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Antibacterial Activity and Chemical Composition of Ajowan (*Carum copticum* Benth. & Hook) Essential Oil

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ABSTRACT

Gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) were employed to determine the chemical composition of essential oil obtained from dry fruits of *Carum copticum*. Thymol (36.7%), γ-terpinene (36.5%) and ρ-cymene (21.1%) were found to be the major constituents of the oil. The anti-bacterial activities of the oil were mainly investigated against food poisoning bacteria (*Salmonella thyphimorium*, *Pseudomonas aeruginosa*, *Entero-pathogenic Escherichia coli*, and *Staphylococcus aureus*) by broth microdilution and agar diffusion methods. The oil exhibited significant anti-bacterial activities against all the examined bacteria. In conclusion, the results of this study showed that the oil of Ajowan is rich in monoterpenes and it may be used as a natural anti-bacterial agent in drug and food industries.

Keywords: *Carum copticum*, Essential oil, Anti-bacterial activity, Gas Chromatography Mass Spectrometry

INTRODUCTION

During the past centuries, essential oils have been used traditionally in preservation of foods against microbial decay (1). Many of these oils are "generally regarded as safe" (GRAS) and have pleasant odors and taste; therefore, they are widely used in food and cosmetic industries as flavoring and perfume (2). Moreover, essential oils are used safely in herbal medicine as anti-microbial compounds (3). Bacterial food-born illnesses referred as food poisoning are mainly caused by *Staphylococcus aureus, Salmonella thyphimorium* and, entero-pathogenic *Escherichia coli* (4). On the other hand, in recent years, resistance to anti-bacterial drugs has increased dramatically. For example, *Pseudomonas aeruginosa* that is usually considered as one of the main causes of nosocomial infections is resistant to most of the known antibiotics (5). Considering the limited diversity of antibiotics, development of new antimicrobial compounds, especially from natural sources, is of great interest.

Ajowan (*Carum copticum* Benth. & Hook.) is an annual herbaceous essential oil bearing plant belonging to the Apiaceae family, which grows in India, Iran, and Egypt (6). It has been reported that Ajowan fruit oil has diuretic, carminative, analgesic, anti-dyspnoea and, anti-inflammatory compounds (7). Traditionally, the water extract of Ajowan is widely used to relieve

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the symptoms of flue in children (8, 9). In addition, methanol extract of this plant was reported to have antibacterial activity against multi-drug resistant *salmonella typhi* (10). The ripening seeds of this plant contain 2-4% essential oil that is rich in monoterpenes like thymol and is mainly used as an antiseptic agent as well as a drug component in medicine (11).

In the present study, the components of *C. copticum* essential oil were analyzed and examined for its inhibitory activities against some pathogenic and food-born bacteria.

MATERIALS AND METHODS

The plant species from which the oil was obtained were collected from Experimental Station Farm of Tarbiat Modares University, Tehran, Iran, and were identified and authenticated by A.R. Khosravi, a plant taxonomist, at Shiraz University, Herbarium, Shiraz, Iran. Voucher specimen (no. 24985) has been deposited in the herbarium.

Oil Isolation

The Ajowan seeds were ground and the resulting powder was subjected to hydrodistillation for 3 hours in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (12). The extracted oil samples were dried over anhydrous sodium sulphate and stored in sealed vials at 4°C for gas chromatography (GC) and GC/ mass spectrometry (MS) analysis.

GC Analysis

GC analysis was performed, using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 3°C/min. Injector and detector (FID) temperatures were 290°C; helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages were calculated by electronic integration of FID peak areas without the use of response factors correction. Linear retention indices for all components were determined by co-injection of the samples with a solution containing homologous series of C_8-C_{22} 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 m x 0.25 mm i.d.); oven temperature was 40°C to 240 °C at a rate of 4°C. Transfer line temperature was 260°C. Carrier gas was helium with a linear velocity of 31.5 cm/s, split ratio 1/60. In addition, ionization energy was 70 eV, scan time 1 s, and mass range 40-300 amu. The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds or with the data published in the literature (13). Mass spectra from the literature were also compared (14).

Antibacterial assay

Five American Type Culture Collection (ATCC) strains including *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (8821M), *Escherichia coli* (ATCC 25922), and *Salmonella typhimurium* (RITCC 1731) were tested by agar diffusion and broth dilution methods. All the tested bacteria were cultured in Mueller-Hinton (MH) broth and incubated at 37°C to reach the turbidity equal to 0.5 McFarland Standard. The number of living bacteria in suspension was measured by culturing 100µl of diluted suspension on MH agar.

Screening the antibacterial activity by agar diffusion method

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Table 1. Chemical compositions (%, w/w) of C. copticum essential oil.

Components	Retention Index [a]	(%)	Identification Methods
α -thujene	932	0.5	MS, RI
α -pinene	941	0.2	MS, RI, CoI
Sabinene	981	0.3	MS, RI
β - pinene	984	2.5	MS, RI, CoI
α -phyllanderene	1000	0.7	MS, RI
α -terpinene	1022	0.7	MS, RI
ρ - cymene	1028	21.1	MS, RI, CoI
β - phyllanderene	1035	0.4	MS, RI
γ - terpinene	1060	36.5	MS, RI, CoI
Terpinene- 4 - ol	1177	0.01	MS, RI
Thymol	1294	36.7	MS, RI, CoI
Carvacrol	1306	0.1	MS, RI
Total		99.7	

[a] The retention Kovats indices were determined on DB-5 capillary column. MS= Mass Spectroscopy, RI= Retention Index, CoI= Co injection with authentic compounds

The agar diffusion method was employed for the screening and determination of antimicrobial activity of the essential oil. 0.1 ml of 0.5 McFarland standard of each of the above-mentioned species was applied to the MH agar with a cotton swab. The plates were allowed to dry for at least 15 min before 10µl of dissolved essential oil (consisting of 5µl pure essential oil in the solvent) was added to each well prepared with biopsy punch. A well containing 5 µl solvent was served as control in each plate. The plates were then incubated at 37°C for 24 hours. The diameters of the inhibition zones were measured in millimeters by vernier calipers. All the tests were performed in triplicate.

Determination of minimum inhibitory concentration (MIC)

MICs were determined using the Broth dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS), with some modifications. Briefly, Mueller-Hinton (MH) Broth was supplemented with 0.002% (v/v) tween 80 (Sigma) to enhance dispersion of the Ajowan oil. Geometric dilutions of the essences, ranging from 80.0 to 0.036 µl /ml, were prepared in 1ml MH broth media in the test tubes. Growth controls consisting of MH broth without essence were included for each tested isolate. After the addition of 1 ml of the inoculums (1.5x106) to each tube, they were incubated at 35-37°C for 18-24 hours. In addition, 2 ml of uninoculated MH broth was included as a sterility control and blank. The growth in each tube was compared with that in the control tube. The MIC was the lowest concentration of Ajowan essential oil that resulted in a clear tube. Ten microlitres from each tube was spot-inoculated onto Nutrient Agar (NA) and incubated overnight at 37°C to determine the MBC. The highest dilution that inhibits the bacterial growth on nutrient agar after overnight incubation was taken as MBC. The experiments were performed three times.

RESULTS AND DISCUSSION

The chemical compositions of *Carum copticum* essential oil are shown in Table 1. Twelve compounds representing 99.7 % of *C. copticum* essential oil were identified. The major constituents of *C. copticum* were thymol (36.7%) and its precursors, γ - terpinene (36.5%), and ρ - cymene (21.1%). The oil was also examined for anti-bacterial

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activities against 4 standard bacteria by the broth microdilution and agar diffusion methods. The essence exhibited significant antibacterial activities against all examined bacteria by agar diffusion method, except *P.aeuroginosa*. Minimal inhibitory and bactericidal concentrations of the essential oil against the examined bacteria are presented in Table 2.

In agar diffusion assay, Ajowan showed inhibitory effects against all the examined bacteria except *P.aeuroginosa*. However, in MIC analysis, the essential oil showed strong inhibitory activity against *P.aeuroginosa* in comparison to that of the disc diffusion method. These data are in contrast with the results of Bazzaz *et al.* (15), who reported no antibacterial activity of the oil against *P.aeuroginosa*. The difference in the results might be due to different constituents of the oil not reported in their work.

Similar to previous studies (7,16), thymol (36.7%) was found to be the major constituent of the oil known as 'Ajwan-ka-phul' (crude thymol), while others reported carvacrol as the major constituent of this oil (17). It has been shown that thymol and its precursors, cymene and terpinene, (18, 19) have strong antimicrobial activities. It has been reported that thymol might induce its antimicrobial action by perturbation of the lipid fraction of the microorganism plasma membrane, resulting in alterations of the membrane permeability and leakage of intracellular materials (20). Although terpinene was the second main constituent identified in the Ajowan oil, no strong anti-bacterial activity was reported from its gamma isomer (21). P-cymene is another major compound identified in Ajowan oil that is a hydrophobic molecule and causes swelling of the cytoplasmic membrane (21). It is not an effective anti-bacterial agent when used alone (23, 24), however, in combination with other phenolic compounds such as carvacrol, it has shown a great anti-microbial activity by incorporating cymene in the lipid bilayer of bacteria, facilitating the transport of phenolic monoterpenes of EOs across the cytoplasmic membrane (25). On the other hand, some studies have shown that the whole EO has a stronger anti-bacterial activity than the individual major components (26, 27), demonstrating that the minor constituents are also important to the anti-microbial activity and may have a synergistic influence (21, 28).

Considering the promising inhibitory and bactericidal activity of the examined ESO, it might be used as a natural food preservative as well as antibacterial substance in nosocomial infections. However, further studies are still required to investigate its application in medicine and food industries.

REFERENCES

1. Evans, W.C. 2002. *Trease and Evans Pharmacognosy*. 15th ed. London: W. Saunder's company Ltd.
2. Lahlou, M. 2004. Essential Oils Fragrance Compounds: Bioactivity and Mechanism of Action. *Flavor Fragrance J.*, **19**: 159-165.
3. Chevallier, A. 2001. *The Encyclopedia of Medicinal Plants*. Dorling Kindersley, London.
4. Abubakar, I., Irvine, L., Aldus, C.F., Wyatt, G.M., Fordham, R., Schelenz S., Shepstone, L., Howe, A., Peck, M. and Hunter, P.R.

Table 2. Anti-bacterial activity of *Carum copticum* essential oil by broth microdilution and agar diffusion methods**.**

Species	Mean diameter of inhibition zones (mm)	*MIC* ($^{V}/_{V}$)	*MBC* ($^{V}/_{V}$)
S.aureus	22	%0.031	%0.031
P.aeuroginosa	0	%1	%2
E.coli	21	%0.031	%0.0 62
S.typhimurium	23	%0.015	%0.031

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2007. A Systematic Review of the Clinical, Public Health, and Cost-effectiveness of Rapid Diagnostic Tests for the Detection and Identification of Bacterial Intestinal Pathogens in Feces and Food . *Health Technology Assessment* (HTA), **11**: 1-216

5. Veesenmeyer, J.L., Hauser, A.R., Lisboa, T. and Rello, J. 2009. *Pseudomonas aeruginosa* Virulence and Therapy: Evolving Translational Strategies. *Crit Care Med*, **37**: 1777-86.
6. Zargari, A. 1996. Medicinal Plants. Tehran University Publications. Vol. **2**, 975 p.
7. Thangam, C. and Dhananjayan, R. 2003. Anti-inflammatory Potential of the Seeds of *Carum copticum*. *Indian J. Pharmacol,* **35**: 388-391.
8. Boskabady, M.I.I. and Shaikhi, J. 2000. Inhibitory Effect of *Carum copticum* on Histamine (H1) Receptors of Isolated Guinea-pig Tracheal Chain. *J. Ethnopharmacol,* **69**: 217-227.
9. Khajeh, M., Yamini, Y., Sefidkon, F. and Bahramifar, N. 2004. Comparison of Essential Oil Composition of *Carum copticum* Obtained by Supercritical Carbon Dioxide Extraction and Hydrodistillation Methods. *Food Chem*, **86:** 587-591.
10. Rani, P. and Khullar, N. 2004. Antimicrobial Evaluation of Some Medicinal Plants for Their Anti-enteric Potential Against Multi-drug Resistant *Salmonella typhi*. *Phytother Res*, **18**: 670-673.
11. Gupta, S. 2002. Handbook of Spices and Packaging with Formulas. Engineers India Research Institute, 277.
12. Anonymous. 1997. European Pharmacopoeia, 3rd Ed, Council of Europe, Strasbourg, France, 121-122.
13. Shibamoto T. 1987. Retention Indices in Essential Oil Analysis. In Capillary Gas Chromatography in Essential Oil Analysis, Sandra P, Bicchi C (Ed). Alfred Heuthing-verlag: New York, 259-275**.**
14. Adams, R.P. 2001. Identification of Essential Oils Components by Gas Chromatography/Quadra pole Mass Spectroscopy. Carol Stream, IL, Allured, 61-367.
15. Bazzaz, B.S.F., Azadbakht, M. and Doust, M.S. 2008. Antibacterial Activity of Essential Oils of Iranian Plants (Mazandaran Province). JEOBP, 11: 436-442.
16. Behravan, J., Ramezani, M., Hassanzadeh, M.K. and Ebadi, S. 2007. Evaluation of Antibacterial Activity of the Essential Oils of Zataria Multiflora, Carum copticum and Thymus vulgaris by a Thin Layer Chromatography-Bioautography Method. JEOBP, 10: 259-264
17. Mohagheghzadeh, A., Faridi, P. and Ghasemi, Y. 2007. *Carum copticum* Benth. & Hook., Essential Oil Chemotypes**,** *Food Chem*, **100**: 1217-1219.
18. Cosentino, S., Tuberoso, C.I.G., Pisano, B., Satta, M., Mascia, V., Arzedi, E. and Palmas, F. 1999. In Vitro Antimicrobial Activity and Chemical Composition of Sardinian *Thymus* essential oils**.** *Lett. Appl. Microbiol*, **29**: 130- 135.
19. Marino, M., Bersani, C. and Comi, G. 1999. Antimicrobial Activity of the Essential Oils of *Thymus vulgaris* L. Measured Using a bioimpedometric Method. *J. Food Prot*, **62**: 1017-1023.
20. Trombetta, D., Castelli, F., Sarpietro, M.G., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G. and Bisignano, G. 2005. Mechanisms of Antibacterial Action of Three Monoterpenes. *Antimicrob. Agents Chemother*, **49**: 2474-8.
21. Burt, S. 2004. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods-a review. *Int. J. Food Microbiol*, **94:** 223-53.
22. Ultee, A., Bennink, M.H.J. and Moezelaar, R. 2002. The Phenolic Hydroxyl Group of Carvacrol is Essential for Action Against the Food-borne Pathogen *Bacillus cereus*, *Appl. Environ. Microbiol*, **68**: 1561-1568.
23. Juven, B.J., Kanner, J., Schved, F. and Weisslowicz, H. 1994. Factors that Interact With the Antibacterial Action of Thyme Essential Oil and Its Active Constituents. *J. App. Bacteriol*, **76**: 626- 631.
24. Dorman, H.J.D. and Deans S.G. 2000. Antimicrobial Agents from Plants: Antibacterial Activity of Plant Volatile Oils. *J. Appl. Microbiol,* **88**: 308- 316.
25. Juliano, C., Mattana, A. and Usai, M. 2000. Composition and In Vitro Antimicrobial Activity of the Essential Oil of Thymus Herba-barona Loisel Growing Wild in Sardinia. *JEOR*, **12:** 516–522.
26. Gill, A.O., Delaquis, P., Russo, P. and Holley, R.A. 2002. Evaluation of Antilisterial Action of Cilantro Oil on Vacuum Packed Ham. *Int. J. Food Microbiol*, **73:** 83- 92.

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27. Mourey, A. and Canillac, N. 2002. Anti-Listeria Monocytogenes Activity of Essential Oils Components of Conifers. *Food Control*, **13**: 289- 292.
28. Karami-Osboo, R., Khodaverdi, M., Akbari, A.F. 2010. Antibacterial Effect of Effective Compounds of *Satureja hortensis* and *Thymus vulgaris* Essential Oils against *Erwinia amylovora. J. Agr. Sci. Tech.,* **12:** 35-45.

بررسی اثر ضد باکتریایی وتجزیه شیمیایی اسانس گیاه دارویی زنیان(*Carum copticum* Benth & Hook.)

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چکیده

در پژوهش حاضر ترکیبات شیمیایی موجود در اسانس میوه گیاه دارویی زنیان(*Carum copticum*) با استفاده از کروماتوگرافی گازی (GC) و کروماتوگرافی گازی متصل به طیف سنج جرمی (GC-MS) بررسی شد. نتایج تجزیه اسانس نشان داد که تیمول (٪۳۶,۷)، ترپینن (٪ ۳۶,۵) و پارا- سیمن (٪ ۲۱,۱) عمده ترین ترکیبات اسانس هستند. در این تحقیق فعالیت ضد باکتریایی اسانس مورد مطالعه عمدتا روی برخی از باکتری های مهم ایجاد مسمومیت غذایی مورد بررسی قرار گرفت. عوامل باکتریایی مورد مطالعه در این پژوهش شامل باکتری های: *Entero- ،Salmonella thyphimorium Pseudomonas aeruginosa pathogenic Escherichia coli ،Staphylococcus aureus* بودند. برای تعیین خاصیت ضد باکتریایی اسانس از روش Broth microdilution و Agar diffusion استفاده شد. اسانس مورد آزمون به طور معنی داری فعالیت ضد باکتریایی روی عوامل باکتریایی مورد آزمایش نشان داد. به طور کلی نتایج این پژوهش نشان داد که اسانس گیاه دارویی زنیان غنی از ترکیبات مونوترپنی است و می توان از آن به عنوان یک عامل ضد میکروبی طبیعی در صنایع غذایی و داروسازی بهره برد.

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