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Chemical Composition and Antimicrobial and Antioxidant Activities of the Essential Oil and Methanol Extract of *Hippomarathrum microcarpum* (Bieb.) from Turkey

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Hippomarathrum microcarpum grows wild in eastern Anatolia, Turkey, and is a plant utilized as food by people. In this study, the in vitro antimicrobial and antioxidant activities of the essential oil and methanol extract from *H. microcarpum* and its essential oil composition were investigated. The essential oil, which has bornyl acetate, caryophyllene oxide, and β-caryophyllene as its main components, exhibited activity against eight bacteria, nine fungi, and a yeast, *Candida albicans*, with minimum inhibitory concentration values ranging from 62.50 to 125 μL/mL; the methanol extract showed weak activity. The antioxidant activity of these extracts was assessed by the β-carotene bleaching test and the 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test. The inhibition of linoleic acid oxidation was very weak for both extracts tested. The inhibition percentages were found to be 22.9 and 33.5% for methanol and essential oil, respectively, at the concentration of 2 g/L. The oil scavenged DPPH at higher concentrations ($IC_{50} = 10.69 \pm 0.05$ mg/mL), but the methanol extract exhibited no activity. The total phenolic content of the methanol extract was found to be 4.7 ± 0.1%.

KEYWORDS: ***Hippomarathrum microcarpum*; antimicrobial activity; antioxidant activity; essential oil; methanol extract; gas chromatography−mass spectrometry (GC-MS)**

INTRODUCTION

The genus *Hippomarathrum* belongs to the family Apiaceae. In Turkey, the genus includes five species: *H. microcarpum*, *H. cristatum*, *H. crassilobum*, *H. scabrum*, and *H. boissieri*. The genus *Hippomarathrum* has erect, much-branched perennial plants of 50−100 cm height. The genus is distrubuted in fields and rocky slopes (*1*). The members of the genus have long been used in folk medicine (*2*). *H. microcarpum* is also utilized as food by the people in eastern Anatolia, Turkey, and is locally called "çakşır" or "çaşır". This wild plant, particularly its stems and leaves, is eaten as a brine-cured food or after cooking in the same way as vegetables.

Recently, there is a growing interest in substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceutics. It has been well-known that essential oils and plant extracts have antimicrobial effects (*3*−*5*). The chemical components of the essential oils and extracts allowed their use in traditional medicine and as a food preservative. In the literature, there is only one paper dealing with the essential oil composition of *H. microcarpum* (*6*). However, the antimicrobial effects of this species and other *Hippomarathrum* species have not been reported in the literature.

The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging, and atherosclerosis (*7*). Although much work has been done on the antioxidant effects of different plant species (*8*−*10*), there is no information about the antioxidant activity of any *Hippomarathrum* species.

As mentioned above, the antimicrobial and antioxidant activities of the aerial part of *H. microcarpum* have not been studied to date. Therefore, the aim of the present work was to study in vitro antibacterial and antioxidant activities of the essential oils and methanol extracts of *H. microcarpum* and to determine the chemical composition of its essential oil by gas chromatography−mass spectrometry (GC-MS).

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MATERIALS AND METHODS

Plant Material. *Hippomarathrum microcarpum* (Bieb.) plants at flowering stage in August 2003 were collected from a rocky mountain area at a height of 1800 m in the vicinity of Köse (which is located at a crossing region between Euro-Siberian and Irano-Turan floristic regions), Gümüşhane, Turkey. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist, Meryem Sengul, in the Department of Biology, Atatürk University, Erzurum, Turkey. Collected plant materials were dried in shadow and ground in a grinder with a 2 mm diameter mesh. A voucher specimen has been deposited at the Herbarium of the Department of Biology, Ataturk University, Erzurum, Turkey (ATA HERB No. 9790).

Chemicals and Standards. Methanol used in preparing the methanol extract was obtained from Sigma (St. Louis, MO). Sodium sulfate needed for isolation of the essential oil was also obtained from Sigma. Nutrient agar (NA), sabouraud dextrose agar, and potato dextrose agar (PDA) used for the disk diffusion assay were obtained from Fluka. Ofloxacin, sulbactam cefoperazone, and netilmicin were obtained from Oxoid. Dimethyl sulfoxide for the microwell dilution assay was obtained from Sigma. Nutrient broth was obtained from Fluka. Amphotericin and Tween 20 used for the minimum inhibitory concentration (MIC) agar dilution assay were obtained from Sigma. Chemicals used in antioxidant activity and total phenolics assays were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). All solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). Pure chemicals used for GC-MS were obtained by Sigma, Fluka, Aldrich, and Merck.

Preparation of the Methanol Extract. The dried and powdered leaves (500 g) were extracted with 1 L of methanol using a Soxhlet extractor (ISOPAD, Heidelberg, Germany) for 72 h at a temperature not exceeding the boiling point of the solvent (*11*). The extract was filtered using Whatman filter paper (no. 1) and then concentrated in vacuo at 40 °Ċ using a rotary evaporator (Büchi Labortechnic AG, Flawil, Switzerland). The residues obtained were stored in a freezer (Nuaire, Inc., Asheville, NC) at −80 °Ċ until further tests.

Isolation of the Essential Oil. Air-dried plant material was subjected to hydrodistillation for 3 h using a Clevenger-type apparatus (Ildam Ltd., Ankara, Turkey) as previously reported (*4*, *5*). The essential oil yield was calculated as 0.74% v/w. The obtained essential oil was dried over anhydrous sodium sulfate and, after filtration, stored at 4 °C until tested and analyzed.

GC-MS Analysis Conditions. The analysis of the essential oil was performed using a Therm-Finnigan Trace GC/Trace DSQ /A1300 (EI quadrapole) (Thermo-Finnigan, San Jose, CA) equipped with an SGE-BPX5 MS capillary column (Scientific Instrument Services Inc., Ringoes, NJ) (30 m × 0.25 mm i.d., 0.25 μm). For GC-MS detection an electron ionization system with an ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The program used was 50−150 °C at a rate of 3 °C/min, held isothermal for 10 min, and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μL were injected manually and in the splitless mode. The components were identified on the basis of the comparison of their relative retention time and mass spectra with those of standards, Wiley7N, TRLIB library data of the GC-MS system, and literature data (*12*). The quantitative data were expressed as area percent. The results were also confirmed by comparison of the compounds' elution order with their relative retention indices on nonpolar phases reported in the literature (*12*).

Antimicrobial Activity. Microbial Strains. The methanolic extracts and the essential oil were tested individually against a range of 40 microorganisms, including 24 bacteria, 15 fungi, and a yeast species. The microorganisms used are listed in **Tables 2** and **3**. Microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine, and Plant Diagnostic Laboratory, Faculty of Agriculture, Ataturk University, Erzurum, Turkey. The identity of the microorganisms used in this study was confirmed by the Microbial Identification System (Sherlock Microbial Identification System version 4.0, MIDI Inc., Newark, DE) in the Biotechnology Application and Research Center at Ataturk University.

Disk Diffusion Assay. The dried plant extracts were dissolved in methanol to a final concentration of 30 mg/mL and sterilized by filtration through 0.45 μm Millipore filters (Schleicher & Schuell, Microscience, Dassel, Germany). Antimicrobial tests were then carried out by the disk diffusion method (*13*) using 100 μL of suspension containing 10^8 colony forming units (CFU)/mL of bacteria, 10^6 CFU/mL of yeast, and 10^4 spores/mL of fungi spread on nutrient agar (NA), sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The disks (6 mm in diameter) impregnated with 10 μL of essential or 10 μL of the methanol solution of the dried plant extracts (300 μg/disk) were placed on the inoculated agar. Negative controls were prepared with the same solvents used to dissolve the plant extracts. Ofloxacin (10 μg/disk), sulbactam (30 μg) plus cefoperazone (75 μg) (105 μg/disk), and netilmicin (30 μg/disk) were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested. The inoculated plates were incubated at 37 °C for 24 h for clinical bacterial strains, 48 h for the yeast, and 72 h for fungi isolates. Plant-associated microorganisms were incubated at 27 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated twice.

Microwell Dilution Assay. The MIC values were determined for the bacterial strains that were sensitive to the essential oil in the disk diffusion assay. The inocula of the bacterial strains were prepared from 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils and extracts of *H. microcarpum*, dissolved in 10% dimethyl sulfoxide (DMSO), were first diluted to the highest concentration (500 μg/mL) to be tested, and then serial 2-fold dilutions were made to obtain a concentration range from 7.8 to 500 μg/mL in 10 mL sterile test tubes containing nutrient broth. The MIC values of the *H. microcarpum* extracts against bacterial strains and *Candida albicans* isolates were determined on the basis of a microwell dilution method (*14*) with some modifications.

The 96-well plates were prepared by dispensing 95 μL of nutrient broth and 5 μL of the inoculum into each well. One hundred microliters from the stock solutions of *H. microcarpum* essential oil prepared at the 500 μg/mL concentration was added into the first wells. Then, 100 μL from the serial dilutions was transferred into the six consecutive wells. The last well containing 195 μL of nutrient broth without compound and 5 μL of the inoculum on each strip was used as a negative control. The final volume in each well was 200 μL. Maxipime (Bristol-Myers Squibb, Princeton, NJ) at a concentration range of 500−7.8 μg/mL was prepared in nutrient broth and used as a standard drug for positive control. The plate was covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker (MS2-Minishaker, IKA, Labortechnik, Staufen, Germany) at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth in each medium was determined by reading the respective absorbance (Abs) at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, VT) and confirmed by plating 5 μL samples from clear wells on nutrient agar medium. The oil tested in this study was screened twice against each organism.

MIC Agar Dilution Assay. The agar dilution method, as described previously by Gul et al. (*15*), was used to determine the MIC values of the fungus isolates. The essential oils of *H. microcarpum* were added aseptically to sterile molten PDA medium, containing Tween 20 (Sigma 0.5%, v/v), at the appropriate volume to produce the concentration range of 7.8−500 μg/mL. The resulting PDA solutions were immediately poured into Petri plates after vortexing. The plates were spot inoculated with 5 μL (10^4 spore/mL) of each fungal isolate. Amphotericin B (Sigma A 4888) was used as a reference antifungal drug. The inoculated plates were incubated at 27 and 37 °C for 72 h for plant and clinical fungus isolates, respectively. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the essential oil at which the absence of growth was recorded. Each test was repeated at least twice.

Antioxidant Activity. *2,2-Diphenylpicrylhydrazyl (DPPH) Assay*. The hydrogen atoms or electron-donating ability of the corresponding extracts and butylated hydroxytoluene (BHT) was determined from the bleaching of purple-colored methanol solution of DPPH. This spec-

trophotometric assay uses the stable radical DPPH as a reagent (*16*−*18*). Fifty microliters of various concentrations of the extracts in methanol was added to 5 mL of a 0.004% methanol solution of DPPH in a 10 mL test tube. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (*I*%) was calculated as

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all of the reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as inhibition percentage against extract concentration. Tests were carried out in triplicate.

β-Carotene−*Linoleic Acid Assay.* In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide formation from linoleic acid oxidation (*17*). A stock solution of β-carotene−linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 mL of chloroform (HPLC grade); 25 μL of linoleic acid and 200 mg of Tween 40 were added as emulsifier because β-carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 mL of distilled water saturated with oxygen was added with vigorous shaking at a rate of 100 mL/min for 30 min; 2500 μL of this reaction mixture was dispersed to test tubes, and 350 μL portions of the extracts, prepared in 2 g/L concentrations, were added. The emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with a positive control BHT and a blank. After this incubation time, the absorbance of the mixtures was measured at 490 nm. Antioxidative capacities of the extracts were compared with those at the BHT and the blank. Tests were carried out in triplicate.

Assay for Total Phenolics. The total phenolic constituents of the *H. microcarpum* extracts were determined using the literature methods involving Folin−Ciocalteu reagent and gallic acid as standard (r^2 = 0.998) (*19*, *20*). The extract solution (0.1 mL) containing 1000 μg of extract was put in a volumetric flask, 46 mL of distilled water and 1 mL of Folin−Ciocalteu reagent were added, and the flask was shaken thoroughly. After 3 min, 3 mL of 2% Na_2CO_3 was added, and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The same procedure was repeated for all of the standard gallic acid solutions (0−1000 μg 0.1 mL^{-1}), and the standard curve was determined using the equation

$$\text{absorbance} = 0.0012 \times \text{gallic acid } (\mu g) + 0.0033$$

Statistical Analysis. SPSS for Windows, version 11.0, was used for statistical analysis. Results were expressed as average ± standard deviation (SD).

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil. As can be seen in **Table 1**, GC-MS analysis of the crude oil resulted in the identification of 46 compounds representing 84.8% of the oil. Bornyl acetate (19.9%), caryophyllene oxide (7.7%), β-caryophyllene (6.3%), and pinocarvone (4.2%) were the main compounds.

The genus *Hippomarathrum* is represented by five subspecies in the Turkish flora: *H. microcarpum*, *H. cristatum*, *H. crassilobum*, *H. scabrum*, and *H. boissieri* (*1*). Within the genus, in Turkey, only the essential oil composition of *H. boissieri* has been investigated (*21*). In this species, β-caryophyllene (25.5%), caryophyllene oxide (9.4%), and α-pinene (8.8%) were found to be the main components. In the literature survey, there has been only one report of the essential oil composition of the species. In the study carried out in Iran (*6*), leaf and flower oils of *H. microcarpum* were analyzed separately. The main components of leaf oil were β-caryophyllene (26.4%), γ-muurolene (19.0%), and linalool (6.1%); the major components of the flower oil were β-caryophyllene (18.5%), γ-muurolene (19.2%), thymol (6.9%), and linalool (5.9%).

Table 1. Chemical Composition of the Essential Oil of *H. microcarpum* (Bieb.)

RI[a]	component	composition (%)	identification[b]
921	α-pinene	0.3	GC-MS, RI, Col
951	2,4(10)-thujadiene	0.5	GC-MS, RI
994	myrcene	0.4	GC-MS, RI, Col
1037	*p*-cymene	0.3	GC-MS, RI, Col
1040	limonene	0.4	GC-MS, RI, Col
1042	β-phellandrene	0.3	GC-MS, RI, Col
1044	1,8-cineole	0.2	GC-MS, RI, Col
1047	*cis*-ocimene	0.8	GC-MS, RI
1057	*trans*-ocimene	tr[c]	GC-MS, RI
1110	linalool	0.3	GC-MS, RI, Col
1145	*cis*-verbenol	1.8	GC-MS, RI
1148	*trans*-verbenol	5.5	GC-MS, RI
1151	camphor	2.5	GC-MS, RI, Col
1155	safranal 1[d]	0.2	GC-MS, RI
1156	safranal 2[d]	1.0	GC-MS, RI
1160	*trans*-pinocamphone	2.3	GC-MS, RI
1162	pinocarvone	4.2	GC-MS, RI
1168	borneol	1.9	GC-MS, RI
1171	*cis*-pinocamphone	0.8	GC-MS, RI
1179	*p*-cymen-8-ol	0.5	GC-MS, RI
1185	α-terpineol	0.4	GC-MS, RI, Col
1186	safranal 4[d]	0.3	GC-MS, RI
1193	verbenone	0.8	GC-MS, RI
1199	*trans*-carveol	0.2	GC-MS, RI, Col
1211	pulegone	2.9	GC-MS, RI
1215	carvone	0.3	GC-MS, RI, Col
1221	piperitone	2.1	GC-MS, RI
1235	bornyl acetate	19.9	GC-MS, RI, Col
1237	sabinyl acetate	0.5	GC-MS, RI
1240	thymol	0.5	GC-MS, RI, Col
1266	piperitenone	1.0	GC-MS, RI
1276	piperitenone oxide	0.3	GC-MS, RI
1278	α-copaene	1.3	GC-MS, RI, Col
1282	β-bourbonene	0.4	GC-MS, RI
1284	β-elemene	3.6	GC-MS, RI
1298	β-caryophyllene	6.3	GC-MS, RI, Col
1313	α-humulene	1.7	GC-MS, RI, Col
1323	germacrene D	3.4	GC-MS, RI
1332	γ-bisabolene	0.6	GC-MS, RI
1335	γ-cadinene	tr	GC-MS, RI
1336	δ-cadinene	2.3	GC-MS, RI
1338	β-sesquiphellandrene	1.4	GC-MS, RI
1347	β-calacorene	0.2	GC-MS, RI
1362	spathulenol	2.6	GC-MS, RI
1364	caryophyllene oxide	7.7	GC-MS, RI, Col
total		84.8	

[a] Retention index, relative to *n*-decane and non-decane on SGE-BPX%. [b] Col, co-injection; compounds listed in order of elution from a BPX5 MS column. [c] Traces (<0.1%). [d] Tentative identification; correct isomer was not identified.

Our findings regarding the essential oil composition of the aerial parts of *H. microcarpum* plants may not seem to be in accordance with that paper, because in our sample bornyl acetate, caryophyllene oxide, and β-caryophyllene were found to be the major components. This variation can be explained by environmental conditions, such climate, location, seasonal factors, and development stage (*22*−*24*).

Antimicrobial Activity. The antimicrobial activities of *H. microcarpum* essential oil and extracts assayed against the microorganisms in the present study were qualitatively and quantitatively assessed by evaluating the presence of inhibition zones, zone diameter, and MIC values. The results are given in **Tables 2** and **3**. The results showed that the essential oil of *H. microcarpum* had antimicrobial activity against eight bacteria, nine fungi, and one yeast species tested. On the other hand, the methanol extract from aerial parts of *H. microcarpum* plants

Table 2. Antimicrobial Activities of *H. microcarpum* (Bieb.) Extract and Essential Oil against the Bacterial Strains Tested

	plant extract (MeOH)		essential oil		antibiotics	
tet bacterium	DD[a]	MIC[b]	DD[c]	MIC[b]	DD[d]	MIC[e] (max)
Acinetobacter baumanii A8			9	250	18 (OFX)	31.25
Bacillus macerans A199	14	62.50	26	15.62	19 (OFX)	15.62
Bacillus megaterium A59					9 (SCF)	15.62
Bacillus subtilis ATCC-6633					28 (OFX)	62.50
Bacillus subtilis A57			12	62.50	28 (OFX)	125
Brucella abortus A77					12 (SCF)	62.50
Burkholdria cepacia A225					22 (SCF)	125
Clavibacter michiganense A227					25 (SCF)	16.62
Enterobacter cloacae A135					20 (NET	31.25
Enterococcus faecalis ATCC-29122			11	125	18 (SCF)	31.25
Escherichia coli A1					20 (OFX)	62.50
Klebsiella pneumoniae A137					12 (OFX)	125
Proteus vulgaris A161					12 (OFX)	125
Proteus vulgaris KUKEM1329			10	250	13 (OFX)	125
Pseudomonas aeruginosa ATCC-9027					22 (NET)	31.25
Pseudomonas aeruginosa ATCC-27859					22 (NET)	15.62
Pseudomonas syringae pv. *tomato* A35					24 (OFX)	125
Salmonella enteritidis ATCC-13076					27 (SCF)	62.50
Staphylococcus aureus A215			8	31.25	22 (SCF)	31.25
Staphylococcus aureus ATCC-29213			9	250	22 (SCF)	62.50
Staphylococcus epidermis A233					12 (SCF)	15.62
Streptococcus pyogenes ATCC-176			8	250	10 (OFX)	62.50
Streptococcus pyogenes KUKEM-676					13 (OFX)	31.25
Xanthomonas campestris A235	20	31.25			20 (SCF)	31.25

[a] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 300 μg/disk of methanol extract. [b] Minimal inhibitory concentration as μg/mL. [c] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 10 μL of essential oil. [d] DD, diameter of disk diffusion (mm); OFX, ofloxacin (10 μg/disk); SCF, sulbactam (30 μg) + cefoperazone (75 μg) (105 μg/disk); and NET, netilmicin (30 μg/disk) were used as positive reference standards antibiotic disks (Oxoid). [e] Maxipine (μg/mL) was used as reference antibiotic in micro well dilution assay (Sigma).

Table 3. Anticandidal and Antifungal Activities of the Extract and Essential Oil of *H. microcarpum* against the Yeast and Fungus Isolates Tested

	plant extract (MeOH)		essential oil		antibiotics	
test microorganism	DD[a]	MIC[b]	DD[c]	MIC	DD[d]	MIC Amp B[e]
yeast						
Candida albicans A117			7	250	(NET)	31.25
fungi						
Alternaria alternate	15	31.25	35	62.50	(NET)	31.25
Aspergillus flavus			20	31.25	(NET)	15.62
Aspergillus variecolor	20	62.50			(NET)	15.62
Fusarium acuminatum					(NET)	62.50
Fusarium oxysporum	21	62.50	35	62.50	(NET)	62.50
Fusarium solani	24	31.25	7	62.50	(NET)	62.50
Fusarium tabacinum					(NET)	62.50
Moniliania fructicola					(NET)	15.62
Penicillum spp.					(NET)	31.25
Rhizopus spp.					(NET)	125
Rhizoctonia solani			35	31.25	(NET)	31.25
Sclorotinia minor			34	61.50	(NET)	125
Sclorotinia sclerotiorum			35	61.50	(NET)	62.50
Trichophyton rubrum			38	31.25	(NET)	31.25
Trichophyton mentagrophytes			35	61.50	(NET)	15.62

[a] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 300 μg/disk of methanol extract. [b] Minimal inhibitory concentration as μg/mL. [c] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 10 μL of essential oil. [d] DD, diameter of disk diffusion (mm); NET, netilmicin (30μg/disk) was used as positive reference standard antibiotic disk (Oxoid). [e] Amphotericin B (μg/mL) was used as reference antibiotic in MIC agar dilution (Sigma).

showed antimicrobial activity on two bacteria and four fungi (**Tables 2** and **3**), implying that the methanol extract of *H. microcarpum* had weak antimicrobial activity. The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oil of *H. microcarpum*, were in the range of 8−26 mm, and 15.62−250 μL/mL, respectively (**Tables 2** and **3**). The maximal inhibition zones and MIC values of the yeast and fungi species sensitive to the essential oil of *H. microcarpum* were 7−38 mm and 31.25−250 μL/mL, respectively (**Tables 2** and **3**). The antimicrobial and antifungal activities of the essential oils and plant extracts of the *Hippomarathrum* species have not been studied up to now. However, it was previously reported that the essential oil or extracts of some members of the Apiaceae family, including *Ferula gummusa*, *Prangos uechtritzii*, and *Ferula persica* (*25*−*27*), had antimicrobial activity.

The antibacterial and antifungal activities of *H. microcarpum* may be attributed to the presence of the main components in the essential oil: bornyl acetate (*28*), caryophyllene oxide (*29*), β-caryophyllene (*30*), and pinocarvone (*5*, *31*).

As seen from **Tables 2** and **3**, the essential oil has a stronger and broader spectrum of antimicrobial activity as compared to

Table 4. Antioxidant Ability of *H. microcarpum* (Bieb.)

extract	DPPH assay (μg/mL)	linoleic acid assay (RAA %)	gallic acid equivalent total phenolic (μg/mg)
methanol	not active	22.9 ± 2.0	47.0 ± 1.0
essential oil	10690.0 ± 0.5	33.5 ± 2.0	
BHT	19.8 ± 0.5	96 ± 2	

the methanol extract tested. Another remarkable result from this study was that Gram-positive bacteria were more sensitive to the essential oil. This finding concurs with a previous paper (*32*). To understand the nature of the aforesaid activities, a comprehensive study is necessary, including pure compounds isoated from the essential oil in particular.

Antioxidant Activity. Antioxidants retard oxidation and are sometimes added to meat and poultry products to prevent or slow oxidative rancidity of fats that cause browning and deterioration. Antioxidant agents are effective following different mechanisms such as free radical scavenging, quenching, or inhibition of reaction mechanisms. The inhibition ability of linoleic acid oxidation and DPPH radical scavenging activities and the amount of total phenolic compounds of methanolic extract and essential oil of *H. microcarpum* have been tested. The results obtained from the above test methods are given in **Table 4**. The relative antioxidant activity (RAA %) of the extracts was calculated from the equation

$$\text{RAA \%} = A_{\text{Sample}}/A_{\text{BHT}} \times 100$$

where A_{BHT} is the absorbance of the positive control BHT and A_{Sample} is the absorbance of the extract. The essential oil obtained from herbal parts exhibited weak activity (10.69 ± 0.8 mg/mL). On the other hand, the methanol extract was not effective at low concentrations, and scavenging action over 20.00 mg/mL concentration was concluded as possessing no activity. In the case of the linoleic acid system, both extracts seemed not to possess any antioxidant ability for preventing the linoleic acid oxidation (**Table 4**). The total phenolic constituent of the methanol extract was found to be 4.7%. As can be seen from **Table 4**, inhibition was due to the radical scavenging ability of the essential oil rather than another mechanism. The major components of the oil were bornyl acetate (19.9%), caryophyllene oxide (7.7%), β-caryophyllene (6.3%), and pinocarvone (4.2%), and none of these chemicals were reported to exhibit strong antioxidant properties (*33*).

To our knowledge, this is the first study to provide data that the essential oil of *H. microcarpum* possesses potential antibacterial, antifungal, and anticandidal activities, with weak antioxidant activity.

These data have provided a wealth of information on the essential oil composition and antimicrobial and antioxidant effects of *H. microcarpum*. A knowledge of wild foods and their chemical compositions, nutritive values, and biological properties could be of great value to further understand and make better use of these foods. Therefore, the other properties of this plant, such as the its nutritive value, should be studied in the future.

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LITERATURE CITED

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