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Original Article

AN EXAMINATION OF THE ANTIOXIDANT AND ANTIBACTERIAL CAPABILITIES, AS WELL AS THE PHENOLIC COMPOUNDS, OF *SILENE MACRODONTA* AND *SILENE CHAETODONTA*

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ABSTRACT

In this study, *Silene macrodonta* Boiss. and *S. chaetodonta* Boiss. plant species belonging to the *Caryophyllaceae* family growing in the Gaziantep region were examined in terms of antioxidant, antimicrobial and phenolic compounds. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) values of *S. macrodonta* and *S. chaetodonta* Boiss. plant species were determined using Rel Assay kits. It was determined that the TAS value of *S. macrodonta* was higher with 5.983 ± 0.156 , the TOS value of *S. chaetodonta* was higher with 15.686 ± 0.188 , and the OSI value of *S. chaetodonta* was higher with 0.341 ± 0.010 . Within the scope of this study, the antimicrobial activity of plant species was examined by the Agar dilution method, and it was determined that *S. chaetodonta* showed higher antimicrobial activity than *S. macrodonta*. Phenolic contents of plants were examined using LC-MS/MS device. For some of the standards used, the presence of phenolic compounds in the plant was detected. As a result of the studies, it was determined that *S. macrodonta* and *S. chaetodonta* plants have high biological activity.

KEYWORDS: *Silene macrodonta*, *Silene chaetodonta*, antioxidant, antimicrobial, phenolic compounds

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1. Introduction

It is known that throughout the ages, various plants have been used by humans as a source of food and also as medicine, and the benefits obtained from these applications are supported by many scientific studies. Primary and secondary metabolites, antioxidants and phenolic compounds of plants have been proven to have a beneficial role in increasing body resistance [1,2]. It is also known that the majority of active ingredients used in medicines are obtained from plants. Therefore, plants are natural resources used in the treatment of many diseases [3]. The term medicinal plants include a variety of plant species, many of which have medicinal activity. Plants have many uses, including as food and medicine, as well as sweeteners, beverages, dyes, fragrances, and cosmetics [4,5]. Considering today's health problems and the use of extracts obtained from various parts of plants in the treatment of

diseases, our biological wealth is important [6,7]. In addition to the contribution to the literature, this study will also contribute to the natural methods used in disease treatment in recent years.

Plants belonging to the *Silene* genus are given different names locally in Anatolia. The *Silene* genus, one of the largest members of the *Caryophyllaceae* family, is an annual, biennial, herbaceous or evergreen perennial plant [8]. Their seeds are generally kidney-shaped and can be of different shapes. *Silene* plants prefer sandy, humus, clayey and moist soils. They grow easily in sunny areas and fertile soils [9].

For this purpose, in this study, *S. macrodonta* and *S. chaetodonta* species growing naturally in Turkey were examined in terms of antioxidant, antimicrobial and phenolic compounds. In this context, the antioxidant potentials of methanol and dichloromethane extracts of

plant samples were determined and their status as natural sources was determined.

2. Materials and Methods

2.1. Collecting plants and taking their extracts

The plant samples used in the study were collected from the province of Gaziantep/Türkiye. The soil and muddy dirty parts of the plants obtained as a result of field studies were cleaned with distilled water and dried in suitable conditions and in the open air. The plant samples dried in the open air were crushed with a grinding machine and turned into powder. The powdered plant samples were subjected to extraction with methanol (MeOH) and dichloromethane (DCM) in a Soxhlet apparatus. The samples were cartridged as 30 g. Then, the extracts were concentrated under pressure with a rotary evaporator and stored at +4 °C until the experiments were carried out.

2.2. Determination of antimicrobial activities of plants

Antimicrobial activity determinations of methanol and dichloromethane extracts of plants were tested using the agar dilution method according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The minimum inhibitory concentration (MIC) of each extract was determined by testing a series of dilutions against standard bacterial and fungal strains.

Bacteria obtained from the American culture collection, for Gram positives, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, for Gram negatives, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606 were used. For fungi; *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135, *Candida glabrata* ATCC 90030, were used. Bacterial strains were pre-cultured in Muller Hinton Broth medium, and fungal strains were pre-cultured in RPMI 1640 Broth medium. The turbidity of bacteria and fungi was prepared according to the McFarland 0.5 scale and a standard inoculum was obtained. All dilutions were done with distilled water and all extracts were tested at concentrations of 800-12.5 µg/mL. The solvents used for the extracts were tested only for antimicrobial activity [10-13].

2.3. Determination of total antioxidant levels (TAS)

Total antioxidant levels of the samples were determined using the Rel Assay Diagnostics-TAS Assay Kit. The kit contains Reagent 1 (Buffer), Reagent 2 (Colored ABTS Radical Solution), Standard 1 (1.00 mmol Trolox Equiv./L) and Standard 2 (1.00 mmol Trolox Equiv./L). In this context, 200 µl of Reagent 1 was added to the wells on the ELISA plate and 12 µl of plant extract was added on it. The first absorbance measurement was made at 660 nm (the first absorbance of the sample) and 30 µl of Reagent 2 was added and incubated for 5 min at 37 °C. After the incubation process, the second absorbance was read at 660 nm. The same measurement procedures were carried out for standard 1 and standard 2 in the kit. The procedures were repeated separately for all plant extracts [14].

2.4. Determination of total oxidant levels (TOS)

Rel Assay Diagnostics-TOS Assay Kit was used to determine the total oxidant levels of the samples. The kit contains Reagent 1 (Assay buffer), Reagent 2 (Prochromogen solution), Standard 1 (Blank solution: distilled water) and Standard 2 (stock stabilized standard solution (SSSS): 800 mM H₂O₂ Equiv./L). Standard 2 was diluted 40 times with distilled water. For this dilution, first 5 µL of Standard 2 was put into an Eppendorf tube and 1 mL of distilled water was added and then vortexed. Then, 5 µL of the prepared solution was put into an Eppendorf tube and 1 mL of water was added and a 20 µM H₂O₂ solution was prepared. This solution was prepared again with the same procedures each time. Then, 200 µL of Reagent 1 was first placed in the well on the ELISA plate and 30 µL of sample was added. The first absorbance was read at 530 nm (the first absorbance). After the measurement process, 10 µL of Reagent 2 was added. Then, it was kept at 37 °C for 5 minutes and the second absorbance was read at 530 nm. The same procedures were repeated for standard 2. The processes were repeated separately for all plant samples [15]. In addition, after determining the TAS and TOS values of the plant samples, the oxidative stress index (OSI), which shows the percentage of the plants' balancing of the oxidant compounds produced in their structures by environmental factors with antioxidant compounds, was calculated [16].

2.5. Determination of phenolic contents

The phenolic composition of plants extracts was analyzed using an LC-MS/MS system. A total of 24 standard compounds were screened during the analysis. The separation process was conducted on a C-18 Intersil ODS-4 analytical column (3.0 mm × 100 mm, 2 µm) maintained at 40 °C. The mobile phase consisted of 0.1% formic acid in water (Phase A) and 0.1% formic acid in methanol (Phase B). The analysis was performed with a flow rate of 0.3 mL/min, and the injection volume for each sample was set at 2 µL [17].

3. Results and Discussions

3.1. Antimicrobial activity findings of plants

Today, microorganisms are among the main factors of many diseases [18]. People use antimicrobial drugs against these microorganisms [19]. However, in recent years, there has been an increase in the number of resistant microorganisms due to unconscious use of antibiotics [20]. In this context, researchers have turned to the discovery of new antimicrobial drugs [21]. In our study, the antimicrobial activities of methanol and dichloromethane extracts of *S. macrodonta* and *S. chaetodonta* plants were determined. The findings obtained at the end of the study are presented in Table 1.

In the antimicrobial activity studies, the effects of plant extracts on 9 microorganisms were tested. As a result of the study, it was determined that plant extracts had effects on microorganisms at concentrations of 100-400 µg/mL. As a result of the antimicrobial activity tests, *S. chaetodonta* extracts showed higher antimicrobial activity than *S. macrodonta*. It was determined that the methanol extract of *S. macrodonta* showed higher antimicrobial activity against *S. aureus*, *S. aureus* MRSA,

Table 1. Antimicrobial activity of plants findings.

	Extracts	A	B	C	D	E	F	G	H	J
<i>Silene macrodonta</i>	MeOH	100	100	200	200	400	200	100	100	100
	DCM	200	200	200	400	400	200	200	200	100
<i>Silene chaetodonta</i>	MeOH	100	100	100	200	200	100	200	100	200
	DCM	100	100	200	200	200	200	200	200	200

*(A) *S. aureus*, (B) *S. aureus* MRSA, (C) *E. faecalis*, (D) *E. coli*, (E) *P. aeruginosa*, (F) *A. baumannii*, (G) *C. glabrata*, (H) *C. albicans*, (J) *C. krusei*
 *400, 200, 100 µg/ml extract concentrations.

E. coli, *C. glabrata*, *C. albicans* than the dichloromethane extract. In the study, methanol and dichloromethane extracts of *S. macrodonta* were used and it was determined that the tested microorganisms were effective at concentrations of 100-400 µg/mL. MeOH and DCM extracts of *S. chaetodonta* were used and it was determined that they were effective at concentrations of 100-200 µg/ml on the tested microorganisms. It was found that methanol extract of *S. chaetodonta* showed higher antimicrobial activity on *E. faecalis*, *A. baumannii*, *C. albicans* microorganisms than dichloromethane extract. No study on the antimicrobial activity of *S. macrodonta* and *S. chaetodonta* was found in previous studies.

In studies on different *Silene* species, it has been reported that the acetone extract of *Silene gallica*, *Silene succulent* and *Silene apetala* is effective against *Staphylococcus aureus*, *Serratia marcescens*, *Acinetobacter baumannii*, *Klebsella sp.*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, and *Alternaria alternata* [22]. In a different study, it was reported that *S. alba*, *S. conoidea*, *S. dichotoma*, *S. italica*, *S. supina* and *S. vulgaris* were effective against *Aspergillus versicolor*, *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Penicillium ochrocloron*, *Penicillium funiculosum*, *Penicillium verrucosum* and *Trichoderma viride* [23]. In this context, in our study, it was determined that *S. macrodonta* and *S. chaetodonta* plants also have antimicrobial activities and can be a natural antimicrobial source against the tested microorganisms.

3.2. TAS, TOS and OSI values of plants

Free radicals are oxidant compounds produced by environmental effects in living organisms [24]. While low levels of these compounds can be easily tolerated, high levels can cause serious damage. The antioxidant defence system functions to suppress oxidant compounds [25]. However, in some cases, the antioxidant defence system is insufficient and oxidative stress occurs [26]. As a result of oxidative stress, serious diseases such as cancer, diabetes, cardiological disorders, and neurodegenerative diseases can be observed [27,28]. Supplemental antioxidants can be used to prevent these possible consequences of oxidative stress. Plants are natural products with high potential as supplemental antioxidants. In our study, the antioxidant potential of *S. macrodonta* and *S. chaetodonta* was determined. The findings are shown in Table 2.

Table 2. TAS, TOS and OSI values of plant samples.

	TAS (mmol/L)	TOS (µmol/L)	OSI (TOS/(TAS*10))
<i>Silene macrodonta</i>	5.983±0.156	13.672±0.241	0.229±0.005
<i>Silene chaetodonta</i>	4.613±0.183	15.686±0.188	0.341±0.010

TAS, TOS and OSI values of *S. macrodonta* and *S. chaetodonta* have not been reported in the literature before. It was determined for the first time in our study. However, antioxidant potentials of different *Silene* species have been reported using different methods [29-31].

According to the data obtained as a result of the study, it was determined that the TAS value of *S. macrodonta* (5.983±0.156) was higher than of *S. chaetodonta* (4.613±0.183). TAS value is an indicator of the whole of antioxidant compounds produced in natural products [32]. TAS, TOS and OSI values of different plant species have been reported in the literature. In this context, the TAS values of *Ferulago platycarpa*, *Rumex scutatus*, *Helianthemum salicifolium*, *Alcea kurdica* and *Dittrichia graveolens* were reported as 5.688, 8.656, 9.490, 3.298 and 6.93 mmol/L, respectively. TOS values were reported as 15.552, 4.951, 14.839, 8.312 and 12.53 µmol/L, respectively. OSI values were reported as 0.273, 0.057, 0.157, 0.252 and 0.18, respectively [33-37]. Compared to these studies, the TAS value of *Silene macrodonta* used in our study was determined to be higher than *Ferulago platycarpa* and *Alcea kurdica*, and lower than *Rumex scutatus*, *Helianthemum salicifolium*, and *Dittrichia graveolens*. The TAS value of *Silene chaetodonta* used in our study was determined to be higher than of *Alcea kurdica*, and lower than TAS of *Ferulago platycarpa*, *Rumex scutatus*, *Helianthemum salicifolium*, and *Dittrichia graveolens*. In this context, it was observed that both *Silene* species used in our study had antioxidant potential.

The TOS value is an indicator of the totality of oxidant compounds produced in natural products [32]. Of the plant samples used in the study, the TOS value of *S. chaetodonta* (15.686±0.188) was found to be higher than of *Silene macrodonta*. The TOS value of *Silene macrodonta* used in our study was determined to be lower than of *Ferulago platycarpa* and *Helianthemum salicifolium* and higher than *Rumex scutatus*, *Alcea kurdica* and *Dittrichia graveolens*. The TOS value of *Silene chaetodonta* used in our study was found to be higher than this of *Ferulago platycarpa*, *Rumex scutatus*, *Helianthemum salicifolium*, *Alcea kurdica* and *Dittrichia graveolens*. In this context, it was determined that both *Silene* species produced high levels of oxidant compounds.

When the OSI values, which show how much plants suppress the oxidant compounds produced as a result of environmental and structural effects, were examined, it was determined that *S. chaetodonta* (0.341±0.010) was higher than *S. macrodonta* (0.229±0.005). It was observed that the OSI value of *Silene macrodonta* used in our study was lower than that of *Ferulago platycarpa* and *Alcea kurdica*, and higher than for *Rumex scutatus*, *Helianthemum*

salicifolium and *Dittrichia graveolens*. It was determined that *Silene chaetodonta* used in our study had higher OSI values than *Ferulago platycarpa*, *Rumex scutatus*, *Helianthemum salicifolium*, *Alcea kurdica* and *Dittrichia graveolens*. In this context, it was determined that both *Silene* species used in our study had high oxidative stress index but they had antioxidant potential.

3.3. Phenolic compounds of plants results

Plants synthesize different bioactive compounds from their bodies. These bioactive compounds are not nutritional but are medically important compounds [38]. In our study, phenolic compounds found in *S. macrodonta* and *S. chaetodonta* were determined. In this context, their presence in the plant body was investigated using 26 standards in the LC-MS device. The findings are shown in Table 3.

Table 3. Phenolic results of plants (mg/kg).

	<i>Silene macrodonta</i> (mg/kg)	<i>Silene chaetodonta</i> (mg/kg)
Acetohydroxamic Acid	22.09277	21.02428
Catechin	2.93871	1.28155
Vanillic Acid	63.80844	42.38556
Syringic Acid	63.01141	63.00583
Thymoquinone	-----	-----
Resveratrol	21.37435	26.86655
Fumaric Acid	57.54169	52.85805
Gallic Acid	120.17844	116.04689
Caffeic Acid	25.22394	30.94562
Hydroxycinnamic Acid	3.33337	25.05602
Hydroxybenzoic Acid	4.53163	3.85523
Protocatechuic Acid	-----	-----
Salicylic Acid	3.87326	4.04783
Oleuropein	-----	-----
Phloridzin	4.87329	4.31575
2-Hydroxy-1,4-naphthoquinone	-----	-----
Myricetin	5.72354	10.35108
Ellagic Acid	90.401241	19.000829
Quercetin	16.54219	40.69954
Butein	2.6828	4.25537
Naringenin	2.67157	3.55054
Silibinin	-----	-----
Luteolin	2.99182	6.70606
Kaempferol	3.15183	2.59392
Alizarin	-----	-----
Curmin	-----	-----

As a result of the study, thymoquinone, protocatechuic acid, oleuropein, 2-hydroxy-1,4-naphthoquinone, silibinin, alizarin and curmin were not determined among the standards used. In addition, it was determined that the phenolic compound present in the highest concentration was gallic acid (120.17844 and 116.04689 mg/kg, respectively) in both *S. macrodonta* and *S. chaetodonta*. Acetohydroxamic

acid has been reported to have antioxidant, cytotoxic and antibacterial activities in the literature [39]. Catechin has been reported to have antioxidant, antimicrobial, anticancer, antidiabetic activities [40]. Vanillic acid has been reported to have hepatoprotective, DNA protective, cytotoxic, antiproliferative and antioxidant activities [41]. Syringic acid has been reported to have hepatoprotective, antimicrobial, antioxidant activities [42]. Resveratrol has been reported to have antioxidant, anticancer, antimicrobial activities [43]. Fumaric acid has been reported to have anti-inflammatory, analgesic, antibacterial, antitumor, antiproliferative activities [44]. Gallic acid has been reported to have anti-inflammatory, anticancer, antioxidant, neuroprotective, antimicrobial activities [45]. Caffeic acid has been reported to have antioxidant, antiviral, anti-inflammatory, antimicrobial activities [46]. Hydroxycinnamic acid has been reported to have antioxidant, antimicrobial, anti-inflammatory, and anticancer activities [47]. Hydroxybenzoic acid has been reported to have anticancer, antiatherogenic, antiproliferative, and antioxidant activities [48]. Salicylic acid has been reported to have anticancer, antioxidant, and antifungal activities [49]. Phloridzin has been reported to have antioxidant activity [50]. Myricetin has been reported to have antioxidant, anticancer, anti-inflammatory, and analgesic activities [51]. Ellagic acid has been reported to have antitumor, antimetastatic, antiangiogenic, antioxidant and antifungal activities [52]. Quercetin has been reported to have antioxidant, antimicrobial, anti-inflammatory, anticancer activities [53]. Butein has been reported to have anticancer, anti-inflammatory, antioxidant activities [54]. Naringenin has been reported to have immunoregulatory, antioxidant, antiallergic activities [55]. Luteolin has been reported to have anti-inflammatory, antiviral, antibacterial and antioxidant activities [56]. Kaempferol has been reported to have antitumor, antioxidant, anti-inflammatory, antimicrobial activities [57]. In this context, the results obtained in the current study have determined that *S. macrodonta* and *S. chaetodonta* may be a natural source due to the compounds determined in them.

4. Conclusions

In the study, antioxidant, antimicrobial activities and phenolic compounds of *S. macrodonta* and *S. chaetodonta* plants were determined. The data obtained as a result of the study show that the plant samples have antioxidant potential and can be used as a natural source. Antimicrobial activity research has determined that both species have antimicrobial potential against microorganisms, but *S. chaetodonta* has higher antimicrobial activity than *S. macrodonta*. In addition, examination of phenolic compound content shows that these two species can be used as their natural source.

Author Contributions:

Conceptualization, N.K. and H.E.; methodology, N.K. and H.E.; validation, N.K. and H.E.; investigation, N.K. and H.E.; resources, N.K. and H.E.; data curation, N.K. and H.E.; writing—original draft preparation, N.K. and H.E.; writing—review and editing, N.K. and H.E.; visualization, N.K. and H.E.; supervision, N.K. and H.E.; project administration,

N.K.; funding acquisition, N.K and H.E. All authors have read and agreed to the published version of the manuscript.

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