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Antimicrobial activity and essential oil composition of *Cuminum cyminum* L. and *Carum carvi* L. seeds from Iran

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Abstract

The essential oils of *Cuminum cyminum* and *Carum carvi* seeds obtained from Iran, were screened for their antimicrobial activity against *Candida albicans* and *Escherichia coli*. Essential oils were obtained by hydro-distillation using Clevenger type apparatus during approximately 3 hours and analyzed using gas chromatography/mass spectrometry (GC/MS). Forty one components were identified in *C. cyminum* oil that represented 99.98% of the oil. The main components of the oil were, α -Terpinene-7-al (33.48%), n-Dodecane (25.54%), Cumin aldehyde (14.56%), 1,8-Cineole (9.4%), α -Terpinene (4.76%) and Sabinene (4.74%). Forty one components were identified in *C. carvi* oil that represented 99.97% of the oil. The main components of the oil were, γ -Terpinene (17.79%), γ -Terpinene-7-al (16.81%), 9-epi-(E)-Caryophyllene (14.92%), Cumin aldehyde (13.68%), α -Terpinene-7-al (7.21%), *p*-Cymene (5.21%) and Limonene (4.40%). Antimicrobial activity of the essential oils were evaluated by disc diffusion methods. The oils showed high antimicrobial activity against *Candida albicans* and *Escherichia coli* two medically important pathogens.

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Introduction

In the recent decades, antimicrobial plant products have gained a special attention because of increase resistance to antibiotics acquired of some microorganisms (Al-Sheddi, 2009). With the growing interest in the use of essential oils in both the food and the pharmaceutical industries, a systematic examination of plant essential oils for these properties has become increasingly important. The use of natural antimicrobial compounds is important not only in the preservation of food but also in the control of human and plant diseases of microbial origin (Baratta *et al.*, 1998).

Cuminum Cyminum and *carum carvi*, belonging to the family Apiaceae, are one of the earliest cultivated herbs in Asia, Africa and Europe. In folk medicine, these plants are used as a carminative for stomach disorders, diarrhea, and colic, as well as particularly in veterinary medicine (Gruenwald *et al.*, 2004). The oil of these plants is especially used as a carminative and astringent. Moreover, essential oil of these plants shows a high antifungal activity against various pathogenic fungi, and effective high antibacterial activity. Therefore they are also used as a fumigant or additive in the storage of foodstuffs (Li & Jiang, 2004).

The aim of this study was to determine the antimicrobial activity and essential oil composition from seeds of *Cuminum cyminum* and *Carum carvi* from Iran.

Materials and methods

Plant seed samples of *Cuminum cyminum* and *Carum carvi* were purchased from local markets. One hundred grams of each seed was crushed and ground in a household grinder.

Essential oil extraction

Essential oil was obtained from the powder of each plant seed (100 g) from *Cuminum cyminum* and *Carum carvi* that were subjected to hydro-distillation using a Clevenger-type apparatus (w/w) for 3 h. Then the oils drying by anhydrous sodium sulfate. The

isolated oils were stored in tightly closed vials at 4°C until analysis.

Essential oil analysis

Essential oil was analyzed by Hewlett – Packard GC/MS (model 7890 series II) operating at 70e V ionization energy, equipped with a HP–5 capillary column phenyl methyl siloxane (30m' 0.25mm, 0.25 µm film thickness) with helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method (Van den dool and Kratz, 1963). using n–alkanes as standard. The compounds were identified by comparison of retention indices (RRI–HP–5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra (Adams, 2001).

Micro –organisms

Standard strain of *Candida albicans* (ATCC No. 10231) and Gram-negative bacteria *E. coli* (ATCC 25922), were obtained from Iranian Research Organization for Science and Technology.

Determination of antifungal/antibacterial activity by the disc diffusion method

In vitro antimicrobial activity of the essential oil of *Cuminum cyminum* and *Carum carvi* seeds were evaluated by disc diffusion method, with determination of inhibition zones (IZ), according to the National Committee for Clinical Laboratory Standards using 100 µL each suspension of the tested microorganisms containing 2.0×10^6 CFU/ml for bacteria and 2.0×10^5 CFU/ml (0.5 McFarland) spore for fungi strain. The bacteria inocula was prepared by suspending overnight colonies from sabouraud dextrose agar (SDA) media, and the *C. albicans* was prepared by suspending colonies from 48 h old potato dextrose agar (PDA) cultures respectively. Fungal or bacterial suspension were seeded into Petri dishes (9 cm) containing 20 ml sterile sabouraud dextrose agar (SDA) or potato dextrose agar (PDA) using a sterile cotton swab. The sterile paper discs (6 mm in diameter) were

individually impregnated with 20 µl of the oil and then placed on the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37°C for 24 h and at 30°C for 48 h for the *C. albicans* strain. After incubation, the mean inhibition zone diameter for each concentration was measured in millimeters. All the studies were performed in triplicate. Blank discs containing 20 µl DMSO were used as negative controls (Alizadeh, 2013).

Statistical analysis

All data were done in three replicate and analyzed by analysis of variance (ANOVA) and mean values were compared with Duncan's multiple range test using SPSS software (version 9.2 for windows).

Result and discussion

Essential oil components

The chemical composition of the essential oil of *Cuminum cyminum* L. and *Carum carvi* seeds (purchased from local markets from Iran) and the retention indices are presented in (Table 1,2). Forty one components were identified in *C. cyminum* oil that represented 99.98% of the oil. The main components of the oil were, α-Terpinene-7-al (33.48%), n-Dodecane (25.54%), Cumin aldehyde (14.56%), 1,8-Cineole (9.4%), α-Terpinene (4.76%) and Sabinene (4.74%). Forty one components were identified in *C. carvi* oil that represented 99.97% of the oil. The main components of the oil were, γ-Terpinene (17.79%), γ-Terpinene-7-al (16.81%), 9-epi-(E)-Caryophyllene (14.92%), Cumin aldehyde (13.68%), α-Terpinene-7-al (7.21%), p-Cymene (5.21%) and Limonene (4.40%). The composition of the essential oil of *Cuminum cyminum* and *Carum carvi* has been examined previously by other researchers. Li and Jiang (2004) reported, Cuminal (36.31%), cuminic alcohol (16.92%), γ-terpinene (11.14%), safranal (10.87%), p-cymene (9.85%) and β-pinene (7.75%) were the major components of the essential oil of the seeds of *Cuminum cyminum* from China. Velazquez *et al.*, (2011) reported, cuminaldehyde (22.03%), γ-terpinene (15.69%) and 2-carene-10-al (12.89%) were the main components of

the essential oil of *Cuminum cyminum*. Mohamadpour *et al.*, (2012) reported, α-pinene (29.2%), limonene (21.7%), 1,8-cineole (18.1%), linalool (10.5%), and α-terpineol (3.17%) as the major compounds of the essential oil of *Cuminum cyminum* from Alborz Mountain. Begum *et al.*, (2008) reported, thymol (48.20%), o-cymene (19.29%), γ-terpinene (17.61%) and trimethylene dichloride (8.81%) were the main components of the essential oil of *C. carvi* essential oil.

Antimicrobial activity

Antimicrobial activities of *Cuminum cyminum* and *Carum carvi* oil was analyzed by disc diffusion method against *Candida albicans* and Gram-negative bacteria *E. coli*. The result of inhibition zones diameters of the essential oil of *Cuminum cyminum* L. and *Carum carvi* against two microorganisms tested are reported in (Table 3). There was considerable variability in size of zone of inhibition depending the different volumes of oil was used. Greater zones of inhibition were observed when 20 µL/disk of essential oils were used. The inhibition zones for *Cuminum cyminum* L. essential oil ranged from 7.7-18.3 mm for *Candida albicans* (ATCC No. 10231) and 7-24 mm for the *E. coli* (ATCC 25922). The inhibition zones for *Carum carvi* L. essential oil ranged from 9.7-24 mm for *Candida albicans* (ATCC No. 10231) and 7-23 mm for the *E. coli* (ATCC 25922). The antimicrobial activity of *Cuminum cyminum* and *Carum carvi* oil exhibited that these oils have great antimicrobial activities against two the tested microorganism. The antimicrobial activity of the essential oil of *Cuminum cyminum* and *Carum carvi* has been examined previously by other researchers. Daneshmandi *et al.*, (2010) reported, *cuminum cyminum* essential oil, has the greatest antibacterial activity against bacillus cereus. Keshavarz *et al.*, (2013) reported, *Carum carvi* essential oil has anti-colitic activity on TNBS-induced colitis in rats. Mohamadpour *et al.*, (2012) reported, the essential oil of *Cuminum cyminum* has a strong inhibitory effect on fungal growth. Some Researchers reported there is relationship between the chemical structures of the most abundant compounds in the

tested essential oils and the antimicrobial activity. In this study antimicrobial activity of *Cuminum cyminum* and *Carum carvi* can be attributed to the presence of 1,8-Cineole, α -Terpinene, γ -Terpinene and Limonene. Because some studies have shown that these compounds have antimicrobial activity (Park, *et*

al., 2012 and Vurren and Viljoen, 2007). These compounds destroy microorganisms through destroying the cell walls and proteins, interfering in the work of membrane enzymes and affecting DNA and RNA replication (Uyar, *et al.*, 2008).

Table 1. Essential oil components of *Cuminum cyminum* analyzed by (GC/MS).

NO	Compound	RI	Percentage of oil
1	α -Thujene	923	0.30
2	α -Pinene	930	0.01
3	Camphene	945	0.45
4	Sabinene	970	4.74
5	β -pinene	975	0.76
6	Myrcene	987	0.48
7	n-Decane	996	1.24
8	α -Phellandrene	1003	0.13
9	α -Terpinene	1014	4.76
10	p-Cymene	1022	0.23
11	Limonene	1025	0.37
12	β -Phellandrene	1026	0.11
13	1,8-Cineole	1028	9.40
14	γ -Terpinene	1057	0.05
15	cis-Sabinene hydrate	1063	0.05
16	Terpinolene	1085	0.05
17	Linalool	1096	0.04
18	cis-p-Menth-2-en-1-ol	1118	0.12
19	1-Terpineol	1135	0.10
20	Terpinene-4-ol	1174	0.09
21	α -Terpineol	1188	0.92
22	cis-dihydroCarvone	1191	0.13
23	n-Dodecane	1196	25.54
24	Cumin aldehyde	1244	14.56
25	α -Terpinene-7-al	1288	33.48
26	γ -Terpinene-7-al	1291	0.49
27	Carvacrol	1301	0.54
28	p-Mentha-1,4-dien-7-ol	1327	0.11
29	Unknown	1336	0.03
30	(E)-Caryophyllene	1416	0.01
31	trans--Bergamotene	1432	0.06
32	(E)- β -Farnesene	1453	0.03
33	9-epi-(E)-Caryophyllene	1467	0.12
34	β -Acoradiene	1470	-
35	ar-Curcumene	1479	0.01
36	Viridiflorene	1494	0.01
37	β -Bisabolene	1505	0.02
38	Myristicin	1518	-
39	(E)-Nerolidol	1560	0.01
40	Caryophyllene oxide	1579	0.10
41	Carotol	1592	0.10
Total			99.98

^aRI, retention indices in elution order from HP – 5 column.

Table 2. Essential oil components of *Carum carvi* analyzed by (GC/MS).

NO	Compounds	RI	Percentage of oil
1	α -Thujene	923	0.32
2	α -Pinene	930	1.44
3	Camphene	945	0.05
4	Sabinene	970	0.65
5	β -pinene	974	2.23
6	Myrcene	987	0.67
7	n-Decane	996	0.21
8	α -Phellandrene	1003	0.04
9	δ -3-Carene	1008	0.04
10	α -Terpinene	1014	0.32
11	<i>p</i> -Cymene	1022	5.21
12	Limonene	1026	4.40
13	1,8-Cineole	1028	0.17
14	(<i>Z</i>)- β -Ocimene	1033	0.28
15	(<i>E</i>)- β -Ocimene	1043	0.10
16	γ -Terpinene	1058	17.79
17	cis-Sabinene hydrate	1063	0.07
18	meta-Cresol	1074	0.15
19	Terpinolene	1085	1.18
20	Linalool	1096	0.13
21	Unknown	1165	0.32
22	Terpinene-4-ol	1174	0.50
23	<i>p</i> -Cymen-8-ol	1182	0.06
24	α -Terpineol	1187	0.18
25	cis-dihydroCarvone	1191	0.89
26	n-Dodecane	1196	0.05
27	Cumin aldehyde	1240	13.68
28	Unknown	1264	0.29
29	Phellandral	1271	0.11
30	α -Terpinene-7-al	1283	7.21
31	γ -Terpinene-7-al	1291	16.81
32	Carvacrol	1300	0.56
33	<i>p</i> -Mentha-1,4-dien-7-ol	1327	2.66
34	Geranyl acetate	1380	0.12
35	(<i>E</i>)-Caryophyllene	1415	0.13
36	<i>p</i> -Cymen-7-ol acetate	1420	3.05
37	9-epi-(<i>E</i>)-Caryophyllene	1463	14.92
38	Myristicin	1519	2.33
39	Elemicin	1554	0.18
40	Dill apiol	1621	0.21
41	α -Bisabolol	1682	0.11
Total			99.97

^aRI, retention indices in elution order from HP – 5 column.

Table 3. Antimicrobial activity for different volumes of essential oils of *Cuminum cyminum* and *Carum carvi*.

Essential Oil	Microorganism	Inhibition Zone (mm)							
		20	10	5	2.5	1.25	0.63	0.32	0.16
<i>C. cyminum</i>	<i>E. coli</i>	24.0 a	18.3 a	16.0 a	15.3 a	11.0 b	10.0 a	8.3 b	7.0 b
<i>C. cyminum</i>	<i>C. albicans</i>	18.3 b	16.3 c	15.0 ab	12.7 bc	11.3 b	11.0 a	10.0 a	7.7 b
<i>Carum carvi</i>	<i>E. coli</i>	23.0 a	18.0 ab	16.0 a	10.3d	10.0 bc	8.7 b	7.3 c	7.0 b
<i>Carum carvi</i>	<i>C. albicans</i>	24.0 a	19.7 a	15.7 a	13.3 b	12.3 a	10.3 a	9.7 a	9.7 a

Each value in the table was obtained by calculating the average of three experiments.

Diameter of inhibition zone including disc diameter of 6 mm.

In each column, means with the same letters are not significantly different at 5% level of Duncan's new multiple range test.

Conclusion

In this study it was found that essential oil of *Cuminum cyminum* and *Carum carvi* had a good antimicrobial activity against *Candida albicans* and *E. coli* and it can be used in aromatherapy and pharmacy, and also in pathogenic systems to prevent the growth of microbes. It seems that the activity of the essential oil of these herbs is due to the interactions of their components, such as 1,8-Cineole, α -Terpinene, γ -Terpinene and Limonene. However, more studies are needed to be done on antimicrobial effect of the essential oil of these species.

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