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Chemical Composition of Volatile Extract and Biological Activities of Volatile and Less-Volatile Extracts of Juniper Berry (*Juniperus drupacea* L.) Fruit

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Volatile chemicals in a dichloromethane extract from a steam distillate of juniper berry fruit (*Juniperus drupacea* L.) and its two column chromatographic fractions (eluted with hexane and ethyl ether) were analyzed by gas chromatography/mass spectrometry. The major compounds in the dichloromethane extract were α-pinene (23.73%), thymol methyl ether (17.32%), and camphor (10.12%). A fraction eluted with hexane contained α-pinene (44.24%) as the major constituent. A fraction eluted with ethyl ether had thymol methyl ether (22.27%) and camphor (19.65%) as the main components. Three samples prepared from the distillate and two additional samples prepared by petroleum ether and ethanol extraction directly from juniper berry fruits exhibited clear antioxidant activities with dose response in both 1,2-diphenyl picrylhydrazyl and β-carotene assays. All samples except the hexane fraction showed comparable activities to that of the synthetic antioxidant *t*-butyl hydroquinone at a level of 200 μg/mL in the two testing systems. The extracts of dichloromethane, petroleum ether, and ethanol exhibited appreciable antimicrobial activities against six microorganisms with minimum inhibitory concentrations ranging from 0.5 mg/mL (volatile extract against *Candida albicans*) to 1.2 mg/mL (ethanol extract against *Aspergillus niger*). The results of the present study suggest that this fruit could be a natural antioxidant supplement for foods and beverages.

KEYWORDS: α-Pinene; antimicrobial activity; antioxidant activity; camphor; juniper berry fruit; volatile chemicals

INTRODUCTION

The fruit of *Juniperus drupacea* (Labill), or juniper berry fruit, has a limited distribution in the Eastern Mediterranean region. *J. drupacea* has been classified as a separate genus *Arceuthos*, but a close relationship with *Juniperus oxycedrus* L. was recently was confirmed by the use of DNA fingerprinting (*1*).

The mature female cone of the juniper berry has long been used for flavoring foods, as seasoning for pickling meats, and in alcoholic beverages. The essential oil of the juniper berry has been used in aroma formulations for perfumery and cosmetics as well as in folk medicine for various diseases, such as bronchitis, arthritis (*2*), and several parasitic diseases (*3*).

Many essential oils have been used as therapeutic agents since ancient times. For example, chamomile oil, celery oil, juniper oil, and coriander oil are used for anti-inflammation. Certain essential oils, including juniper, have been scientifically proven to possess medicinal activities such as anti-inflammatory (*4*), antiarthritic (*2*), antiviral (*5*), antitumor (*6*), antihyperglycemic (*7*), and anticarcinogenic (*8*). Recent studies reported that various essential oils, including jasmine, parsley seed, rose, and ylang-ylang exhibit potent antioxidant activities (*9*). The discovery of the antioxidant activity of essential oils, suggesting that essential oils possess great health benefits (*10*), has gained considerable attention among researchers (*11*). Moreover, due to recent safety concerns over synthetic compounds, there has been increasing interest in the use of natural plant substances, including essential oils, for food and medicinal therapy.

In the present study, volatile chemicals of the dried fruit of Syrian *J. drupacea*, obtained by steam distillation followed by dichloromethane extraction, were analyzed by gas chromatography/mass spectrometry (GC/MS). Also, both the volatile extract and the extracts obtained by direct extraction of the same fruit with petroleum ether and ethanol were tested for antimicrobial activity and antioxidant activity. Syrian *J. drupacea* was chosen to study because a previous report on the chemical

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composition of an essential oil from the Syrian *J. drupacea* fruit (*12*) suggested the possible use of this oil for some medicinal purposes.

MATERIALS AND METHODS

Chemicals and Materials. Tertiary butyl hydroquinone (TBHQ) was purchased from Sigma Chemical Co. (St. Louis, MO). Dichloromethane and ethanol were purchased from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). 1,2-Diphenylpicrylhydrazyl (DPPH) and β-carotene were bought from TCI AMERICA (Portland, OR). All solvents were from VWR International (Brisbane, CA). All authentic volatile chemicals were obtained from Takata Koryo Co., Ltd. (Osaka, Japan) as a gift.

Pure cultures of the bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and fungi (*Aspergillus niger*, *Aspergillus parasiticus*, and *Candida albicans*) used for the antimicrobial activity test were provided by the Department of Microbiology, National Research Center (NRC) (Dokki, Giza, Egypt). Mother cultures of each microorganism were set up 24 h before the assays to reach the stationary phase of growth. The tests were assessed by inoculating Petri dishes from the mother cultures with proper sterile media, with the aim of obtaining a microorganism concentration of 10^5 colony forming units (cfu/mL). The fruit of *J. drupacea* L. (juniper berry fruit) was purchased from reliable commercial markets in Egypt.

Isolation and Fractionation of Volatile Extract. The dried crushed juniper berry fruit (200 g) was placed in a 3 L round-bottomed flask with 1 L of deionized water. The solution was steam distilled for 4 h. The distillate (900 mL) was extracted with 100 mL of dichloromethane using a liquid−liquid continuous extractor for 6 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed by a rotary flash evaporator. The distillation was stopped when the volume of extract was reduced to ~1 mL. The solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.5 mL (volatile extract). The volatile extract was stored under nitrogen in a sealed vial at −5 °C until use. The experiment was repeated 3 times.

The volatile extract (0.5 g) was fractionated with a silica gel column (particle size of 30−60 mm). The column was eluted with 100 mL each of hexane (hexane fraction) and ethyl ether (ethyl ether fraction) in series. Each eluate was concentrated to 0.5 mL by the method described previously. The fractions were stored under nitrogen in a sealed vial at −5 °C until further experiment.

Preparations of Petroleum Ether and Ethanol Extracts from Juniper Berry Fruit. The dried crushed juniper berry fruit (200 g each) was soaked in 100 mL each of petroleum ether (bp 40−60 °C) and ethanol (95%) in two different 500 mL Erlenmeyer flasks at room temperature for 7 h. After the extract was filtered, the filtrate was condensed to 0.5 mL by the method described previously. The condensed extracts were stored in the dark at −5 °C until further experiments.

DPPH Radical Scavenging Assay. Radical scavenging activity assays of samples prepared from the dried juniper berry fruit were performed by a previously reported method (*13*). A methanol solution of DPPH (6×10^{-5} M) was prepared immediately before the assay. Various concentrations of each juniper sample (50, 100, 150, and 200 μg/mL) were added to a 1 mL DPPH solution. The reaction mixtures were shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance of the samples was measured by a spectrophotometer at 517 nm. In this assay, TBHQ was used as a standard antioxidant to validate the assay. The experiment was repeated 3 times.

Determination of Antioxidant Activity by β-Carotene Bleaching Assay. The antioxidant activity of the samples also was examined by a β-carotene/linoleic acid system reported previously (*14*). Briefly, 1 mL of a chloroform solution of β-carotene (1 mg/mL), 40 μL of linoleic acid, and 400 μL of Tween 80 (water soluble vitamin E) were placed in a round-bottomed flask. After chloroform was removed under a nitrogen stream, 100 mL of distilled water was added slowly to the residue in the flask, which was subsequently agitated to give a stable emulsion. An aliquot of 4.5 mL of this emulsion was transferred to a 10 mL test tube, and then 500 μL of appropriately diluted juniper samples (50−200 μg/mL) was added. The tubes were placed in a water bath at 50 °C, and the absorbance was measured after 120 min at 470 nm. A blank sample was prepared by adding 500 μL of distilled water to the control reaction mixtures, and the absorbance was measured immediately after preparation at 470 nm.

Analysis of Chemicals in Volatile Extract and Its Fractions. Volatile compounds in the volatile extract and its hexane and ethyl ether fractions were identified by comparison with the Kovats gas chromatographic retention index (KI) (*15*) and by the mass spectral fragmentation pattern of each GC component as compared to those of authentic compounds. An Agilent model 6890 gas chromatograph equipped with a 30 m × 0.25 mm i.d. (d_f = 0.25 μm) bonded phase DB-5 fused silica capillary column (Agilent, Folsom, CA) and a flame ionization detector (FID) was used to obtain the KI, which also was compared to published data (*16–18*). The oven temperature was programmed from 35 to 220 °C at 3 °C/min and held for 40 min. The linear helium carrier gas flow rate was 29 cm/s. The injector temperature was 200 °C, and the detector temperature was 250 °C.

An Agilent model 6890 GC instrument interfaced to an Agilent 5791A mass selective detector was used for mass spectral analysis of the GC components at a MS ionization voltage of 70 eV. A 30 m × 0.25 mm i.d. (d_f = 0.25 μm) DB-Wax bonded phase fused silica capillary column (Agilent, Folsom, CA) was used for GC. The linear velocity of the helium carrier gas was 30 cm/s. The injector and detector temperature was 250 °C. The oven temperature was programmed from 35 to 180 °C at 3 °C/min and held for 40 min. Identification of the GC components also was confirmed with NIST mass spectra library data.

Antibacterial and Antifungal Activity Tests. The microbial activities of the volatile extract, ethanol extract, and petroleum ether extract were tested using the disk-diffusion method. After a Whatman No. 1 filter paper disk (5 mm diameter) was soaked in the testing samples (10 μL), the disk was placed on the surface of the cold solid medium in Petri dishes and then inoculated with the testing organisms.

Sterile trypticase soy agar (TSA) and potato dextrose agar (PDA) media, which were inoculated with the microorganisms tested previously, were allowed to stand for 1 h at 50 °C and then incubated at 370 °C for 24 h for bacteria, at 270 °C for 48 h for yeast, and at 270 °C for 72 h for mold according to a previously reported method (*19*). After incubation, the degree of growth inhibition was determined by measuring the growth inhibition zone in a Petri dish. The degree of growth inhibition of testing samples was evaluated by comparison with those of the controls (gentamycin sulfate and nystatin for bacteria and fungi, respectively). Each assay was repeated 3 times.

Determination of Minimum Inhibitory Concentration (MIC). The MIC is defined as the minimum level of a sample that inhibits the growth (populations) of microbial colonies by ~90%. The MIC was determined by the microdilution agar plate method (*20*). Tempered TSA (90 mL) was agitated vigorously with various concentrations of the volatile extract, ethanol extract, or petroleum ether extracts, each at 3.0, 2.8, 2.5, 2.2, 2.0, 1.5, 1.0, 0.6, 0.5, 0.25, 0.15, 0.10, 0.05, and 0.025 mL/100 mL concentration ranges. Approximately 15 mL of each concentration mixture was transferred with 1 mL of microorganism inocolum to the agar plates. The plates were incubated for 24 h at 37 °C for bacteria, 48 h at 27 °C for yeast, and 72 h at 27 °C for mold. After incubation, the number of colonies in each plate was determined. Each assay was repeated 3 times.

Statistical Analysis. All results were expressed as mean ± standard deviation (n = 3), and the statistical significance was assessed by the Student's t test. The confidence limits were added at $p < 0.05$.

RESULTS AND DISCUSSION

Chemicals Found in Volatile Extract and Its Hexane and Ethyl Ether Fractions. The yield (w/w) of volatile extracts from juniper berry fruit was 3.5 ± 0.05%. The yields (w/w) of petroleum ether and ethanol extracts from juniper berry fruit were 4.67 ± 0.21 and 10.69 ± 0.94%, respectively.

A total of 73 volatile chemicals were identified in the volatile extract. **Table 1** shows the chemicals identified in the volatile

Table 1. Compounds Identified in Volatile Extract of *J. drupacea* Fruit and List of Each Column Chromatographic Fraction

compound	KI[a]	GC peak area %		
		volatile extract	hexane fraction	ethyl ether fraction
Hydrocarbons				
(*E*)-4-octene	843	0.30	ND	0.56
heptane	724	0.01	2.50	0.16
Alkyl-alcohols, -Aldehydes, -Ketones, and -Acid				
hexanal	851	0.20	ND	0.44
1-hexenol	858	0.10	ND	0.85
5-hydroxypentanal	891	0.01	ND	0.63
heptanal	901	0.80	ND	1.70
2-methyl-4-heptanone	926	1.50	ND	3.60
1-octene-3-ol	982	0.01	ND	0.50
dodecanoic acid	1590	0.10	ND	0.12
pentadecanal	1712	2.00	1.53	1.70
hexadecanone	1807	0.09	ND	2.00
ethylhexadecanoate	1838	0.12	ND	1.00
hexadecanol	1876	0.12	ND	1.00
octadecanaldehyde	2048	0.09	ND	0.12
Alkyl Esters				
methylbutyrate	746	4.05	0.29	0.24
buten-2-ol acetate (2-methyl-3-)	790	2.00	0.09	0.06
allylbutyrate	881	*b*	ND	0.27
furfurylhexanoate	1394	*b*	1.01	ND
butyllaurate	1673	0.72	ND	2.73
Terpenes and Terpenoids				
α-pinene	939	23.73	44.24	12.29
exo-5-norbonene-2-ol	946	0.01	ND	0.13
(*Z*)-β-ocimene	1037	1.20	8.93	*b*
stemone	1129	0.07	3.27	*b*
camphor	1141	10.12	ND	19.65
cis-β-terpineol	1156	0.12	0.12	0.45
sabinene hydrate acetate	1157	0.39	ND	0.32
trans-β-terpineol	1162	0.16	ND	0.12
borneol	1185	2.67	0.87	0.32
fenchyl acetate (EXO)	1194	3.86	1.75	0.90
thymol methyl ether	1238	17.32	0.11	22.27
neoisopulegol acetate	1298	0.38	0.20	0.40
γ-terpinen-7-al	1301	0.01	*b*	0.16
trans-verbenyl acetate	1339	0.50	1.11	ND
terpinen-4-ol acetate	1341	0.12	0.80	ND
cyperene	1413	0.12	3.96	ND
β-cedrene	1417	0.40	ND	1.00
dehydroaromadendrene	1471	0.03	ND	0.15
γ-gurjunene	1478	0.01	ND	0.65
γ-muurolene	1499	*b*	ND	0.12
germacrene D	1538	0.01	ND	0.14
α-cadinene	1547	0.10	1.11	ND
germacrene B	1561	0.99	0.30	ND
β-calacorene	1586	0.40	2.15	ND
gleenol	1591	0.08	0.14	0.29
cedranone (5)	1603	0.09	ND	0.20
10-epi-γ-eudesmol	1609	0.14	ND	0.23
1-epi-cubenol	1636	0.36	ND	0.50
3-isothujopsanone	1638	0.78	ND	1.25
β-cedren-9-ol	1647	ND	1.10	0.85
khusinol	1647	0.95	*b*	1.80
(*Z*)-nerolidol acetate	1673	5.64	1.13	2.66
α-bisabolol	1662	ND	ND	0.21
α-cadinol	1681	0.09	ND	0.07
β-farnesol	1695	5.64	1.46	2.80
α-caryophyllene alcohol	1707	0.17	0.22	0.35
(*E*)-farnesol	1724	0.14	0.27	0.13
laurenene	1855	3.13	7.66	0.46
rimuene	1879	4.07	7.02	1.02
nootkatoone	1811	*b*	ND	1.79
diethyl-2-hydroxyglutarate	1821	0.09	ND	1.01
phyllocladene	2014	ND	ND	1.10
abietatriene	2048	0.09	ND	0.02
abietadiene	2082	0.08	0.07	ND
abietal (4-EPI)	2289	1.35	5.29	0.09
abietal	2316	0.04	0.86	3.62
Aromatic Compounds				
benzaldehyde	961	0.02	ND	0.15
2,6-dimethoxyphenol	1368	0.01	0.58	ND
dihydroeugenol	1377	*b*	0.10	ND
vanilline	1398	0.14	0.21	ND
trimethylphenylbutenone	1765	0.25	0.13	0.37
6-methoxyeugenol	2213	ND	0.59	0.23

[a] KI on DB-5. [b] GC peak area % <0.01; ND: not detected.

extract and their hexane and ethyl ether fractions. The GC peak area % and KI on the DB-5 column of the samples also are shown. The major chemicals identified in the volatile extract were α-pinene (23.73%), thymol methyl ether (17.32%), camphor (10.12%), (*Z*)-nerolidyl acetate (5.64%), β-farnesol (5.64%), rimuene (4.07%), methylbutyrate (4.05%), fenchyl acetate (EXO) (3.86%), laurenene (3.13%), borneol (2.67%), 4-epi-abietal (1.35%), and (*Z*)-β-ocimene (1.2%).

The main constituents found in the hexane fraction were α-pinene (44.24%), (*Z*)-β-ocimene (8.93%), laurenene (7.66%), rimuene (7.02%), 4-epi-abietal (5.29%), cyperene (3.96%), and β-calacorene (2.15%). The major components identified in the ethyl ether fraction were thymol methyl ether (22.27%) and camphor (19.65%), followed by α-pinene (12.29%), abietal (3.62%), β-farnesol (2.8%), and (*Z*)-nerolidyl acetate (2.66%).

After fractionation by hexane and ethyl ether, α-pinene, (*Z*)-β-ocimene, cyperene, β-calacorene, laurenene, and 4-epi-abietal

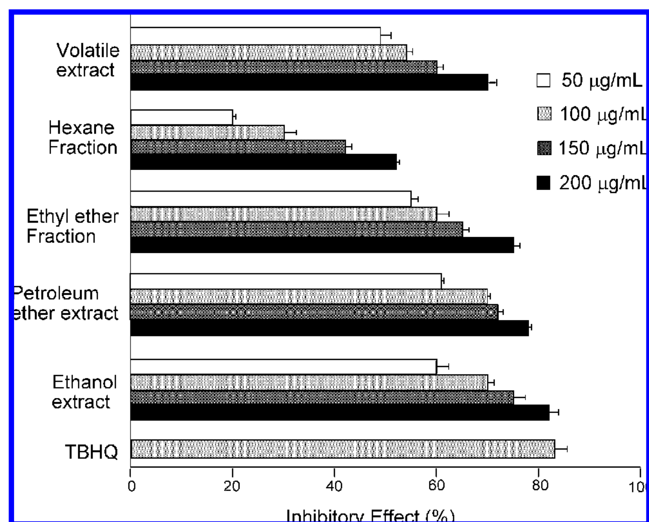

Figure 1. Results of DPPH antioxidant assay on samples prepared from juniper berry fruit and the standard TBHQ.

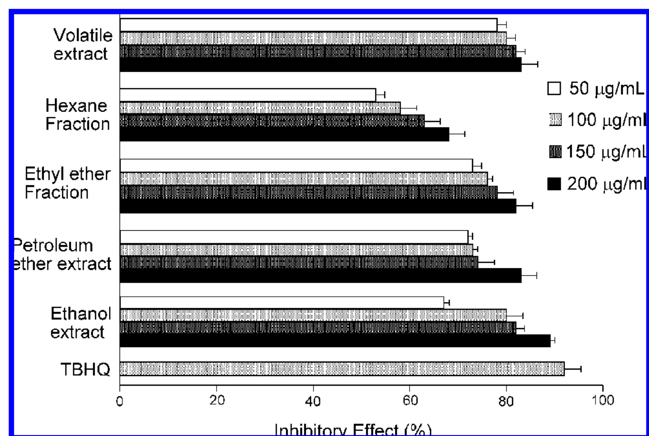

Figure 2. Results of β-carotene bleaching assay on samples prepared from juniper berry fruit and the standard TBHQ.

Table 2. Inhibitory Activity (MZI and MIC) of Extracts Obtained from *J. drupacea* Fruit against Microorganisms

microorganism	volatile extract		petroleum ether extract		ethanol extract	
	MZI[a]	MIC[b]	MZI	MIC	MZI	MIC
S. aureus	17.7	0.6	14.0	0.8	10.0	0.9
P. aeruginosa	20.1	0.6	16.0	0.7	12.0	0.8
E. coli	16.0	0.7	14.0	0.8	9.0	1.0
A. niger	15.3	0.8	13.0	0.9	8.0	1.2
A.parasiticus	19.0	0.6	16.0	0.7	13.0	0.8
C. albicans	21.3	0.5	17.0	0.7	14.0	0.8

[a] Units of mm. [b] Units of mg/mL.

were found in higher concentrations in the hexane fraction than in the original volatile extract. Camphor and thymol methyl ether were found in much higher concentrations in the ethyl ether fraction than in the original volatile extract, whereas they were found only in trace amounts in the hexane fraction. These results suggest that some specific components can be concentrated by fractionation with different organic solvents.

There are no reports on the composition of volatile chemicals found in this fruit prior to the present study. It should be noted that the results obtained in the present study are closely related to those of related juniper species. For example, the *J. oxycedrus* ssp. *Oxycedrus* berry oil from Lebanon reportedly contained a high level of α-pinene (27.4%) (*21*). Volatile chemicals obtained from the berries of Italian *J. oxycedrus* L. by supercritical carbon dioxide extraction also were dominated by α-pinene (10.5%) (*22*). More recently, a large amount of α-pinene (24.9%) was found in *Juniperus communis* berry oil from Spain (*23*). The existence of a close relationship between *J. drupacea* and *J. oxycedrus* recently was confirmed by a DNA fingerprinting method (*1*).

Antioxidant Activity of Samples Prepared from Juniper Berry Fruit. The antioxidant activity was evaluated using DPPH and β-carotene assays. DPPH is a stable radical that loses its purple color when it accepts an electron from an antioxidant molecule (*24*). The DPPH assay offers a convenient, accurate, and simple method of titrating the oxidizable groups of antioxidants (*25*).

Figure 1 shows the results of the antioxidant test obtained by DPPH assay. The standard compound, TBHQ, showed an 83.2 ± 2.8% radical scavenging activity at a level of 100 μg/mL, indicating that this method is valid. All samples exhibited satisfactory antioxidant activities at the levels tested (50, 100, 150, and 200 μg/mL). The decreasing order of antioxidant activity in the samples was the ethanol extract > petroleum ether > ethyl ether fraction of the volatile extract > volatile extract > hexane fraction of the volatile extract. The highest activity (84.67 ± 2.9%) was obtained by the ethanol extract at a level of 200 μg/mL. Clear dose-dependent activity also was observed in the case of ethanol and petroleum ether extracts as well as the volatile extract and its fractions. The volatile extract exhibited 60.8 ± 2.8 and 69.0 ± 4.0% antioxidant activities at levels of 150 and 200 μg/mL, respectively. The petroleum ether extract exhibited antioxidant activities (60−77%) at all four levels tested. The ethyl ether fraction of the volatile extract also showed moderate activity.

One recent report indicated that the scavenging abilities of various essential oils for the DPPH radical ranged from 39% for angelica seed oil to 90% for jasmine oil at a level of 200 μg/mL (*9*). The radical scavenging activity of these oils might be due to the presence of phenolic compounds such as eugenol, thymol, thymol methyl ether, and carvacrol as well as some phenolic acids and flavonoids (*26*).

Figure 2 shows the results of antioxidant activity tests conducted by the β-carotene bleaching assay. The standard compound, TBHQ, showed 89.9 ± 3.0% antioxidant activity at a level of 100 μg/mL, indicating that this method is valid. All samples tested exhibited an appreciable antioxidant activity with clear dose responses. The ethanol extract showed the highest activity among the extracts. It inhibited the bleaching by 88.9 ± 1.40% at a level of 200 μg/mL. At the same level (200 μg/mL), the petroleum ether extract (83.6 ± 1.9%), volatile extract (83.1 ± 4.1%), ethyl ether fraction of the volatile extract (83.1 ± 2.9%), and hexane fraction of the volatile extract (68.9 ± 5.0%) also possessed clear antioxidant activities.

A previous study reported that the antioxidant effect of natural plants was due to the presence of compounds with phenolic hydroxyl groups (*27*). Therefore, the antioxidant activity of the volatile extract may be due to the presence of compounds with phenolic groups, such as camphor, eugenol, and vanillin (**Table 1**).

A previous report showed that thymol inhibited hexanal oxidation by nearly 99% at a level of 5 μg/mL over 30 days (*28*), while camphor and farnesol reportedly possessed moderate antioxidant activities (*29*). The presence of phenolic compounds, including flavonoids, also may play a significant role in the antioxidant activity of the ethanol and petroleum ether extracts because most flavonoids have been isolated from natural plants by extraction with polar solvents such as methanol, ethanol, and water (*30, 31*). The results of this study suggest that the extracts from juniper berry fruit can be one source of natural antioxidants in addition to that of flavor and fragrance.

Antibacterial and Antifungal Tests. Table 2 shows the results of the antibacterial and antifungal tests (MZI) on three extracts (volatile, petroleum ether, and ethanol), along with the lowest concentration of their inhibition (MIC). The presence of bacteria or fungi was observed in three extracts after incubation on growth media. The volatile extract was found to have a higher antimicrobial activity against all tested microorganisms than other extracts (ethanol and petroleum ether extracts). The greatest inhibition zones were noted for strains of *C. albicans* (21.3 mm), *P. aeruginosa* (20.1 mm), *A. parasiticus* (19 mm), and *S. aureus* (17.7 mm). Moderate activity was observed for the rest of the tested strains (16 and 15.3 mm for *E. coli* and *A. niger*, respectively).

The ethanol and petroleum ether extracts showed some lower inhibitory activity than the volatile extracts against the tested microorganisms. Comparing the results of growth inhibition zones for all three extracts, the petroleum ether extract possessed antimicrobial properties halfway between those of the volatile and ethanol extracts.

The antibacterial activities obtained in the present study were consistent with previously reported results in a different juniper berry fruit (*J. oxycedrus* ssp. *Oxycedrus*) grown in Greece (*18*). The essential oil of the heartwood of Sardinian *J. oxycedrus* exhibited inhibitory activity against Gram-positive bacteria, in particular against the screened *Blastomycetes* (*32*).

All extracts tested exhibited appreciable inhibition against all strains with the MIC ranging from 0.5 mg/mL (volatile extract against *C. albicans*) to 1.2 mg/mL (ethanol extract against *A. niger*). The decreasing order of MIC among the three extracts against the strains tested was ethanol extract > petroleum extract > volatile extract. The volatile extract possessed strong antimicrobial properties against phylogenetically distant organisms (*C. albicans*), Gram-positive bacterium (*S. aureus*), and Gram-negative bacterium (*E. coli*).

Antimicrobial activities of extracts from juniper berry fruit are difficult to correlate to specific chemicals due to their complexity and variability of constituents. However, α-pinene, which is the major component of volatile extract (23.73%) and its hexane fraction (44.24%), reportedly possessed a potent antimicrobial activity similar to hydrogen peroxide (*33*). Therefore, the antimicrobial activities of these samples may be due to the presence of high levels of α-pinene. Also, some research reports a relationship between the chemical structures of the most abundant compounds in the tested extracts or essential oils and their antimicrobial activity (*34*). It also was hypothesized that mono- and sesquiterpenes with phenolic hydroxyl groups were able to form hydrogen bonds with the active sites

of target microorganisms and contribute to the overall antimicrobial effect of the essential oils (*35*).

There have been several reports on the antimicrobial activities of samples prepared from natural plants, such as herbs and spices (*36, 37*). However, the chemical constituents and antioxidant/antimicrobial activities of the fruit of *J. drupacea* L. have not been reported prior to the present study. This study suggests that juniper berry fruit is an excellent source of natural antioxidant and antimicrobial chemicals.

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