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Isolation of *Bacillus* spp. from Soil and an Evaluation of Their Sensitivity towards Different Extracts and Essential Oils of Cumin (*Cuminum cyminum* L.)

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ABSTRACT

Throughout the present study, some *Bacillus* spp. were isolated from soil, and the effectiveness of the medicinal plant *Cuminum cyminum* L.'s essential oil extracts were tested against the isolated bacteria. The *Bacillus* spp. were identified through 16S rDNA sequence analysis and the antibacterial activity of various organic solvent extracts as well as the essential oils of *C. cyminum* L. determined *in vitro* using agar diffusion method and Minimal Inhibitory Concentration (MIC) tests. The hydrodistilled essential oil was analyzed through GC-MS. Twenty-seven compounds representing 92.61% of the total oil were identified. Oxygenated monoterpenes and scsquiterpene hydrocarbons constituted the major components of the oil. The inhibition zones of essential oil (extracted through organic solvent) against the tested bacteria were found within the range of 14.4 to 20.2 mm. Organic extracts of *C. cyminum* L. also revealed a great potential of antibacterial activity against *Bacillus* spp. Among all the extracts, ethanol extract showed the highest activity against *Bacillus megaterium* with an inhibition zone of 22.9 mm and MIC value of 500 µg ml^{-1}. In most cases, the essential oil and organic extracts exhibited either similar or higher antibacterial activity in comparison with the standard drug Erythromycin. The results finally suggest that the essential oil as well as organic extracts of *C. cyminum* L. can act as sources of natural antimicrobial agents with potential applications in food and pharmaceutical industries.

Keywords: Antibacterial activity, *Bacillus* spp., *C. cyminum* L., Extracts and essential oil.

INTRODUCTION

Human pathogenic bacteria form some of the most serious threats to man's health. The screening of plant extracts as well as plant products as regards antimicrobial activity has proved that higher plants present a potential source of novel antibiotic prototypes (Afolayan, 2003). The trend of using natural products has been on the increase and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobial substances (Hosseinzadeh *et al*., 2007; Guiamet *et al.,* 2008; Borrego *et al*., 2012). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Parekh and Chanda, 2007). There exist extensive

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scientific literature on the antimicrobial potential of spices reviewed by researches (Chaudhry and Tariq, 2008). Essential oils are complex mixtures of biologically active substances used for a long time as flavoring agents and constituents of a number of commercial products. Recent scientific literature has revealed the antimicrobial, antifungal and antioxidant potential of essential oils. Hence, use of essential oil/extracts has, as a consequence, to take advantage of all the active compounds present in essential oil/extracts (Al-Reza *et al.,* 2011).

Members of the aerobic spore-forming genus *Bacillus* spp. and other closely related species can be recovered from almost every environment in the biosphere. *Bacillus* spp. and related genera have been associated with such food spoilages as ropy bread (Sorokulova *et al.,* 2003) besides causing several human infections that account for a range of diseases (Callegan *et al.,* 2006; Tena *et al.,* 2007), and incidents of food borne illnesses (Dierick *et al.,* 2005). There is a considerable interest in using *Bacillus subtilis* producing lipopeptide antibiotics like Iturin A and Surfactin as a biocontrol agent and repressive activity over plant pathogens (Bais *et al.,* 2004). *Bacillus* spp. produces many kinds of antibiotics which share a full range of such antimicrobial activities as Bacitracin, Pumulin and Gramicidin (Todar, 2005).

Its seeds being used in various food applications, *C. cyminum* L. (Family: Apiaceae), commonly known as "cumin" is widely cultivated in the world. *C. cyminum* L. seed is commonly used as a spicy food in the form of powder for imparting flavor to different food preparations (Kafie *et al.,* 2002). It benefits from a variety of medicinal properties. The seeds are used (in traditional medicine) as carminative, and to such maladies as treat hoarseness or stomach pain. In addition, *C. cyminum* L. is used as antispasmodic, carminative, and as appetite stimulant (Morton, 1976). *C. cyminum* L. oil acts as a highly antifungal agent against various pathogenic fungi (Rahman *et al.,* 2000; Sheikh *et al*., 2010). It is also used as either a fumigant or additive in food storage (Tunc *et al*., 2000). Many pharmacological effects have been reported with respect to this spicy plant as anti-diabetic, immunologic, anti-epileptic, anti-tumor and antimicrobial activities. *C. cyminum* L. used in the medicinal preparations is supposed to be produced with such a high quality that it encompasses all the properties needed in the final product making it optimally suitable for ultimate use (Gohari and Saeidnia, 2011).

Therefore, the aims followed in the present study were: (1) to determine the chemical composition of the essential oil through Gas Chromatography–Mass Spectrometry (GC-MS); and (2) to evaluate the antibacterial activity of the essential oil and various organic (hexane, chloroform, ethyl acetate and methanol) extracts from seeds of *C. cyminum* L. against a diverse range of foodborne pathogenic and spoilage *Bacillus* spp. bacteria with emphasis on the possible future use of the essential oil as well as plant extracts as alternatives to chemical bactericides utilized in food preservation.

MATERIALS AND METHODS

Isolation from Soil, and Identification of Bacteria

A 20 g sample of freshly collected soil was suspended in sterile NaCl (0.9%) and maintained on a rotary shaker for 45 minutes at its maximum possible speed. The suspension was serially diluted, plated on PCA (Plate Count Agar) medium (pH 7.0, Sigma) and incubated at 30°C under aerobic conditions for 15 days. Most representative colonies were randomly collected from plates, purified by being streaked twice and stored as stock cultures in 20% (v/v) glycerol at -80°C for their genetic identification by 16S rDNA sequencing as previously described (McCaig *et al.,* 2001). PCR was performed in a final volume of 25 µl containing buffer 10X, 1.0 unit of

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*Taq*DNA polymerase (Amersham Biosciences), 0.2 mM each of dNTPs, 200 nM of each primer 63F 5'*CAGGCCTAACACATGCAAGTC* (Marchesi *et al*., 1998) and 1389R 5'ACGGGCGGTGTGTACAAG (Osborn *et al.*, 2000) as well as 50 ng of template DNA. The thermal cycler (Bio Rad ICycler 170-8740) was programmed for the initial denaturation step (94°C) of 5 minutes, followed by 44 cycles of 1 minute denaturation along with 1 minute. primer annealing (37°C) and 2 minutes. primer extension (72°C), followed by the 7 minutes primer extension (72°C) step. The amplified DNA was visualized through gel electrophoresis. The most similar bacterial species were found in the GenBank through BLAST search (http://www.ncbi.nlm.nih.gov). Neighbor-joining phylogenetic trees were constructed based on 16S rDNA sequences using Jalview version 2.7.

Collection of Plant Seeds and Preparation of Essential Oil

Seeds of *C. cyminum* L. were collected from local market of Kushtia, Bangladesh in March 2011. The specimen voucher number had been deposited in Bangladesh National Herbarium. The seeds of *C. cyminum* L. were dried in shade at room temperature for 7 days. The air-dried seeds (200 g) of cumin were subjected to hydro distillation for 3 hours using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4°C for further analysis.

Preparation of Organic Extracts

The air-dried seeds of *C. cyminum* L. were first pulverized into powdered form. The dried powder (50 g) was then extracted with hexane, chloroform, ethyl acetate and methanol separately at room temperature for 7 days and the solvents evaporated through vacuum rotary evaporator and at a temperature of 50°C. The extraction process yielded hexane (7.3 g), chloroform (6.2 g), ethyl acetate (7.4 g) and ethanol (6.5 g) of the extracts, respectively. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, St. Louis, MO, USA).

GC-MS Analysis

The GC-MS was carried out using total ion monitoring mode on a Varian 3800 Gas Chromatograph interfaced to a Varian Saturn ion trap 2200 GC-MS spectrometer. The temperatures of transfer line and ion source were set at 280 and 275°C respectively. Ions were obtained through electron ionization mode. A VF-5 capillary column (30 m length, 0.25 mm ID and 0.25 µm film thickness) was made use of. A 20% split injection mode was selected with a solvent delay time of 3 minutes, and with an injection volume of 0.2 µl. The initial column temperature was adjusted at 50°C for 1 minute, programmed at 8°C min^{-1} up to 200°C and heated until 280°C at 10°C min^{-1}. Injection port was set at 250°C. Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. Molecular ions (Mass range: 40-500 m z^{-1}) were monitored for identification. The relative contents of the oil constituents were expressed as percentage through peak area normalization. Identification of components of the essential oil was based on their retention indices, relative to a homologous series of *n*-alkane (C_8-C_{20}) on the VF-5 capillary column under the same operating conditions and computer matching with the GC-MS spectra from the Wiley 6.0 MS and literature data (Adams, 2007).

Antibacterial Assay

The dried extracts were dissolved in the same solvent used for their initial extraction and sterilized through filtration using 0.22 µm

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sterile Millipore filter (Millipore Corp., Billerica, MA, USA). The antibacterial test was carried out through agar disc diffusion method (Murray *et al*., 1995) using 100 µl of standardized inoculum suspension containing 10^7 CFU ml^{-1} of bacteria. The essential oil was diluted 1:5 (v/v) with ethanol and aliquots of 10 µl spotted onto the sterile Whatman No. 1 filter paper discs (6 mm diameter); while 10 µl of 50 mg ml^{-1} of each organic extract (500 µg $disc^{-1}$) being applied on the filter paper discs and placed on the inoculated LB agar medium. Negative controls were prepared using the same solvents initialy employed to dissolve the samples. Standard antibiotics, Amoxicillin and Erythromycin (10 µg $disc^{-1}$) from Sigma-Aldrich Co., St. Louis, MO, USA) were used as positive control for the tested bacteria. The plates were incubated micro aerobically at 37ºC for 24 hours. Antibacterial activity was evaluated through a measurement of the diameter of the zones of inhibition against the tested bacteria. Each assay of the experiment was replicated three times.

Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) of nanoemulsions was assessed according to Al-Reza *et al.* (2011). Active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks, inoculated in LB medium at 37°C for 24 hours. The nanoemulsions were incorporated into LB broth medium to get the final concentration ranging from 0 to 1,000 µg ml^{-1}. Finally, 20 µl inoculums of each bacteria strain (10^7 CFU ml^{-1}) were transferred to each tube with the tests being performed in a volume of 2 ml. The control tube contained only organisms and not the nanoemulsion. The culture tubes were incubated at 37°C for 24 hours. The lowest concentration of the test samples, which did not show any visual growth of tested organisms after macroscopic evaluation, was determined as MIC, which was expressed in µg ml^{-1}.

RESULTS AND DISCUSSION

Identification of Bacteria

DNA's extracted from soil isolates were subjected to PCR to be amplified for identification of bacterial species. Amplified DNA products were confirmed through Gel Electrophoresis and by visualization of their band patterns. Based upon the 16S rDNA sequences, the bacteria were confirmed as the *Bacillus* spp that will follow. The molecular identities of the isolates were compared with those of the known sequences in the public databases in NCBI and the BLAST results given in Table 1. The sequences express maximum similarity of 100% for *Bacillus megaterium*, *Bacillus simplex*, *Bacillus cereus* and *Paenibacillus* BF38 while 99% for *Terribacillus sp*. 3LF and *Paenibacillus sp*. L32 as presented in Table 1. Phylogenetic constructed is presented in Figure 1.

Table 1. List of bacteria identified throuhout study.

Name of strains	Name of bacteria	Accession number	(%)Similarity
Hb42	*Bacillus megaterium*	FJ614260	100
Xb17	*Bacillus simplex*	FJ225298	100
Xb10	*Terribacillus sp*. 3LF	AM931170	99
Hb21	*Bacillus cereus*	EU741083	100
Db8	*Paenibacillus sp.* L3	DQ196465	99
Db26	*Paenibacillus sp*.BF38	AM934687	100

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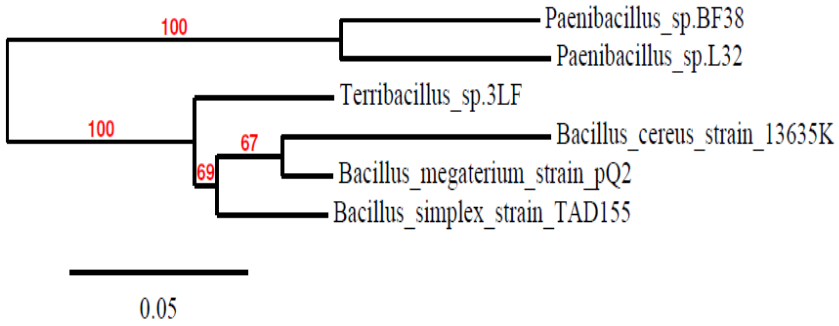

Figure 1. Phylogenetic tree of identified bacteria. The phylogenetic tree is shown in Phylogram. Bootstrap values (expressed as percentages of 100 replications) are shown at branch points; values greater than 50% were considered significant. The bar represents the unit length of the number of nucleotide substitutions per site.

Chemical Composition of the Essential Oil of *C. cyminum* L.

The essential oils obtained from spices have been known to possess biological activities, notably antimicrobial properties, since ancient times. With the growing interest in the use of essential oils in food and pharmaceutical industries, test of plant extracts for these properties has gained pronounced and increasing importance (Jirovetz *et al.,* 2002). *C. cyminum* L. has been a traditional spice since early times. The identified compounds, qualitative and quantitative analytical results through GC-MS, are presented in Table 2. Twenty-seven constituents, accounting for some 96.39% of the total oil compositions were identified. The oil contains a complex mixture of mainly oxygenated mono- and sesquiterpenes, as well as mono- and sesquiterpene hydrocarbons. The major compounds detected in the seed's oil were ethaneperoxoicacid,1-cyano-1-(2-ph) (17.11%), benzene,1-[(2-bromophenoxy)methyl]-3 (12.48%), 2-methylbicyclo[4.3.0]non-1(6)-ene (8.34%), limonene (7.73), linalool (6.19%), tritetracontane (6.03%), cartol (5.57%), γ-terpinene (4.92%), and eucalyptol (4.01%). Several papers have reported that all these compounds possess significant antioxidant activity in several model systems (Bettaieb *et al.,* 2010). It is also possible that some minor components might be involved in some type of synergism with the other existing active compounds. If one excludes the case of some phenolic components whose antimicrobial and antioxidant activities are well known and widely documented (Yanishlieva *et al.,* 1999) and some other instances of pure compounds, nothing would be left over to be known, as regards the effectiveness of most of the remaining components (Aeschbach *et al.*, 1994). However, it is noteworthy that the composition of the essential oils from a particular species of plant can widely differ depending upon the harvesting seasons ,extraction methods, and geographical origin, and while in the composition of their components to considerable extent different parts of the same plant can also differ widely (Burt, 2004).

Antibacterial Activity of Various Extracts and Essential Oil of *C. cyminum* L.

The antimicrobial activities of extracts obtained from spices, herbs and other aromatic plants or parts thereof, using either organic solvents or steam distillation have been recognized for many years. Plants and

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Table 2 Chemical composition of the essential oil of *C. cyminum* L. seeds.

Sl. No.	Compound	RT [a]	% RA [b]	Identification [c]
1.	Eucalyptol	7.725	4.01	RI, MS
2.	Trifluoroacetyl-alpha-fenchol	9.824	tr[d]	RI, MS
3.	1,3,3-Trimethylcyclohex-1-ene-4-carboxa	10.784	3.08	RI, MS
4.	Ethaneperoxoicacid,1-cyano-1-(2-ph)	11.750	17.11	RI, MS
5.	2-Methylbicyclo[4.3.0]non-1(6)-ene	12.094	8.34	RI, MS
6.	1-Cyclohexene-1-carboxyldehyde,4-(1-	12.304	0.46	RI, MS
7.	Nonadecane	12.388	3.30	RI, MS
8.	Benzene,1-[(2-Bromophenoxy)methyl]-3	12.494	12.48	RI, MS
9.	1H-Indene,1-ethyledene-	12.949	0.93	RI, MS
10.	Phenol,2-methoxy-6-(1-propyl)-	13.063	0.50	RI, MS
11.	Hydroxylamine,O-decyl-	13.430	tr	RI, MS
12.	Naphthalene	14.467	0.50	RI, MS
13.	Caryophyllene	14.603	0.34	RI, MS
14.	Limonene	14.747	7.73	RI, MS
15.	2-Hexayl-1-octanol	14.837	3.07	RI, MS
16.	β-Farnesene	14.938	0.68	RI, MS
17.	Trans-Z-alpha-Bisabolene epoxide	15.190	0.74	RI, MS
18.	Farnesanol	15.274	tr	RI, MS
19.	Acoradiene	15.475	0.54	RI, MS
20.	Tritetracontane	16.531	6.03	RI, MS
21.	Caryophyllene oxide	17.165	0.69	RI, MS
22.	Linalool	18.325	6.19	RI, MS
23.	1-Eicosanol	19.349	0.83	RI, MS
24.	Diisononylphthalate	46.687	1.15	RI, MS
25.	Linalyl acetate	47.413	2.67	RI, MS
26.	Cartol	48.957	5.57	RI, MS
27.	γ-Terpinene	49.145	4.92	RI, MS
	Total identified		92.61%	

[a] Retention Time relative to *n*-alkanes on VF-5 capillary column; [b] Relative area (peak area relative to the total peak area); [c] MS: Comparison of mass spectra with MS libraries, RI: comparison of retention index with bibliography, [d] Trace amount (< 0.3%).

plant extracts have been used since antiquity in folk medicine and food preservation, providing a range of compounds possessing pharmacological activity (Deans and Svoboda 1990). Various publications have documented the antibacterial activity of essential oil constituents and plant extracts (Bhattacharjee *et al*., 2006; Al-Reza *et al*., 2010).

Throughout the present study, the antibacterial activity of some essential oils and of various extracts (ethanol, chloroform, hexane and ethyl acetate) of *C. cyminum* L. seeds against *Bacillus* spp. was qualitatively assessed through an observation of the inhibition zones. The oil exhibited a noticeable antibacterial effect against the tested bacteria, with diameter of inhibition zones ranging from 14.4±0.7 to 20.2±0.5 mm, as indicated in Table 3. Various organic extracts also revealed a substantial antibacterial activity against all the bacteria, at a concentration of 500 µg $disc^{-1}$ (Table 3). Ethanol extract exhibited the strongest antibacterial effect against *B. megaterium, Paenibacillus* sp. BF38 and *B. cereus* with their respective diameter zones of inhibition of 22.9±0.4, 20.1±0.6 and 18.8±0.3 mm, whereas chloroform extract demonstrating the strongest effect against *B. cereus* (inhibition zone 21.8±0.3 mm), as compared with standard drug Erythromycin (10 µg $disc^{-1}$) On the other hand, hexane and ethyl acetate extracts revealed notable

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Table 3. Antibacterial activity of the essential oil and various organic extracts of *C. cyminum* L.[a]

	Zone of inhibition (mm)						
	Extracts					Antibiotics	
Name of bacteria	Essential oil	EtOH	$CHCl_3$	Hexane	EtOAc	Amoxicillin	Erythromycin
Bacillus megaterium	14.4±0.7	22.9±0.4	19.6±0.7	21.9±0.7	18.6±0.7	12.6±0.5	13.3±0.9
Bacillus simplex	15.8±0.5	15.8±0.5	15.8±0.5	18.8±0.5	20.8±0.7	10.3±0.5	14.5±0.8
Terribacillus sp. 3LF	14.6±0.6	17.6±0.6	20.6±0.4	16.6±0.6	14.6±0.6	10.3±0.4	13.8±0.6
Bacillus cereus	20.2±0.5	18.8±0.3	21.8±0.3	22.2±0.8	12.8±0.4	10.3±0.2	14.6±0.5
Paenibacillus sp. L32	17.6±0.7	18.4±0.7	14.4±0.7	15.2±0.9	14.4±0.7	12.5±0.6	10.4 ±0.4
*Paenibacillus sp.*BF38	15.6±0.4	20.1±0.6	18.6±0.4	14.2±0.4	12.6±0.4	10.1±0.7	14.2±0.3

* Diameter of inhibition zones (mm) around the discs (6 mm) impregnated with 10 µL of 1:5 (v/v) dilution with ethanol.

*Various organic extracts (500 µg $disc^{-1}$).

The standard antibiotics were: Amoxicillin and Erythromycin (10 µg $disc^{-1}$)

Values are given as mean±SD of the triplicate experiment.

antibacterial effects with inhibition zones within the ranges of 14.2±0.4 to 22.2±0.8 and 12.6±0.4 to 20.8±0.7 mm, respectively. In some cases, the oil and organic extracts (ethanol, chloroform, hexane and ethyl acetate) exhibited higher antibacterial activity as compared with Amoxicillin while Erythromycin exhibiting higher activity in some other cases than the essential oil and solvent extracts. In a recent survey, pharmacological studies have been conducted on the ethanol, chloroform, hexane and ethyl acetate extracts of defatted *C. cyminum* L. seeds to evaluate their effects on the central nervous system and as well on their analgesic effectivity. Besides, *C. cyminum* L. seeds are some of the popular spices regularly utilized as flavoring agents in a number of ethnic cousines. In Iranian ancient medicine, the fruits of the plant have been used for the treatment of toothache, diarrhea and epilepsy (Sheikh *et al*., 2010). Some researchers have noted *C. cyminum* L. as an emerging alternative antimicrobial agent as regards human health (Janahmadi *et al.,* 2006; Sheikh *et al*., 2010). According to the present investigation, *C. cyminum* L. seeds are shown to possess antibacterial potential and can be used as potential antibacterial agents against human pathogenic and soil bacteria. Comparing results reported in (Table 3) with the previous data regarding antibacterial activity of essential oil (Tena *et al.,* 2007) it appears that the oil content of *C. cyminum* L. exhibited highly pronounced activity. Most of the tested bacteria were found sensitive to the oil. The diluted (500 µg ml^{-1}) oil showed good activity against most tested organisms. This activity could be attributed to the presence of oxygenated monoterpene and sesquiterpene hydrocarbons with the findings being in agreement with those of the previous reports (Sartoratto *et al*., 2004; Shunying *et al.,* 2005). The results showed that the ethanol solvent of the essential oil of *C. cyminum* L. possessed a considerable antimicrobial activity. This result is also in line with the previously obtained results (Afolayan and Meyer, 1995).

Minimal Inhibitory Concentration (MIC)

As shown in (Table 4), the MIC values for the oil were found to be lower for *B. megaterium*, *B. simplex* ,*Terribacillus sp*. 3LF and *B. cereus* (62.5-125 µg ml^{-1}) than for *Paenibacillus sp*. L32 and *Paenibacillus sp*. BF38 (250 - 500 µg ml^{-1}). On the other hand, the MIC values of the organic extracts of ethanol, chloroform, hexane and ethyl acetate against the bacteria tested were

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Table4. Minimal Inhibitory Concentration (MIC) of essential oil and organic extracts of *C. cyminum* L.

Microorganism	EO [a]	EtOH [b]	MIC (µg ml^{-1}) Organic extracts		
			$CHCl_3$ [c]	Hexane [d]	EtOAc [e]
Bacillus megaterium	62.5	500	125	125	250
Bacillus simplex	62.5	125	62.5	62.5	500
Terribacillus sp. 3LF	125	250	62.5	250	62.5
Bacillus cereus	125	125	500	500	250
Paenibacillus sp. L32	250	62.5	500	250	500
Paenibacillus sp. BF38	500	500	250	250	500

[a] Essential Oil; [b] Ethanol extract; [c] Chloroform extract; [d] Hexane extract, [e] Ethyl Acetate extract.

found within the range of 62.5-500 µg ml^{-1} (Table 4). Hexane extract showed higher antibacterial activity compared with chloroform, ethanol and ethyl acetate extracts. In this study, *Bacillus* spp. were found to be more susceptible to the essential oil. The antibacterial activity of the organic extract could be attributed to the presence of some bioactive phytochemicals (alkaloids, flavonoids, steroids, terpenoids, etc.) in *C. cyminum* L. These findings are in agreement with the previous report (Agarwal *et al.,* 2010) indicating that *C. cyminum* L. does cotain those compounds.

CONCLUSIONS

The organic extract of *C. cyminum* L. seeds benefit from the important property of antimicrobial activity against *Bacillus* spp. Oxygenated monoterpenes and scsquiterpene hydrocarbons constituted the major components of these essential oils which demonstrate significant antibacterial behavior. In this regard, the use of *C. cyminum* L. seeds and their volatile compounds as natural preservatives in food products may be an alternative to the use of chemical additives. Our results could provide useful data for the utilization of this oil, in food, pharmaceutical or cosmetic industry.

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جداسازی گونه‌های باسیل (*Bacillus spp*) از خاک و برآورد حساسیت این گونه نسبت به عصاره و همچنین روغنهای اصلی موجود در زیره (*Cuminum cyminum* L.)

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چکیده

جداسازی گونه‌های باسیل (*Bacillus spp*) از خاک و برآورد حساسیت این گونه نسبت به عصاره و همچنین روغنهای اصلی (Essential oils) موجود در زیره (*Cuminum cyminum*) مورد آزمایش قرار گرفتند. گونه‌های باسیل از طریق تجزیه توالی 16 RDNA و فعالیت ضد باکتریائی عصاره‌های زیره (استخراج شده با استفاده از حلال‌های مختلف آلی) و همچنین روغنهای اصلی آن به صورت آزمایشگاهی (In vitro) و در حالیکه از روش پخش آگار (Agar diffusion method) و همچنین تست‌های کمترین غلظت بازدارنده (Minimum Inhibitory Concentration

(MIC) استفاده می‌شد مورد آزمایش قرار گرفتن عصارهٔ روغنی اصلی که با تقطیر به دست آمده بود با استفاده از گاز کروماتوگرافی – اسپکتروسکپی جرمی (Gas Chromatography Mass Spectroscopy (GC-MS) مورد تجزیه و تحلیل قرار گرفت. میزان ۹۲/۶۱ درصد از کل روغنهای اصلی شناسائی شد. هیدروکربنهای مونوترپین ویسسکوی ترپین (Monterpene and Sesuiterpene) اکسیژنه اجزاء تشکیل دهنده عمدهٔ روغنهای اصلی زیره بودند نواحی بازدارنده (Inhibitionxones) روغنهای علیه باکتریها در محدودهٔ ۱۴/۴ تا ۲۰/۲ میلی‌متر تخمین زده شدند. عصاره‌های آلی زیره نیز پتانسیل فعالیت ضد باکتری زیادی (در مقابل Bacillus spp) از خود بروز دادند. در میان تمامی عصاره‌ها، عصارهٔ اتانول بیشترین فعالیت علیه باسیل مگاتریوم (*Bacillus megaterium*) با ناحیهٔ بازدارندهٔ ۲۲/۹ میلی‌متر و کمترین غلظت بازدارنده (MIC) به میزان ۵۰۰ Mg/ml را از خود بروز داد. در بیشترین موارد، روغنهای اصلی و عصاره‌های آلی زیره فعالیت ضد باکتریائی مشابه و یا زیادتری در قیاس با داروی استاندارد اری‌ترومایسین(Erythromycin) داشتند. نتایج نهایتاً نشان می‌دهند که روغنهای اصلی و همچنین عصارهٔ آلی *C.cyminum* می‌توانند در سمت عوامل طبیعی ضد میکرب (با پتانسیل‌هائی کاربردی) در صنایع غذائی و دارویی نقش ایفا کنند.

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