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Scientific paper

Potential Biochemical Properties of Endemic *Onosma mutabilis*

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Abstract

The *Onosma L.* (Lithospermae, Boraginaceae) genus contains many plant species with therapeutic properties due to its rich phytochemicals. *Onosma mutabilis* Boiss. & Hausskn. ex Boiss. (*O. mutabilis)* is the species for which there is not enough information on its characteristics.

Objective: The total phenolic content, antioxidant activity, possible bioactive compounds, and antibacterial activities of ethanolic extracts of leaf, stem, root, and flower parts of endemic *O. mutabilis* were investigated.

Conclusions: The total phenolic content of all *O. mutabilis extracts* was in the range of 9.2–31 mg gallic acid equivalent (GAE)/g of extract. According to the results of antioxidant activity, the IC_{50} antioxidant capacity values determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method were between 4.39–29 μg/mL, while the equivalent trolox antioxidant activity determined by the cupric reducing antioxidant values (CUPRAC) was 0.45–0.78 mmol of trolox equivalents (TE)/g of extract. Bioactive compounds have been analysed using gas chromatography coupled with mass spectrometry (GC/MS) and were found to contain 29 different chemical components. All plant extracts tested showed effective antibacterial activity against *A. baumannii* (ATCC 02026) (62.5 μg/mL MIC value) when compared to the reference drug Ampicillin (125 μg/mL).

Keywords: *Onosma mutabilis*, phenolic compounds, antioxidant activity, antibacterial activity.

1. Introduction

Onosma L. (1762: 196) (Lithospermae, Boraginaceae) is a large genus in the world. It is distributed from the northwest of Africa to Europe and Asia and mainly in Turkey and Iran[1,2]. The total number of *Onosma* species known from Turkey is 103 [3,4]. When the high rate of endemism in Turkey (57.84%) was taken into account, it was seen that Turkey was the centre of diversity of the Onosma genus. *Onosma* species is widely used worldwide in traditional medicine. The various parts of *Onosma* species are known to be used for the treatment of various disorders such as bronchitis, hemorrhoids, tonsillitis, pain relief, and relief of blood disorders in Turkey.[5,6]

On the other hand, antioxidant enzymes produced by our body's defence system are critical to maintain the oxidant-antioxidant balance. In addition, plant-derived antioxidant substances have been reported to be effective against degenerative diseases caused by oxidative stress.[7] For this reason, the determination of the effects of therapeutically effective plants on free radical-induced oxidative damage attracts the attention of many researchers. Despite the unique bioactive composition of plants, the phytochemical content of approximately 15% was investigated and the biological activity of 6% was screened.[8] Antimicrobial compounds isolated from medicinal plants are effective against different bacteria.[9,10] In addition, the emergence of multidrug-resistant pathogens in recent times has had adverse effects on public health. This encourages new research and the development of more effective drugs to replenish therapeutic drug reservoirs.[11] How-

ever, the chemical components and antioxidant and antimicrobial properties of endemic plants grown in various countries and used for medicinal purposes still need to be discovered. Therefore, active research on plants is necessary to identify potential candidates as safer and more effective agents in the future.

To our knowledge, only one study has been conducted to determine the phytochemical content of *O. mutabilis*. In the study performed by Jabbar et al.[12] different extraction solvents were used, and it was reported that 18 different bioactive species were detected. Cytotoxicity studies have been carried out on different cell lines, but no studies have been carried out on the antibacterial activities of the plant.

In this study, we have identified the phenolic compound, chemical composition, antioxidant and antibacterial activity of the ethanolic extract obtained from roots, stems, flowers, and leaves of endemic *O. mutabilis*.

2. Experimental

2. 1. Chemicals and Instruments

The Folin-Ciocalteu reagent and ethanol (99%) were supplied from Merck (Darmstadt, Germany), gallic acid (3,4,5-trihydroxybenzoic acid, abbreviated as GA), anhydrous sodium carbonate (Na_2CO_3) was obtained from Fluka (USA). DPPH (1,1-diphenyl-2-picrylhydrazyl radical), BHT (2,6-Di-tert-butyl-4-methylphenol), and Mueller-Hinton broth (Sigma 70192) and Resazurin dye (Sigma R7017) were obtained from Sigma-Aldrich (St. Louis, MO). The soxhlet apparatus was supplied by Isolab (Wertheim, Germany). A Rotary evaporator (Buchi B-491, Germany), UV-1601 spectrophotometer (UV-1601, Shimadzu, Japan), GC/MS (GC: 7890 A, MS: 5975 C, Agilent, USA) were used throughout this work.

2. 2. Plant Materials

The samples of *O. mutabilis* were identified and collected by Dr. Riza Binzet from Mersin (Location: C5 Mersin, Mersin-Gözne, around Darısekisi, rocky slopes and scrub, 36°58'10.91"N 34°34'11.79"E, 780 m) (Fig. 1).

2. 3. Preparation of Plant Extracts

Fresh leaves, roots, stem, and flower samples of *O. mutabilis* were air dried in the shade at room temperature (25 °C) for three weeks. Then the leaves, roots, stems and flowers samples were reduced to powder separately with a blender (Blender 8011ES Model HGB2WTS3, 400 W) and kept in glass bottles at room temperature. Ten grams of powdered samples were extracted in 300 mL of ethanol solvent using the Soxhlet extraction method for 6 hours. Ethanol was evaporated at 50–60 °C using a Rotary Evaporator with bath water. Stock solutions were prepared at concentrations of 1 mg/mL of each part of the plant. Extracts were kept before analysis in a sealed vial at +4 °C.

2. 4. Determination of the Total Phenolic Content

The content of phenolic compounds in extracts obtained from different parts of the plant analysis according to the Folin-Ciocalteau method.[13] 1 mL of Folin-Ciocalteau reagent was added to 1 mL of ethanolic plant solution (1 mg/mL). The sample was kept in the dark for five minutes. Then 2 mL of Na_2CO_3 solution (20%, (w/v)) and 2 mL of water were added to the reaction medium. After incubation at room temperature for 30 minutes in the dark, the absorbance was measured at 714 nm. The total polyphenol content was calculated using the gallic acid calibration curve and reported as mg of gallic acid equivalent per gram of extract (mg GAE/g E).

2. 5. DPPH Radical Scavenging Assay

The free radical scavenging of the ethanolic extracts obtained from different parts of *O. mutabilis* by the DPPH· test according to the method established by Ilokiassanga et al.[14] First, a stock solution of dried plant extracts was prepared with ethanol at a concentration of 1 mg/mL. The solutions of ethanolic extracts of *O. mutabilis* prepared in each concentration range (100–1000 μg/mL) were analysed. 100 μL of the extract solutions were mixed with 100 μL of freshly prepared DPPH (0.2 mM). The mixture ob-

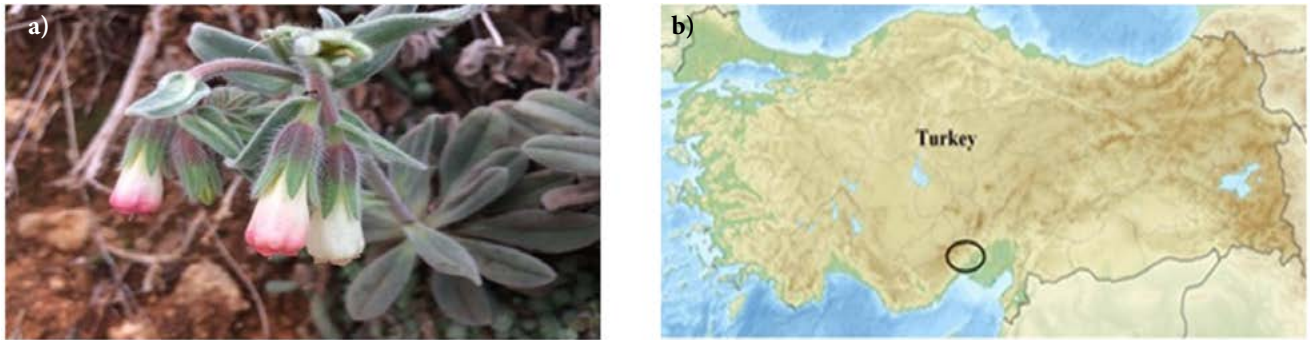

Fig. 1 (a) Habitus and (b) map of the distribution of *O. mutabilis*.

tained was slightly shaken and incubated at room temperature for 30 min in the dark. BHT was used as a reference. The absorbance values of the sample solutions and BHT were measured at 517 nm using ELISA (Thermo Scientific TM Multiskan TM FC). The tests were repeated 3 times. The percent inhibition of the DPPH free radical scavenging activity was calculated with Eq. (1):

$$\% \text{ Inhibition} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100 \quad (1)$$

Radical scavenging activity was indicated as IC_{50}, which shows the concentration of plant extracts required to inhibit 50% of the free radicals DPPH.

2. 6. Cupric Reducing Antioxidant Capacity (CUPRAC) Assay

CUPRAC assay,[15] and the results were expressed as Trolox equivalents. In detail, 1 mL of 1.0×10^{-2} M copper chloride solution, 7.5×10^{-3} M neocuproin solution and 1 M (pH = 7) ammonium acetate buffer are added to the test tube, respectively. Then Trolox and distilled water are added. Solutions prepared with a total volume of 4.1 mL are kept closed for 30 minutes under room conditions. At the end of this period, the absorbance values are measured at 450 nm against the reference solution without a sample.

2. 7. Antibacterial activity

The antibacterial activity and minimum inhibitory concentration (MIC) values of extracts obtained from different parts of the plant were tested using REMA.[16] The following five bacteria were tested in this study: *Staphylococcus aureus* ATCC 25925, *Bacillus subtilis* ATCC 6633, *Aeromonas hydrophila* ATCC 95080, *Escherichia coli* ATCC 25923, *Acinetobacter baumannii* ATCC 020226.

2. 8. Determination of MIC Values for *O. mutabilis*

Antibacterial activity was evaluated using the microdilution assay in 96-well sterile polystyrene microplates. Extract at concentrations of 1000 µg/mL was prepared by dissolving in DMSO and filtered through a 0.22 µm microporous filter. Each well in the microplate was filled with 100 µL of Mueller-Hinton broth (Sigma 70192). The working solutions of the extracts with serial twofold dilutions were adjusted to 500–0.24 µg/mL. Ampicillin was used as the standard drug in the study and the dilution of the standard drug was carried out in the same way. The bacterial suspension was prepared from standard bacterial strains at 0.5 McFarland density. This suspension was then diluted with sterile distilled water (1/20). 10 µl of this suspension was added to the corresponding wells. Thus, the final bacterial density in the wells was adjusted to $5x10^5$ CFU/mL (CLSI 2012). The working solution of Resazurin (resazurin sodium salt, Sigma R7017) was prepared in 0.01% (w/v) distilled water and sterilised by passing through a 0.22 µm membrane filter. 10 µL of sterilised resazurin was added to the wells. Plates were covered with a plastic film (ThermoFisher Scientific MicroAmp® optical adhesive film, 4360954) to prevent evaporation. The plates were then incubated at 37 °C for 24 hours. At the end of the period, the colour change in the plates was visually evaluated. The change in resazurin from blue to pink or colourless was considered bacterial growth. The MIC value was determined as the lowest concentration of plant samples that prevents the growth of bacteria that prevented resazurin from turning blue to pink or colourless. All antibacterial activity assays were repeated three times.

2. 9. Determination of Bioactive Compounds

The essential compounds of *O. mutabilis* were analysed with a7890A GC system with an inert MSD of 5975C and a capillary column [Agilent Technologies 19091S-433-HP5-MS]. The injection temperature was 285 °C. The volume of injection was 2 µL. The GC temperature programme was used as follows: At 40 °C, holding there for 5 min, 40 to 220 °C at a rate of 4°C/min and holding at 220 °C for 5 min, and then increased from 220 to 280 °C at a rate of 5°C/min and holding there for 15 min, from 280 to 300 °C at a rate of 15 °C/min and holding there for 5 min. Spectra were obtained in the range of 50–550 m/z. Helium gas was used as the carrier gas with a flow rate of 1 mL/min. The maximum temperature was 325 °C. Total analysis time: 82.5 min. The chemical components of the extract were identified by matching the retention times and mass spectral fragmentation patterns with those of the compounds resulting from data from the NIST/EPA/NIH mass spectral library (NIST05a.L).

3. Results and Discussion

3. 1. Total Phenolic Content Analysis

Composed of an aromatic hydroxyl core, plant phenolics are one of the most important groups of compounds that work as primary antioxidants and free radical scavengers. Spectrophotometric measurements were performed based on the blue colour of the phosphomolybdic-phosphotungstic-phenol complex formed in the Folin-Ciocalteu method,[17] which is widely used in the determination of the total phenolic content. The total phenolic content of the *O. mutabilis* extracts, expressed as gallic acid equivalents, are shown below in Fig. 2

In this study, the findings we obtained for the phenol content of *O. mutabilis* are compatible with the literature. In a study with *O. mutabilis* grown in Iran, the total phenolic content of the methanolic extract of this plant species was determined to be 37.24 mg equivalent rutin equivalents/g extract.[12] Sarikurkcu et al.[18,19] determined the total

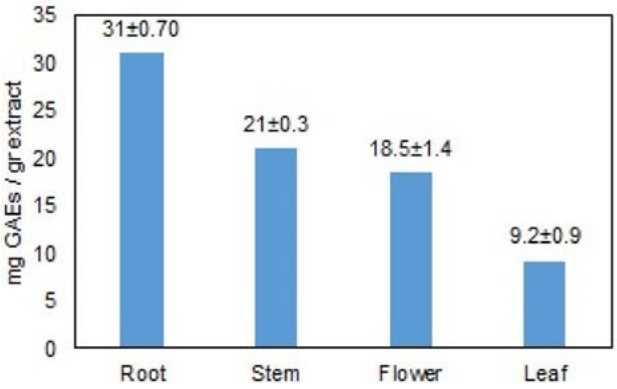

Fig. 2 Total phenolic contents of the ethanolic extracts of different parts of *O. mutabilis.* The values presented represent the mean of three experiments ± SD.

phenolic content of the methanolic extracts of *O. gigantea* and *O. rascheyana* to be 9.12 µmol GAEs/g and 31.55 mg GAE / g, respectively. Furthermore, Kirkan et al.[20] *Onosma tauricum var. tauricum* species and showed that the total phenolic content of this plant is 16.20 µmol GAEs/g. In a study with another Onosma species (*Onosma chlorotricum*) the total phenolic content was determined as 56.10 mg GAE / g of dry extract.[21] Emsen et al.,[22] analysed the ethyl acetate extracts of *O. bozakmanii* and determined the total phenolic content as 36.29 µg GAE/mg extract.

3. 2. Antioxidant Activity

The antioxidant activity of the ethanolic extracts of *O. mutabilis* was evaluated using DPPH and CUPRAC methods. The DPPH method is widely used because its assay is reliable, simple, fast, and sensitive and determines the antioxidant activity in vitro of several natural bioactive compounds.[23,24] Basically, in this method, there is a decrease in the strong absorbance of DPPH at 517 nm due to the reaction of proton transfer to the DPPH free radical by the antioxidant.[25]

Table 1 shows the IC_{50} values of the ethanolic extract of different parts of *O. mutabilis*. Free radical scavenging ability is expressed as the IC_{50} value. The IC_{50} value is the amount of antioxidant required to reduce 50% of the initial concentration of DPPH.[26] A low IC_{50} value means high free radical scavenging activity.[27] The IC_{50} values of the roots, stems and flowers were the highest with 5.37, 4.39 and 8.02 µg/mL, respectively. The phenolic content of the plant is proportional to the concentration of the extract and indicates that it has very high antioxidant activity.[28]

While the CUPRAC method shows the ability of the extract to reduce Cu metal, the results are proportional to the total amount of copper reduced by antioxidant compounds through electron transfer. The CUPRAC assays were expressed as mmol of Trolox equivalent/g of extract.

The extracts of *O. mutabilis* gave CUPRAC values with a total antioxidant capacity ranging between 0.45 and 0.78 as mmol Trolox equivalent/g. The highest antioxidant capacity determined by the CUPRAC analysis was recorded for the root extract. This is followed by flower, leaf, and stem extracts, respectively (Table 1). The highest reduction potential determined by the CUPRAC assay was also observed in the root extract.

Table 1. Antioxidant activities (DPPH, and CUPRAC) of ethanolic extracts from different parts of *O. mutabilis**.

Sample	DPPH assay (IC_{50} µg/mL)	CUPRAC (mmol TE/g extract)
Root	5.37±0.23	0.78±0.22
Stem	4.39±0.29	0.45±0.26
Flower	8.02±0.57	0.52±0.34
Leaf	29±0.63	0.67±0.36
BHT	1.75±0.18	0.64±0.28

* The values presented are the mean of three experiments ± SD.

Our results support previous studies on the antioxidant capacities of other medicinal plants in the Boraginaceae family. Researchers used methanol extracts, unlike us, in the research carried out on different types of this plant. Jabbar[12] investigated the antioxidant activity of *O. mutabilis* methanol extracts using the DPPH method and reported an IC_{50} value of 3.54 mg/mL, respectively.

Saravanakumar et al.[29] investigated the free radical scavenging activity of the methanolic extract of *O. bracteosa* plant with the DPPH test and showed that the IC_{50} value was 4.58 mg/mL. Furthermore, Sarikurkcu et al.,[30] determined the antioxidant activities of methanolic extracts of *O. frutescens* with DPPH and CUPRAC tests as 1.14 and 0.53 mg/mL, respectively, and showed that they have high antioxidant potential. Kumar et al.,[31] recorded the DPPH IC_{50} value of methanolic *O. hispidum* root extract as 2.73 µg/mL. Kirkan et al[32]., determined that methanol extracts of *O. cappadocica* showed high activity based on the DPPH scavenging test and the CUPRAC test. In addition, another study by Kirkan et al.[20] showed that methanolic extracts of *O. tauricum var. tauricum* exhibited a high antioxidant potential when tested with the DPPH and CUPRAC methods.

It can be said from the results that the ethanolic extracts of *O. mutabilis,* especially the roots and stem, have quite high antiradical activities, with radical scavenging values close to that of the standard. The difference in free radical scavenging activity in various parts of *O. mutabilis,* such as the root, stem, flower, and leaves, may be related to its chemical composition. It is difficult to compare the results of different methods used to determine antioxidant activity, such as CUPRAC and DPPH.[33] Therefore, the results are not given comparatively.

3. 3. Antibacterial Activity

The MIC values of the extracts, compared to the standard bacterial strains used in the study, were determined to be in the range of 250–31.25 µg/mL. The test-

ed extracts had higher antibacterial activity against *A. baumannii* with a 62.5 μg/mL MIC value, compared to the reference drug ampicillin with a 125 μg/mL MIC value.

Based on the results, it was determined that the MIC values of the extracts against *A. hydrophila* were 62.5 μg/mL. It was determined that the results showed a lower antibacterial effect compared to the reference drug, but a result close to the MIC value (31.25 μg/mL) of ampicillin. Although the MIC results of the plant extracts (31.25 μg/mL) for *B. subtilis*, a Gr (+) bacterium, were higher than the MIC results of the other four bacteria, the activity was found to be lower when compared to the MIC value of ampicillin (0.9 μg/mL). The plant extracts were defined to show low activity against standard bacterial strains of *S. aureus* and *E. coli* (Table 2).

Table 2. MIC (μg/mL) values of extracts and reference drugs tested against standard bacterial strains.

	S. aureus (ATCC25925)	*E. coli* (ATCC25923)	*A. baumannii* (ATCC02026)	*B. subtilis* (ATCC6633)	*A. hydrophila* (ATCC95080)
Root	125	125	62.5	31.25	62.5
Stem	125	125	62.5	31.25	62.5
Flower	250	125	62.5	31.25	62.5
Leaf	125	125	62.5	31.25	62.5
Ampicillin	31.25	15.62	125	0.9	31.25

A lot of research is focused on studying the antimicrobial activity of various parts of plants of the family Boraginaceae. In various studies, root extracts from different species of *Onosma* have been shown to be effective against Gr (+) bacteria.[34] In our study, the MIC value of root extracts of *O. mutabilis* against *S. aureus* was 125 μg/mL and against *B. subtilis* was 31.25 μg/mL. In other studies, the MIC values of *O. dichroanthum* root extracts against Gr (+) bacteria were in the range of 0.156–0.312 mg/mL.[34] Dousti and Nabipor[21] showed that by the MIC assay *O. chlorotricum* Boiss methanol extracts showed higher antibacterial activity against Gr (+) bacteria than Gr (-) bacteria. Halim et al.,[35] reported that *O. Bracteatum* extracts inhibited Gr (+) bacteria more than G (-) bacteria.

3. 4. Chemical Composition Analysis

Using GC-MS analysis of *O. mutabilis* flower, leaf, stem, and root extracts, a total of 29 compounds with high-quality peaks were detected (Table 3). The results showed that there are different compounds in the flower, leaf, stem, and root parts of the plant, and that the rates of these compounds varied by their peak areas. In our study, based on the results of the GC-MS analysis of *O. mutabilis* flower, leaf, stem and root extracts, fatty acids such as butanoic acid, hexadecanoic acid, 14-pentadecanoic acid, linoleic acid, 9,12,15-octadecatrienoic acid, octadecanoic acid and derivatives of fatty acids such as ethyl linoleate and hexanamide have been found.

Butanoic acid was found only in the leaf part of the plant; hexadecanoic acid was found in all parts of the plant, but in different concentrations. Relative rates of hexadecanoic acid, which has a strong antimicrobial and anti-inflammatory[36,37] effect, are mainly flower, leaves, roots, and stem, respectively. Hexanamide and 14-pentadecanoic acid were found only in the root of the plant and linoleic acid was detected only in the flower of the plant. Furthermore, ethyl linoleate was found in the flower and root part of the plant; 9,12,15-octadecatrienoic acid was detected in the flower and leaves of the plant. Octadecanoic acid was found in all parts of the plant. Fatty acids are compounds with important structural functions. Studies have shown that fatty acids such as stearic acid, oleic acid, and linoleic acid reduce inflammation due to their antioxidant properties, albeit indirectly, in vascular endothelial cells. Therefore, it has been suggested that treatment using these parts of the plant could reduce the risk of atherosclerosis and cardiovascular disease.[38] Phytol is an important diterpene with antimicrobial, antioxidant and anticancer activities.[36,39,40] Neophytadiene, another important bioactive compound found in the flower and stem parts of *O. mutabilis*, has analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant effects.[41] The compound 14ß-Pregnane, found in the root part of *O. mutabilis* at a concentration of 1%, has a steroid structure and is a defence chemical with preventive and therapeutic effects against diabetic retinopathy.[42] Another bioactive compound detected based on GC-MS results is β-Sitosterol, commonly known as phytosterol. Phytosterols, found in plant cell membranes, are chemically similar to mammalian cell-derived cholesterol. It has been shown in many in vitro and in vivo studies that β-sitosterol has various biological effects, including anxiolytic and sedative effects, analgesic, immunomodulatory, antimicrobial, anticancer, anti-inflammatory, and lipid-lowering effects; it is also hepatoprotective and showed a protective effect against nonalcoholic fatty liver disease.[43] Hydrocarbons, another important group of organic compounds, are found in the flower, leaf, stem and root extracts of *O. mutabilis*. Hexadecane, tri-tetracontane, heptadecane, octadecane, nonadecane, tricosane, hexacosane, tetra-

Table 3. Phytochemical contents of flower, leaf, stem, and root samples of *O. mutabili*s analyzed by GC-MS.

Compound	Chemical Formula	Flower	Leaf	Stem	Root	t_R (min)	CAS NO
		%					
Butanoic acid	$C_4H_8O_2$	–	2.65	–	–	9.67	016844-99-8
1*H*-Indole	C_8H_7N	3.40	1.24	1.24	2.99	18.21	000120-72-9
Decaborane	$B_{10}H_{14}$	–	5.37	–	–	29.1	017702-41-9
Hexadecane	$C_{16}H_{34}$	–	–	–	0.43	35.74	000638-36-8
Tri-tetracontane	$C_{43}H_{88}$	–	–	–	0.20	35.85	007098-21-7
Heptadecane	$C_{17}H_{36}$	–	–	–	1.35	37.23	000629-78-7
Octadecane	$C_{18}H_{38}$	–	–	–	4.06	39.60	000593-45-3
Neophytadiene	$C_{20}H_{38}$	2.45	6.84	–	–	41.03	000504-96-1
Hexanamide	$C_6H_{13}NO$	–	–	–	1.57	43.51	998195-79-6
Hexadecanoic acid	$C_{16}H_{32}O_2$	10.5	4.07	0.85	8.69	44.18	000057-10-3
14*ß*-Pregnane	$C_{21}H_{36}$	–	–	–	1.00	44.55	998433-89-7
1,7-Dimethyl Phenanthrene	$C_{16}H_{14}$	–	–	–	8.63	45.85	000483-87-4
Phytol	$C_{20}H_{40}O$		4.63	–	–	47.51	000150-86-7
Azaperone	$C_{19}H_{22}FN_3O$	–	–	–	3.51	48.08	001649-18-9
Linoleic acid	$C_{18}H_{32}O_2$	2.06	–	–	–	48.23	998405-19-4
Ethyl linoleate	$C_{20}H_{36}O_2$	4.81	–	–	5.23	48.55	000544-35-4
14-Pentadecenoic acid	$C_{15}H_{28}O_2$	–	–	–	7.52	48.66	017351-34-7
9,12,15- Octadecatrienoic acid	$C_{18}H_{36}O_2$	14.0	3.97	–	–	48.73	001191-41-9
Octadecanoic acid	$C_{18}H_{36}O_2$	4.71	17.1	17.1	10.04	49.22	000111-61-5
Hexacosane	$C_{26}H_{54}$	–	–	3.12	–	51.42	000630-01-3
Tetracosane	$C_{24}H_{50}$	–	–	4.40	–	54.14	000646-31-1
Nonadecane	$C_{19}H_{40}$	4.04	1.73	5.75	0.74	56.83	000629-92-5
Tricosane	$C_{23}H_{48}$	2.63	1.32	0.57	–	57.65	000638-67-5
Heneıcosane	$C_{21}H_{44}$	–	–	5.82	–	58.08	000629-94-7
Docosane	$C_{22}H_{46}$	–	–	9.82	–	58.48	000629-97-0
Heptacosane	$C_{27}H_{56}$	5.45	–	–	–	59.72	000593-49-7
Octacosane	$C_{28}H_{58}$	–	–	8.51	–	60.73	000630-02-4
Eicosane	$C_{20}H_{42}$	0.68	7.31	4.63	–	61.78	000112-95-8
β-Sitosterol	$C_{29}H_{50}O$	1.49	–	–	–	68.91	000083-46-5

cosane, eicosane, heneicosane, heptacosane, docosane, and octacosane are among the hydrocarbons detected based on GC-MS results. Among these compounds, eicosane is interesting for its antibacterial activity,[44] heneicosane for its antimicrobial effect,[45] and tetracosane, heptadecane, hexadecane for its antioxidant and antimicrobial properties.[44,46,47] Decaborane, which is found in the leaf part of *O. mutabilis*, attracts attention due to its toxic and volatile properties.[48] 1H-Indole, an aromatic organic compound, was detected in all parts of the plant, including flowers, leaves, stems, and roots. In the study by Jabbar in 2021 that evaluated the phytochemical content, antioxidant properties and toxicity of *O. mutabilis*, the plant was examined as a whole and the contents of flowers, leaves, stems, and roots of the plant were not compared in terms of phytochemicals. However, according to our results, the parts of the flower, leaves, stem and root of the plant contain different bioactive species at different rates. On the contrary, among the 29 compounds found in our study, unlike Jabbar's previous report, many different compounds have been detected, mainly phytol, neophytadiene, 14ß-Pregnane, 1H-Indole, linoleic acid, ethyl linoleate, 14-pentadecenoic acid, 9,12,15-octadecatrienoic acid, octadecanoic acid, tricosane, hexacosane, tetracosane, heneicosane, heptacosane, docosane, and octacosane. Therefore, endemic *O. mutabilis* can be considered as a bioactive agent with superior potential for pharmacological and chemical applications.

4. Conclusion

Ethanolic extracts obtained from different parts of *O. mutabilis* collected from the Mersin region of Turkey have antioxidant and antibacterial effects due to the large number of bioactive compounds (hexadecanoic acid and β-sitosterol, etc.). Our results show that there is a positive correlation between the amount of phenolic substances and free radical scavenging activities. In our study, the root and stem showed the highest antioxidant activity, respectively. Furthermore, it was found that the root, stem, leaf, and flower extracts of the plant were effective against *A. baumannii* bacteria known as a nosocomial infection agent. Due to the limited information on the anticancer, anti-inflammatory, antifungal, and many other molecular-level properties of the plant, more studies are needed for its pharmaceutical and industrial use.

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5. Reference

1. L. Cecchi, A. Coppi, F. Selvi. *Phytotaxa.* **2016,** *288*, 201–213. **DOI:**10.11646/phytotaxa.288.3.1
2. M. Firat, R. Binzet. *Adansonia* **2021,** *43*, 185-195. **DOI:**10.5252/adansonia2021v43a16
3. R. Binzet, Ö**.** Eren. *Phytotaxa* **2018,** *356*, 117-130. **DOI:**10.11646/phytotaxa.356.2.2
4. R. Binzet. *Turk J Bot* **2016,** *40*, 194-200. **DOI:**10.3906/bot-1410-23
5. A. Tosun, K. E. Akkol, O. Bahadir, E. Yeşilada. *J. Ethnopharmacol.* **2008,** *120*, 378-381. **DOI:**10.1016/j.jep.2008.09.007
6. U. Ozgen, M. Ikbal, A. Hacimuftuoglu, P. J. Houghton, F. Gocer et al. *J. Ethnopharmacol.* **2006,** *104,* 100-103. **DOI:**10.1016/j.jep.2005.08.052
7. V. López, S. Akerreta, E. Casanova, J. M. García-Mina, R. Y. Cavero et al. *Plant Food Hum. Nutr.* **2007,** *62,* 151-155. **DOI:**10.1007/s11130-007-0056-6
8. R. Verpoorte. *J. Pharm. Pharmacol.* **2000,** *52*, 253-262. **DOI:**10.1211/0022357001773931
9. L. Yarmolinsky, M. Bronstein, J. Gorelick. *Isr. J. Plant Sci.* **2015,** *62*, 294-297. **DOI:**10.1080/07929978.2015.1067076
10. Z. Wang, Y. Zhou, X. Shi, X. Xia, Y. He et al. *Food Biosci.* **2021,** *42*, 101206. **DOI:**10.1016/j.fbio.2021.101206
11. N. Aelhidar, A. Nafis, A. Kasrati, A. Goehler, J. A. Bohnert et al. *Ind. Crop. Prod.* **2019,** *130*, 310-315. **DOI:**10.1016/j.indcrop.2018.12.097
12. A. A. Jabbar. *Food Sci. Nutr.* **2021,** 9: 5755-5764. **DOI:**10.1002/fsn3.2544
13. P. Ersan, Ö. Sönmez, B. Gözmen. *J. Iran. Chem. Soc.* **2020,** *17*, 871-879. **DOI:**10.1007/s13738-019-01824-x
14. S. B. Iloki-Assanga, L. M. Lewisluján, C. L. Laraespinoza, A. A. Gilsalido, D. Fernandezangulo et al. *BMC Res. Notes* **2015,** *8*: 1-14. **DOI:**10.1186/s13104-015-1388-1
15. R. Apak, K. Güçlü, M. Özyürek, S. Çelik. E. *Microchim. Acta* **2008,** *160*, 413-419. **DOI:**10.1007/s00604-007-0777-0
16. A. W. Bauer, W. M. Kirby, J. C. Sherris, M. Turck. *Am. J. Clin. Pathol.* **1966**, *45*, 493-496. **DOI:**10.1093/ajcp/45.4_ts.493
17. V. L. Singleton, R. Orthofer, R. M. Lamuela-Raventos. *Methods Enzymol.* **1999,** *299*, 152-178. **DOI:**10.1016/S0076-6879(99)99017-1
18. C. Sarikurkcu, B. Kirkan, M. S. Ozer, O. Ceylan, N. Atilgan, M. Cengiz, B. Tepe. *Ind. Crop. Prod.* **2018,** 115, 323-329. **DOI:**10.1016/j.indcrop.2018.02.040
19. C. Sarikurkcu, E. Demir, M. S. Ozer, R. Binzet. *Int. J. Plant Pharm.* **2022,** 2, 128-135.
20. B. Kirkan, C. Sarikurkcu, M. S. Ozer, M. Cengiz, N. Atılgan, O. Ceylan, B. Tepe. *Ind. Crop. Prod.* **2018,** 125, 549-555. **DOI:**10.1016/j.indcrop.2018.09.043
21. B. Doustı, F. Nabıpor. *J. Biotechnol. Comp. Biol. Bionanotechnol.* **2021,** 102, 377-386. **DOI:**10.5114/bta.2021.111095
22. B. Emsen, B. Surmen, H. S. Karapinar. *Plant Biosyst.* ***2022,*** 1-10. **DOI:**10.1080/11263504.2023.2165561
23. H. Koraqi, FC Ajazi, K. Kimete Lluga-Rizani, S. Kazlauskaite. *Croat. J. Food Sci. Techno. l* **2022,** *14*: 1-8. **DOI:**10.17508/CJFST.2022.14.1.12
24. M. M. Ebulue. ASEAN *J. Sci. Eng.* **2022,** *3*, 69-78. **DOI:** 10.17509/ajse.v3i1.45017
25. J. R. Soares, T. C. P. Dins, A. P. Cunha, L. M. Ameida. *Free Rad. Res.* **1997,** *26*, 469-478. **DOI:**10.3109/10715769709084484
26. T. H. Lee, C. H. Lee, P. Y. Ong, S. L. Wong, N. Hamdan et al. *S. Afr. J. Bot.* **2022,** *148:* 170-179. **DOI:**10.1016/j.sajb.2022.04.026
27. F. B. Mukeba, J. B. Mukoko, M. M. Mayangi et al. *Eur. J. Med. Plants* **2020,** *31*: 33-47. **DOI:**10.9734/ejmp/2020/v31i2030355
28. H. Kikuzaki, M. Hisamoto, K. Hirose, K. Akiyama, H. Taniguchi. *J. Agric. Food Chem.* **2002,** *50*: 2161-8. **DOI:**10.1021/jf011348w
29. K. Saravanakumar, C. Sarikurkcu, R. T. Sarikurkcu, M. H. Wanga. *Ind. Crop. Prod.* **2019,** *142*, 111878. **DOI:**10.1016/j.indcrop.2019.111878
30. C. Sarikurkcu, S. S. Sahinler, B. Tepe. *Ind. Crop. Prod.* **2020,** 154, 11263. **DOI:**10.1016/j.indcrop.2020.112633
31. N. Kumar, A. Singh, D. K. Sharma, K. Kishore. *Int. J. Pharm. Biol. Sci.* **2017,** 7 (3), 30-35.
32. B. Kirkan, C. Sarikurkcu, A. S. Tepe. *Biointerface Res. Appl. Chem.* **2023,** 13 (1): 1-10. **DOI:**10.33263/BRIAC131.088
33. M. Rafi, L. Wulansari, D. A. Septaningsih, T. F. Purnomo, R. Auliatifani, K. Khaydanur et al. *J. Trop. Life Sci.* **2021,** *11*, 375-382. **DOI:**10.11594/jtls.11.03.14
34. P. Z. Moghaddam, M. Mazandarani, M. R. Zolfaghari, M. T. Badelehand, E. A. Ghaemi E. *Afr. J. Microbiol. Res.* **2012,** *6*, 1776-1781. **DOI:**10.5897/AJMR11.1225
35. A. Halim, M. A. Zeb, M. Sajid, T. U. Rahman, K. F. Khattak, S. Ullah, et al. *J. Microbiol. Exp.* **2015,** 3: 00074. **DOI:**10.15406/jmen.2015.02.00074
36. M. Saha, P. K. Bandyopadhyay. *Microb. Pathog.* **2020,** *141*: 103977. **DOI:**10.1016/j.micpath.2020.103977
37. V. Aparna, K. V. Dileep, P. K. Mandal, P. Karthe, C. Sadasivan et al. *Chem. Biol. Drug Des.* **2012,** *80*, 434-439. **DOI:**10.1111/j.1747-0285.2012.01418.x
38. D. Richard, K. Kefi, U. Barbe, P. Bausero, F. Visioli. *Pharmacol. Res.* **2008,** *57*, 451-455. **DOI:**10.1016/j.phrs.2008.05.002
39. C. C. M. P. Santos, M. S. Salvadori, V. G. Mota, L. M. Costa, A. A. C. Almedia et al. *J. Neurosci.* **2013,** 949452. **DOI:**10.1155/2013/949452
40. Y. Song, S. K. Cho. *Biochem. Anal. Biochem.* **2015,** *4*: 1-7. **DOI:**10.4172/2161-1009.1000211
41. S. Vats, T. Gupta. *Physiol. Mol. Biol. Plants* **2017,** *23*, 239-248. **DOI:**10.1007/s12298-016-0407-6
42. D. Durak, Y. Kalender. *Folia Biol.* **2007,** *55*, 133-141. **DOI:**10.3409/173491607781492551
43. S. Babu, S. Jayaraman. *Biomed. Pharmacother.* **2020,** *131*, 110702. **DOI:**10.1016/j.biopha.2020.110702

44. O. Boussaada, S. Ammar, D. Saidana, J. Chriaa, I. Chraif et al. *Microbiol. Res.* **2008,** *163*, 87-95. **DOI:**10.1016/j.micres.2007.02.010
45. V. Vanitha, S. Vijayakumar, M. Nilavukkarasi, V. N. Punitha, E. Vidhya, P. K. Praseetha. *Ind. Crop. Prod.* **2020,** *154*, 112748. **DOI:**10.1016/j.indcrop.2020.112748
46. T. Rhetso, R. Shubharani, M. S. Roopa, V. Sivaram. *Future J. Pharm. Sci.* **2020,** *6*, 1-9. **DOI:**10.1186/s43094-020-00100-7
47. S. Yogeswari, S. Ramalakshmi, R. Neelavathy, J. Muthumary. *Glob. J. Pharmacol.* **2012,** *6*, 65-71. **DOI:**10.1080/00431672.2012.666178
48. G. B. Dunks, K. Palmer-Ordonez, E. Hedaya, P. Keller, P. Wunz. *Inorg. Synth.* **1984,** *22*, 202-207. **DOI:**10.1002/9780470132531.ch46

Povzetek

Rod *Onosma L.* (Lithospermae, Boraginaceae) vsebuje številne rastlinske vrste, ki imajo zaradi številnih fitokemikalij terapevtske lastnosti. *Onosma mutabilis* Boiss. & Hausskn. ex Boiss. (*Onosma mutabilis*) je vrsta, za katero ni dovolj podatkov o njenih značilnostih.

Cilj: Raziskali smo skupno vsebnost fenolov, antioksidativno aktivnost, možne bioaktivne spojine in antibakterijske aktivnosti etanolnih izvlečkov listov, stebla, korenin in cvetnih delov endemične *O. mutabilis*.

Zaključki: Skupna vsebnost fenolov v vseh ekstraktih *O. mutabilis* se je gibala med 9,2 in 31 mg ekvivalentov galne kisline (GAE)/g ekstrakta. Glede na rezultate antioksidativne aktivnosti so bile vrednosti antioksidativne kapacitete IC_{50}, določene z metodo 1,1-difenil-2-pikrilhidrazil (DPPH), med 4,39 in 29 µg/ml, medtem ko je bila ekvivalentna antioksidativna aktivnost trolox, določena z merjenjem reducirajoče antioksidativne vrednosti bakrovih ionv (CUPRAC), 0,45–0,78 mmol trolox ekvivallentov (TE)/g ekstrakta. Bioaktivne spojine so bile analizirane z uporabo plinske kromatografije v povezavi z masno spektrometrijo (GC/MS) in ugotovljeno je bilo, da vsebujejo 29 različnih kemičnih sestavin. Vsi testirani rastlinski izvlečki so pokazali učinkovito antibakterijsko delovanje proti *A. baumannii* (ATCC 02026) (vrednost minimalne inhibitorne koncentracije (MIC) 62,5 µg/ml) v primerjavi z referenčnim zdravilom ampicilin (125 µg/ml).