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Antioxidant and antimicrobial activity of *Artemisia fragrans* Willd essential oil and methanol extracts

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This study was designed to examine the chemical composition and the antioxidant and antimicrobial activities of both essential oil and methanol extracts of *Artemisia fragrans*. Gas chromatographic and gas chromatography / mass spectrometry analyses of the essential oil showed 22 constituents, representing 98.42% of the oil; the major components – 1,8-cineole (27.64%), camphor (26.99%), α-thujone (18.71%), and β-thujone (9.16%) – constituted 82.50% of it. The samples were screened for their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl and β-carotene / linoleic acid assay methods. The highest radical-scavenging activity was shown by the polar sub-fraction of the methanol extract (half-maximal inhibitory concentration, IC_{50} = 94.7 μg/mL), which was also higher than that of butylated hydroxytoluene (IC_{50} = 19.7 μg/mL). In the β-carotene bleaching test, the nonpolar fraction of the methanol extract gave the best inhibition result (91.5%). The results support the traditional usage and also possible use of plant volatile oil and extracts in the food, pharmaceutical and cosmetic industries.

Key words: *Artemisia fragrans*, antioxidant and antimicrobial activities, minimum inhibitory concentration, 1,8-cineole, camphor

INTRODUCTION

Artemisia is a large genus within the family Asteraceae (Compositae), containing 300 species of aromatic and medicinal plants that are usually found as small fragrant shrubs or herbs in the Northern Hemisphere [1, 2]. Many *Artemisia* species have a characteristic scent or taste, caused by monoterpenes and sesquiterpenes; the favorable properties of these compounds have contributed greatly to their application in folk medicine. In addition, *Artemisia* plants, plant sections, extracts and essential oils are used as spices for flavoring various food products, in the preparation of pharmaceuticals and cosmetics, and in food preservation [3]. Several biological activities have been reported for *Artemisia* species, including anti-malarial, antiviral, anti-tumor, anti-pyretic, anti-hemorrhagic, anti-coagulant, anti-anginal, anti-oxidant, anti-hepatitis, anti-ulcerogenic, antispasmodic and others [4]. Many related and different *Artemisia* species, such as *A. annua* [5] and *A. khorassanica* [6] have been tested, and the antimicrobial effects of these species have been established. *Artemisia fragrans* Willd, commonly known as Chao, is a species growing in Armenia, Iran, Russia, and neighboring domains. This perennial herb grows wild in the Azerbaijan, Mazandaran, Qazvin, and Tehran provinces of Iran [7] and is famed for its strong fragrance. The species possesses antibacterial property [8]. Previous phytochemical studies on this species revealed the presence of several sesquiterpen lactones with germacrane, eudesmane, guaian and elemane frameworks [9–14].

To the best of our knowledge, reports concerning the chemical composition of the essential oil and antimicrobial profiles of *A. fragrans* are scant, and there is no report regarding its antioxidant activities in the literature. Thus, the present research reports (1) the chemical composition of the essential oil of *A. fragrans* cultivated in Iran; (2) the *in vitro* profiles of the antioxidant activity of the plant products (essential oil and the extracts); (3) the total phenolic content of the plant extracts, expresssed as gallic acid equivalents; and (4) the antimicrobial potentials of the essential oil and the extracts of the plant.

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EXPERIMENTAL

Plant material

The aerial parts of *Artemisia fragrans* Willd were collected in October 2011 from the Kashan area (Isfahan province, central Iran) at an altitude of ca. 950 m. The aerial part (leaves and flowers / inflorescences) were dried in shade (at room temperature). An authenticated specimen of the plant (herbarium No. KBGH 6214) was deposited in the herbarium of the Kashan Research Botanical Garden, Research Institute of Forests and Rangelands, Kashan, Iran.

Solvents and chemicals

1,8 Cineol and camphor were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Gentamicin, rifampin and nystatin were purchased from Himedia (Mumbai, India). Analytical grade methanol, dimethyl sulphoxide (DMSO), HPLC grade chloroform, anhydrous sodium sulphate, Tween 40, and all cultures media were obtained from Merck (Darmstadt, Germany). Ultra pure water was used for the experiments.

Isolation of the essential oil

The air-dried aerial parts (150 g) of the plant were powdered, and the volatile fraction was obtained by hydrodistillation, which was carried out for 4.5 h, using an all-glass Clevenger-type apparatus (yield of 0.90% w/w), as recommended by the European Pharmacopeia [15]. The obtained essential oil was dried over anhydrous sodium sulfate, and after filtration, it was stored in an amber vial at low temperature (+4 °C) before analysis.

GC and GC-MS analyses

The essential oil from the aerial parts of *A. fragrans* was analyzed using an Agilent HP-6890 gas chromatography system (Agilent Technologies, Palo Alto, CA, USA), with an HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with a flame ionization detector. The oven temperature was maintained at 60 °C for 3 min initially and then increased at the rate of 3 °C/min to 280 °C. Injector and detector temperatures were set at 220 °C and 290 °C, respectively. Helium was used as a carrier gas at a flow rate of 1 mL/min, and 1.0 μL of diluted samples (1/1000 in *n*-pentane, v/v) was injected manually in the splitless mode. The percentages of the peak areas were used for obtaining quantitative data.

GC/MS analysis of the oil was carried out on an Agilent HP-6890 gas chromatography system (Agilent Technologies, Palo Alto, CA, USA), with an HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (ionization energy: 70 eV) operating under the same conditions as described for gas chromatography. Retention indices were calculated for all components using a homologous series of n-alkanes injected under conditions equal to those of samples.

The identification of compounds

The identification of components of volatile oil was based on retention indices (RI) relative to *n*-alkanes and computer matching with the Wiley 275L. and Wiley 7n.L. libraries, in addition to comparisons of the fragmentation pattern of the mass spectra with data published in the literature [16].

Preparation of methanol extracts

Thirty grams of the powdered aerial parts of the plant were Soxhlet-extracted with 400 mL of methanol for 8 h [17]. The solvent was removed using a rotary evaporator and the residue, after drying in a vacuum oven at 50 °C, yielded 4.0 g (13.2%) of dried extract. This extract was suspended in water and extracted with chloroform (4 × 100 mL) to obtain 1.94 g (8.53%) of polar and 0.69 g (3.03%) of nonpolar sub-fractions. The extracts were concentrated, dried, and kept in the dark at +4 °C prior to use.

Antioxidant assays

DPPH radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay usually involves hydrogen atom transfer reaction, but, based on kinetic data, an electron transfer mechanism has also been suggested for this assay [18, 19]. Radical-scavenging activities (RSAs) of essential oil, its major components (1,8-cineole, camphor) and extracts of the plant were determined using a published DPPH radical scavenging activity assay method [20] with minor modifications.

Briefly, a 10 mg/mL stock solution of each essential oil, 1,8-cineole, camphor, extracts and the synthetic standard antioxidant BHT were prepared in methanol. Dilutions were made to obtain concentrations ranging from 1 to 5×10^{-10} mg/mL. Diluted solutions (2 mL each) were mixed with 2 mL of a freshly prepared 80 μg/mL DPPH methanol solution and allowed to stand for 30 min in the dark at room temperature for any reaction to take place. Absorbance values of these solutions were recorded on an ultraviolet and visible (UV–Vis) spectrometer (Cintra 6, GBC, Dandenong, Australia) at 517 nm using a blank containing the same concentration of oil, or extracts, or BHT without DPPH. Inhibition of free radical DPPH in percent (I%) was calculated as follows:

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

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (half-maximal inhibitory concentration, IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate, and IC_{50} values were reported as mean values ± standard deviation (SD) of triplicates.

β-Carotene-linoleic acid bleaching assay

In this assay, antioxidant activity was determined by measuring the inhibition of volatile organic compounds and conjugated diene hydroperoxides arising from linoleic acid oxidation. The method described by Miraliakbari and Shahidi [21] was used with slight modifications. A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 mL chloroform, 25 μL of linoleic acid and 200 mg Tween 40. The chloroform was evaporated under vacuum, and 100 mL of oxygenated distilled water were then added to the residue. The samples (2 g/l) were dissolved in dimethyl sulfoxide (DMSO), and 350 μL of each sample solution were added to 2.5 mL of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50 °C for 2 h, together with two blanks, one contained the antioxidant BHT as a positive control and the other contained the same volume of DMSO instead of the samples. The test tube with BHT maintained its yellow colour during the incubation period. The absorbance values were measured at 470 nm on an ultraviolet and visible (UV–Vis) spectrometer (Cintra 6, GBC, Dandenong, Australia). Antioxidant activities (inhibition percentage, 1%) of the samples were calculated using the following equation:

$$I\% = (A_{\beta\text{-carotene after 2-h assay}} / A_{\text{initial } \beta\text{-carotene}}) \times 100,$$

where $A_{\beta\text{-carotene after 2-h assay}}$ is the absorbance of β-carotene after 2 h assay remaining in the samples and $A_{\text{initial } \beta\text{-carotene}}$ is the absorbance of β-carotene at the beginning of the experiments. All tests were carried out in triplicate, and inhibition percentages were reported as mean values ± SD of triplicates.

Determination of total phenolic contents

Total phenolic constituents of the polar and nonpolar subfractions of the methanol extract of *A. fragrans* were determined by literature methods involving Folin-Ciocalteu reagent and gallic acid standard [22].

Solutions of the extract (0.1 mL) containing 1 000 μg of the extracts were taken individually in a volumetric flask, 46 mL of distilled water and 1 mL Folin-Ciocalteu reagent were added, and the flask was thoroughly shaken. After 3 min, 3 mL of 2% Na_2CO_3 solution were added and the mixtures were allowed to stand for 2 h with intermittent shaking. The absorbance values were measured at 760 nm.

The same procedure was repeated for all the standard gallic acid solutions (0–1 000 mg/0.1 mL) and a standard curve was obtained with the following equation:

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

Total phenols of the extract, as gallic acid equivalents, were determined by using the absorbance of the extract measured at 760 nm as input to the standard curve and the equation. All tests were carried out in triplicate, and phenolic contents as gallic acid equivalent values were reported as means ± SD of triplicates.

Antimicrobial activity

Microbial strains

The essential oil, its major components (1,8-cineole and camphor), and the extracts of *A. fragrans* were individually tested against a set of 10 microorganisms. The following microbial strains were provided by Iranian Research Organization for Science and Technology (IROST) and used in this research: *Escherichia coli* (American Type Culture Collection or ATCC 10536), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (Persian Type Culture Collection or PTCC 1188), *Proteus vulgaris* (PTCC 1182), *Salmonella paratyphi-A serotype* (ATCC 5702), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16404). Bacterial strains were cultured overnight at 37 °C in nutrient agar (NA) and fungi were cultured overnight at 30 °C in sabouraud dextrose agar (SDA).

Disk-diffusion assay

Determination of the antimicrobial activity of dried extracts and essential oil of *A. fragrans* was accomplished by the agar disc diffusion method [23]. The dried plant extracts were dissolved in DMSO to a final concentration of 30 mg/mL and filtered using 0.45-μm Millipore filters for sterilization. The inocula of the microbial strains were prepared from 12-h broth cultures and the suspensions were adjusted to 0.5 McFarland standard turbidity. Antimicrobial tests were carried out using the disk-diffusion method [24] with 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 the mueller hinton (MH) agar, sabouraud dextrose (SD) agar, and potato dextrose (PD) agar mediums, respectively. The disks (6 mm in diameter) were impregnated with 10 μL of the essential oil, its major components (1,8-cineole and camphor), or the extracts solutions (300 μg/disk) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 24 h at 37 °C for bacterial strains and 48 h and 72 h at 30 °C for yeast and mold isolates, respectively. Gentamicin (10 μg/disk), and rifampin (5 μg/disk) were used as positive controls for bacteria and nystatin (100 U/disk) for fungi. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial activities, and each assay was repeated twice.

Microwell dilution assay

Bacterial strains and yeast sensitive to the essential oil, its major components (1,8-cineole and camphor), and extracts of the plant in the disk-diffusion assay were studied for their minimal inhibition concentration (MIC) values using microwell dilution assay method [25]. The inocula of the microbial strains were prepared from 12-h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oil, its major components, and extracts of *A. fragrans* dissolved in 10% DMSO were first diluted to the highest concentration (500 μg/mL) to be tested, and then serial two-fold dilutions

were made in a concentration range from 7.8 to 500 μg/mL in 10 mL sterile test tubes containing brain heart infusion (BHI) broth for bacterial strains and sabouraud dextrose (SD) broth for yeast. 96 well plates were prepared by dispensing 95 μL of the culture media and 5 μL of the inoculum into each well. A 100 μL aliquot from the stock solutions of the plant extracts initially prepared at the concentration of 500 μg/mL was added into the first well. Then, 100 μL volumes from their serial dilutions were transferred into six consecutive wells. The last well containing 195 μL of the culture medium without the test materials and 5 μL of the inoculum on each strip were used as the negative control. The final volume in each well was 200 μL. Gentamicin and rifampin for bacteria and nystatin for yeast were used as standard drugs for positive control in conditions identical to tests materials. The plates were covered with sterile plate sealers. Contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by the presence of a white pellet on the well bottom and confirmed by plating 5 μL samples from clear wells on NA medium. The MIC value was defined as the lowest concentration of the plant extracts required for inhibiting the growth of microorganisms. All tests were repeated two times.

MIC agar-dilution assay

MIC values of the plant essential oil and its major components (1,8-cineole and camphor) for the fungus isolate (*A. niger* ATCC 16404) sensitive to them were evaluated based on the agar-dilution method described by Gul et al. [26]. Appropriate amounts of the oil and these compounds were added aseptically to sterile molted SDA medium containing Tween 20 (0.5%, v/v) to produce the concentration range of 7.8–500 μg/mL. The resulting SDA agar solutions were immediately mixed and poured into petri plates. The plates were spot inoculated with 5 μL (10^4 spores/mL) of fungus isolate. Nystatin was used as the reference antifungal drug, and the inoculated plates were incubated at 30 °C for 72 h. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. The MIC was defined as the lowest concentration of the oil and components needed to inhibit the growth of microorganisms. Each test was repeated at least twice.

RESULTS AND DISCUSSION

Chemical composition of the volatile oil

The components of the oil were identified by their retention times, retention indexes relative to C_9–C_{28} *n*-alkanes, and comparison of their mass spectra with those of authentic samples or with data already available in literature. The percentage composition of the identified compounds was computed from the area of the gas-chromatography peak without any correction factor and calculated relatively.

The hydrodistillation of the dried flowering aerial parts of *A. fragrans* Willd. gave a light yellow-colored oil, with a yield of 0.90% (w/w). Twenty-two components were identified in the oil, representing 98.42% of the total composition (Table 1). The major constituents of the essential oil were 1,8-cineole (27.64%), camphor (26.99%), α-thujone (18.71%),

Table 1. **Chemical constituents of the essential oil of *Artemisia fragrans* Willd**

No.	Compound[a]	Composition, %	RI[b]	RI[c]
1	Camphene	1.24	943	954
2	**1,8-Cineole**	**27.64**	**1 030**	**1 031**
3	**α-Thujone**	**18.71**	**1 110**	**1 102**
4	**β-Thujone**	**9.16**	**1 120**	**1 114**
5	**Camphor**	**26.99**	**1 148**	**1 146**
6	Pinocarvone	0.91	1 165	1 165
7	3-Thujanol	2.09	1 170	1 169
8	Terpinen-4-ol	1.95	1 181	1 177
9	Myrtenol	1.56	1 201	1 196
10	Cis-Piperitol	0.50	1 214	1 196
11	Carvone	0.41	1 250	1 243
12	Piperitone	0.61	1 260	1 253
13	neoiso-3-Thujyl acetate	0.29	1 272	1 284
14	3-Thujyl acetate	2.43	1 300	1 296
15	α-Copaene	0.31	1 381	1 377
16	β-Caryophyllene	0.30	1 424	1 419
17	(E)-β-Farnesene	0.25	1 467	1 457
18	Germacrene-D	0.98	1 487	1 485
19	α-Curcumene	0.70	1 492	1 482
20	Bicyclogermacrene	0.27	1 503	1 500
21	Spathulenol	0.67	1 589	1 578
22	Viridiflorol	0.45	1 595	1 593
	Total	98.42		

[a] Compounds listed in order of elution from HP-5MS column. [b] Relative retention indices to C8-C24 n-alkanes on HP-5MS column.
[c] Literature retention indices (Adams, 2007).

and β-thujone (9.16%); the oil contains 14 monoterpenoids (94.5%) and 8 sesquiterpenoids (3.9%). The essential oil of *A. fragrans* is rich in monoterpenoids.

Previous studies showed that bornane derivatives and 1,8-cineole are major characteristic components of many species of *Artemisia* genus. Camphor (a bornane derivative) and 1,8-cineole were the major constituents of the essential oils of *A. annua*, [27] *A. diffusa*, [28] *A. haussknechtii*, [29] four *Artemisia* species growing in western Canada [30] and three *Artemisia* species from Turkey [31].

1,8-cineole and camphor, the most abundant components of this oil, were also found to be the major components of the essential oil obtained from leaves and flowers of *A. fragrans* collected from the northwestern part of Iran [32] and from its aerial parts at the flowering stage collected from Mazandaran (a province in the north of Iran) [13]. Furthermore, 1,8-cineole and α-thujone were also detected in considerable amounts in the oil of *A. fragrans* collected from Tabriz region of Iran [12].

Shafaghat et al. [8] reported that the essential oil from *A. fragrans* leaves and roots contained chrysanthenon (23.8%), 1,8-cineole (23.7%), β-caryophyllene (9.6%), p-cymene (7.7%), filifolide-A (5.7%) and filifolone (5.7%), and camphor (67.0%) and camphene (16.9%) as the main constituents, respectively.

According to a previously published report [33], the two major constituents of the essential oil obtained from the aerial parts of *A. fragrans* and *A. austriaca* cultivated in Tabriz were 1,8-cineole (11.48% and 27.97%, respectively) and camphor (54.92% and 40.59%, respectively), which are in agreement with the results presented here, except for the amounts of the compounds. Although thujone derivatives, which are toxic components, are found in the essential oil of *A. fragrans* cultivated in Tabriz and in the sample used in this study, these derivatives are not found in the essential oil of *A. austriaca*.

Several compounds such as chrysanthenone, filifolone, p-cymene and filifolide-A [8, 13], myrtenal [33], α-terpinolene, γ-terpinene and carvacrol [32] previously found in *A. fragrans* essential oil, have not been found in our sample. It would also be noteworthy to point out that the constituents of the plants essential oils are normally influenced by several factors such as geographical, climatic, seasonal and experimental conditions.

Antioxidant activity

The essential oil, its major components (1,8-cineole and camphor) and the methanol extract fractions of *A. fragrans* were subjected to screening for their possible antioxidant activities using the DPPH and β-carotene-linoleic acid assays. The results from the radical-scavenging assays for the essential oil, its major components (1,8-cineole and camphor), the extract fractions, and the positive control (BHT) are presented in Table 2.

The polar extract of the plant showed the best RSA, with an IC_{50} value of 94.73 ± 0.23 μg/mL, followed by the nonpolar extract (467.74 ± 0.03 μg/mL). Literature review shows the presence of different phenolic compounds such as cirsilineol, apigenin, 6-methoxytricin, acacetin, caffeoylquinic acid derivatives, and flavonoids in plants belonging to the *Artemisia* family [34, 35]. The presence of these compounds in the polar extract of *A. fragrans* may be the main cause of its high RSA and high total phenolic contents.

The essential oil did not show a significant activity in the DPPH-scavenging test and only 61% inhibition was achieved at a concentration of 10 mg/mL. In the case of inhibition of the linoleic acid assay, both the polar extract and the essential oil of *A. fragrans* were not able to effectively inhibit linoleic acid oxidation; these showed only 24.41% and 0.56% inhibitions, respectively, both values being significantly lower than that of the positive control BHT. In this assay, the polar extract of the plant showed the highest inhibition percentage (91.59%), which is close to the result from the synthetic antioxidant BHT (96.73%, Table 2). The high bleaching activity of the plant extract obtained in this test may be a consequence of the presence of allyl and / or benzyl containing compounds. Occurrence of compounds with allylic and / or benzylic hydrogens, such as terpenoids, was also reported in plants of the *Artemisia* genus [36, 37].

The plant oil showed weak antioxidant abilities in terms of preventing linoleic acid oxidation and reducing DPPH radicals. Alma et al. [38] reported that phenolic compounds, such as thymol and carvacrol, and essential oils rich in phenolic compounds showed potent antioxidant and DPPH-rad-

Table 2. **Antioxidant activity of the essential oil, its major components (1,8-cineole, camphor), and methanol extract subfractions of *A. fragrans*, and BHT in DPPH free radical scavenging activity and β-carotene / linoleic acid bleaching assay methods**

Sample	DPPH IC_{50}, μg/ml	β-carotene / linoleic acid Inhibition, %
Polar subfraction	94.73 ± 0.23	24.41 ± 0.24
Non-polar subfraction	467.74 ± 0.03	91.59 ± 0.35
Essential oil	ND[a]	0.56 ± 0.32
1,8-Cineole	na[b]	na[b]
Camphor	na[b]	na[b]
BHT	19.72 ± 0.82	96.73 ± 0.57
Negative control	NA[c]	2.49 ± 0.26

[a] ND, Not Determined. [b] not active. [c] NA, Not Applicable.

ical-scavenging activities. These compounds were not found in our oil and it is the base of fair antioxidant activity.

The essential oils obtained from several *Artemisia* species collected in western Canada were tested against DPPH radical and β-carotene-linoleic acid assays and all of them showed weak antioxidant activity in both systems, which is in agreement with our present data [30].

To determine the antioxidant nature of the oil, its main components, e. g. 1,8-cineole and camphor, were tested individually, and they did not show any antioxidative activity in all the methods used. There are a few reports regarding the antioxidant activity of the essential oils of some plants containing 1,8-cineole and camphor as their major components [31, 39]. All these reports confirm that 1,8-cineole and camphor are not active in both DPPH and β-carotene–linoleic acid tests. Thus, weak antioxidant activities observed for the essential oils of plants should be attributed to their main components.

Amount of total phenolic constituents

The total phenolic contents of the plant extracts were determined using a colorimetric assay based on the reduction of the Folin-Ciocalteu reagent. The results, expressed as gallic acid equivalents, were 95.5 ± 0.64 μg/mg (9.5%, w/w) and 34.5 ± 0.29 (3.4%, w/w) for the polar and the nonpolar extracts of the plant, respectively. These values are comparable to the values reported in literature for other *Artemisia* species, such as *A. arborescens* (16.7 μg/mg), *A. campestris* (103.4 μg/mg) and *A. herba-alba* (35.8 μg/mg of the ethyl acetate fraction) [40]. Summarizing, it can be observed that the content of phenolics in the extracts correlates with the antioxidant activity. It seems clear that the presence of polar phenolics is another factor in free radical-scavenging activity. Additionally, the highest activity, seen for the polar extract, reflects the radical-scavenging characteristics of these phenolics. The key role of phenolic compounds as scavengers of free radicals is emphasised in several reports [41, 42]. Moreover, radical-scavenging activity is one of the several mechanisms contributing to overall activity, thereby creating synergistic effects.

Antimicrobial activity

The antimicrobial activity of the essential oil of *A. fragrans*, its major components (1,8-cineole and camphor), and the methanol fractions were evaluated against a set of 10 microorganisms and their potencies were assessed qualitatively and quantitatively by the presence or absence of inhibition zones, the corresponding zone diameters, and MIC values. The results given in Table 3 indicate that at the tested concentrations, the essential oil of the plant has notable antimicrobial activity against all tested microorganisms. However, the polar extract of the plant showed only moderate antibacterial activity against *K. pneumoniae*, and the nonpolar extract did not show any activity against the microorganisms tested. Shafaghat et al. [8] also reported similar antibacterial activity on the essential oil of this plant.

1,8-Cineole and camphor, as the major components of the essential oil, were also tested for comparison; 1,8-cineole, the same as present in the essential oil, had considerable antimicrobial activity, whereas camphor was moderately active against one species of yeast and a few bacteria.

Results obtained from the disk-diffusion method indicate that *A. niger* is the most resistant microorganism to the two main components of the plant's essential oil, although the results of the antimicrobial activity assays indicated that 1,8-cineole showed high inhibitory activities against *C. albicans* with an MIC value of 31.25 μg mL^{-1}. The other sensitive microorganism was *P. vulgaris*, which had an MIC value of 62.5 μg mL^{-1}.

In general, the gram-positive strains of bacteria tested appeared to be more sensitive to the oil and its main compounds. These results agree with those reported by other authors [43, 44]. However, this study also records a significant susceptibility of some of the examined gram-negative bacteria. This result may be explained by the high content of 1,8-cineole (27.64%) and camphor (26.99%) in the essential oil of *A. fragrans*

Table 3. **Antimicrobial activity of the essential oil, its major components (1,8-cineole, camphor), and methanol extracts subfractions of *A. fragrans***

Test microorganism	Essential oil		1,8-cineole		Camphor		Extracts				Antibiotics					
							$CHCl_3$		H_2O		Rifampin		Gentamicin		Nystatin	
	DD[a]	MIC[b]	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
B. subtilis	18	500	30	250	11	250	–	–	–	–	13	15.6	21	500	NA	NA
E. coli	12	500	20	500	–	–	–	–	–	–	11	500	20	500	NA	NA
S. aureus	20	500	18	500	10	250	–	–	–	–	10	250	21	500	NA	NA
K. pneumonia	17	500	20	500	11	250	–	–	13	500	7	250	22	250	NA	NA
S. epidermidis	12	500	22	250	–	–	–	–	–	–	40	250	35	500	NA	NA
S. dysenteriae	15	500	15	250	13	125	–	–	–	–	8	250	18	500	NA	NA
P. vulgaris	14	500	11	62.5	–	–	–	–	–	–	10	125	23	500	NA	NA
S. paratyphi-A serotype	12	500	12	500	–	–	–	–	–	–	–	–	21	500	NA	NA
C. albicans	28	500	11	31.2	10	125	–	–	–	–	NA	NA	NA	NA	33	125
A. niger	16	500	–	–	–	–	–	–	–	–	NA	NA	NA	NA	27	31.2

[a] DD (Disc Diffusion Method), Inhibition zones in diameter (mm) around the impregnated discs.

[b] MIC (Minimal Inhibition Concentrations as μg/mL), NA (Not Applicable).

analyzed in the present study. According to literature, the essential oils of various plants, such as *A. santonicum* and *A. spicigera*, containing a relatively high proportion of oxygenated monoterpenes showed varied antimicrobial activities [31, 45]. In the present study, the essential oil of *A. fragrans* was characterized by a high content of oxygenated monoterpenes (Table 1). Therefore, the antimicrobial activities of the oil, particularly against *C. albicans*, can be attributed to the oxygenated monoterpenes contained within. Oxygenated monoterpenes, such as camphor, 1,8-cineole, and terpinen-4-ol, which were detected in the oil of *A. fragrans* as major components, were reported to have an antibacterial activity [46, 47]. On the contrary, camphor is also known to possess slight antifungal [48] and antibacterial activities [49].

CONCLUSIONS

According to literature data, this is the first study on the antioxidant activity of the essential oil and extracts of *A. fragrans* indicating good to moderate antioxidant activity for the plant. Furthermore, the antimicrobial tests showed that *A. fragrans* possesses essential oil with significant antimicrobial properties. The antimicrobial activity could be influenced by the oxygenated monoterpenes (such as 1,8-cineole and camphor), but other components can also contribute to this activity. An *in vivo* assay is also necessary to confirm the antimicrobial and antioxidant activities of *A. fragrans*, which could be usefully applied in the food, pharmaceuticals, and cosmetics industries. Isolation of the oxygenated monoterpenes responsible for the antimicrobial activity would be an interesting future study topic aimed at identifying the molecule generating the desirable efficacy.

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ARTEMISIA FRAGRANS WILLD ETERINIŲ ALIEJŲ IR METANOLINIŲ EKSTRAKTŲ ANTIOKSIDANTINIS IR ANTIMIKROBINIS AKTYVUMAS

Santrauka

Buvo tiriamas *Artemisia fragrans* eterinių aliejų ir metanolinių ekstraktų antioksidantinis ir antimikrobinis aktyvumas. Dujų chromatografijos ir masių spektrometrijos būdu nustatyta bei identifikuota 22 komponentai, iš viso sudarantys 98,42 % eterinių aliejų.