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Original Article

Antimicrobial Activity of Essential Oil from Seeds of *Carum carvi* and Its Composition

Jaripa Begum[1], M Nazrul Islam Bhuiyan[1*], Jasim Uddin Chowdhury[1], M Nuzmul Hoque[1] and M Nural Anwar[2]

[1]*Bangladesh Council of Scientific & Industrial Research (BCSIR), Chittagong Cantonment, Chittagong 4220, Bangladesh,* [2]*Department of Microbiology, University of Chittagong, Chittagong 4331, Bangladesh*

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The essential oil of *Carum carvi* L. seeds was screened for its antimicrobial activity against ten pathogenic bacteria and six phytopathogenic fungi. The essential oil showed promising inhibitory activity against all the test bacteria, even at 2 µl/disc. The minimum inhibitory concentration (MIC, 100-300 ppm) and minimum bactericidal concentration (MBC, 200-400 ppm) values of essential oil were determined. The antifungal screening of the essential oil showed 100% inhibition of radial mycelial growth of all the test fungi at 100 ppm. The MIC and minimum fungicidal concentration (MFC) values were found to vary from 50-300 ppm and 200-400 ppm respectively. The essential oil extracted by hydrodistillation from seeds of *C. carvi* was analyzed by gas chromatography-mass spectrometry (GC-MS). About 10 compounds had been identified in the seeds oils, accounting for more than 99.7% of the oils. The main components of the seeds oil were thymol (48.20%), o-cymene (19.29%), γ-terpinen (17.61%) and trimethylene dichloride (8.81%).

Keywords: Antimicrobial activities, *Carum carvi,* Essential oil, GC-MS, Thymol

Introduction

Caraway (*Carum carvi* L.) is a widely cultivated spice native to Europe, Asia and North Africa. It also appears wild in Iceland, Scandinavia, throughout Russia, in Siberia, Persia, the Caucasus and the Himalayas[1-3] . The renowned Greek physician Dioscorides suggested the oil was an excellent remedy for pale-faced girls. In addition, studies have shown that caraway oil has antibacterial and larvicidal properties[4]. Caraway has a long history of use as a household remedy especially in the treatment of digestive complaints where its antispasmodic action soothes the digestive tract and its carminative action relieves bloating caused by wind and improves the appetite[5-7] . It is often added to laxative medicines to prevent griping[6]. The seed is antiseptic, aromatic, anaesthetic, anodyne, antianxiety, diuretic, mildly expectorant, fungicidal, muscle relaxant, soporific, tonic, emmenagogue, expectorant, galactogogue and stimulant[5,8-11]. It can be chewed raw for the almost immediate relief of indigestion and can also be made into infusions[6]. The seed is also used in the treatment of bronchitis and are an ingredient of cough remedies, especially useful for children and for mothers for increasing breast milk[7]. A tea made from the seeds is a pleasant stomachic and carminative, it has been used to treat flatulent colic[11-12]. The seed is used in Tibetan medicine where it is considered to have an acrid taste and a heating potency. It is used to treat failing vision and loss of appetite[13]. An essential oil from the seed is used in perfumery, for scenting soap, as a parasiticide etc.[8,14-15].

The main components of *C. carvi* oil are carvone, limonene, germacrene D, and *trans*-dihydrocarvone[4]. Gas chromatography GC and gas chromatography-mass spectrometry (GC-MS) studies showed the presence of carvone (60%) and limonene (35%) as the major chemical constituents of the essential oil of *C. carvi*[16]. Arganosa *et al.*[17] reported the major chemical constituents of the seed oil is carvone. The herb oil of caraway was found to consist of germacrene D (75%) with â-caryophyllene, â-elemene, humulene, germacrene A and B, and two cadinenes[18]. The essential oil content may vary between 2 and 7% of the air dried fruit weight[1]. Seed content of carvone varies between 1.3 and 3.5%. Caraway seeds contained the main components divided into carvone (47–62%) and limonene (34-50%)[19].

There is paucity of information regarding the antimicrobial activity and composition of the essential oil of caraway seeds in Bangladeshi local variety. Considering the above facts the present work has been undertaken to study antimicrobial activity of *C. carvi* seeds essential oil and its compositions of local variety.

Materials and Methods

Plant material

Carum carvi (caraway) seeds materials were collected from the experimental fields of BCSIR Laboratories, Chittagong, during December 2007. One voucher specimen (J-421) was deposited in the herbarium of BCSIR Laboratory, Chittagong.

[*]***Corresponding author:***
Mohammad Nazrul Islam Bhuiyan, Scientific Officer, Medicinal & Aromatic Plant Research Divison, Bangladesh Council of Scientific & Industrial Research (BCSIR) Chittagong Cantonment, Chittagong 4220, Bangladesh
Tel (Office): (031) 681761, 681763, Ext. 45; Cell: 01552 404192; E-mail: nazrul119@yahoo.com

Extraction of essential oil

The collected materials were crashed, dried and then grinned. The grinned materials were soak in distil water. This water mixture is placed with Clevenger-type apparatus. The materials were hydrodistilled using Clevenger-type apparatus[20]. Distillation of the materials was run for 4 h. The oil collected was then dried over anhydrous sodium sulphate and their physical characters like colour and odour were recorded. The oils were then stored in sealed container under refrigeration prior to analysis.

Determination of antimicrobial activity of essential oil

The essential oil obtained from *C. carvi* was tested for its antibacterial activity against ten potential pathogenic bacteria, *viz*., *Bacillus subtilis* BTCC 17, *B. cereus* BTCC 19, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* CRL (ICDDR, B), *Shigella dysenteriae* AE 14396, *S. sonnei* CRL. (ICDDR, B), *Salmonella typhi* AE 14612, *S. paratyphi* AE 14613, *Staphylococcus aureus* ATCC 6538 and *Vibrio cholerae,* AE 14748, and six phytopathogenic fungi, *viz*., *Alternaria alternata* (Fr.) Kedissler., *Botryodiplodia theobromae* pat., *Colletotrichum corchori* Ikata (Yoshida), *Curvularia lunata* (Wakker) Boedijin, *Fusarium equiseti* (Corda) Saccc. and *Macrophomina phaseolina* (Maubl) Ashby.

Antibacterial activity

The *in vitro* sensitivity of the bacteria to the test materials was done by disc diffusion method[21]. Mueller-Hinton medium was used for culture of bacteria. Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic ampicillin (20 ìg/disc, BEXIMCO Pharma Bangladesh Ltd., Dhaka).

Antifungal activity

In vitro antifungal activities of the essential oil of *C. carvi* seeds and nystatin were determined by poisoned food technique[22]. Sabouraud agar medium was used for culture of fungi. Linear mycelial growth of fungus was measured after 3-5 days of incubation. The percentage inhibition of radial mycelial growth of the test fungus was calculated as follows:

$$I = \frac{C - T}{C} \times 100$$

Where, I = percentage of inhibition; C = diameter of the fungal colony in the control and T = diameter of the fungal colony in treatment. All the results were compared with the standard antifungal antibiotic Nystatin (100 ppm). A control set was also maintained in each experiment. Each experiment was repeated thrice. The essential oil and standard antifungal and antibacterial agents were dissolved separately in specific volume of 30% dimethyl sulfoxide (DMSO) before used.

Determination of the MIC and the MBC

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values of essential oil against 10 test bacteria were determined by micro- and macrodillution broth technique[23] using Mueller-Hinton medium .The MIC and MFC values of essential oil against six test fungi were also determined by micro- and macrodilution broth technique[23] using Sabouraud medium. During MIC, MBC and MFC experiments, essential oil of 50 to 500 ppm concentrations were used.

GC-MS analysis of C. carvi essential oil

The essential oils from rhizomes of *C. carvi* was analyzed by gas chromatography-mass spectrometry (GC-MS) electron impact ionization (EI) method on GC-17A gas chromatograph (Shimadzu, Japan) coupled to a GC-MS QP 5050A mass spectrometer (Shimadzu, Japan); fused silica capillary column (30 m x 2.5 mm; 0.25 mm film thickness), coated with DB-1 (J&W); column temperature 100ºC (2 min) to 250ºC at the rate of 3ºC/min; carrier gas, helium at constant pressure of 90 kPa. Acquisition parameters full scan; scan range 40-350 amu. The essential oil composition was identified by comparing the mass spectra from NIST Library (NIST 147 and NIST 27).

Results and Discussion

The essential oil from the seeds of *C carvi* was screened for their antibacterial activity against pathogenic bacteria and compared to that of standard antibacterial antibiotic ampicillin. The results of the sensitivity test are shown in the Table 1. Inhibition zone of 27 to 45 mm was recorded even at 2 µl/disc concentration. The inhibitions were found better against all test bacteria except for *S. dysenterae*, which was equal to standard ampicillin. The MIC value of the essential oil varied between 100 and 300 ppm against the test bacteria. The essential oil showed the lowest MIC value (100 ppm) against *S. dysenterae*. The MBC values of essential oil varied between 200 and 400 ppm against test bacteria. The oil exhibited lowest MBC value (200 ppm) against *S. dysenterae* and *V. cholerae*. The results are in concurrence with the results of the essential oils of other plants reported by many authors[24-27].

The antifungal activity of essential oil of *C. carvi* against six fungi was recorded and the results are presented in the Table 2. Hundred percent radial mycelial growth inhibition was recorded against the all six test fungi at a concentration of 100 ppm, which was far better than that of the standard nystatin. The MIC values were found to vary from 50 to 300 ppm against the test fungi (Table 3). The essential oil of *C. carvi* exhibited the lowest MIC values (50 ppm) against *A. alternata, C. lunata, B. theobromae* and *M. phaseolina.* The MFC values of the essential oil were found to vary between 200 and 400ppm (Table 4). The oil exhibited the lowest MFC values (200 ppm) against all the test fungi except *C. corchori* (400 ppm). Similar antifungal activities of essential oils of other plants have also been reported by many authors[28-29].

Table 1. *Antibacterial activity of essential oil of Carum carvi seeds*

Bacterium	Antibacterial activity (Diameter of zone of inhibition in mm)				
	Essential oil (µl/disc)				Ampicillin (20µg/disc)
	2	5	10	15	
Gram-positive organism					
Bacillus cereus	30	35	38	43	22
Bacillus megaterium	38	42	47	52	22
Bacillus subtilis	38	40	43	46	25
Staphylococcus aureus	29	34	38	45	20
Gram-negative organism					
Escheriachia coli	31	33	36	40	13
Pseudomonas species	29	32	36	41	19
Salmonella typhi	27	32	35	39	30
Shigella dysenteriae	35	39	42	46	35
Shigella sonnei	45	48	52	59	30
Vibrio cholerae	35	38	42	47	24

Table 2. *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of essential oil from C. carvi seeds against 10 bacterial tests organisms*

Bacterium	Bacterial growth in Mueller-Hinton broth (Essential oil concentration ppm)							
	50	100	200	300	400	500	MIC (ppm)	MBC (ppm)
Gram-positive organism								
Bacillus cereus	+	+	+	-	-	-	300	400
Bacillus subtilis	+	+	-	-	-	-	200	300
Bacillus megaterium	+	+	-	-	-	-	200	300
Staphylococcus aureus	+	+	+	-	-	-	300	400
Gram-negative organism								
Escheriachia coli	+	+	-	-	-	-	200	300
Pseudomonas sp.	+	+	+	-	-	-	300	400
Salmonella typhi	+	-	-	-	-	-	200	300
Salmonella paratyphi	+	+	-	-	-	-	200	300
Shigella dysenteriae	+	-	-	-	-	-	100	200
Vibrio cholerae	+	+	-	-	-	-	200	200

(+) = Growth; (-) = No growth

Table 3. *Antifungal activity of essential oil of Carum carvi seeds*

Fungus	Antifungal activity (Percent radial mycelial growth inhibition)				
	Essential oil (ppm)				
	100	250	500	750	Nystatin (100 ppm)
Alternaria alternata	100	100	100	100	56
Botryodiplodia theobromae	100	100	100	100	82
Colletotrichum corchori	100	100	100	100	42
Curvularia lunata	100	100	100	100	72
Fusarium equiseti	100	100	100	100	46
Macrophomina phaseolina	100	100	100	100	71

Table 4. *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of essential oil from C. carvi seeds against six fungal tests organisms*

Fungus	Fungal growth in Sabouraud broth medium (Essential oil concentration in ppm)						MIC	MFC
	50	100	200	300	400	500	(ppm)	(ppm)
Alternaria alternata	-	-	-	-	-	-	50	200
Botryodiplodia theobromae	-	-	-	-	-	-	50	200
Colletotrichum corchori	+	+	+	-	-	-	300	400
Curvularia lunata	-	-	-	-	-	-	50	200
Fusarium equiseti	+	-	-	-	-	-	100	200
Macrophomina phaseolina	-	-	-	-	-	-	50	200

(+) = Growth; (-) = No growth

Essential oil from the seeds of *C. carvi* from Bangladesh was analyzed by GC-MS. The oil yield was 0.80% respectively. Table 5 reported the composition of the seeds oils of *C. carvi*. Ten compounds were identified in seeds oil, which were characterized by the presence of a high content of thymol (48.20%), o-cymene (19.29%), γ-terpinen (17.61%), trimethylene dichloride (8.81%), β-pinene (3.08%), 2-(1-cyclohexenyl) cyclohexanone (0.68%), β-phellandrene (0.67%), 3-carene (0.57%), α-thujene (0.55%) and linalool (0.54%). Results showed that the seeds oil was a complex mixture of numerous compounds; many of which are present in trace amounts. It is worth mentioning here that there is great variation in the chemical composition of the seeds of *C. carvi* oils. Thymol (48.20%) is the most important and main component in seeds oil but it is totally absent in all the reported oils.

Table 5. *Constituents of seeds essential oil of Carum carvi*

Name of constituent	Amount (%)
α-Thujene	0.55
β-Pinene	3.08
o-Cymene	19.29
β-Phellandrene	0.67
γ-Terpinen	17.61
3-Carene	0.57
Linalool	0.54
2-(1-Cyclohexenyl)cyclohexanone	0.68
Thymol	48.20
Trimethylene dichloride	8.81

So, we conclude that thymol is the first reported component in *C. carvi* seed oils. Comparison of seeds oil of Bangladesh with those reported from different region of the world showed that our oil is especially different to others[4,17-19]. Carvone and limonene, which have been reported as major constituents in the seeds oil, were absent in our oil. This confirms that the variation in the cultivar reported is not due to geographic divergence and ecological conditions but it is due to different chemotype. On the basis of above fact it may be concluded that *C. carvi* growing widely in Bangladesh, may be utilized as a source of natural thymol.

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