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Chemical Composition and Antimicrobial Activity of *Pimpinella affinis* Ledeb. Essential Oil Growing in Iran

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Abstract: The chemical composition of the essential oil obtained from the fruits of *Pimpinella affinis* Ledeb. (Apiaceae) was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques. Twenty-four components were identified in the essential oil of *P. affinis* Ledeb., whose major constituents were geijerene (17.68 %), limonene (12.86 %), pregeijerene (9.92 %), germacrene D (8.54 %) and trans- β-cimene (4.94 %). The essential oil was evaluated for antibacterial and antifungal activities. The oil showed antimicrobial activity against all the tested microorganisms, excepted Pseudomonas aeruginosa. Maximum activity was observed against fungal microorganisms.

Key words: *Pimpinella affinis* Ledeb., Apiaceae, essential oil, antimicrobial

INTRODUCTION

The Apiaceae Lindl. (Umbelliferae) comprise 300-455 g enera and 3000-3750 species distributed in the northern hemisphere (Heywood, 1999; Rechinger, 1972). Its members include economically important vegetables (e.g., carrot, parsnip, celery) and condiments (e.g., coriander, anise, caraway, cumin, parsley and dill). They have distinctive flavors which are largely due to diverse volatile compounds in the fruits and leaves that not only account for their extensive culinary use but also wide applications in traditional medicine (Mozaffarian, 1996). *Pimpinella* is represented in Iran by 20 sp. (5 endemic), 2 subspecies and 4 varieties representing a total of 26 taxa (Askari *et al*., 2005) *Pimpinella affinis* [syn. *P. reuteriana* Boiss., *P. griffithiana* Boiss., *P. ambigua* W. D. Koch ex Wolff, *P. multiradiata* (Boiss.) Korov., *P. korovinii* R. Kamelin] presents in different regions of Iran, Iraq, Soiree and Israel. It is a biennial aromatic plant, 20-110 cm in height, with white umble inflorescences and ellipsoid fruits. It grows wild in the center and north of Iran. *Pimpinella affinis* fruits have been used in Iran folk medicine as carminative, appetizers, sedative and agents to increase milk secretion (Mozaffarian, 1996).

To the best of our knowledge, there are no previous reports concerning the volatile constituents of *P. affinis*. The aim of this study was to determine of the quantity and quality of essential oil from the fruits of *P. affinis* Ledeb. from Iran and its antimicrobial activity.

MATERIALS AND METHODS

Plant material: The fruits of *P. affinis Ledeb.* were collected in July 2006, from the the north of Iran. A voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

Isolation of the essential oil: The fruits (100 g) were dried at 25°C in the shade and subjected to hydrodistillation, using a Clevenger-type apparatus for 4 h. The oil was dried with anhydrous sodium sulphate, weighed and stored at 4-6°C until use.

GC Analysis: The oils from the fruits of *P. affinis* Ledeb. was analysed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 ×0.25 mm i.d., film thickness 0.25 μm; J°W Scientific); oven temperature, held at 40°C for 5 min and then programmed to 260°C at a rate of 4°C min^{-1}; injector and detector (FID) temperatures, 270°C; carrier gas, helium at a linear velocity of 32 cm/s. Percentages were calculated by area normalization method without the use of response factor correction. The retention indices were calculated for all compounds using a homologous series of *n*-alkanes.

GC-MS Analysis: GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30×0.25 mm i.d., film thickness 0.25 μm; J and W Scientific); oven temperature programme, 50-260°C

at a rate of 4°C min^{-1}; transfer line temperature, 270°C; carrier gas, helium at a linear velocity of 31.5 cm/s; split ratio, 1:60; ionization energy, 70 eV; scan time, 1 s; mass range, 40-300 amu.

Identification of components: The linear retention indices for all the compounds were determined by coinjection of the sample with a solution containing the homologous series of C8-C22 *n*-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature (Adams, 1995) and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database.

Antimicrobial activity: The antimicrobial and antifungal activities of the essential oil was determined against *Staphylococcus aureus* (ATCC 29737), *Echerichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), Saccharomyces cerevisiae (ATCC 16404) and *Candida albicans* (ATCC 14053). Bacterial and fungal strains were tested on soybean casein digest agar and Sabouraud dextrose agar, respectively. Sterilized paper disks were loaded with different amount of the essential oil (0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 mg mL^{-1}) and applied on the surface of agar plates. All plates were incubated at 37°C for 24 h for bacteria; at 25°C for 24 h for *C. albicans*. The MIC was defined as the lowest drug concentration, resulting in a clear zone of growth inhibition around the disk after conventional incubation period. Total 23 Paper disks containing different concentrations of fluconazole and gentamycin (Sigma Chemical Co.) were applied over the test plates as a comparative positive control.

RESULTS AND DISSCUSION

The hydrodistillation of the fruits of *P. affinis* Ledeb. gave an oil in 0.9 % (w/w) yield, based on the dry weight of the plant. Thwenty-four components were identified representing 97.62 % of the total oil. The qualitative and quantitative essential oil compositions are presented in Table 1, where compounds are listed in order of their elution on the DB-5 column. The major constituents of the oil were geijerene (17.68 %), limonene (12.86 %), pregeijerene (9.92 %), germacrene D (8.54 %) and trans- β-ocimene (4.94 %). It is well known that pregeijerene quickly isomerizes to geijerene. In this work, only hydrodistilled oil was used, in which pregeijerene was present approximately up to 10% and amenable for isolation by preparative G.C. It was interesting to note that the sesquiterpenes were dominated by pregeijerene, geijerene, germacrene D. The result of this research is in accordance with other earlier studies on *Pimpinella* sp. that all found to be rich in limonene (Baser *et al.*, 1996; Askari *et al.*, 2006).

Table 1: Chemical composition of the essential oil from the fruits of *Pimpinella affinis* Ledeb

Compound	RI	Composition (%)
α-Pinene	937	0.89
Myrcene	984	0.56
Limonene	1024	12.86
cis-β-Ocimene	1035	1.88
trans-β-Ocimene	1040	4.94
Linalool	1085	0.32
Geijerene	1143	17.68
Terpinene-4-ol	1166	0.35
Decanal	1192	2.59
Geraniol	1240	0.65
Pregeijerene	1285	9.92
Delta elemene	1337	0.57
Methyl cinnamate	1342	2.72
Geranyl acetate	1370	2.00
Methyl eugenol	1403	2.15
á-Humulene	1446	1.56
Germacrene D	1480	8.54
Bicyclogermacrene	1493	2.89
d-Cadinene	1536	0.35
Nerolidol (stereochemistry is unknown)	1544	1.92
Spathulenol	1564	2.21
τ-Cadinol	1642	1.83
β-Bisabolol	1672	0.78
Farnesol (stereochemistry is unknown)	1699	1.85
Monoterpene hydrocarbons		21.13
Oxygenated monoterpenes		5.47
Sesquiterpene hydrocarbons		41.51
Oxygenated sesquiterpenes		8.59

RI-Retention indices determined on DB-5 column

The results obtained in the antimicrobial assay are shown in Table 2. the oil showed antimicrobial activity against all the tested microorganisms, excepted *Pseudomonas aeruginosa*. Maximum activity was observed against fungal microorganisms Saccharomyces cerevisiae (MIC = 2 mg mL^{-1}) and *Candida albicans* (MIC = 2 mg mL^{-1}). Moderate inhibitory activity of the oil against *Staphylococcus aureus* and *Echerichia coli* were also determined with MIC value of 32 and 64 mg mL^{-1} respectively. No activity was observed against *Pseudomonas aeruginosa*. In the present study Gram-positive bacteria *Staphylococcus aureus* was more susceptible than Gram-negative bacteria strains. It has frequently been reported that Gram-negative bacteria were resistant to the inhibitory effects of essential oils and their components. This resistance has been attributed to the presence of cell wall lipopolysaccharides, which can screen out the essential oils; the lipids are thus prevented from accumulating on the transporting cell membrane and from entering the cells.

Table 2: Antimicrobial activity (MIC) of essential oil of *Pimpinella affinis* Ledeb. (mg mL^{-1})

	MIC (mg mL^{-1})		
Strains	Essential oil	Gentamycin	Fluconazole
Staphylococcus aureus (ATCC 29737)	32	4×10^{-3}	ND
Echerichia coli (ATCC 8739)	64	1×10^{-3}	ND
Pseudomonas aeruginosa (ATCC 9027)	-	8×10^{-3}	ND
Saccharomyces cerevisiae (ATCC 16404)	2	ND	10×10^{-3}
Candida albicans (ATCC 14053)	2	ND	10×10^{-3}

[a]ND, not determined

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