ORIGINAL ARTICLE

Chemical composition and antimicrobial activity of the volatile oil of *Eucalyptus sargentii* Maiden cultivated in central Iran

Javad Safaei-Ghomi, Hossein Batooli[1]
The Essential oil Research Institute, University of Kashan, 51167 Kashan, [1]Isfahan Research Center of Agriculture and Natural Sources, Kashan Station, Kashan, Iran

Eighteen components were identified from the leaves oil of *Eucalyptus sargentii* Maiden, which were collected in the autumn from Kashan (Isfahan Province, Iran) by GC and GC-MS, representing 98.0% of total oil. The main constituents of the oil were 1,8-cineole (55.48 %), α-pinene (20.95 %), aromadendrene (6.45 %), and trans-pinocarveol (5.92%). *In vitro* antimicrobial activity of the oil of *E. sargentii* was tested against three Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*), five Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Shigella dysenteriae*) bacteria, and two fungi (*Aspergillus niger* and *Candida albicans*). The results of the bioassay showed that the oil exhibited moderate to high antimicrobial activity.

Key words: 1,8-cineole, antimicrobial activity, *Eucalyptus sargentii* Maiden, volatile oil, α-pinene

INTRODUCTION

The essential oils and various extracts of plants have been of great interest for their potential uses as alternative remedies for the treatment of many infectious diseases. The antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies.[1,2] The genus *Eucalyptus* (Myrtaceae) includes about 700 species of evergreen shrubs and trees native of Australia and Tasmania and naturalized in various tropical and subtropical countries.[3] This plant is mainly cultivated for paper, pharmaceutical, and cosmetic industries. Several species of Eucalyptus are also used in folk medicine as antiseptic for infections of the upper respiratory tract such as the common cold, influenza, and sinus congestion.[4-8] The presence of mono- and sesquiterpenes in the Eucalyptus oil has been reported.[9,10] The major component usually includes the monoterpene 1,8-cineole with known biological activity.[11] Although, the essential oil composition of *E. sargentii* has been reported,[12,13] there is no report on the antimicrobial activity of its essential oil from Iran. Thus, in the present study, *in vitro* antimicrobial activity of the oil of this plant was investigated for the first time.

MATERIALS AND METHODS

Plant Material

The leaves of *E. sargentii* were collected from cultivated sample in Kashan Botanical Garden (Kashan, Isfahan Province, Iran) at an altitude of ca. 1000 m in November 2008. A voucher specimen of the plant has been placed in the herbarium of Kashan Research Botanical Garden, Kashan, Iran.

Isolation Procedure

Leaves were air-dried and crushed in a grinder. Essential oil was obtained by hydrodistilation for 4 h using a British type Clevenger apparatus. The oil was dried over anhydrous sodium sulfate and stored at low temperature (+4°C) until analysis.

GC/FID and GC/MS Analysis

The essential oil was analyzed using a Hewlett Packard 6890 gas chromatograph equipped with a FID detector and a HP-5MS fused capillary column (30 m × 0.25 mm film thickness, 0.25 μm). The operating conditions were as follows: injector and detector temperatures were set at 220ºC and 230ºC, respectively, oven temperature was kept at 50ºC for 2 min and programmed to 130ºC at a rate of 3ºC /min, and kept constant at 130ºC for 3 min, then programmed to 270ºC at a rate of 5ºC /min, and kept constant at 270ºC for 3 min. Helium was the carrier gas with a flow rate of 1 ml/min, diluted sample (1/1000 in n-pentane, v/v) of 1.0 μL was injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data with no use of correction factors. GC/MS analysis of the oil was performed on a Hewlett Packard 5973

Address for correspondence: Dr. J. Safaei-Ghomi, Essential Oil Research Institute, University of Kashan, 51167 Kashan, I. R. Iran.
E-mail: safaei@kashanu.ac.ir
Received: 07-06-2009; **Accepted:** 09-01-2010; DOI: 10.4103/0973-8258.69176

mass selective detector in the electron impact mode (70eV); resolution, 1000, coupled with a Hewlett Packard 6890 gas chromatograph operating under the same conditions as described above. The quadrupole mass spectrometer was scanned over the 45–465 amu. The temperature of the interface, the quadrupole, and the ion source were 250°C, 150°C, and 230°C, respectively.

Identification of Components

Essential oil was analyzed by GC and GC/MS systems using a non-polar column and identification of components in the oil was based on retention indices (RI) relative to n-alkanes and computer matching with the WILEY 275.L library, as well as by comparison of the fragmentation pattern of the mass spectra with data published in the literature.[14,15] The percentage composition of the samples was computed from the GC-FID peak areas without the use of correction factors.

Bioassay Procedure

The essential oil was tested against 10 microorganisms including *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29737, *Escherichia coli* ATCC 10536, *Klebsiella pneumoniae* ATCC 10031, *Staphylococcus epidermidis* ATCC 12228, *Shigella dysenteriae* PTCC 1188, *Proteus vulgaris* PTCC 1182. Microorganisms were provided by Iranian Research Organization for Science and Technology (IROST). The *in vitro* antimicrobial activity of the essential oil was evaluated by the disc diffusion method, using mueller hinton agar for bacteria, sabouraud dextrose agar, and potato dextrose agar for yeast and mold, respectively.[15] Paper discs (6 mm in diameter) were impregnated with 10 µl of oil and placed on the inoculated plates. These plates, after standing at 4°C for 2 h, were incubated at 37°C for 24 h (for bacteria), and at 30°C for 48 h (for yeast), and 72 h (for mold). Gentamicin (10 µg/disc) and Tetracycline (30 µg/disc) were used as positive controls for bacteria and Nystatin (100 IU/disc) for fungi. Growth inhibition zones (including disc diameter of 6 mm) were measured after incubation periods. Minimum inhibitory zones (MIC) values were measured by the microdilution broth susceptibility assay recommended by NCCLS.[16] 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 oil were dissolved in 10% dimethylsulfoxide (DMSO) and diluted to the highest concentration (500 µg/ml) to be tested, then serial twofold dilutions were made in a concentration range from 7.8 to 500 µg/ml in 10 ml sterile test tubes containing brain heart infusion broth (BHI) for bacteria and sabouraud dextrose broth for yeast.

MIC values of the essential oil against microorganisms were determined based on a micro-well dilution method. The 96-well plates were prepared by dispensing into each well 95 µl of BHI broth (for bacteria) and sabouraud dextrose (for yeast) and 5 µl of the inoculum. A 100-µl aliquot from the stock solutions of the essential oil initially prepared at the concentration of 500 µg/ml was added into the first wells. Then, 100 µl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl of BHI broth without compound and 5 µl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µl. After incubation at 37°C for 24 h (for bacteria) and at 30°C for 48 h (for yeast), the MIC values were determined. All experiments were performed in duplicate. The same procedure carried out for positive controls, Gentamicin (10 µg/disc), Tetracycline (30 µg/disc), and Nystatin (100 IU/disc).

RESULTS AND DISCUSSION

Essential Oil Analysis

The oil yield was 1.4% (V/W) based on the dry weight of the plant. Eighteen compounds consisting up to 99.46% of the essential oil were identified by GC and GC-MS analysis. 1,8Cineole (55.48%), α-pinene (20.95%), aromadendrene (6.45%), and trans-pinocarveol (5.92%) were the major components representing 88.80% of the total oil [Table 1]. Oxygenated monoterpenes such as 1,8-cineole were the most abundant constituents of the plant essential oil. Other terpens that occurred in significant amounts included α-pinene, a hydrocarbon monoterpene, and hydrocarbon

Table 1: The percentage composition of the essential oil of *Eucalyptus sargentii* M. from central Iran (Kashan area)

Compound[a]	(%)[b]	Retention indices[c]
α-pinene	20.95	923
1,8-cineole	55.48	1024
endo-fenchol	0.52	1104
trans-pinocarveol	5.92	1130
pinocarvone	2.11	1152
trans-P-mentha-1(7),8-dien-2-ol	0.3	1179
α-terpineol	0.95	1181
α-gurjunene	0.19	1397
β-gurjunene	0.23	1417
aromadendrene	6.45	1425
allo-aromadendrene	1.10	1445
β-selinene	0.29	1471
viridiflorene	0.46	1480
epiglobulol	0.6	1552
ledol	0.31	1561
spathulenol	0.24	1573
globulol	2.58	1581
viridiflorol	0.78	1589
Total	99.46	–

[a]As identified by GC-FID/MS software; names according to Wiley 7.0 mass spectra library, and by comparing their retention indices. [b]Percentage of each component; [c]Kovats retention indices measured relative to n-alkanes (C6-C24) on the HP 5MS capillary column.

Table 2: Antimicrobial activity of the *Eucalyptus sargentii* M. essential oil from Kashan area microorganism antibiotics

	Essential oil (µg/ml)		Tetracycline (30 µg/disc)		Gentamicin (10 µg/disc)		Nystatin (100 IU/disc)	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
Bacillus subtilis	24	500	18	7.8	21	500	nt	nt
Staphylococcus epidermidis	20	125	39	250	35	500	nt	nt
Staphylococcus aureus	27	125	24	250	21	500	nt	nt
Klebsiella pneumoniae	16	62.5	22	250	22	250	nt	nt
Escherichia coli	15	62.5	20	500	20	500	nt	nt
Shigella dysenteriae	13	31.25	25	250	18	500	nt	nt
Proteus vulgaris	14	250	20	125	23	500	nt	nt
Pseudomonas aeruginosa	_	_	8	500	23	500	nt	nt
Aspergillus niger	_	_	nt	nt	nt	nt	27	31.25
Candida albicans	17	125	nt	nt	nt	nt	32	125

IZ, Inhibition zones diameter (mm) including diameter of sterile disc (6 mm); MIC, minimum inhibitory concentration values are given as µg/ml for essential oil and standards. (-), Inactive; (<14), resistance; (14–17), moderately active; (>17), highly active; nt, not tested.

and oxygenated sesquiterpenes. Literature review showed two reports concerning the essential oil composition of *E. sargentii*.[12,13]

According to the GC and GC-MS results of the essential oil of *E. sargentii* collected in the spring from Kashan,[12] 1,8-cineole (75.5%), α-pinene (8.3%), and β-eudesmol (4.1%) were the main constituents of the oil. Compared to our work which, was carried out on plants collected in the autumn season, the amount of α-pinene was higher while the content of 1,8-cineole was higher in the spring. Although β-eudesmol has been reported as one of the major components in the spring oil,[12] this compound was not found in the autumn oil. The second report on the essential oil composition of this plant from South Australia shows 1,8-cineole (48.56%) and α-pinene (11.95%) as major components,[13] which is similar to our results. On the basis of the results obtained, we conclude that the leaves of *E. sargentii* from Kashan is rich in 1,8-cineole and α-pinene. The differences in chemical composition and percent of each constituent among this and previous works may be attributed to several factors such as climatic, seasonal, and experimental conditions.[17]

Antimicrobial Activity

The essential oil of *E. sargentii* was tested against three Gram-positive and five Gram-negative bacteria, as well as a mold and yeast. The results of the bioassay [Table 2] showed that the oil exhibited moderate to high antimicrobial activity against all the bacteria and yeast tested, except two microorganisms, *P. aeruginosa* and *A. niger*. Maximum activity of the leaves essential oil was observed against three Gram-negative bacteria *K. pneumonia*, *E. Coli*, and *S. dysenteriae*, but this oil had less activity on the growth of Gram-positive bacteria. Three bacterial strains, *B. subtilis*, *S. epidermidis*, and *S. aureus*, were found to be less sensitive to the oil. It was reported that greater antimicrobial potency could be ascribed to the oxygenated terpenes.[18] The percentage of oxygenated monoterpenes in *E. sargentii* essential oil was 65.28% from which 1,8-cineole was the main oxygenated monoterpene. Based on these results, the activity of the essential oil could be attributed to the effect of these compounds together with other minor ones that enhance the overall effect (synergism effect).[19]

ACKNOWLEDGMENT

Financial support made by the Research Affairs Office of the University of Kashan, Kashan, I. R. Iran, is gratefully acknowledged.

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Source of Support: Research Affairs Office of the University of Kashan, Kashan, I. R. Iran, **Conflict of Interest:** None declared.