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DETERMINATION OF THE ANTIMICROBIAL AND ANTIOXIDANT CAPACITY OF *MENTHA DUMETORUM* ESSENTIAL OIL

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ABSTRACT. This study evaluated the antimicrobial and antioxidant properties of *Mentha dumetorum* essential oil. The oil was extracted by steam distillation and analyzed using GC-MS. The major components identified were linalool (21.76%), linalyl acetate (20.76%), elemol (11.63%), eucalyptol (7.99%), and α-terpineol (7.82%). Antimicrobial activity was tested using agar well diffusion and microdilution methods against seven bacterial and three yeast strains. The oil exhibited strong antibacterial and antifungal activities, with inhibition zones ranging from 8.56-12.04 mm and 19.41-22.88 mm, respectively. Minimum inhibitory concentration values confirmed significant activities against *Staphylococcus epidermidis* and *Candida* species. Antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), as well as copper(II) and iron (III) ions, reducing antioxidant power activity assays. Radical scavenging activity was relatively weak compared to Trolox, while reducing power was significant, especially in copper(II) and iron(III) ions, reducing antioxidant power activity analyses. These findings suggest that the bioactive constituents of *M. dumetorum* contribute to its antimicrobial and antioxidant reducing potential. The oil shows promise as a natural antimicrobial agent and may enhance oxidative stability in pharmaceutical, cosmetic, or food applications. Further studies should investigate toxicological safety, pharmacological efficacy, and potential synergistic effects with existing antimicrobial drugs.

KEY WORDS: Agar well, Antimicrobial activity, Essential oil, *Mentha dumetorum*, Minimum inhibitory concentration

INTRODUCTION

According to statistics published by the World Health Organization (WHO), microbial infections are the second most common cause of death worldwide. The prolonged and widespread use of antimicrobials has resulted in an increase in pathogenic microorganisms that are resistant to multiple drugs. Consequently, infectious diseases have become more frequent, and the number of microorganism species resistant to approved antimicrobial drugs has grown [1]. For example, penicillin resistance, which is commonly seen in respiratory tract infections, varies between 0% and 51% worldwide; while 8% to 65% of *Escherichia coli*, known to cause urinary tract infections, has developed resistance to ciprofloxacin [2]. In a study conducted during the COVID-19 pandemic, it was reported that the majority of *Klebsiella pneumoniae* isolates isolated from intensive care units were resistant to ampicillin and ceftazidime [3]. Therefore, there is an

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increasing need for initiatives aimed at discovering effective potential therapies with minimal side effects that could serve as alternatives to the agents currently used in treatment [4].

Plants play a vital role in the pharmaceutical industry because they contain biologically active components [5]. Many of the synthetic drugs used today are plant-derived or synthetic derivatives of plant-derived components. The resistance mechanisms developed by pathogenic microorganisms against antimicrobial drugs necessitate the discovery of new antimicrobial agents, and studies on plants are increasing day by day for this purpose [6].

Many plants belonging to the Lamiaceae family are rich in secondary metabolites such as essential oils and are used in medicine, pharmacy, food, and cosmetics [7]. Essential oils obtained from various species belonging to the Mentha genus in this family are known to have antimicrobial, antiviral, fungicidal, antioxidant, and insecticidal properties [8, 9]. The content and yield of essential oils vary depending on the species of the plant, the part of the plant used, the production technique, the geographical structure of the region where it is grown, and climatic conditions [10-12]. Research conducted on Mentha species in Turkey has focused on determining the components of their essential oils and how these components vary according to different geographical and climatic conditions.

Mentha dumetorum (M. Dumetorum) is a natural hybrid species of *Mentha longifolia* and *Mentha aquatica*. Based on literature information, the antimicrobial activity observed in *M. aquatica* and *M. longifolia* has sparked interest in the antimicrobial activity of *M. dumetorum* essential oil [4-13-14]. Although studies on the *M. dumetorum* species are limited in number, these studies have focused on the isolation, characterization, and antioxidant activity of compounds in the plant's extracts, as well as the determination of the chemical components of the essential oil. No previous studies have been conducted on the determination of the antimicrobial and antioxidant activities of *M. dumetorum* essential oil. Therefore, this study aims to determine the antimicrobial effect of *M. dumetorum* essential oil on seven standard bacteria and three yeast strains and to evaluate its antioxidant activities using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging activities, CUPRAC, and FRAP reduction power activity analyses.

EXPERIMENTAL

Test plant

The essential oil obtained from the *M. dumetorum* plant, collected from the Isparta region, was utilised in the study conducted at the Phytotherapy Research Laboratory of the University of Health Sciences. M. *dumetorum* used in this study were cultivated under controlled conditions from registered cultivars in the experimental fields of the Faculty of Agriculture, University of Isparta/Turkey, Applied Sciences by Prof. Dr. İsa TELCİ, and the corresponding species certification data are recorded in the university inventory.

Test microorganisms

Staphylococcus aureus ATCC 25823, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606 bacterial strains, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 750 yeast strains were used. All strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

Chemicals and reagents

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma-Aldrich, Germany), 2,2-Diphenyl-1-picrylhydrazyl (DPPH•, Sigma-Aldrich, Germany), Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Sigma-Aldrich, Germany), Copper (II) chloride (Sigma-Aldrich, Germany), Neocuproine (2,9-Dimethyl-1,10-phenanthroline, Sigma-Aldrich, Germany), Ethanol (Sigma-Aldrich, Germany), HCl (Hydrochloric acid, Sigma-Aldrich, Germany). Ammonium acetate, (ISOLAB, Germany), 2,4,6-Tripyridyl-S-triazine (2,4,6-Tris(2-pyridyl)-1,3,5-triazine, Sigma-Aldrich, Germany), FeSO4 (Iron(II) sulfate, Sigma-Aldrich, Germany), $FeCl_3$ (Iron(III) chloride, Sigma-Aldrich, Germany), Potassium peroxydisulfate (ISOLAB, Germany), Dipotassium phosphate, (Sigma-Aldrich, Germany) Sodium acetate trihydrate (Sigma-Aldrich, Germany).

Mueller Hinton Agar (MHA, Condalab, Madrid, Spain), Mueller Hinton Broth (MHB, Condalab, Madrid, Spain), Trypticasein Soy Agar (TSA, Condalab, Madrid, Spain), Sabouraud Dextrose Agar (SDA, Condalab, Madrid, Spain), 2,3,5-triphenyl tetrazolium chloride (TTC-, Sigma-Aldrich, Austria).

Instruments and apparatus

Clevenger Apparatus (İldam cam, Turkey), refrigerator (Bosch, Turkey), Gas Chromatography–Mass Spectrometry (Thermo Fisher Scientific, Milan, Italy), Microplate reader (Synergy Neo2, BioTek, USA), McFarland Densitometer (Biosan Den, Latvia), Vortex mixer (VELP, Italy), Incubator (JEIO TECH, Korea), Micropipette (ISOLAB, Germany).

Extraction of essential oil

Essential oil was obtained from plant material using the water distillation method with a Clevenger apparatus. First, 50 grams of *M. dumetorum* plant material was added to a 2 L glass flask, 1 L of distilled water was added and mixed, and distillation was initiated by boiling. The essential oil was carried by the steam of the boiling water and collected in the burette of the Clevenger apparatus. The boiling process was continued until the amount of collected essential oil remained constant (approximately 2.5 h). The pure water and essential oil collected in the burette were allowed to separate for a while, and the essential oil was collected. Throughout the distillation process, anhydrous sodium sulphate (Na_2SO_4) was added to the volatile oil phase obtained from the Clevenger apparatus to remove trace amounts of residual water present in the oil phase. The obtained oil was stored in an amber coloured vial at +4 °C in a refrigerator until further analysis [15].

Analysis of essential oil components

Volatile oil component analysis was carried out using a Gas Chromatography-Mass Spectrometry (GC-MS) system (Thermo Fisher Scientific ISQ7000). The analysis was performed using a TG-1MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The samples were prepared by dilution with hexane at a ratio of 1:20 (v/v). Injection was performed in split mode with a split ratio of 1:100. The column flow rate was set to 1.0 mL/min, with a purge flow of 3.0 mL/min and a split flow of 100 mL/min. The inlet temperature was set at 340 °C, and 1 µL of the prepared sample was injected into the system. The oven temperature program was initially set to 40 °C for 2 min, then ramped at a rate of 5 °C per minute to 340 °C and held at this temperature for 8 min. The total analysis time was 70 min. The mass spectrometer operated in electron ionization (EI) mode. The transfer line, ion source, and ion optics temperatures were set at 340 °C, 300 °C, and 271 °C, respectively. The scan range was m/z 50-649, and the detector was operated at a dynode

voltage of -10.0 kV. Data acquisition and processing were conducted using Chromeleon 7 software. Compound identification was performed by comparing the acquired mass spectra with those in the flavor2.hp, MAINLIB, and NIST spectral libraries.

Preparation of test microorganisms

The bacteria stored at -80 ℃ used in the study were activated one day in advance on Tryptic Soy Agar (TSA) medium (37 ℃ for 24 h), while the yeasts were activated on Sabouraud Dextrose Agar (SDA) medium (27 ℃ for 24 h). The bacterial and yeast concentrations were adjusted to 0.5 McFarland (10^6 CFU/mL) using Mueller Hinton Broth (MHB) medium and a McFarland device (BioSan) with the aid of a sterile pipette [16, 17].

Determination of antimicrobial effects using the agar well method

Mueller-Hinton Agar (MHA) was used for bacteria, and Sabouraud Dextrose Agar (SDA) for yeasts in the method. An agar punch was used to create equal holes on the solid media prepared in petri dishes; it was sterilized with alcohol and heat after each use. In the method, the essential oil was used in its pure form, and 10 µL was added to the agar wells inoculated with microorganisms. As positive controls, tigecycline was used for bacteria, and voriconazole (20 µL at a concentration of 512 µg/mL) was used for yeasts. The culture media were incubated at 37 ℃ for bacteria and 27 ℃ for yeasts for 24 h. At the end of incubation, the circular diameter (inhibition zones) from the well edge to the point where microbial growth began was measured in mm using a caliper [18].

Determination of antimicrobial effects using the microdilution method

The Minimum Inhibitory Concentration (MIC) values of the essential oil used in the study were determined using the microdilution method [13, 14]. In the microdilution method, sterile, flat-bottomed (Brand) plates with 96 U-shaped wells were used. Except for the first well of the plate, 50 µL of MHB medium was added to all wells for bacteria, and 50 µL of RPMI medium was added for yeasts. Then, 50 µL of volatile oil was added to the first and second starting columns, and serial dilutions ranging from 1 to 0.0019 µL/mL were performed using an automatic pipette, starting from well 2 and continuing to well 11. 50 µL of bacterial suspension at a concentration of 10^6 cfu/mL was added to each well. The last well was left as a growth control. The same procedure was applied for tigecycline and voriconazole, which were used as positive controls, and serial concentrations ranging from 256 µg/mL to 0.125 µg/mL were obtained. All prepared plates were incubated in an incubator at 37 and 27 ℃ for 24 h. After 24 h, 50 µL of a 2 mg/mL 2,3,5-triphenyl tetrazolium chloride (TTC-Merck) solution was added to each well, and the MIC values were determined based on the color change in the plates after 30 min of incubation.

Antioxidant activity studies

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity

The DPPH$^{\bullet}$ radical scavenging activity is based on the reduction of the DPPH$^{\bullet}$ radical by antioxidants present in the samples, leading to its conversion into the non-radical, DPPH form, accompanied by a corresponding decrease in absorbance at 571 nm [19]. The DPPH$^{\bullet}$ solution was prepared in ethanol at a concentration of 0.1 mM. The results were compared with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Ten µL of sample (essential oil) and standard solutions at different concentrations were added to 190 µL of DPPH$^{\bullet}$ solution. The mixture was shaken and incubated at room temperature in the dark for 30 min. The change in

color of the DPPH$^{\bullet}$ solution from purple to yellow or colourless indicates the scavenging DPPH$^{\bullet}$ radical activity. The DPPH• radical scavenging activities of the samples and the standard were expressed as percentage (%) radical scavenging activity, calculated using the equation provided below.

DPPH• radical scavenging activity: $[(A_0-A_1)/(A_0)] \times 100$

Where A_0: Absorbance of the control (DPPH$^{\bullet}$); A_1: Absorbance of the standard (Trolox) or samples.

The IC_{50} values were determined using the linear regression equation obtained from the plot of percentage (%) radical scavenging activity versus sample concentration at various concentrations. The concentrations that scavenged 50% of the DPPH$^{\bullet}$ radical (IC_{50} values) were calculated using the graph curve equation.

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

The 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging activity is based on the absorbance reading at 734 nm of the colorless ABTS molecules formed as a result of the reduction of the green-colored $ABTS^{\bullet+}$ radical cation in the sample [20]. The $ABTS^{\bullet+}$ radical is formed after incubating a 7 mM ABTS solution in a 2.45 mM potassium persulfate solution for an average of 12-16 h. This radical gives a dark green color. This solution is diluted with buffer to adjust the absorbance at 734 nm to ~0.7. The results were compared with the Trolox as a standard. 10 µL of the Trolox standard and samples (volatile oil) at different concentrations were taken, 190 µL of $ABTS^{\bullet+}$ solution was added, and the mixture was left to stand at room temperature for 30 min. The $ABTS^{\bullet+}$ radical scavenging activity of the samples was calculated using the equation given below.

$ABTS^{\bullet+}$ Radical scavenging activity: $[(A_0-A_1)/(A_0)] \times 100$

Where A_0: Control ($ABTS^{\bullet+}$) absorbance; A_1: Absorbance of the standard (Trolox) and samples

The IC_{50} values were determined using the linear regression equation obtained from the plot of percentage (%) $ABTS^{\bullet+}$ radical scavenging activity versus sample concentration at various concentrations. The concentrations that scavenged 50% of the $ABTS^{\bullet+}$ radical (IC_{50} values) were calculated using the graph curve equation.

Copper (II) ion reducing antioxidant activity

The antioxidant activity of copper (II) ions is evaluated based on the reduction of the blue complex formed by Cu^{2+} ions in the samples with Neocuproine to a yellow complex in the presence of antioxidants [21]. For analysis, a 7.5 mM Neocuproine (2,9-dimethyl-1,10-phenanthroline) solution is prepared with ethanol, a 10 mM copper (II) chloride solution, and a 1M ammonium acetate buffer at pH 7.0. Trolox standard at different concentrations was used as a positive control. Fifty µL each of copper (II) solution, neocuproine solution, and ammonium acetate buffer were added to the wells. The mixture was incubated at 37 °C for 15 minutes. 100 µL of volatile oil solutions and standard substance solutions at different concentrations were added. The samples were kept in the dark at 37 °C. The activity resulted in the formation of an orange-colored complex. At the end of incubation, the absorbance values were read at 450 nm against a reference solution without the sample. The reduction power capacities of the samples were calculated as mmol Trolox equivalent (mmol TEAC/g sample) per gram of sample (volatile oil) using the calibration graph equation obtained by using different concentrations of Trolox, which was used as the standard.

Iron(III) ion reducing antioxidant power activity

The determination of the reduction capacity of iron (III) is based on the principle that the [Fe (III)-TPTZ] complex formed by the reaction of Fe (III) with 2,4,6-tripyridyl-S-triazine (TPTZ) is reduced to the dark blue-violet Fe(II)-TPTZ complex under the influence of antioxidants, leading to an increase in absorbance values [22]. The FRAP reagent was prepared by taking 1 mL each of a 10 mM 2,4,6-tripyridyl-S-triazine solution and a 20 mM $FeCl_3$ solution and mixing them with 10 mL of a 300 mM acetate buffer (pH 3.6). Positive controls were prepared using iron (II) sulfate ($FeSO_4$) solutions at different concentrations. For each sample (volatile oil) and standard, 10 µL was added to 300 µL of freshly prepared FRAP reagent at different concentrations. The reaction mixtures were incubated at 37 °C for 4 min. Absorbance values were read at 593 nm. The reduction power of the plant extracts was calculated as mmol Fe^{2+} equivalent per gram of sample (essential oil) (mmol Fe^{2+}/g sample) using the calibration graph equation obtained by using different concentrations of iron(II) sulfate as the standard.

RESULTS AND DISCUSSION

In this study, the antimicrobial and antioxidant activities of *M. dumetorum* essential oil obtained from *M. dumetorum* by hydrodistillation were determined. Additionally, the volatile oil components were analyzed by GC-MS. Although sufficient studies have been conducted on the biological activities of *M. longifolia* L. and *M. aquatica* L., the species from which *M. dumetorum* volatile oil is obtained, studies on *M. dumetorum* are quite limited. Since there are no studies on the components, antimicrobial activity, and antioxidant capacity of *M. dumetorum* essential oil, the discussion section compares it with the essential oils of other plants in the same family (Lamiaceae).

Hydrolysis and GC-MS analysis of essential oil

The relative percentage amounts and retention times of the chemical components of *M. dumetorum* essential oil are given in Table 1. As can be seen in Table 1, the chemical component with the highest amount was analyzed as linalool with a percentage of 21.76 and linalyl acetate with a percentage of 20.76. These two components are followed by elemol at 11.63%, eucalyptol at 7.99%, α-terpineol at 7.82%, geranyl acetate at 3.44%, geraniol at 3.41%, and ß-caryophyllene at 3.1%. The remaining components were found to be a total of 49 chemical compounds, with concentrations ranging from 2% to 0.1%.

When examining the species from which *M. dumetorum* is obtained, it is observed that *M. aquatica* essential oils are rich in various oxygenated monoterpenes and monoterpene ketones, primarily menthofuran, 1,8-cineole, linalool, linalyl acetate, carvone, pulegone, and menthone [23]. In *M. longifolia* essential oils, several components have been identified, with major components including piperitone oxide, carvone, pulegone, menthone, and limonene. Thymol is also among the important components [24]. The essential oil obtained from *M. dumetorum* collected from Tokat province was reported to contain 24 chemical components and to be rich in carvone (39.69%), eucalyptol (14.34%), dihydrocarvone (12.78%), and limonene (7.79%) [25].

Antimicrobial analysis of essential oil

The antimicrobial activity of *M. dumetorum* essential oil was determined against 7 standard bacterial strains and 3 standard yeast strains, tested using agar well and microdilution methods. The antimicrobial activity results obtained for the bacteria used in the study are presented in Tables 2 and 3, while the effects on fungi are presented in Tables 4 and 5.

Table 1. Chemical components of *M. dumetorum* essential oil.

No.	Peak Name	Retention Time (min)	Relative Area (%)	No.	Peak Name	Retention Time (min)	Relative Area (%)
1	α-Pinene	8.858	0.4	26	Carvyl acetate	21.489	0.27
2	Sabinene	9.939	0.55	27	ß-Elemene	21.986	0.4
3	L-ß-Pinene	9.939	0.93	28	Valencene	22.513	0.3
4	Myrcene	10.436	1.4	29	ß-Caryophyllene	22.69	3.1
5	Eucalyptol	11.555	7.99	30	α-Humulene	23.493	0.3
6	Limonene	11.633	0.47	31	(E)-ß-Famesene	23.493	0.56
7	trans-ß-Ocimene	11.892	0.74	32	D-Germacrene	24.03	1
8	ß-Ocimene	12.218	1.3	33	Peruviol	24.918	0.47
9	Terpinolene	13.391	0.37	34	d-Cadinene	25.02	0.31
10	Linalool	13.629	21.76	35	Elemol	25.472	11.63
11	1-Octen-3-yl-acetate	13.976	0.31	36	Farnesol (Z,E-)	26.186	0.19
12	L- α -Terpineol	15.367	0.49	37	Caryophyllene oxide	26.322	0.08
13	α -Terpineol	16.14	7.82	38	Guaiyl acetate	26.595	0.12
14	Nerol	17.19	1.84	39	8-epi-.gama.-eudesmol	27.129	0.07
15	Geraniol	18.027	3.41	40	Eremophilene	27.377	0.09
16	Linalyl acetate	18.163	20.76	41	T-Cadinol	27.628	0.23
17	Isobornyl acetate	18.881	0.13	42	ß-Eudesmol	27.805	1.21
18	Lavandulyl acetate	19.017	0.12	43	α -Eudesmol	27.928	1.31
19	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	19.122	0.15	44	6-epi-shyobunol	28.72	0.18
20	Dihydroedulan II	19.235	0.11	45	Hexadecamethyl-cyclooctasioxane	29.441	0.13
21	Limonen-10-yl acetate	20.405	0.12	46	Octadeamethyl-cyclononasiloxane	32.842	0.04
22	Piperitenone oxide	20.483	0.07	47	Palmitic acid	34.322	0.08
23	Terpinyl acetate	20.483	0.09	48	Cyclodecasiloxane, eicosamethyl-	35.577	0.1
24	Neryl acetate	20.904	1.88	49	Phytol	37.25	0.07
25	Geranyl acetate	21.381	3.44				

Table 2. Inhibition zone diameters (mm) of *M. dumetorum* essential oil and tigesiclin on bacterial strains.

Strains	*M. dumetorum* essential oil	Tigesiclin (512 µg/mL)
Staphylococcus aureus ATCC 29213	9.13	28
Escherichia coli ATCC 25922	10.24	26.25
Salmonella enterica ATCC 13076	11.87	24.94
Staphylococcus epidermidis ATCC 12228	12.04	20.71
Acinetobacter baumannii ATCC 19606	11.00	22.84
Pseudomonas aeruginosa ATCC 27853	-	4.68
Bacillus subtilis ATCC 6633	8.56	15.72

-: No zone formation.

Table 3. MIC values of *M. dumetorum* essential oil and tigesiclin against bacterial strains.

Strains	*M. dumetorum* essential oil		Tigesiclin (µg/mL)
	(µL/mL)	(%) v/v	
Staphylococcus aureus ATCC 29213	0.0078	0.2	0.5
Escherichia coli ATCC 25922	0.0039	0.1	0.5
Salmonella enterica ATCC 13076	0.0078	0.2	2
Staphylococcus epidermidis ATCC 12228	< 0.0019	< 0.048	1
Acinetobacter baumannii ATCC 19606	0. 0625	1.56	2
Pseudomonas aeruginosa ATCC 27853	0.5	12.50	32
Bacillus subtilis ATCC 6633	1	25	64

Table 4. Inhibition zone diameters (mm) of *M. dumetorum* essential oil and voriconazole on yeast species.

Strains	*M. dumetorum* essential oil	Voriconazole (512 µg/mL)
Candida albicans ATCC 10231	21.16	-
Candida tropicalis ATCC 750	19.41	11.59
Candida parapsilosis ATCC 22019	22.88	15.08

-: No zone formation.

Table 5. MIC values of *M. dumetorum* essential oil and voriconazole against yeast strains.

Strains	*M. dumetorum* essential oil		Voriconazole(512 µg/mL)
	(µL/mL)	(%) v/v	
Candida albicans ATCC 10231	0.0312	0.78	4
Candida tropicalis ATCC 750	0.125	3.13	16
Candida parapsilosis ATCC 22019	0.0312	0.78	4

According to the study findings, *M. dumetorum* essential oil was found to produce inhibition zone diameters of varying sizes against all strains except *P. aeruginosa* ATCC 27853. Among the tested bacterial strains, the highest inhibition zone diameter was obtained against the *S. epidermidis* ATCC 12228 strain and recorded as 12.04 mm. The lowest inhibition zone diameter was determined to be 8.56 mm against the *B. subtilis* ATCC 6633 strain. Four different Gram (-) and three different Gram (+) bacteria were used in the study. No significant difference was observed in the inhibition zones formed against Gram (-) and Gram (+) bacteria. Among the tested yeast strains, the highest inhibition zone diameter was obtained against the *C. parapsilosis* ATCC 22019 strain and recorded as 22.88 mm. The 21.16 mm inhibition zone diameter formed by the essential oil against the *C. albicans* ATCC 10231 strain was a notable finding, as voriconazole, used in the control group, was ineffective. The lowest MIC value of the essential oil among the tested bacteria was detected against the *S. epidermidis* ATCC 12228 strain as < 0.0019 µL/mL. The lowest MIC value of the essential oil among the tested yeasts was determined to be 0.0312 µL/mL against *C. albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019 strains. The data obtained using the well agar method were found to be consistent with those obtained using the microdilution method. When the antimicrobial effect of the essential oil against the tested Candida strains was evaluated in terms of inhibition zone, it was observed that it formed a higher inhibition zone diameter against Candida strains than against bacterial strains. When evaluated in terms of MIC values, it was determined that the MIC values of the essential oil on bacteria were lower. Furthermore, based on the MIC values obtained for the essential oil against bacterial and yeast strains, it can be said that the essential oil is more effective than control

antimicrobial agents. In conclusion, it was determined that the essential oil exhibits highly effective antifungal and antibacterial properties against the tested yeast and bacterial strains.

Rasooli and Rezaei [26], evaluated the antimicrobial activities of *M. longifolia* essential oil on *E. coli* and *S. aureus* in their study and suggested that its bactericidal property is due to the high level of limonene (13.7%) in its composition. In our study, the detection of limonene at a low level of 0.47% in the GC-MS analysis of *M. dumetorum* essential oil suggests that other components may also contribute to its antimicrobial activity against *E. coli* and *S. aureus*. In their study, Mimica-Dukic *et al.* [13], reported that the essential oils of *M. aquatica*, *M. piperita*, and *M. longifolia* exhibited varying levels of antimicrobial activity against *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228 strains, and no antimicrobial activity against *P. aeruginosa* ATCC 27853 strain, which is parallel to the antimicrobial activity of *M. dumetorum* essential oil. The major components of *M. aquatica* essential oil are 16.94% menthofuran, 14.15% 1,8-cineole, and 9.36% e-caryophyllene; the major components of *M. piperita* essential oil are 39.63% menthol, 10.44% menthyl acetate, and 8.93% menthone; The major components of *M. longifolia* essential oil were identified as 38.79% piperitone, 11.20% menthone. In the same study, it was stated that the very low MIC values of *M. piperita* and *M. longifolia* essential oils against *C. albicans* were significant when compared to bifonazole. In our study, it was found that the MIC values of *M. dumetorum* essential oil against the three Candida species tested were significantly lower than those of voriconazole, indicating a parallel antifungal activity.

In their study, Mkaddem *et al.* [27], evaluated the antimicrobial activity of *M. longifolia* and *Mentha viridis* essential oils based on inhibition zone diameter and obtained inhibition zones of 19 to 21 mm against *C. albicans*. It is thought that the observed antifungal activity may be related to the main components pulegone and carvone. In our study, it was determined that the essential oil of *M. dumetorum* formed an inhibition zone of 21.16 mm against the *C. albicans* ATCC 10231 species. Although neither essential oil showed any antimicrobial activity against *E. coli* and *S. aureus* in the same study, the antimicrobial activity of *M. dumetorum* essential oil against these species is among the findings obtained in our study. In their study, Li *et al.* [28], found that *M. longifolia* L. essential oil exhibited antibacterial activity against various pathogenic bacteria, with an MIC value of 1.576 mg/mL for *E. coli* ATCC 25922, and the main components of the essential oil were analyzed as carvone (47.39%) and limonene (12.48%). In our study, the MIC value of *M. dumetorum* essential oil against *E. coli* ATCC 25922 was found to be 0.0039 (µL/mL), and its main components were identified as linalool (21.76%) and linalyl acetate (20.76%). Bukhari [29], reported in his study that the minimum inhibitory concentrations of *M. longifolia* essential oil against *E. coli* ATCC 25922 and *C. tropicalis* ATCC 13803 were 3.9 and 62.5 µg/ml, respectively. In our study, the minimum inhibitory concentrations of *M. dumetorum* essential oil against *E. coli* ATCC 25922 and *C. tropicalis* ATCC 750 were determined to be 0.0039 and 0.125 µL/mL, respectively. Tourabi *et al.* [14], found that Moroccan *M. longifolia* L. essential oil contains high levels of piperitenone oxide (53.43%), caryophyllene (20.02%), (-) germacrene D (16.53%), and demonstrated strong antimicrobial activity against *P. aeruginosa* and strong antifungal activity against *C. albicans*. In silico results indicated that piperitenone oxide was responsible for the antibacterial activity, while cis-Muurola-4(15),5-dien and piperitenone oxide were responsible for the antifungal effect. In our study, the fact that *M. dumetorum* essential oil did not form an inhibition zone against *P. aeruginosa* and that the piperitenone oxide ratio was detected at a very low level of 0.07% in the GC-MS analysis supports the findings of Tourabi and coworkers. Due to the increasing number of microorganisms resistant to synthetic antimicrobial agents, the difficulties in treating the diseases they cause are increasing day by day. This study showed that *M. dumetorum* essential oil is significantly effective against the tested bacterial and yeast strains. Thus, this study highlights the need for further research on the production, analysis, and use of this effective essential oil against disease-causing microorganisms in humans. It is hoped that future studies on the antimicrobial activity of *M. dumetorum* essential oil will contribute significantly to human health.

Antioxidant activity of essential oil

The antioxidant activities of essential oils were determined using DPPH• and ABTS•+ radical scavenging activities, as well as CUPRAC and FRAP reduction power activities. The antioxidant activity results of *M. dumetorum* essential oil are presented in Table 6.

Table 6. Findings on the antioxidant activity of *M. dumetorum* essential oil.

	DPPH$^{\bullet}$	ABTS$^{+\bullet}$	CUPRAC	FRAP
	(IC_{50} mg/mL)	(IC_{50} mg/mL)	(µmol TE/g numune)	(µmolFe^{+2}/g numune)
M.dumetorum essential oil	200 ± 0.18	17.66±0.42	6.87±0.6	55.0±4.0
Trolox (Standard)	0.10 ± 0.006	0.10±0.0003		

When comparing the radical scavenging activity findings of the essential oil with Trolox, which is used as a standard for positive control, it was observed that the radical scavenging activity of *M. dumetorum* essential oil was much lower than that of Trolox. However, it was observed that the reducing power activity of *M. dumetorum* essential oil was higher than Trolox.

Since there is no previous study on the antioxidant capacity of *M. dumetorum* essential oil, the discussion section was compared with the essential oils of other plants in the same family (Lamiaceae). In our study, the antioxidant capacity of *M. dumetorum* essential oil was found to be low in both ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ radical scavenging activities when compared to Trolox, but its FRAP reducing power activity was found to be high. Previous studies on *Mentha* species have also yielded similar results. When the antioxidant activities of the essential oils of *Mentha spicata* and *M. longifolia* were examined, it was reported that these oils exhibited only low ABTS$^{\bullet+}$ radical scavenging activity, but their DPPH$^{\bullet}$ radical scavenging activity was much lower [30]. In a study conducted by Akşit and colleagues [31], it was reported that the polar fractions of the water extract of *M. dumetorum*, which were isolated and characterized, exhibited significant antioxidant properties.

Our findings have lower radical scavenging activity compared to Trolox are consistent with some data in the literature on the volatile oils of other plants belonging to the Lamiaceae family. In a study on the antioxidant activity of *Origanum vulgare* (oregano) essential oil, the EC_{50} value of the oil obtained from the flowers was found to be 0.68 mL/mL in the DPPH radical scavenging test, while the stem oil had a lower capacity. It was reported that the activity in this study was due to a correlation between carvacrol content and antioxidant activity [32].

The main components of *M. dumetorum* essential oil include linalool, linalyl acetate, and Elemol. Linalool is known as a monoterpene with antioxidant properties and may contribute to reducing power activity. However, the radical scavenging capacity of linalool may be lower compared to standard antioxidants such as Trolox. This could explain why the radical scavenging activity of *M. dumetorum* essential oil is low, but its reducing power activity is high [33].

In a study on the antioxidant properties of linalool and geraniol, it was demonstrated that these compounds reduce oxidative stress by decreasing intracellular reactive oxygen species and increasing the antioxidant capacity of cells. Additionally, these compounds were found to reduce the secretion of pro-inflammatory cytokines and alter the expression of genes associated with iron metabolism. These effects suggest that linalool may contribute to its reducing power activity [34]. It has been reported in the literature that linalool's radical scavenging capacity is lower than that of standard antioxidants such as Trolox. This difference may explain why essential oils containing linalool have low radical scavenging activity but high reducing power activity [35].

In conclusion, the antioxidant profile of *M. dumetorum* essential oil is similar to that of other members of the Lamiaceae family. The results obtained in different antioxidant tests may vary depending on the specific mechanisms of action of the essential oil components and the nature of the tests. The study indicates that while Mentha species essential oils may not have as strong

radical scavenging abilities as standard antioxidants, they exhibit high reducing power activity. This could be attributed to components like linalool and geraniol present in the essential oils, which contribute to their reducing power. However, their radical scavenging capacity may be somewhat limited.

CONCLUSIONS

This study is the first to determine the biological activities of *M. dumetorum* essential oil. It was found to exhibit significant antimicrobial activity against the bacterial and yeast strains tested in the study. Among the main components of the essential oil, Linalool, Linalyl acetate, and Elemol were observed to contribute to its reducing power activity, but their radical scavenging activity was limited. The antioxidant capacity of *M. dumetorum* essential oil is similar to that of other members of the Lamiaceae family, and the variable results obtained in different antioxidant tests suggest that the chemical compound content of the essential oil may be related to specific mechanisms of action and the nature of the test methods. Future research should focus on the standardization of the essential oil, its toxicological profile, and the clinical evaluation of its pharmacological potential. Additionally, investigating its efficacy against resistant pathogens and its potential synergistic effects with other antimicrobial agents could contribute significantly to the development of new-generation natural antimicrobial compounds.

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