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Investigation of the Chemical Composition, Antimicrobial, and Antioxidant Activity of Endemic *Onosma halophila* Boiss. & Heldr.

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Abstract
The *Onosma halophila* Boiss. & Heldr. belongs to the Boraginaceae family and it is endemic species from Turkey that distributes in Salt Lake (Tuz Gölü) and the surrounding salty steppes. In this study, the chemical content, antimicrobial, and antioxidant activity of endemic *O. halophila* were determined for the first time. Thirty-one components were identified by GC–MS analysis in *O. halophila*. Antimicrobial activity was tested against a total of eight microorganisms, including three Gram-positive, three Gram-negative bacterial strains, and two fungal strains, using the Micro dilution technique. The obtained extracts showed strong antifungal and antibacterial activity. The MIC value of extracts samples against the tested strains ranged from 15.625 to 125 µg/mL. In addition, it was determined that the extracts had different levels of antioxidant activity. The IC50 values were determined 45.20–1760 µg/mL for DPPH radical scavenging assay, 3.125–1016 µg/mL for H_2O_2 radical scavenging assay, and 147.12–1837 µg/mL for superoxide radical scavenging assay, respectively. As a result, it has been determined that *O. halophila* has the potential to be used in complementary medicine and various ethnobotanical fields in future due to the important components it contains.

Introduction

Onosma L. (Lithospermae, Boraginaceae) is a large genus with about 170–230 species in the world. *Onosma* species are distributed from the northwest of Africa to Europe and Asia and mainly in Turkey and Iran [1, 2].

It is knowing that the various parts of *Onosma* species are used for the treatment of different disorders such as bronchitis, tonsillitis, hemorrhoids, blood disorders, pain relief, and wound healing in traditional medicine at Turkey [3, 4]. *Onosma* species have biological components such as shikonin-alkannin derivatives, alkaloids, naphthoquinones, polyphenols, terpenoids, and fatty acids [5]. These compounds are used for various purposes such as dyes, food, and medicine [6, 7]. Therefore, it is very important to reveal the chemical contents of *Onosma* taxa.

Reactive oxygen species like hydroxyl radical, superoxide anion has an effect on the metabolism. As a result of the increase of free radicals due to radiation, chemical pollutants, toxins, deep-fried fast foods and stress, a condition called oxidative stress occurs. Oxidative stress plays an important role in the pathogenesis of various diseases such as aging, cancer, diabetes, high blood pressure, and nervous system diseases [8–13]. Therefore, in order to prevent these negative situations caused by oxidative stress, reactive oxygen species should be eliminated or kept at a certain level. Antioxidants destroy free radicals or keep them in balance inside the cell. Compounds obtained from plants can provide resistance to oxidative stress by scavenging free radicals and inhibiting lipid peroxidation. Also phytochemicals have ability to scavenge free radicals, donate hydrogen atoms, or chelate metal cations [14]. Phytochemicals are inexpensive, easy to access, and effective antioxidant agents compared to synthetic antioxidant molecules. Despite the developments in the pharmacological industries, the resistance of bacteria

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to drugs is increasing. As a result of the increase in multi-resistant bacterial diseases, new infections may occur in hospitals and this may result in high mortality. For this reason, the resistance of bacteria to drugs poses a great risk for both economic and human health. In order to eliminate these negative situations, it is important to find and use new alternative and effective antimicrobial agents. The use of phytochemicals with antimicrobial properties is of great importance in drug-resistant bacterial treatments. Plants have antimicrobial agent potential due to the phytochemicals they contain such as tannin, essential oil, and flavonoid components [15].

In this study, the aerial parts of the halophyte endemic *Onosma halophila* distributed around the Tuz gölü from Turkey were extracted with different solvents. In addition, the chemical components, antioxidant, and antimicrobial activities of the obtained extracts were determined.

Materials and Methods

Chemicals

All chemicals are analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), potassium hexacyanoferrate $K_3Fe(CN)_6$, $FeCl_3$, hydrogen peroxide (H_2O_2), and potassium dihydrogen phosphate (KH_2PO_4), were purchased from Merck Milipore. Riboflavin, nitroblue tetrazolium (NBT), methionine, ascorbic acid, and trichloro acetic acid (TCA) were purchased from (Sigma-Aldrich, Germany).

Plant Material

O. halophila samples were collected by Dr. Rıza Binzet from Aksaray (Collection location: Aksaray, Eskil to Cihanbeyli 2–5 km, salty steppe. 38°23′ N 33° 22′ E, 950 m) in June 2020 (Fig. 1), The plant was identified by Dr. Riza Binzet. The voucher specimens are deposited in the Mersin University Herbarium (MERA), Mersin, Turkey.

Fig. 1 The habitus and flower structure of *O. halophila*

Preparation of Plant Samples

For experimental studies, the above-ground vegetative parts of the samples were collected from the field and the collected parts were dried in the shaded area for about 4 weeks, and the dried samples were then used in the grinding mill (Blender 8011ES Model HGB2WTS3 400 W) in Mersin University Advanced Technology Education, Research and Application Center (MEITAM) and ground into powder and stored in a sterile dark bottle at + 4 °C until extraction studies.

Preparation of the Extracts

Soxhlet Extraction

Thirty grams of air dried and powdered aerial parts of *O. halophila* was extracted with 250 mL pure solvents (HPLC grade) by using the Soxhlet apparatus for 6 h. In our study, four different solvents were used as ethanol, ethyl acetate, methanol, and petroleum ether, respectively. The extracts were filtered with Whatman No.1 filter paper. Then, the obtained extracts were concentrated under vacuum at 40 °C by using a rotary evaporator and stored at + 4 °C for the use of the future analysis.

Subcritical Water Extraction

In order to perform the subcritical water extraction, the HPLC column with the dimensions of 250 mm × 4.6 mm was emptied and used in the extraction studies. In order to provide subcritical extraction conditions, the desired pressure and temperature conditions were obtained by using ISCO 260D brand syringe pump and GC oven. In order for the plant to be extracted to interact more with the solvent and to increase the mass transfer rate of the active ingredient, the dried plant samples were brought to certain dimensions using a pestle and then passed through a 1-mm sieve and made ready for extraction. 1.3 g of the sample, which was brought to a certain size, was weighed and mixed with a certain amount of sea sand to show a homogeneous distribution in the extraction column and then placed in the extraction column to form a fixed bed. The extraction pressure was set to 50 bar and the temperature to 110 °C, and static extraction was carried out for 30 min in order to ensure the sample-solvent balance in the first stage. In the second step, elution was carried out by using subcritical water as solvent for 30 min at constant flow rate under dynamic extraction conditions. At the exit of the column, the obtained plant extract was collected in glass bottle and stored for analysis.

GC–MS Analysis of the Plant Extract

The analyses of the extracts obtained by the subcritical extraction method were carried out using the Agilent 6890 GS system equipped with a mass detector HP-5MS silica capillary column (30 m × 0.25 mm ID; film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.8 mL/min, split 5: 1. The injector transfer line temperature was set to 250 °C. The oven temperature was kept at 50 °C for 1 min, gradually raised to 250 °C at 5 °C/min and subsequently held isothermal for 5 min. The extracts were dissolved using hexane (1:100 hexane, v:v). The furnace temperature program was kept the same as the GC conditions and the residence time of the components was calculated using the hydrocarbon standard (C_{10}–C_{40} *n*-alkanes, injected under the same conditions as the samples). The chemical components of the extracts were identified by comparison with Wiley7Nist05 and the GC/MS library.

Antimicrobial Activity of Plant Extracts

Antimicrobial Test Microorganism

The antimicrobial activity of the extracts from *O. halophila* was investigated against *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC BAA-1901, *Candida parapsilosis* ATCC 22019, and *Candida metapsilosis* CBS 2916: CBS-KNAW.

Antimicrobial Test of Plant Samples

The antimicrobial susceptibility test was evaluated using the modification microdilution assay in 96-well sterile polystyrene microplates [32]. The fungal and bacterial cell inoculum was prepared from the stock culture grown in Tryptic Soy Agar (TSA, Merck, Darmstadt, Germany) at 28 °C for 24 h and Mueller–Hinton Agar (MHA, Merck, Darmstadt, Germany) 37 °C for 24 h. The antimicrobial susceptibility test was performed as described by our previous study [16].

Evaloution of the Antioxidant Potential of the Plant Samples

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity of the samples was determined by modifying the method specified by Shekhar et al. [17]. Briefly 0.02 g DPPH was dissolved in 50 mL methanol solutions. 3 mL of freshly prepared DPPH solutions was added to the 1 mL of various concentration of the samples (62.5–1000 µg/mL) and incubated in the dark for 30 min. Then the absorbance was measured at 517 nm. Methanol was used as the blank. Ascorbic acid was used as a positive control. All solution was freshly prepared. Inhibition (%) of DPPH free radical DPPH was calculated as in Eq. (1).

$$\text{Inhibition}\ (\%) = \left[\left(A_c - A_s\right)/A_c\right] \times 100 \quad (1)$$

A_c is the absorbance of control solution and A_s is the absorbance of the sample solution.

Hydrogen Peroxide (H_2O_2) Scavenging Assay

The ability of the samples to the scavenge hydrogen peroxide (H_2O_2) was determined by based on the method of Ruch et al. [18]. 1 mL of samples (100–400 µg/mL) was transferred into the bottles and their volume was made up to the 2 mL with 40 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solutions (40 mM). The reaction mixture was vortexed and incubated for 10 min at room condition. After the incubation, the absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. Phosphate buffers was used as blank. All solutions were freshly prepared. The ability of the extracts to scavenge the H_2O_2 was calculated using the Eq. (1).

Reducing Power Assay

The reducing power activity was investigated for the samples using determinated method by Oyaizu [19]. Different concentrations of the *O. halophila* extracts (62.5–1000 µg/mL) were added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3FE(CN)_6$] solution. The reaction mixtures were incubated at 50 °C for 20 min at water bath. After the incubation, 2.5 mL 10% trichloroacetic acid (TCA) solution was added to reaction mixtures. Reaction mixtures were centrifuged at 3000 rpm for 10 min. Then 2.5 mL of the mixture was taken and mixed 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%). The reaction mixtures were incubated for 15 min at room temperature and the absorbance was measured at 700 nm. All solutions were freshly prepared. Ascorbic acid was used as positive control.

Superoxide Scavenging Assay

The superoxide scavenging activity of the plant extract samples was investigated based on the determinated method by Martinez et al. [20]. 3 mL of reaction mixture containing 50 mM sodium phosphate (pH 7.8), 1×10^{-2} mM methionine,

Table 1 Chemical composition of compounds of *O. halophila* from Turkey

No	Compound	Molecular formula	Molecular weight	Retention time (RT)	Area (%)
1	1-Bromo-3,5-di-tert-butybenzene	$C_{14}H_{21}Br$	269.23	50.907	1.52
2	9,17-Octadecadienal	$C_{18}H_{32}O$	264.40	51.459	0.57
3	Venlafaxine	$C_{17}H_{27}NO_2$	277.20	51.750	1.15
4	Ticlopidine	$C_{14}H_{14}ClNS$	263.78	52.243	1.02
5	Amitriptyline	$C_{20}H_{23}N$	277.20	55.270	11.65
6	Terbinafine	$C_{21}H_{25}N$	291.20	56.516	11.88
7	Tribenzylamine	$C_{21}H_{21}N$	287.40	56.872	0.62
8	1-Chlorooctadecane	$C_{18}H_{37}Cl$	288.94	61.122	0.38
9	gamma-Himachalene	$C_{15}H_{24}$	204.35	65.888	0.24
10	Nonadecanoic acid	$C_{19}H_{38}O_2$	298.30	66.375	0.24
11	Erucyl amide	$C_{22}H_{43}NO$	337.30	68.820	2.82
12	*n*-Pentacos-3-ene	$C_{25}H_{50}$	350.40	71.004	5.63
13	11,13-Dimethyl-12 tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.46	71.758	0.68
14	Bornylamine	$C_{10}H_{19}N$	153.20	72.405	0.47
15	Longiborneol	$C_{15}H_{26}O$	153.26	72.654	0.60
16	*N*-(3-(Trifluoromethyl)benzyl)pyrazine-2-carboxamide	$C_{13}H_{10}F_3N_3O$	281.23	73.717	0.24
17	Cembrane	$C_{20}H_{40}$	280.53	74.328	1.88
18	Octaosane	$C_{28}H_{58}$	394.50	74.726	10.44
19	Pyridine-3-carboxamide, oxime, *N*-(2-trifluoromethylphenyl)	$C_{13}H_{10}F_3N_3O$	281.23	75.569	0.71
20	Bacchotricuneatin c	$C_{20}H_{22}O_5$	342.40	76.174	0.81
21	Eicosane	$C_{20}H_{40}$	366.31	78.305	0.55
22	1-Tricosene	$C_{23}H_{48}$	324.60	81.997	3.53
23	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	888.20	82.394	1.35
24	Betulinol	$C_{30}H_{50}O_2$	442.4	83.029	1.42
25	Apo-11-lycopenal	$C_{15}H_{22}O$	218.2	83.101	0.95
26	14-beta-H-Pregna	$C_{21}H_{36}$	288.5	83.783	1.43
27	Z,E-2,13-Octadecadien-1-ol	$C_{18}H_{34}O$	266.3	85.736	22.63
28	Bemegride	$C_8H_{13}NO_2$	155.1	86.745	2.75
29	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-mehtyl ester	$C_{14}H_{21}NO_3$	251.2	87.285	1.95
30	2-Methylheptadecane	$C_{18}H_{38}$	254.30	88.787	1.25
31	2-Ethylacridine	$C_{15}H_{13}N$	207.10	91.339	0.82
32	Not known compounds	–	–	–	7.82

3×10^{-6} mM riboflavin, 100 µM/L EDTA, and 1×10^{-4} M NBT was mixed with extracts at different concentrations (62.5—1000 µg/mL). These mixtures were illuminated under fluorescent lamp for 15 min. The absorbance was measured at 560 nm. Illuminated reaction mixture without samples was used as control. The superoxide radical scavenging activity of plant samples was calculated by the Eq. (1).

Results and Discussion

GC–MS Analysis

In previous studies, it has been reported that *Onosma* taxa mostly contain flavonoids, phenolics, apigenin 7 glucosides, alkannine, shikonin, and naphthoquinones [26–37]. In this study, chemical composition of *O. halophila* was examined by GC–MS. The molecular formula, molecular weight, retention time (RT), and the area (%) of the analyzed components are shown in Table 1. 31 components in *O. halophila* were characterized by GC–MS analysis. The major components of the extracts were determinated as Z, E-2, 13-Octadecadien-1-ol (22.63%), Terbinafine (11.68%),

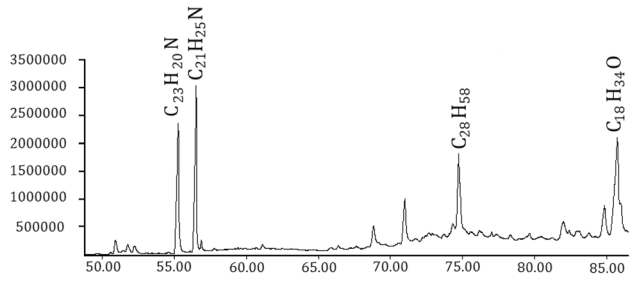

Fig. 2 GC–MS analysis results of *O. halophila* ethanolic extract sample

Antimicrobial Activity

It has been seen in the studies that *Onosma* species has antimicrobial activity against gram (−) and gram (+) bacterial strains [38–45]. But there are no reports in the literature regarding the antimicrobial activities of *O. halophila*. Therefore, the data presented here are the first record for the literature. Extractions of plants mixed with different solvents were carried out using the extraction technique, and the MIC values of the obtained extracts against bacterial and fungal species are given in Table 2. The extracts obtained showed strong antifungal activity against

Table 2 Antimicrobial activities of extracts presented as MIC values (µg/mL)

Microorganisms	Plant extract				Antibiotics	
	Ethanol	E. acetate	Methanol	P. ether	Ampicilin	Antifungal agent (fluconazole)
S. aureus	125	125	125	125	**0.976****	–
S. pneumoniae	**62.5***	125	125	**62.5***	**0.976****	–
E. faecalis	125	125	125	**62.5***	**0.976****	–
E. coli	**62.5***	**62.5***	125	**62.5***	**31.25***	–
P. aeruginosa	**62.5**	125	125	125	**31.25***	–
K. pneumoniae	125	125	125	125	**31.25***	–
C. metapsilosis	125	**31.25***	**62.5***	**15.625***	–	**0.976****
C. parapsilosis	**62.5***	**15.625***	**62.5***	**62.5***	–	**0.976****

–: not determinated

*effective; **more effective; we tested ten tubes

Amitriptyline (11.65%), and Octacosane (10.44%), respectively (Fig. 2).

In the literature review, it was noted that some chemical components in *O. halophila* have important biological activities. It has been reported that defined bioactive compounds such as terbinafine, betulinol, venlafaxine, amitriptyline, and eicosane have pharmacological properties such as antifungal, anticancer, antidepressant, antioxidant, antitumor, and hemolytic activity [21–24]. Also, Z,E-2,13-Octadecadien-1-ol and 14-beta-H-Pregna identified in *O. halophila*, are types of pheromone and is only found in insect species. This compound is not only a defense chemical, but also a sex pheromone and acts as a reproductive facilitator [25]. It is interesting that the pheromone species Z,E-2,13-Octadecadien-1-ol and 14-beta-H-Pregna were detected in this plant sample. Based on observations from field studies, it is thought that the source of these compounds may be from the eggs and larvae of Lepidoptera and Hemiptera insect species. Because this taxa lay their eggs under the leaf and stem epidermis of *O. halophila* and complete their larval development in these tissues.

C. parapsilosis and *C. metapsilosis* species that cause candidemia in intensive care units today. The MIC value of extract samples against the tested *Candida* species ranged from 15.625 to 62.5 µg/mL. Ethly acetate extract of the *O. halophila* has shown strong antifungal activity compared to the methanolic and ethanolic samples. We think that this is due to the membrane structure of the tested *Candida* species and their interaction with the phytochemicals released as a result of the extraction. Also in this study, the MIC value of *O. halophila* extracts against the gram (+) and gram (−) bacteria ranged from 62.5 to 125 µg/mL.

Antioxidant Activity

In this study, the antioxidant activity of *O. halophila* was revealed for the first time with this study. Studies have shown that *Onosma* taxa have antioxidant potential due to the components such as pyrrolizidine alkaloids, shikonin, deoxyshikonin, and pyrrolidine [46]. In our study, the investigation of the antioxidant activity of *O. halophila* was performed by using different assays. The antioxidant activity at extracts of the *O. halophila* obtained by using

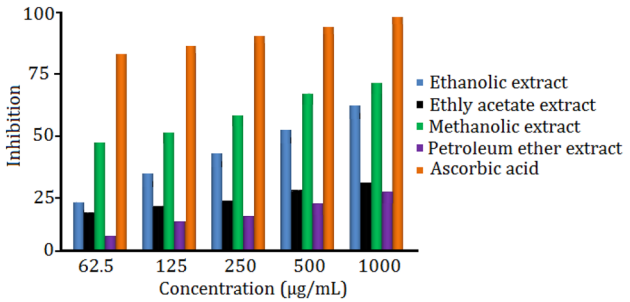

Fig. 3 DPPH radical scavenging of the *O. halophila* extracts

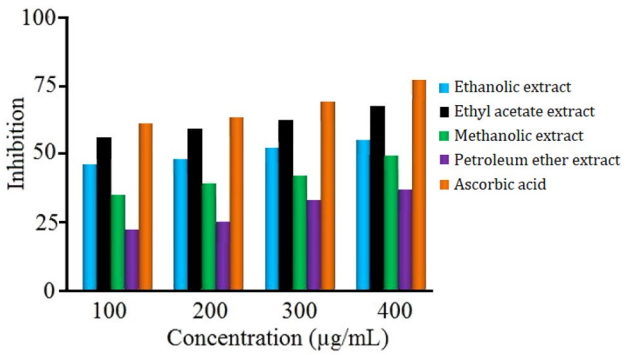

Fig. 4 The hydrogen peroxide scavenging activity of *O. halophila* extracts

different solvents were investigated according to the DPPH, superoxide, hydrogen peroxide scavenging, and reducing power activity test.

DPPH Radical Scavenging Assay Activity

The extracts obtained as a result of our study showed different antioxidant activity. The DPPH radical scavenging activity of *O. halophila* extracts is shown in Fig. 3. Ethanolic, ethyl acetate, methanolic, and petroleum ether extract of *O. halophila* and ascorbic acid showed at 61.19%, 29.84%, 69.63%, 21.46%, and 96.12% of inhibition at 1000 µg/mL, respectively. In the result of the works, we think that the low DPPH radical scavenging activity of ethyl acetate and petroleum ether extracts is due to the low contents of the phenolic and flavonoid compound released during the extraction. The IC50 values of ethanolic, ethyl acetate, methanolic, and petroleum ether extracts of *O. halophila* were determined as 45.2 µg/mL, 589 µg/mL, 273.1 µg/mL, and 1760 µg/mL.

Hydrogen Peroxide Scavenging Assay Activity

Hydrogen peroxide is a lipophilic molecule and it passes cell membranes, after entering the cell, reacts with ions like Fe^{2+} or Cu^{2+} or biomolecules in the cell and make to hydroxyl or superoxide radicals (Eq. 2–4) [47]. As a result, hydroxyl or superoxide radical may cause on the cells like causes tissue damage and cell death. Antioxidant molecules like plants phytochemicals can convert H_2O_2 to the other structures, and therefore, it can prevent the negative effects of hydrogen peroxide (Eq. 5).

$$Fe^{+3} + O^{2\cdot} \rightarrow Fe^{2+} + O_2 \quad (2)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{-} \quad \text{(Fenton reaction)} \quad (3)$$

$$O2^{\cdot -} + H_2O_2 \rightarrow HO^{\cdot} + OH^{-} + O_2 \quad \text{(Haber–Weiss reaction)} \quad (4)$$

$$H_2O_2 + \text{Antioxidant molecule} \rightarrow H_2O + {}^1\!/_2\, O_2 \quad (5)$$

The H_2O_2 scavenging activities of different extracts obtained in this paper were investigated and the obtained extracts showed activity at different rates depending on the concentration. The hydrogen peroxide scavenging activity of the samples is given in Fig. 4. IC50 values of ethanolic, ethly acetate, methanol, and petroleum ether extracts of *O. halophila* were determined as 19.29 µg/mL, 3.125 µg/mL, 600 µg/mL, and 1016 µg/mL, respectively.

Reducing Power Assay Activity

Reducing power activity of the plant extracts measures the reductive ability of antioxidant properties of phytochemical to the transform Fe^{+3} to Fe^{+2}. The yellow color of the reaction mixture changes to the with the range green and blue color depending on the reducing power of the compounds at plant extracts. Higher absorbance values indicate higher reducing ability. Methanolic extract of *O. halophila* has strong reducing power ability than the other plant extract samples (Table 3).

Superoxide Radical Scavenging Assay Activity

Hydrogen peroxide, superoxide radical and hydroxyl radical are interconverted via Fenton reaction and Haber-Weiss reactions. Hydroxyl and superoxide radicals are toxic to cells as they induce lipid peroxidation. Also superoxide radical has a important role of the pathogenesis diseases. Therefore, destruction of the superoxide at organism is very important [48]. The superoxide radical scavenging activity was performed according to the capacity of the samples to capture the superoxide radicals produced in the riboflavin-light-NBT system and prevent the formation of blue formazan [49]. The production of blue formazan was followed by monitoring the absorbance at 560 nm. Ethanolic, ethly acetate, methanolic, and petroleum ether extract of *O.*

Table 3 Plant extract samples' absorbance value at 700 nm

Concentration	Ethanolic extract	Ethly acetate extract	Methanolic extract	Petroleum ether extract	Ascorbic acid
62.5 µg/mL	0.099	0.006	0.172	0.091	0.350
125 µg/mL	0.136	0.010	0.274	0.115	0.412
250 µg/mL	0.162	0.013	0.284	0.174	0.826
500 µg/mL	0.189	0.015	0.504	0.186	1.084
1000 µg/mL	0.221	0.108	0.834	0.196	1.210

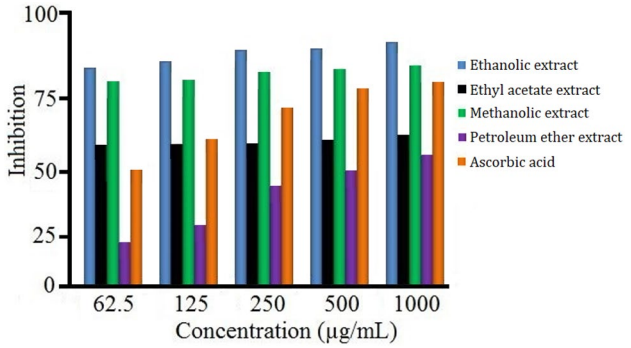

Fig. 5 Superoxide radical scavenging activity of *O. halophila* extracts

halophila gave at 88.44%, 54.19%, 79.52%, and 46.88% of the inhibition at 1000 µg/mL (Fig. 5). Ethanolic and methanolic extracts showed strong superoxide scavenging activity than ascorbic acid at all concentrations. IC50 values for ethanol, ethyl acetate, methanol, and petroleum ether extracts of *O. halophila* were determined as 147.12 µg/mL, 503.41 µg/mL, 235.88 µg/mL, and 1837 µg/mL, respectively.

Conclusion

In this paper, the chemical compositions and biological activities of endemic *O. halophila* were determined for the first time. As a result of our study, it was seen that *O. halophila* plant contains important compounds such as terbinafine and venlafaxine which are used as antifungal and antidepressant. *O. halophila* extract has showed strong antifungal activities against *C. parapsilosis and C. metapsilosis species* that cause candidemia in intensive care units today. The antifungal activity of the *O. halophila* could be attributed to the presence of terbinafine. In addition, in this article, it was determined that *O. halophila* extracts have antioxidant activity. These *in vitro* antioxidant test shows that plant extracts are an important source of natural antioxidants that may be helpful in preventing the progression of various oxidative stresses. However, the components responsible for antioxidant activity are currently unclear. Therefore, more research is needed to identify the antioxidant compounds found in the plant extract. As a result, it has been determined that *O. halophila* has the potential to be used in complementary medicine and various ethnobotanical fields in future due to the important components it contains.

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Author Contributions All authors contributed equally to this work.

Data Availability The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This study does not involve any animal or human testing.

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