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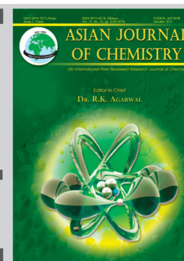

Free Radical Scavenging Activity and Chemical Composition of Essential Oils from *Chaerophyllum sativum* Against Food-Related Microorganisms

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Essential oil from aerial part of *Chaerophyllum satyvum* was obtained by hydrodistillation and analyzed by GC and GC-MS. The 95.9 % containing 42 compounds were identified. The main identified compounds were linalool (49.8 %), limonene (7.8 %), bicyclogermacrene (4.9 %) and α-santalol (4.6 %). The oxygenated monoterpens was main compounds of the oil. The antibacterial activity of the oil were tested by the disk diffusion method against three Gram-positive (*Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli*, *Candida albicans*) bacteria with the 1-4 mg mL^{-1} and 1-0.5 mg mL^{-1} MIC, respectively.

Key Words: Chaerophyllum sativum, Essential oil, Antibacterial activity.

INTRODUCTION

Prior to the development of modern medicine, the traditional systems of medicine that have evolved over the centuries within various communities, are still maintained as a great traditional knowledge base in herbal medicines. Plant materials play a major role in primary health care as therapeutic remedies in many countries[1,2]. Plants still continue to be an important source of drugs for north and central provinces of Iran[3]. Majority of the world population[4-6]. It has been well known since ancient times that plants and spices have antimicrobial activity. There has been a considerable interest to use plants and spices for the elimination of microorganisms because of increasing antibiotic resistance of microorganisms.

The family Apiaceae Lindl. (Umbelliferae Juss.) comprises about 455 genera and around 3750 species. The genus *Chaerophyllum* belongs to the tribe *Apiaceae scandiceae*, subtribe Scandicinae. Comprising about 30 species it is considered the largest and most diverse genus in the subtribe.

Herbal remedies are considered the oldest forms of health care known to mankind on this adjusted at 1/50. The injector and detector (FID) temperatures were kept at 250 ºC and 280 ºC, respectively. GC-MS analysis was performed on a Thermoquest-Finnigan Trace GC-M instrument equipped with a DB-1 fused silica capillary column (60 m 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was raised up from 60 to 250 ºC at a rate 5 ºC/min and then kept at 250 ºC for 10 min. Transfer line temperature was 250 ºC. Helium was used as a carrier gas at a flow rate of 1.1 mL/min with a split ratio of 1/50. A quadrupole mass spectrum was scanned over 45465 amu with an ionizing voltage of 70 eV and an ionizing current of 150 A.

EXPERIMENTAL

The aerial parts of *Chaerophyllum sativum,* which growing wild in Iran, is collected on September 2010 from Ramsar, Iran. A voucher specimen has been deposited in Islamic Azad University, Tonekabon Branch, Iran.

Oil isolation procedure: Air-dried aerial parts of the plant (100 g) were hydrodistilled for 4.5 h using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulfate and kept in a sealed vial at 4 ºC. The essential oil of *Chaerophyllum sativum* was obtained in the yield of 0.1 % (w/w).

GC and GC-MS analysis: GC analysis was performed on a Thermoquest-Finnigan Trace GC instrument equipped with a capillary DB-1 fused silica column (30 m 0.25 mm i. d., film thickness 0.25 µm). The oven temperature was raised from 60 to 250 ºC at a rate of 5 ºC/min, then held at 250 ºC for 10 min. Nitrogen was used as a carrier gas at a flow rate of 1.1 mL/min. Split ratio was adjusted at 1/50. The injector and detector (FID) temperatures were kept at 250 ºC and 280 ºC, respectively. GC-MS analysis was performed on a Thermoquest-Finnigan Trace GC-MS instrument equipped with a DB-1 fused silica capillary column (60 m 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was raized up from 60 ºC to 250 ºC at a rate 5 ºC/min and then kept at 250 ºC for 10 min. Transfer line temperature was 250 ºC. Helium was

used as a carrier gas at a flow rate of 1.1 mL/min with a split ratio of 1/50. A quadrupole mass spectrum was scanned over 45 465 amu with an ionizing voltage of 70 eV and an ionizing (Fig. 1).

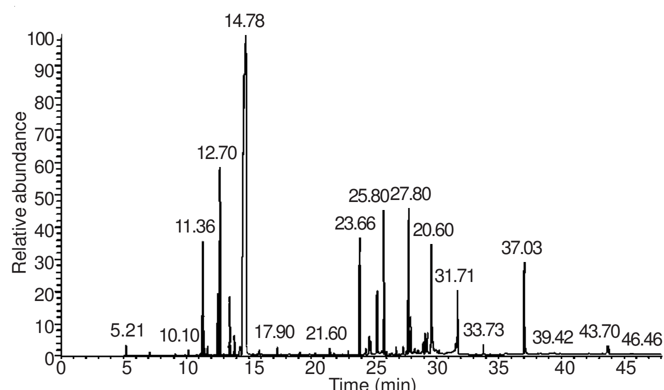

Fig. 1. GC and GC-MS spectra of *Chaerophyllum satyvum*

Identification of oil components: The constituents of the oil were identified by calculation of their retention indices under temperature programmed conditions for *n*-alkanes (C_6 -C_{24}) and the oil on a DB-1 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with a computer library (Wiley 7.0)

Antibacterial activity: *In vitro* antimicrobial activity of essential oils was assessed against the following bacterial and yeast strains: *Staphylococcus aureus* (PTCC1431), *Bacillus cereus* (PTCC 1015), *Bacillus subtilis* (isolated and biochemically characterized in our laboratory), *Escherichia coli* (PTCC1399) and *Candida albicans* (PTCC 5027) as Gram-negative bacteria. The inhibition effect on bacterial growth was determined by the disc diffusion method. The essential oil (15 µL) was applied on the paper discs (the disc diameter was 6 mm). Each concentrated extract was dissolved in its own solvent (100 mg/mL) and 25 µL of each solution was delivered on the disc and left for complete evaporation of the solvent. Then disc papers were placed in the inoculated plates. After 24 h of incubation at 37 ºC the diameter of growth inhibition zones were measured. Methanol and DMSO were used as negative and a standard reference antibiotic (ampicillin and penicillin) as positive controls. All experiments were performed in triplicate with a computer library (Wiley 7.0) current of 150 A (Fig. 1).

Evaluation of antibacterial activity: Antimicrobial activity of essential oils was evaluated by broth dilution susceptibility tests. Micro-broth dilution method were used according to the standard protocols of NCCLS with some modifications to determine the minimum concentration of each antimicrobial agent required for inhibition (MIC) or killing (MBC) of the test microorganism.[7-9]

The inoculants of the microbial strains were prepared from freshly cultured bacteria and yeast strains that were adjusted to 0.5 McFarland standard turbidity using sterile normal saline, and then were further diluted (1:100) by sterile Mueller-Hinton broth just before adding to the trays. Essential oils serial dilutions were made in a concentration range from 64 to 0.125 mg/mL in sterile plastic micro-dilution trays containing Mueller-Hinton broth supplemented by 0.5 % Tween 80. MICs were recorded after 22 h incubation at 37 ºC. Minimum bactericidal concentrations were determined by sub-culturing of 100 µL from each negative well and from the positive growth control onto a Nutrient agar plate. MBCs were defined as the lowest concentration that could kill 99.9 % of the test strains. Each experiment was done in duplicate[10-12].

RESULTS AND DISCUSSION

The essential oil of *Chaerophyllum sativum* was obtained and forty-two compounds were identified. The main identified compounds were linalool (49.8 %), limonene (7.8 %), bicyclogermacrene (4.9 %) and α-santalol (4.6 %). The oxygenated monoterpens was main compounds of the oil (Table-1).

TABLE-1
ESSENTIAL OIL COMPOSITION OF *Chaerophyllum sativum*

RI	Area (%)	Compound
611.574	0.21	Methyl cyclopentane
941.176	0.14	α-Pinene
979.608	0.06	Sabinene
986.667	3.32	β-Pinene
990.98	0.17	Myrcene
1001.06	0.25	*n*-Octanal
1029.23	1.88	*p*-Cymene
1035.56	7.79	Limonene
1047.18	0.04	δ3-Caren
1062.68	1.52	γ-Terpinen
1076.41	0.75	*cis*-Linalool oxide (furanoid)
1092.25	0.53	*cis*-Linalool oxide (furanoid)
1108.77	49.8	Linalool
1140.7	0.03	*trans*-*p*-Mentha-2,8-dien-1-ol
1145.96	0.09	(s)-3-Ethyl-4-methylpentanol
1197.19	0.24	α-terpineol
1261.07	0.07	2E-decenal
1289.29	0.07	Thymol
1305.22	0.06	*cis*-Pinocarvyl acetate
1388.06	0.04	E-methyl cinnamate
1403.54	0.09	β-Elemene
1440.16	3.46	E-caryophyllene
1459.45	0.16	Aromadendrene
1470.47	0.5	γ-Decalactone
1473.62	0.27	α-Humulene
1494.49	1.6	α-*trans*-bergamotene
1499.61	0.05	D-Germacrene
1508.68	0.04	E,E-α-Farnecene
1516.53	4.65	Bicyclogermacrene
1526.03	0.09	*trans*-Cycloisolongifol-5-ol
1542.56	0.04	β-Bazzanene
1591.32	0.1	Isospathunelol
1599.17	4.99	Spathunelol
1606.96	1.05	Caryophyllene oxide
1615.65	0.05	Viridiflorol
1620.43	0.13	Carotol
1677.39	4.9	α-Santalol
1765.44	0.18	Occidentalol acetate
1773.27	1.69	β-Atlantol
1955.78	0.05	Hexadecanoic acid
2016.67	3.92	z-Farcarionol
2334.38	0.89	Pentacosane

According to the results obtained in (Table-2), essential oil have moderate to high activity against Gram-negative (*Escherichia coli, Candida albicans*) bacteria. As it demonstrate

TABLE-2
ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL

	Essential oil	
	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	4.0	16
Escherichia coli	1.0	2
Bacillus cereus	4.0	16
Bacillus subtilis	1.0	4
Candida albicans	0.5	4

the essential oil has an activity against three Gram-positive (*Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis*).

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