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CARUM COPTICUM L. ESSENTIAL OILS AS NATURAL ANTIMICROBIAL AND ANTIOXIDANT

M KAZEMI

Department of Horticulture, Science and Research Branch,
Islamic Azad University, Tehran, Iran

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Abstract

Carum copticum L. comprises several relevant species for food, cosmetic, perfumery and pharmaceutical industries. GC/MS analysis of the enential oil of *C. copticum* revealed γ- terpinene as a major component of *C. copticum*, with its contribution of 33.85%. Essential oils (EOs) exhibited a significant antimicrobial activity against all tested microbial strains. In addition, the *C. copticum* oil demonstrated the highest DPPH radical scavenging activity. These results clearly show the antimicrobial and antioxidant effects of the plant essential oil.

Introduction

Ajowan (*Carum copticum* L.) is an annual herbaceous plant belonging to the Umbelliferae, which grows in India, Iran, and Egypt with white flowers and small, brownish seeds (Khajeh *et al.* 2004). Natural products with their diverse biological and pharmacological activities represent a gold mine for scientists searching for lead compounds for the treatment of health disorders and antioxidant activity (Lis-Balchin and Deans 1997). Recently, investigation of natural products for the discovery of active compounds with antimicrobial and antioxidant properties from plant origin that can be applied to the food industry has gained interest (González-Lamothe *et al.* 2009). Among plant extracts, essential oils (EOs) and their components are gaining increasing interest in the food, cosmetic and pharmaceutical industries because of their relatively safe and wide acceptance by consumers, and exploitation for potential multi-purpose functional uses (Riahi *et al.* 2013). Therefore, our primary objective was to characterize the EOs of *C. copticum*, and compare their antimicrobial and antioxidant activities.

Materials and Methods

Seeds of *Carum copticum* were purchased from of Ilam during July-June, 2013-2014. identified by Dr. Esmaeili and voucher specimens has been (No. 323), in the private herbarium of Dr. F. Esmaeili. Essential oils were isolated by hydrodistillation for 3 hrs using a Clevenger-type apparatus, (European pharmacopeia 2013). The obtained EOs were dried over anhydrous Na_2SO_4 and stored in a sealed dark vials at 4°C.

Composition of the essential oils was determined by gas chromatography (GC) and mass spectrophotometry (GC/MS). The GC analysis was done on an Agilent Technologies 7890 GC equipped with a single injector and a flame ionization detector (FID). The analysis was carried out on fused silica capillary HP-5 column (30 m × 0.32 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250 and 280°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min; oven temperature program was 60 - 210°C at the

*Corresponding author: <mokazemi12@gmail.com>.

rate of 4°C/min and then programmed to 240°C at the rate of 20°C/min and finally held isothermally for 8.5 min; split ratio was 1 : 50. GC/MS analysis was carried out by use of Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness 0.25 µm) coupled with 5975-C mass spectrometer. Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 230 and 280°C, respectively. Mass range was from 45 to 550 amu. Oven temperature program was the same given above for the GC. The constituents of the EOs were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C_8 - C_{25}) and the oil on a HP-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature (Adams 2007). For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

The efficacy of the essential oils to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated using a spectrophotometry method (Cuendet *et al.* 1997) on the basis of bleaching of the bluish-red or purple colour of DPPH solution as a reagent. Briefly, a 50 µl volume of various dilutions of each sample was mixed with 5 ml of 0.004% methanol solutions of DPPH followed by 30 min incubation at ambient temperature. Thereafter, the sample absorbance was recorded against control at 517 nm. The inhibition percentages were measured using Eq. 1. The antioxidants activity of the test samples in concentration providing 50% inhibition were considered as IC_{50} (µg/ml).

$$\text{Inhibition } \% = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}} \times 100 \quad (1)$$

Butylated hydroxy anisole (BHA) and ascorbic acid were used as positive controls. All experiments were repeated three times and the average results and standard deviations were calculated.

Gram-positive bacteria *viz*. *Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis* and in case of Gram-negative bacteria: *Shigella shiga, Shigella sonnei, Pseudomonua aeruginosa* were taken. Fungal strains *viz*. *Candida parapsilosis*, *Aspergillus niger* and *fumigatus* were taken for anti-microbial study.

The agar disc diffusion assay was employed for the determination of antimicrobial activity of the essential oil (Zhu *et al.* 2005). Briefly, a suspension of the test organism (2×10^8 CFU/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually impregnated with 15 µl of the diluted oil aliquots (200 µg/ml stock), then placed on the inoculated plates, for 2 hrs at 4ºC. The plates were incubated at 37ºC for 24 hrs for bacteria, and at 30ºC for 48 hrs for fungal strain, using a spread restraint method for epiphytes at 30ºC for 48 hrs (Uzela and Cetinb 2004). The diameters of the zone of inhibition (DD) were measured in millimeters. Each test was carried out in triplicate, repeated three times, and the average was calculated for the inhibition zone diameters.

On the other hand, a broth micro-dilution method was used to determine the MIC (Yu *et al.* 2007). All tests were performed in Mueller Hinton broth and Sabouraud Dextrose broth in cases both for bacteria and fungi, respectively supplemented with ethanol at a final concentration of 0.5% (v/v). Serial dilutions of the oils were prepared in a 96-well plate, ranging from 0.05 to 200.00 µg/ml. The final concentration of each strain was adjusted to 5×10^4 CFU/ml. The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth.

To determine MBC, broth was taken from each well and incubated in Mueller Hinton Agar at 37ºC for 24 hrs for bacteria. The MBC were determined by serial of 10 µl into microtiter plates

containing 100 µl of broth per well and further incubation for 24 hrs at 37ºC. The MBC was defined as the lowest concentration of the essential oil at which the incubated microorganism was completely killed (Yu *et al.* 2007). Each test was carried out in triplicates and repeated three times.

The results are presented as mean ± Sd and statistically analyzed by oneway analysis of variance (ANOVA) followed by Duncan's test.

Results and Discussion

The *Carum copticum* essential oil was obtained in yield of 0.96 ± 0.05% (v/w) dried mass. Results obtained by the GC/MS chemical analysis of the essential oil are presented in Table 1. Ten compounds were identified in *C. copticum* oil, which accounted for 86.26% of the total oil; the major constituent was γ- terpinene (33.85%), followed by thymol (16.35%), α-pinene (9.65%) and carvacrol (8.93%). In a study by Rasooli *et al.* (2008) indicated the major constituent of the essential oil of *C. copticum* was *p*-cymene and in other studies, γ-terpinene was the second most abundant constituent of oil (Khajeh *et al.* 2004). These differences in the chemical composition of oils might arise from several environmental (climatic, seasonal and geographical), genetic differences and different chemotypes and the nutritional status of the plants.

Table 1. Chemical compositions (%, v/w) of *Carum copticum* essential oil.

	Components	*Carum copticum*[a] (%)	Retention index[b]	Identification methods
1	Myrcene	0.32	523	MS, RI
2	α-thujene	1.32	932	MS, RI
3	α-pinene	9.65	941	MS, RI, CoI
4	*β*- pinene	3.51	948	MS, RI, CoI
5	α-phyllanderene	0.54	1000	MS, RI
6	*p* - cymene	5.91	1028	MS, RI, CoI
7	*β*-phyllanderene	1.05	1035	MS, RI
8	γ- terpinene	33.85	1060	MS, RI, CoI
9	Thymol	21.18	1294	MS, RI, CoI
10	Carvacrol	8.93	1306	MS, RI
Total		86.26	Yield	0.96±0.05

[a] Percentage composition determined on column.

[b] The retention Kovats indices were determined on HP 5 capillary column in reference to n-alkanes.

MS = Mass spectroscopy, RI = Retention index, CoI = Co injection with authentic compounds.

The results from the antimicrobial activity tested by micro dilution method are summarized in Table 2. *C. copticum* essential oil exhibited significant antimicrobial activity against all tested strains. Inhibition values ranged as follows: MIC 1.5 ± 0.10 µg/ml and MBC 6.0 ± 0.63 µg/ml for bacteria, and MIC 1.5 ± 0.1 µg/ml and MBC 5.5 ± 0.15 µg/ml for fungi. The *C. copticum* oil showed the strongest activity against both type of microorganisms.

Generally, the fungi appear to be more sensitive compared to bacteria, which could be explained by their different cell organization. EO of *C. copticum* expressed higher antibacterial activity than both the antibiotics tested (streptomycin and netilmycin). In our study, most of the

Table 2. Antimicrobial activity of the essential oils from *Carum copticum* using MIC and MBC test.

Microorganisms	The essential oil of *C. copticum*			Antibiotic		
	Zone inhibition[a]	MIC[b]	MBC[b]	DD[c]	MIC	MBC
Gram-positive bacteria						
Bacillus cereus	15±0.12	2.5±0.21	2.5±0.18	20±0.11	2.5±0.05[f]	2.5±0.12[f]
B. subtilis	15±0.13	2.5±0.12	2.5±0.32	20±0.13	2.5±0.05[f]	2.5±0.10[f]
Staphylococcus aureus	20±0.04	5.5±0.06	6.0±0.63	25±0.55	5.5±0.21[f]	5.5 ±0.12[f]
Gram-negative bacteria						
Shigella shiga	20±0.41	5.5±0.01	5.5±0.26	20±0.15	5.5±0.64[f]	5.5±0.06[f]
Escherichia coli	10±0.94	1.5±0.1	1.5±0.33	15±0.01	3.0±0.19[f]	3.0±0.53[f]
Shigella sonnei	25±0.10	4.0±0.12	4.5±0.06	25±0.06	4.5±0.00[f]	4.0±0.05[f]
Pseudomonas aeruginosa	25±0.85	5.0±0.83	5.5±0.28	25±0.64	5±0.59[f]	5±0.08[f]
Fungal strains						
Candida parapsilosis	35±0.30	1.5±0.11	1.5±0.10	20±0.85	3.5±0.65[d]	4.0±0.05[d]
Aspergillus niger	20±0.06	2.0±0.11	2.5±0.32	25±0.15	5.5±0.53[d]	5.5±0.95[d]
A. fumigatus	25±0.17	5.5±0.64	5.5±0.15	25±0.07	4.5±0.07[d]	4.5±0.34[d]

DD (mm) : Disc diffusion; a : Tested at a concentration of 1.2 µg/disc. b : Values given as µg/ml. c : Tested at a concentration of 5.0 µg/disc. d : Streptomycin. f : Netilmycin. All tests were performed in triplicate. Statistically, the differences were significant at $p \leq 0.01$ or at least at $p \leq 0.05$ (in a few cases), using DMRT.

antimicrobial activity in essential oils from *C. copticum* appears to be associated with thymol, γ- terpinene and *p* - cymene, these results agree with those reported by Marino *et al.* (1999). Concerning the *C. copticum* essential oil and its major constituent all results were statistically significant ($p \leq 0.01$).

Table 3. DPPH radical scavenging activity of *C. copticum* seed essential oil and its main constituents. Butylhydroxyanisole and ascorbic acid were used as positive controls.

Sampels	IC_{50} (µg/ml)
C. copticum essential oil	18.12 ± 0.94
γ - terpinene	16.01± 0.43
Thymol	18.11± 0.85
BHA	15.55± 0.17
AA	11.05± 0.05

Values are mean ± Sd of three replications.

The results presented in Table 3 revealed that *C. copticum* essential oil and its main constituents exhibited a remarkable activity. In particular, γ - terpinene showed clearly a higher activity (IC_{50} = 16.01± 0.43 µg/ml). Butylated hydroxyamide anisole (BHA) and ascorbic acid (AA) as positive controls exhibited IC_{50} values equal to 15.55 ± 0.17 µg/ml and 11.05 ± 0.05 µg/ml, respectively. Our findings revealed that the presence of chemical compounds such as thymol and γ - terpinene could be attributed by strong antioxidant activity.

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