–181. [CrossRef]
[38] Mammadov R, Düşen O, Uysal (Demir) D, Köse, E. Antioxidant and antimicrobial activities of extracts from tubers and leaves of *Colchicum balansae* Planchon. J Med Plant Res 2009;3:767–770.
[39] Iloki-Assanga SB, Lewis-Luján LM, Lara-Espinoza CL, Gil-Salido AA, Fernandez-Angulo D, Rubio-Pino JL, Haines DD. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of Bucida buceras L. and Phoradendron californicum. BMC Res Notes 2015;8:1–14. [CrossRef]
[40] Salama MM, Ezzat SM, Sleem AA. A new hepatoprotective flavone glycoside from the flowers of Onopordum alexandrinum growing in Egypt. Z. Naturforsch C 2011;66:251–259. [CrossRef]
[41] Abd-Allah WES, Radwan HM, Shams K A, Ismail SI, Ali SM. The lipid and volatile oil of the seed and aerial parts of Onopordum alexandrinum Boiss. growing in Egypt and their antioxidant activity. Egyp Pharm J 2012;11:49.
[42] Angelov G, Georgieva S, Petkova-Parlapanska K. Antioxidant activity of extracts from cotton thistle (Onopordum acanthium L.). Sci Technol 2012;2:19–23.
[43] Parzhanova AB, Petkova NT, Ivanov IG, Ivanova SD. Evaluation of biologically active substance and antioxidant potential of medicinal plants extracts for food and cosmetic purposes. J Pharm Sci Res 2018;10:1804–1809.
[44] Oueslati MA, Gunenc A, Rigane G, Ghazghazi H, Valencia C, Salem RB, et al. Chemical composition, antioxidant and cytotoxic activities of Onopordum acanthium L. Crude oil and defatted meal. Rev Roum Chim 2019;64:503–510. [CrossRef]
[45] Valizadeh E, Zonouz NF, Zand A, Shahbazi S, Malekian A. Evaluation of antioxidant potentials of extracts of cotton thistle ('Onopordum leptolepis' DC.) obtained by various solvents. Aust J Crop Sci 2011;5:1163–1166.
[46] Baştürk A, Peker S. Antioxidant Capacity, Fatty Acid Profile and Volatile Components of the Onopordum Anatolicum and Onopordum Heteracanthum Species Seeds Grown in Van, Türkiye. J Inst Sci Technol 2021;11:2810–2822. [CrossRef]
[47] Petkova N, Mihaylova D. Flower heads of Onopordum tauricum Willd. and Carduus acanthoides L-source of prebiotics and antioxidants. Emir J Food Agric 2016;28:732–736. [CrossRef]
[48] Olszowy M. What is responsible for antioxidant properties of polyphenolic compounds from plants?. Plant Physiol Biochem 2019;144:135–143. [CrossRef]
[49] Mihaylova D, Georgieva L, Pavlov A. In vitro antioxidant activity and phenolic composition of Nepeta cataria L. extracts. Int J Agric Technol 2013;1:74–79.
[50] Ozay C, Mammadov R. Screening of some biological activities of Alyssum fulvescens var. fulvescens known as Ege madwort. Acta Biol Hung 2017;68:310–320. [CrossRef]
[51] Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants 2019;8:96. [CrossRef]
[52] Koc S, Isgor BS, Isgor YG, Shomali Moghaddam N, Yildirim O. The potential medicinal value of plants from Asteraceae family with antioxidant defense enzymes as biological targets. Pharm Biol 2015;53:746–751. [CrossRef]
[53] Sharififar F, Moshafi MH, Dehghan-Nudehe G, Ameri A, Alishahi F, Pourhemati A. Bioassay screening of the essential oil and various extracts from 4 spices medicinal plants. Pak J Pharm Sci 2009;22:317–322.
[54] Choudhary MI, Thomsen WJ. Bioassay techniques for drug development. Florida, USA: CRC Press; 2001.
[55] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine Shrimp: A convenient general bioassay for active plant constituents. Plant Med 1982;45:31–34. [CrossRef]
[56] Osukoya O, Fadaka A, Adewale O, Oluloye O, Ojo O, Ajiboye B, ... Kuku A. In vitro anthelmintic and antioxidant activities of the leaf extracts of Theobroma cacao L. AIMS Agric Food 2019;4:568–577. [CrossRef]
[57] Kaska A, Deniz N, Çiçek M, Mammadov R. Evaluation of antioxidant properties, phenolic compounds, anthelmintic, and cytotoxic activities of various extracts isolated from Nepeta cadmea: an endemic plant for Türkiye. J Food Sci 2018; 201883:1552–1559. [CrossRef]

[58] Olivaro C, Escobal M, de Souza G, Mederos A. Chemical characterisation and in vitr o anthelmintic activity of phenolic-rich extracts from the leaves and branches of Maytenus ilicifolia, a native plant from South America. Nat Pro Res 2022;36:3168–3172. [CrossRef]

[59] Tchetan E, Olounladé PA, Azando EVB, Khaliq HA, Ortiz S, Houngbeme A, et al. Anthelmintic activity, cytotoxicity, and phytochemical screening of plants used to treat digestive parasitosis of small ruminants in Benin (West Africa). Animals 2022;12:2718. [CrossRef]