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Chemical composition, antimicrobial and antioxidant activity of essential oils from cumin and ajowan

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Abstract. *Patil SD, Maknikar PP, Wankhade SJ, Ukesh CS, Rai MK. 2016. Chemical composition, antimicrobial and antioxidant activity of essential oils from cumin and ajowan. Nusantara Bioscience 8: 60-65.* Plant essential oils have gained importance as alternative remedies for treatment of many infectious diseases and food preservatives. In the present study, we have determined the chemical composition of the essential oils (EOs) from two Indian spices *Cuminum cyminum* (cumin) and *Trachyspermum ammi* (ajowan) of family Apiaceae by gas chromatography-mass spectrometry (GC-MS). Moreover, the antimicrobial potential of these oils was evaluated against several Gram-positive and Gram-negative bacteria using disc diffusion and broth microdilution methods. A Total of 20 major chemical components were analyzed by GC-MS studies and were found to be cuminaldehyde (36.67%) and caren-10-al (21.34%) in case of cumin essential oil while *p*-cymene (15.54%) and thymol (15.48%) were found to be present in ajowan essential oil. Both the EOs exhibited potent antibacterial effect against most of the tested pathogens. Furthermore, cumin and ajowan EOs demonstrated remarkable antibacterial activity against *Salmonella typhi* with an inhibition zone diameter of 54 and 60 mm respectively with identical MIC value of 12.5 µl/ml. Ajowan EO was found to exhibit wide spectrum activity against both the Gram-positive and Gram-negative organisms when compared with cumin. Both the essential oils were more potent than standard antibiotic chloramphenicol except cumin against *Escherichia coli* and *Enterobacter aerogenes*. Antioxidant activity of cumin was weaker (12.36%) and ajowan was stronger (71.68%) than standard ascorbic acid (20.24%) at 1000 µg/ml concentration when assessed by DPPH radical scavenging assay. Our study suggests that spice essential oils have significant potential in controlling the human and foodborne pathogens.

Keywords: *Cuminum cyminum, Trachyspermum ammi*, essential oils composition, antimicrobial activity, antioxidant activity

INTRODUCTION

The outbreak of population across the globe has opened a huge market for the food industries. The need has urged the entrepreneurs and consequently the scientific community to look beyond the horizons of the nations for the possible chances of growth across the border. Therefore, the transit of foodstuffs specially packaged ones has emphasized on their safety and durability. This is inevitable when there are reports on the outbursts of the food-borne illnesses caused by the pathogenic bacteria and their enterotoxins produced (Gachkar et al. 2007). The efforts have been made to look for the natural resources which not only render the pleasant tastes to the foodstuffs apart from their significant aroma but also enhance their quality and durability. This is obvious when the priority is to look for the natural and non-toxic preservatives. Naturally, among the rest of natural resources, spices are the most expected choices. The spices being the most common in kitchens of India are known for their medicinal values apart from their taste enhancing abilities (Vasanthi and Parameswari 2010). Indian subcontinent is home for some of the world's most exotic spices that have been widely practiced in the folklore medicines in India since antiquity.

Spices are aromatic plants characterized by their characteristic aroma by the virtue of their essential oil (EO) contents. They have diverse applications in medicines (Pisseri et al. 2008), rituals, kitchens, perfumes, cosmetics, etc. and have been well documented in Ayurveda, Siddha, and Unani medicines but were lacking the scientific evidence which would support them. The efforts have been made by researchers in the past to justify the use of spices in traditional medicinal practices. Spices have been screened extensively for their antimicrobial properties (Wong and Kittis 2006; Gachkar et al. 2007; Goñi et al. 2009; Wang et al. 2011; Packiavathy et al. 2012; Patil et al. 2015), food preservation capacity (Burt, 2004) as well as their potential antioxidant properties (Wong and Kittis 2006; Padmashree et al. 2007; Sarikurkcu et al. 2010).

The family Apiaceae (formerly Umbelliferae) members are angiospermic temperate herbs with some approximately 300 genera and 3000 species. These plants have been identified with their properties to induce apoptosis, antimicrobial, cyclooxygenase inhibitory efficiencies and antitumor effects (Pae et al. 2002). The present work was intended to identify the active components present in the essential oils of seeds from two Apiaceae family members *viz.* cumin (*Cuminum cyminum*) and ajowan (*Trachyspermum ammi*) by gas chromatography (GC) and

mass spectrometry (MS) analyses and was further assessed for their antimicrobial potential against some of the pathogenic bacteria along with their free radical scavenging efficacy.

MATERIALS AND METHODS

Plant materials

The seeds *C. cyminum* and *T. ammi* were purchased from the D Mart of Amravati city, Maharashtra, India.

Extraction of essential oils

Plant materials were pulverized before extraction of EOs. The 100 g seed powder of each spice was subjected for hydrodistillation in Clevenger-type of apparatus for 3 h. The extracts were dehydrated with treatment of anhydrous sodium sulfate and the EOs obtained were refrigerated in sealed glass vials at 4 °C for further analysis (Palmeira-de-Oliveira et al. 2012).

GC-MS analysis

GC-MS analyses of EOs were performed by using JEOL, GC-MS (AccuTOF™ GCx Time-of-Flight GCxGC Mass Spectrometer), USA. The reaction was carried out in split mode with a split ratio of 1:50 and 70 ml/min flow rate. Ion source and transfer line temperatures were kept at 230°C and 220°C respectively. The mass spectrometer was operated with Electron Impact Ionization mode at 70eV. The mass spectra were generated by centroid scan and mass range was kept from 50 to 700 amu which is sufficient for most of the organic compounds. Identification of chemical constituents of the EOs was carried out by matching their recorded spectra with the mass spectral data bank of NIST library given by the instrument software.

Antimicrobial activity of essential oils

The antimicrobial activity of EOs was assessed against nine bacterial strains obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India including two Gram-positive bacterial strains *viz. Streptococcus mutans* (MTCC 497); *Streptococcus pyogenes* (MTCC 442) and seven Gram-negative bacterial strains *viz. Proteus vulgaris* (MTCC 426); *Escherichia coli* (MTCC 443); *Klebsiella pneumoniae* (MTCC 109); *Salmonella typhi* (MTCC 734); *Salmonella paratyphi* A (MTCC 735); *Enterobacter aerogenes* (MTCC 111) and *Pseudomonas aeruginosa* (MTCC 424). The strains were maintained on nutrient agar slopes and refrigerated at 4 ºC as stock cultures until further use.

The antimicrobial assay was performed by using disc diffusion method as described by Gulluce et al. (2007). A loopful of each bacterial stock culture was inoculated into sterile Mueller-Hinton broth (MHB) and incubated at 37 ºC for 18 h. These cultures were further adjusted to 0.5 McFarland turbidity standards on Shimadzu UV-Visible spectrophotometer with sterile MHB to obtain 10^8 CFU/ml bacterial suspensions and were further used as inocula. The disc diffusion assay was performed by swabbing each bacterial inoculum aseptically onto the Mueller Hinton agar (MHA) plates. The sterile paper discs (6 mm dia.) were impregnated with 20 µl of each EO and placed on the surface of MHA plates previously inoculated with different bacterial strains. The plates were kept undisturbed for 15 min until the EOs are thoroughly diffused throughout the plates. Chloramphenicol discs (10 mcg/disc) were used as a positive control. Plates in triplicate were incubated at 37 ºC for 18 h and the mean diameter of their inhibitory halos <10 (mm) around each EO disc was recorded as a measure of antibacterial potential against respective bacteria.

Minimum inhibitory concentration determination

The minimum inhibitory concentration (MIC) for each EO was assessed by 96 well broth microdilution method in MHB as per the guidelines are given by Clinical and Laboratory Standards (CLSI 2006). The suspensions of overnight old bacterial cultures were adjusted at 0.5 McFarland turbidity standards to get 10^8 CFU/ml and used as inocula. The EOs of *C. cyminum* and *T. ammi* were dissolved in 10% dimethylsulfoxide (DMSO) to render the proper dissolution of EOs with MHB (Hajlaoui et al. 2010) and their twofold serial dilutions were prepared in the range of 50 to 0.37 µl/ml. The plates were incubated at 37 ºC for 18 h and the highest dilution of EO (lowest concentration), showing no visible growth was regarded as the MIC. Further confirmation of MIC value was made by spot inoculation method.

Antioxidant assay

The antioxidant assay of cumin and ajowan was executed using reducing capability of 2,2,diphenyl-2-picrylhydrazyl (DPPH) as per the method described by Bounatirou et al. (2007). In brief, five diverse concentrations of EOs were prepared (100, 250, 500, 750 and 1000 mg/l) in methanol. 2 ml methanol DPPH (24 µg/ml) solution was treated with 50 µl aliquot of each EO previously prepared in methanol. The reaction mixture was left undisturbed at room temperature and after a reaction time of 30 min, the absorbance of each sample was read at 517 nm on Shimadzu UV-Visible spectrophotometer. Ascorbic acid was used as standard antioxidant for comparison. The reactions were performed in triplicate and the radical scavenging efficacy of each EO was estimated by the formula as follows:

$$\text{Scavenging efficacy \%} = A_0\text{-}A_1/A_0 \times 100$$

Where,
A_0 = absorbance of control
A_1 = absorbance of sample

RESULTS AND DISCUSSION

The composition of cumin and ajowan EOs was assessed using GC-MS analysis and the details of its ten major peaks are presented in Table 1 and 2. The hydrodistillation of cumin seed oil gave a yield of 4.5% (v/w) and its GC-MS analysis deciphered highest content of cuminaldehyde and caren-10-al with 36.67% and

21.34% concentrations respectively (Table 1). In addition to this, the next eight components with lower amounts were β-pinene (18.76%), γ-terpinene (16.86%), terpinen-4-ol (2.44%), α-thujene (1.88%), α-pinene (1.41%), *p*-cymene (0.30%), carbicol (0.19%) and α-terpineol (0.09%).

The hydrodistillation of ajowan seeds gave a yield of 5.1% (v/w) essential oil. GC-MS analysis of ajowan seed oil revealed *p*-cymene being the principal constituent with a concentration of 15.54% followed by β-pinene (10.75%) and carvacrol (10.72%). In addition to this, other components present were α-phellandrene (8.65%), sabinene (4.24%), α-pinene (4.68%), β-phellandrene (7.56%), α-terpinene (6.71%), γ-terpinene (9.27%), and 10.72% carvacrol (Table 2).

The results of antibacterial effects of EOs against nine pathogenic bacteria determined by the disc diffusion method are shown in Table 3. Of the two EOs, ajowan EO exhibited better inhibitory potential against *S. mutans, E. coli, S. paratyphi* A, *P. vulgaris* and *P. aeruginosa* as compared to the cumin seed oil, whilst the cumin EO produced higher inhibitory action against *S. typhi* than ajowan. Cumin EO could not produce the destructive effects against pathogens as compared to the ajowan nonetheless, it was able to show the profound inhibitory halos around the EO paper discs than the standard antibiotic chloramphenicol (10 mcg) used. This was more prominent against the bacteria *viz. S. mutans, P. vulgaris, S. paratyphi* A. This proves that both cumin and ajowan could be used as alternative medicines in combating infections caused by such pathogens. Results of MICs of cumin and ajowan EOs are represented in Table 4. MIC values of cumin EO were identical against the test organisms (25 µl/ml) except *S. typhi* which was more sensitive (12.5 µl/ml). Similarly, ajowan EO also inhibited test pathogens with similar effects (MIC 12.5 to 25 µl/ml).

The reducing efficacy of DPPH in the presence of antioxidant is one of the several methods proposed for antioxidant assay whose characteristic color transformation from purple to yellow is measured spectrophotometrically at 517 nm. Scavenging efficacy of cumin and ajowan EOs was compared with the ascorbic acid (Figure 1), a standard commercial synthetic antioxidant. From the data it was evident that ajowan EO exhibited potent antioxidant efficacy (71.6%) than the standard ascorbic acid (20.24%), on the contrary cumin (12.36%) exhibited very weak antioxidant efficacy.

Table 1. Major chemical components in cumin EO

Peak no.	RT (Min.)	Compound name	Peak area (%)
1	6.48	α-Pinene	1.41
2	7.48	β-Pinene	18.76
3	8.51	p-Cymene	0.30
4	9.26	γ-Terpinene	16.86
5	11.56	Terpinen-4-ol	2.44
6	12.12	Cuminaldehyde	36.67
7	12.82	α-Thujene	1.88
8	12.88	Caren-10-al	21.34
9	14.32	Carbicol	0.19
10	15.87	α-Terpineol	0.09

Table 2. Major chemical components in ajowan EO

Peak no.	RT (Min.)	Compound name	Peak area (%)
1	8.03	α-Phellandrene	8.65
2	8.25	Sabinene	4.24
3	8.75	α-Pinene	4.68
4	9.08	β-Phellandrene	7.56
5	9.29	α-Terpinene	6.71
6	9.47	β-Pinene	10.75
7	9.65	*p*-Cymene	15.54
8	8.85	γ-Terpinene	9.27
9	10.34	Thymol	15.48
10	10.62	Carvacrol	10.72

Table 3. Activity of spice essential oils against pathogenic bacteria using disc diffusion test

Name of organism	Inhibitory halos (mm)		
	Cumin (20 µl)	Ajowan (20 µl)	Chloram-phenicol (10 mcg)
S. mutans (MTCC 497)	28	64	25
S. pyogenes (MTCC 442)	28	42	27
P. vulgaris (MTCC 426)	27	52	26
E. coli (MTCC 443)	14	66	20
K. pneumoniae (MTCC 109)	16	40	26
S. typhi (MTCC 734)	60	54	28
S. paratyphi A (MTCC 735)	31	52	27
E. aerogenes (MTCC 111)	14	48	23
P. aeruginosa (MTCC 424)	26	50	26

Table 4. Minimum inhibitory concentration (MIC) of EO against pathogenic bacteria

Name of organism	MIC µl/ml	
	Cumin	Ajowan
S. mutans	25	12.5
S. pyogenes	25	25
P. vulgaris	25	12.5
E. coli	25	12.5
K. pneumoniae	25	25
S. typhi	12.5	12.5
S. paratyphi A	25	12.5
E. aerogenes	25	25
P. aeruginosa	25	12.5

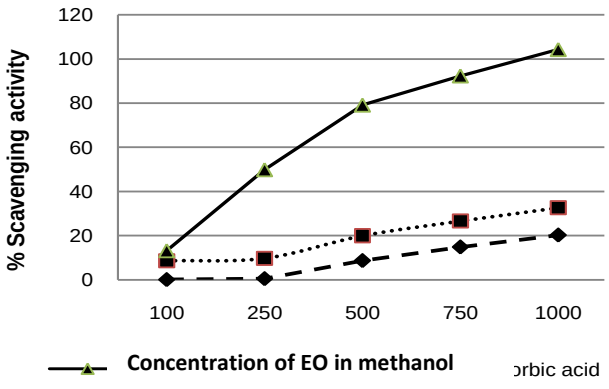

Figure 1. DPPH scavenging assay of ajowan and cumin essential oil compared with standard ascorbic acid

Ever since from the historic time, importance of medicinal plant extracts and essential oils in traditional medicine system have been well documented. Particularly, essential oils are known to possess wide spectrum activities including spasmolytic, antimicrobial, immunomodulant, antioxidant (Pisseri et al. 2008), anticandidal (Rosato et al. 2008; 2009) and antibacterial activity (Iacobellis et al. 2005; Pereira et al. 2014). Antibacterial and antioxidant activity are the promising qualities of essential oil. In recent years, the development of multi-drug resistance among pathogens is becoming a major threat in response to synthetic drugs. Furthermore, numerous synthetic drugs tested contain antibacterial agents, which reduces the microbial count, however, application of such antibacterial drugs can lead to several undesirable side effects (Sullivan et al. 2001; Rafii et al. 2008) by disturbing the ecological balance of human intestinal microflora (Edlund and Nord 1999).

Spices are regularly used in Indian foodstuffs not only because of its unique aroma and flavor but also as traditional medicine and food preservative capacity. Spice derived EOs are highly potent antimicrobials (Kamble and Patil 2008) and are superior antimicrobials than spice extracts (Aggarwal and Goyal 2012). Antimicrobial capacities are attributed to chemical constituents of EO (Singh et al. 2004). By recognizing the use of essential oils in food preservation system along with its pharmacological importance, in the present study cumin and ajowan seeds were purchased from the local market in order to focus primarily on the probable composition of EOs and their contribution in antibacterial and antioxidant properties.

The chemical component cuminaldehyde (36.67%) was the major constituent of cumin EO in the current study. There are other reports which have shown the principal chemical component of cumin as cuminaldehyde with 39.48% whilst γ-terpinene and O-cymene ordered second and third with 15.21% & 11.82% concentrations respectively (Hajlaoui et al. 2010). Oroojalian et al. (2010) acknowledged cuminaldehyde as the major component too with 30.2% concentration. Iacobellis et al. (2005) reported *p*-mentha-1, 4-dien-7-al as the major component with a concentration of 27.4% whereas cuminaldehyde secured second rank with a concentration of 16.1%. Beis et al. (2000) also reported the cuminaldehyde (27.6 %) as the major constituent of cumin EO followed by γ-terpinene (17.25%), *p*-mentha-l.3-dien-7-al (15.18%), β-pinene (10.22%) and *p*-mentha-l,4-dien-7-al (9.48%). It is well-known fact that composition of herb and spice essential oils varies with the determining factors, such as type of cultivar, harvest time, extraction method, geographical origin and storage conditions (Hajlaoui et al. 2010). However, the results of ajowan EO analysis showed that it is a rich source of *p*-cymene (15.54%), thymol (15.48%), β-pinene (10.75%), carvacrol (10.72%) and γ-terpinene (9.27%). It was evident that the percent chemical composition of ajowan EO in our study greatly differed from previous study where *p*-cymene (22.9%), γ-terpinene (23.92%) and thymol (50.07%) reported as major constituents (Moazeni et al. 2012). Paul et al. (2011) also reported thymol as the major constituent of ajowan EO with 49.64%.

The antibacterial effect of cumin EO showed broad-spectrum activity against both the Gram-positive and Gram-negative organisms especially *S. typhi* and *S. paratyphi* A. In addition to this many researchers have reported a potential antimicrobial activity exhibited by cumin seed EO (Iacobellis et al. 2005; Oroojalian et al. 2010; Allahghadri et al. 2010; Derakshan et al. 2010). Our findings suggest that the antibacterial activity of cumin and ajowan EOs may probably be due to their major chemical constituents. Presence of γ-terpinene and *p*-cymene in cumin EO and *p*-cymene, thymol, β-pinene, and carvacrol in ajowan have been previously reported for their antibacterial activities (Ultee et al. 1999; Yang et al. 2014). This antibacterial effect of EO components may fluctuate while using alone and in combination (Fu et al. 2007). The variable results in susceptibility pattern of test pathogens towards EOs could be attributed to loss of cytoplasmic components, intracellular ATP, K and distorted cell surface morphology (Paul et al. 2011) and rate of penetration through the cell wall and cell membrane structure (Cox et al. 2000).

Cumin EO induced moderate scavenging effects at lower concentrations (100 and 250 mg/l), conversely, it was weaker than the standard (ascorbic acid) at higher concentrations (500, 750 & 1000 mg/l). Fakoor and Rasooli (2008) have also shown that cumin EO produce lower scavenging activity as compared to the positive control (Trolox). Similar reports have also been published by Hajlaoui et al. (2010) and such lower antioxidant activity of cumin EO could be attributed to the prevalence of monoterpenic chemical constituents such as γ-terpinene and *p*-cymene present in the EO. It is well recognized that the presence of higher monoterpenic contents can be ineffective in producing the desired antioxidant effect (Hajlaoui et al. 2010). The chemical constituents of cumin EO as determined by GC-MS assay (Table 1) revealed the prevalence of γ-terpinene (35.42%) which is a monoterpene and could be the reason for exhibiting lower antioxidant potential by cumin EO. This finding is consistent with the reports of other researchers who observed identical pattern exhibited due to presence of monoterpenic components (Ruberto et al. 2000).

The scavenging ability of DPPH has been utilized by many researchers to estimate the antioxidant activity of diverse natural products (Padmashree et al. 2007). The free radical DPPH shows antioxidant property by accepting an electron or hydrogen atom to become a stable product (Soares et al. 1997). Highest antioxidative activity (71.68%) of ajowan EO was produced at 1000 mg/l concentration and was three times greater than the effect produced by standard ascorbic acid (20.24%) suggesting its powerful antioxidative effect. This is in agreement with the reports by Gurdip et al. (1998) who also observed better antioxidant activity of ajowan EO over standard synthetic antioxidant butylated hydroxytoluene (BHT) used in their study. Results of high antioxidant activity obtained by Huang et al. (2011) are also consistent with the present finding. They reported that abundance of *p*-cymene and carvacrol is crucial in the antioxidant activity of ajowan

EO. They further added that out of the 25 different EOs studied, ajowan was superior in its antioxidant potential.

From the results obtained in the present study, it can be concluded that EOs from both the members of Apiaceae family, *i.e.* cumin and ajowan have significant antimicrobial and antioxidant activities. In addition to this, superior antimicrobial activity of cumin and ajowan EOs over standard antibiotic chloramphenicol suggest its use as an alternative to the commercial antibacterial agents. Spices like cumin and ajowan would be the better sources for the production of natural antioxidant and food preservatives (antimicrobial agents). Further, extensive studies on the investigation of antimicrobial nature of such oils would be useful for the development of new generation antimicrobials.

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