



Research Article

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

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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: $IC_{50} = 0.26 \pm 0.01$ mg/mL, ABTS: $IC_{50} = 0.19 \pm 0.003$ mg/mL, β -Carotene/linoleic acid: $72.98 \pm 1.31\%$, 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*.

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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

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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 diken, boğa diken, deve diken, 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-ethylbenzothiazoline-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 IC₅₀

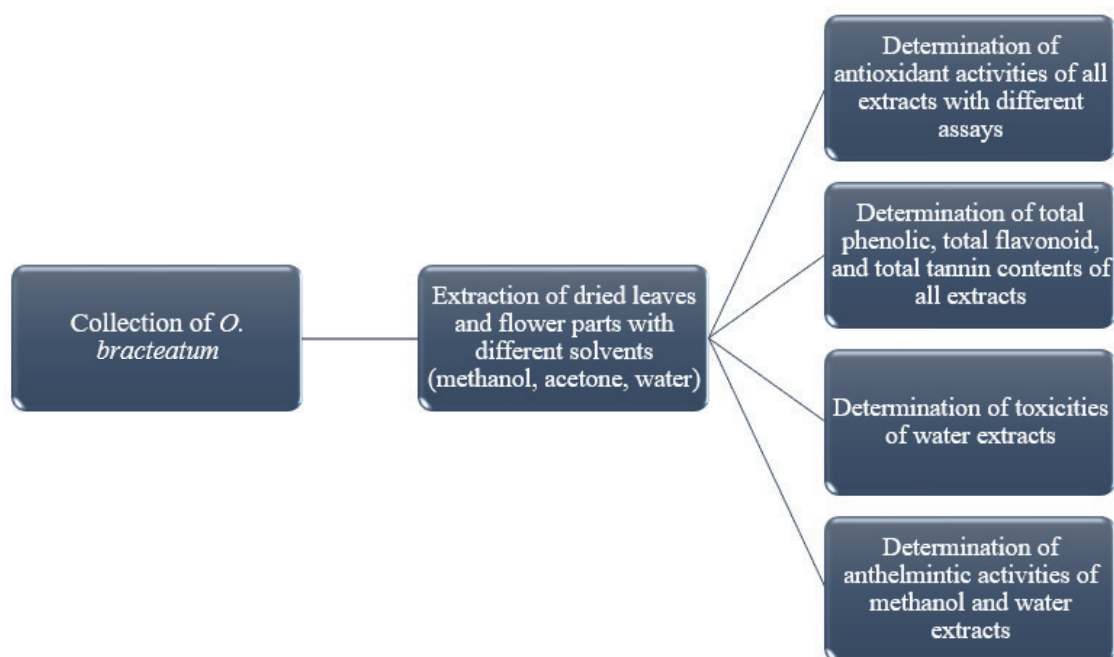


Figure 1. The experiment procedure.

(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 \pm 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 Table 1.

According to the results of DPPH ($IC_{50} = 0.26 \pm 0.01$ mg/mL) and ABTS ($IC_{50} = 0.19 \pm 0.003$ mg/mL) radical scavenging assays as well as the β -Carotene/linoleic acid (72.98 \pm 1.31%) and CUPRAC (34.02 \pm 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 \pm 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 μ g/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 \pm 0.24 mg GAE/g), leaf acetone (32.17 \pm 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 \pm 0.01 ^b	0.19 \pm 0.003 ^b	72.98 \pm 1.31 ^b	34.02 \pm 1.87 ^a	3.96 \pm 0.65 ^{a,b}
LOA	0.90 \pm 0.01 ^d	0.25 \pm 0.002 ^d	61.79 \pm 6.06 ^{b,c}	13.18 \pm 0.37 ^c	4.17 \pm 0.03 ^a
LOW	0.96 \pm 0.01 ^d	0.37 \pm 0.01 ^c	21.59 \pm 6.04 ^d	4.74 \pm 0.01 ^d	1.18 \pm 0.07 ^c
FOM	0.66 \pm 0.01 ^c	0.22 \pm 0.004 ^c	59.18 \pm 4.88 ^{b,c}	17.99 \pm 0.29 ^b	1.91 \pm 0.06 ^c
FOA	1.27 \pm 0.03 ^e	0.39 \pm 0.01 ^e	57.67 \pm 8.40 ^c	14.43 \pm 0.32 ^c	1.43 \pm 0.11 ^c
FOW	0.90 \pm 0.01 ^d	0.54 \pm 0.02 ^f	21.27 \pm 0.37 ^d	6.43 \pm 0.08 ^d	3.39 \pm 0.19 ^b
BHA	0.01 \pm 0.002 ^a	0.04 \pm 0.02 ^a	90.33 \pm 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 compatible 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 LC₅₀ (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. LC₅₀ values of leaf water extract of *O. bracteatum* were found as 0.16±0.0009 mg/mL. The LC₅₀ 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 LC₅₀ < 1000 µg/mL. Habibatni et al. [35] revealed that unlike the butanol extract of *O. acanthium* with an LC₅₀ value of fewer than 500 µg/mL, LC₅₀ 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.

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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.

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