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Diversity in chemical composition and antibacterial activity of essential oils of cumin (*Cuminum cyminum* L.) diverse from northeast of Iran

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Abstract

Essential oils (EOs) of various accessions of green cumin (*Cuminum cyminum* L.) fruit collected from thirty three farms in North, Central and South Khorasan provinces (Northeast Iran) were investigated for their chemical composition and antibacterial activity. The EO of all samples was extracted by hydrodistillation and analyzed using GC and GC–MS. The EO yields ranged from 1.4–2.2 mL/100 g relative to the dried fruits. GC and GC-MS analyses revealed 17 compounds, constituting 95.2%–99.9% of total the EOs. The major constituents of the EOs were safranal (16.8%–29.0%), γ-terpinene (14.1%–19.6%), γ-terpinene-7-al (13.5%–25.5%), cuminaldehyde (17.5%–22.3%), β-pinene (6.8%–10.4%), and *p*-cymene (4.1%–8.8%). The antibacterial activity of the EOs was individually evaluated against four positive-Gram (*Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Listeria monocytogenes*) and two negative-Gram ones (*Proteus vulgaris* and *Salmonella typhimurium*) using disc diffusion and serial dilution methods. The inhibition zones and MIC values for bacterial strains which were sensitive to the EOs of green cumin were in the range of 8.2–33.2 mm and 31–250 µg/mL, respectively. Results obtained in this study revealed that there is a high potential of the EO composition variability among Khorasan cumin accessions. The results can be used in cumin selection programs for production of aromatic cumin and cumin with suppressing effects on food-borne pathogens.

Keywords: Biological activity; *Cuminum cyminum* L.; cuminaldehyde; spice; variation.
Abbreviations: EO_Essential Oil; DIZ_Diameter of Inhibition Zone; MIC_Minimum Inhibitory Concentration; MBC_Minimum Bactericidal Concentration; GC_Gas Chromatography; GC/MS_Gas Chromatography/Mass spectrometry; PCR-RFLP_Polymerase Chain Reaction-Restriction Fragment Length, DMSO_Dimethyl Sulfoxide.

Introduction

Green cumin (*Cuminum cyminum* L.) belongs to the family Apiaceae is a small annual and herbaceous plant. Cumin is naturally found in Iran, Turkey, India, Pakistan, Argentina, China, Central America and other regions. In addition, this plant is being newly cultivated in Iran, India, and China and in the countries bordering the Mediterranean Sea (Thippeswamy and Naidu, 2005; Ghasemi Pirbalouti, 2010). The major production area of green cumin in Iran includes South, Central and North Khorasan provinces. In 2010, the cultivated area of green cumin was 40,000 hectares, with an average yield of 500 to 1500 kg/ha in rainfed and irrigated conditions, respectively. (Ghasemi Pirbalouti, 2010). In Iran, green cumin requires a long, hot summer, and a growing season between 125 to 175 days (in Khorasan), with daytime temperatures around 30 °C. Green cumin is relatively drought-tolerant, and is mostly grown in Mediterranean climates (Kamkar et al., 2007). The plants prefer a mild climate, and can be cultivated up to an elevation of 3,800 m (Sahana et al., 2011). In Khorasan, it is sown in February to April, and requires fertile, well-drained soil (Kamkar et al., 2007).

Cumin is the second most popular spice in the world after black pepper. Cumin fruits are used as spice and some species economically important and are used as culinary herbs, flavoring agents in perfumery and cosmetics (Hajlaoui et al., 2010). All the cumin varieties are used as a stimulant, a carminative, an astringent, and as remedy against indigestion, colic, flatulence and diarrhea (Norman, 1990; Zargari, 1989), and also to stimulate breast milk production in Iranian traditional medicine (Mahdavi Maymandi and Mirtajodini, 2010). Due to the high essential oil content in the fruit, for example Khorasan cumin fruit contains volatile oil (2%–3%), green cumin is an important medicinal and aromatic plant that have medicinal properties, including antimicrobial (Rehman et al., 2000; Gachkar et al., 2007), antioxidant (Gachkar et al., 2007; Hajlaoui et al., 2010; Einafshar et al., 2012), antitumor (Soleymani et al., 2011), anti-nociceptive (Sayah et al., 2002), anti-inflammatory (Soleymani et al., 2011), epileptic activity (Janahmadi et al., 2006), and hypoglycaemic effect (Dhandapani et al., 2002).

Recent findings have shown that some of the medicinal and aromatic plants characteristics can be affected by genetic (cultivar or landrace) and ecological factors including precipitation, temperature, plant competition and nitrogen concentration in the soil (Letchamo et al., 1995; Ghasemi Pirbalouti et al., 2011). The composition of the EO of cumin therefore may vary with genetic, environmental conditions, extraction-method and geographic origin including climate, edaphic, elevation and topography. Knowledge of population diversity is a main prerequisite and the first step in plant breeding (Allard, 1999). A local population of plant is a suitable germplasm for improving plant breeding programs. Currently, in maintaining the natural structure of green cumin, agricultural production technology in Asia has been founded (Sheidai and Ahmadian, 1996). Cumin's distinctive flavor and strong warm aroma is related to its EO content. To our knowledge, no documented reports on diversity of chemical composition and antibacterial activity of the EOs of various populations of green cumin are available. The aims of this study were (i) to determine the variation of chemical constituents of different accessions of green cumin collected from various geographical regions of Iran and (ii) to evaluate the antibacterial activity of the EOs of the different accessions.

Results and discussion

Essential oil yield

The yields of yellow EO of the studied accessions of green cumin ranged between 1.4 to 2.2 mL/100 g relative to the dried fruits (Table 2). The results of current study indicated that there were significant differences ($p \leq 0.05$) between different accessions for EO yield (Table 2). The highest EO yield (2.20 mL/100 g) was obtained from the Boshroyeh accession, whereas the Ghayen accession produced the lowest EO yield (1.36 mL/100 g). The yield of the EOs extracted from other ecotypes that have been reported by other researchers were 3.8% from China (Li and Jiang, 2004), 5.3% from Bulgaria (Jirovetz et al., 2005), 1.6% from Tunisia (Rebey et al., 2012), 1.4 to 2.8% from Turkey (Beis et al., 2000) and 2.0 to 3.3% (v/w) from India (Sowbhagya et al., 2008). The lower EO yield in our study can be attributed partly to genetic factor (ecotype), farming system and environmental condition of the studied region. In Khorasan growers produce cumin as dry farming with few supplement irrigation. EO biosynthesis is strongly influenced by several intrinsic (genotype, ontogeny) and extrinsic (environmental) factors (Lawrence, 1986).

Chemical composition of EOs

The chemical constituents identified by GC-FID and GC-MS, are presented in Table 2 and Fig 1. GC and GC-MS analysis resulted in the identification of 17 constituents of the EOs. The EO analysis detected four major compounds, *viz.* safranal (16.8±6.79 to 29.0±3.7%), γ-terpinene-7-al (13.52±2.75 to 25.47±6.89%), cuminaldehyde (17.5±3.0 to 22.29±1.3%), and γ-terpinene (14.1±1.4 to 19.6±6.2%). Their sum constituted the bulk of the EOs and ranged from 95.2% up to 99.9% of total EO. The identified aldehyde compounds ranged from 34.4% in the Bajestan accession to 49.8% of the total EO in the Ferdos accession. Generally, monoterpene hydrocarbons and sesquiterpenes hydrocarbons in Khorasan accessions were lower than those of other ecotypes reported elsewhere. Other researchers (Kumar and Baslas, 1978; Eikani et al., 1999; Beis et al., 2000; Rehman et al., 2000; Rebey et al., 2012), however, have reported a different percentage of monoterpenes and sesquiterpenes components. In general, cuminaldehyde, menthane derivatives, ɣ-terpinene, *p*-cymene and β-pinene are the major constituents of volatile oils of green cumin (Lis-Balchin et al., 1998). The authors are not aware of any published report on green cumin composition and this work is the first report on chemical constituents on Khorasan accessions of green cumin. The results of the comparison among chemical compositions of essential oil of Khorasan accessions for individual compounds are presented in the following sections.

Safranal

The results showed that there were no significant differences among landraces for safranal content. The highest value (29.0±3.7%) was related to the Ferdos accession while the lowest (16.8±6.79%) was found in the Mahvalat accession (Table 2). Few reports on safranal in green cumin oil are available. Safranal percentages in the EOs of green cumin reported for Razavi Khorasan accession (Oroojalian et al., 2010), and China ecotype (Li and Jiang, 2004) were 9.4 and 10.9%, respectively. Higher value of safranal in EOs of cumin in present study compared with other reports may be related to genetic factor, environmental conditions of the studied region, especially arid conditions and low irrigation in farming system in Khorasan.

ɣ-Terpinene

Statistical analysis indicated that there were no significant differences among accessions for ɣ-terpinene (Table 2). The highest and lowest values of γ-terpinene obtained from the Esfarayen accession with 19.6±6.2% and the Sabzevar accession with 14.1±1.4%, respectively. This component in other cumin ecotypes were reported as 11.4% from China (Li and Jiang, 2004), 15.3% from Bulgaria (Jirovetz et al., 2005), 25.6 to 34.2% from Tunisia (Rebey et al., 2012), 17.2% from Turkey (Beis et al., 2000), 12.8% from Iran (Oroojalian et al., 2010), and 12.8% from Italy (Iacobellis et al., 2005). Results of previous studies indicated the major constituent in the EO of the Spanish cumin (Viuda-Martos et al., 2007) and the chemotype of cumin from Tunisia (Rebey et al., 2012) was γ-terpinene.

Cuminaldehyde

In our study we found significant differences ($p \leq 0.05$) among the accessions for cuminaldehyde (Table 2). The comparison of means indicated that the highest value (22.3%) was found in Nayshaboor accession and the lowest (17.5%) in Bajestan accession. The odor characteristic of cumin is mainly attributed to cuminaldehyde in the fruit which forms nearly 20-40% of the oil (Sahana et al., 2011). The volatile oil from Mexico contained 62.7% of cuminaldehyde, whereas reports from the Mediterranean and Indian regions indicated 47.4 and 43%, respectively (Sahana et al., 2011). Cuminaldehyde percentages reported by other researchers were from 15.3% to 36.3% in the EOs of different ecotypes (Eikani et al., 1999; Sowbhagya et al., 2008; Jirovetz et al., 2005; Beis et al., 2000; Li and Jiang, 2004; Rebey et al., 2012; Iacobellis et al., 2005). The content of cuminaldehyde in the EO from Tunisia ecotype of green cumin increased up to 23.53% under moderate water deficit levels (Rebey et al., 2012).

Table 1. Environment conditions of various regions of C. cyminum L. cultivated in Khorasan provinces, Northeast of Iran

Region	Province	Altitude (m asl [1])	Latitude	Longitude	P[a]	T[b]	RH[c]	pH	E.C.[d]	O.C.[e]	N[f]	P[g]	K[h]	Zn[i]	Mn[j]	Fe[k]	Cu[l]
Gonabad	Central Khorasan	1056	34° 21′ N	58° 41′ E	131	17.1	40	7.9*	6.5	0.35	0.018	22.5	178	0.48	7.2	3.2	0.63
Bajestan	Central Khorasan	1370	34° 31′ N	58° 11′ E	193	17.5	40	8.1	9.1	0.09	0.011	2.7	160	0.46	5.5	2.4	0.57
Sabzevar	Central Khorasan	978	36° 12′ N	57° 43′ E	189	17.7	40	8.2	12.2	0.21	0.029	31.3	217	0.58	6.8	4.6	0.56
Mahvalat	Central Khorasan	940	34° 54′ N	58° 50′ E	131	17.1	40	7.8	12.7	0.31	0.023	3.8	194	0.48	6.4	3.7	0.53
Bardeskan	Central Khorasan	985	35° 16′ N	57° 57′ E	170	18	40	7.9	3.9	0.5	0.043	8.4	314	0.65	6.9	4.2	0.57
Nayshaboor	Central Khorasan	1213	36° 16′ N	58° 48′ E	238	14.1	40	8.2	3.8	0.14	0.012	8.1	147	0.43	5.1	2.7	0.66
Ferdos	South Khorasan	1293	34° 01′ N	58° 10′ E	135	17.5	35	8.0	5.8	0.24	0.019	7.9	164	0.54	6.9	3.3	0.55
Sarayan	South Khorasan	1405	33° 51′ N	58° 51′ E	135	17.5	35	8.1	6.1	0.14	0.011	6.3	277.0	0.39	5.2	3.2	0.55
Boshroyeh	South Khorasan	885	33° 54′ N	57° 27′ E	89	19.2	38	7.9	16.9	0.24	0.021	13.7	173	0.53	7.01	3.3	0.72
Ghayen	South Khorasan	1432	33° 43′ N	59° 10′ E	168	14.5	40	8.1	2.2	0.29	0.021	21.2	302	0.49	4.6	2.9	0.61
Esfarayen	North Khorasan	1216	37° 03′ N	57° 29′ E	235	13	45	8.0	2.8	0.57	0.04	7.7	257	0.63	6.9	4.7	0.73

[a] Average annual precipitation (mm); [b] Average annual temperature (°C); [c] Average annual relative humidity (%); [d] Electrical conductivity (dSm^{-1}); [e] Organic carbon (%); [f] Total nitrogen (%); [g] Available P ($mgkg^{-1}$); [h] Available K ($mgkg^{-1}$); [i] Zinc ($mgkg^{-1}$); [j] Manganese ($mgkg^{-1}$); [k] Iron ($mgkg^{-1}$); [l] Copper ($mgkg^{-1}$).* Soil characteristics are based on average of samples taken from three farms in each region.

Table 2. Effect of various accessions on chemical compositions and yield of the EOs from C. cyminum L. fruits .

Compound	RI[a]	GC peak area %										ANOVA
		Esfarayen	Ghayen	Boshroyeh	Sarayan	Ferdos	Nayshaboor	Bardeskan	Mahvalat	Sabzevar	Bajestan	Gonabad	
Monoterpenes Hydrocarbons													
α-Thujene	933	0.22±0.10*	0.11±0.12	0.29±0.07	0.24±0.03	0.22±0.60	0.21±0.09	0.24±0.05	0.16±0.14	0.19±0.04	0.19±0.22	0.32±0.06	p > 0.05
α-Pinene	944	0.36±0.22	0.37±0.04	0.41±0.14	0.39±0.06	0.37±0.05	0.28±0.08	0.38±0.13	0.25±0.14	0.34±0.05	0.46±0.21	0.48±0.12	p > 0.05
Comphene	955	0.43±0.06	0.47±0.06	0.47±0.15	0.45±0.07	0.43±0.06	0.53±0.06	0.40±0.00	0.43±0.06	0.53±0.06	0.41±0.00	0.42±0.06	p > 0.05
β-Pinene	986	9.25±3.17	7.87±1.00	8.65±2.94	8.32±1.42	7.61±0.56	7.19±0.43	7.15±1.69	6.79±0.75	9.62±2.46	10.39±5.07	9.89±2.35	p > 0.05
Sabinene	981	0.28±0.03	0.31±0.10	0.37±0.15	0.27±0.04	0.30±0.10	0.41±0.16	0.27±0.05	0.37±0.15	0.40±0.12	0.26±0.03	0.31±0.10	p > 0.05
Myrcene	994	0.87±0.27	0.90±0.21	1.01±0.42	0.88±0.09	0.87±0.17	0.81±0.01	0.86±0.20	0.95±0.30	0.73±0.10	1.17±0.69	1.02±0.12	p > 0.05
α-Phellandrene	1008	1.86±0.43	1.17±0.31	1.20±0.26	1.09±0.21	1.19±0.15	1.18±0.43	1.27±0.16	1.49±0.89	1.17±0.31	0.98±0.57	1.64±0.49	p > 0.05
α-Terpinene	1020	0.11±0.09ab	0.10±0.06ab	0.17±0.07a	0.08±0.11ab	0.13±0.03ab	0.12±0.02ab	0.12±0.04ab	0.13±0.21ab	0.04±0.06b	0.15±0.07ab	0.17±0.03a	p ≤ 0.05
p-Cymene	1026	8.84±2.69a	6.96±1.35ab	6.69±1.08ab	7.69±2.02ab	4.14±0.74ab	7.80±0.36ab	4.89±1.93ab	6.79±3.49ab	5.02±1.73ab	6.60±3.38ab	5.64±0.92b	p ≤ 0.05
β-Phellandrene	1030	0.17±0.06ab	0.34±0.33ab	0.19±0.08ab	0.45±0.53ab	0.54±0.39ab	0.10±0.01b	0.82±0.46a	0.11±0.01b	0.54±0.71ab	0.10±0.00b	0.19±0.16ab	p ≤ 0.05
γ-Terpinene	1059	19.59±6.20	17.22±1.20	19.41±4.63	18.33±0.89	15.00±0.87	16.49±0.72	15.39±3.99	16.89±6.47	14.07±1.36	19.37±8.06	18.02±2.73	p > 0.05
Terpinolene	1089	0.15±0.02	0.22±0.05	0.13±0.02	0.16±0.07	0.17±0.07	0.17±0.04	0.15±0.05	0.13±0.04	0.21±0.04	0.13±0.02	0.14±0.06	p > 0.05
Oxides													
α-Pinene oxide	1093	0.79±0.70	1.41±0.36	1.05±1.04	1.18±0.27	1.35±0.02	0.87±0.22	1.22±0.34	1.58±0.76	0.83±0.31	1.39±0.79	1.36±0.32	p > 0.05
Aldehydes													
Cuminaldehyde	1233	21.83±3.48a	20.50±1.58ab	20.63±2.12ab	20.54±0.65ab	20.09±1.41ab	22.29±1.26a	20.43±1.34ab	20.44±0.89ab	21.62±2.41ab	17.54±2.99b	20.25±2.24ab	p ≤ 0.05
Safranal	1274	20.21±5.56	24.89±2.47	24.26±4.61	20.94±3.78	28.97±3.71	25.85±1.70	26.58±5.10	16.83±7.91	25.27±7.81	16.82±6.69	26.78±1.27	p > 0.05
Alcohols													
ɣ-Terpinene-7-al	1286	14.25±3.50b	16.13±1.79ab	13.76±2.76b	19.40±5.62ab	16.69±0.53ab	15.74±1.45b	18.65±1.98ab	25.47±6.89a	20.83±4.47ab	22.23±6.73ab	13.52±2.75b	p ≤ 0.05
Sesquiterpenes Hydrocarbons													
α-Cedrene	1411	0.65±0.15a	0.09±0.14de	0.22±0.20bcde	0.27±0.11 bcde	0.17±0.16cde	0.38±0.04abcd	0.02±0.01e	0.53±0.39ab	0.45±0.14abc	0.19±0.05bcde	0.42±0.24abcd	p ≤ 0.05
EO yield (mL/100 g)		1.81±0.51ab	1.36±0.32b	2.2±0.48a	1.82±1.01ab	1.79±0.52ab	1.76±0.63ab	1.81±1.00ab	1.72±0.56ab	1.40±0.44b	1.64±0.46ab	1.44±0.94b	p ≤ 0.05

¶ Values in each row having similar letter are not statistically different at $p \leq 0.05$. Letters are used only when locations showed statistical differences for specific compounds. †Retention index: Kovats retention index relative to n-alkanes on non-polar column HP-5MS, ‡ Mean ± SD (n=3); The components were identified by their mass spectra and retention indices (RIs) with that of the Wiley and NIST mass spectral databases and the previously published data.

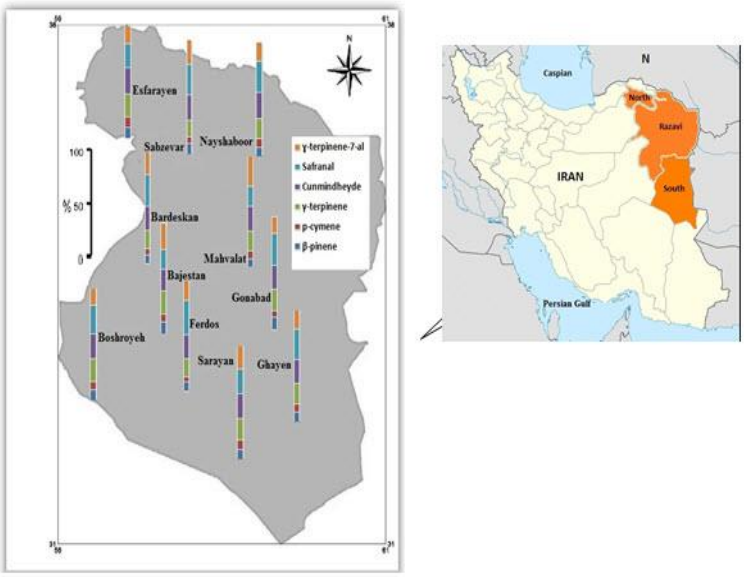
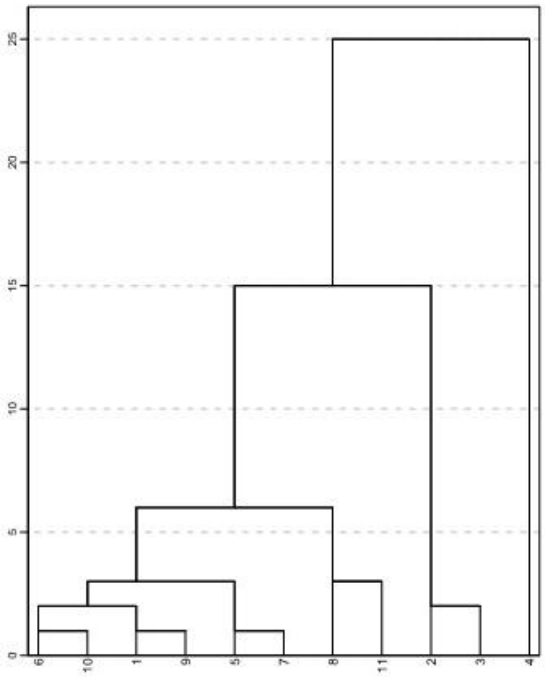


Fig 1. Sampling locations of cultivated populations of Cuminum cyminum L. in Northeast of Iran (Percentages of the main compounds in essential oils of various accessions of green cumin fruits).



1. Gonabad; 2. Bajestan; 3. Sabzevar; 4. Mahvalat; 5. Bardeskan; 6. Nayshaboor; 7. Ferdos; 8. Sarayan; 9. Boshroyeh; 10. Ghayen; 11. Esfarayen.

Fig 2. Dendogram obtained by hierarchical cluster analysis of the percentage composition of EOs from different accessions of green cumin.

ɣ-Terpinene-7-al (p-mentha-1,4-dien-7-al)

The results of variance analysis indicated that there were significant differences ($p \leq 0.05$) between different accessions for γ-terpinene-7-al (Table 2). The highest value (25.5%) was found in the Mahvalat accession and the lowest (13.5%) in the Gonabad accession. Again, few published reports on γ-terpinene-7-al in green cumin oil are available. Kan et al. (2007) found relatively similar amounts of cuminaldehyde (19.9-23.6%) and ɣ-terpinene-7-al (13.9-16.9%) in India. They suggested that fruits of green cumin should be harvested at the fully ripe or mature stage for an optimum volatile oil yield and composition. Results a study by Eikani et al. (1999) indicted the values of ɣ-terpinene-7-al in the EOs extracted from green cumin using supercritical CO_2 and steam-distilled methods were 41.0% and 27.4%, respectively. In addition, this compound in other species of cumin was identified, for example the percentages of ɣ-terpinene-7-al in the EO of *Carum carvi* L. Iranian ecotype were 14 to 17% (Razzaghi-Abyaneh et al. 2009; Fatemi et al., 2011), and in the EOs *of Bunium persicum* [Boiss.] Fedtsch Pakistani ecotype (Thappa et al., 1991) and Iranian ecotype (Oroojalian et al., 2010) were 36.8% and 10.5%, respectively.

Correlation and hierarchical cluster analysis

A correlation analysis of the main oil compounds content was done to determine the relationship among the different geographic and environmental conditions. The compounds, such as safranal, γ-terpinene-7-al, cuminaldehyde, γ-terpinene, β-pinen, and *p*-cymene that were present in the EOs of various accessions were used for analysis (Table 3). A highest positive correlation ($p \leq 0.05$) was between the amount of copper and γ-terpinene (0.67). In contrast, the highest negative correlation ($p \leq 0.05$) was between the amount of copper and ɣ-terpinene-7-al (-0.77). Cu has important functions in plant metabolism, especially in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Marschner, 1995). The highest positive correlation ($p \leq 0.05$) was between the relative γ-terpinene ($C_{10}H_{16}$) and *p*-cymene ($C_{10}H_{14}$) levels (Table 3), compounds that have similar structure with only a difference is in hydrogen molecules. The hierarchical cluster analysis of all identified components grouped the EOs of 11 accessions into three distinctive clusters (Fig 2). The first cluster was formed by the EOs of eight accessions (Gonabad, Bardeskan, Nayshabor, Ferdos, Sarayan, Boshroyeh, Ghayen, and Esfarayen) that contained *p*-cymene (8.84%), γ-terpinene (19.6%), safranal (29.0%), and cuminaldehyde (22.3%). The second cluster was formed by the EOs of two accessions (Bajestan and Sabzevar) which contained high concentrations of β-pinene (10.4%). The third cluster was formed by the γ-terpinene-7-al rich the EO of one accession (Mahvalat).

Antibacterial tests

The *in vitro* antibacterial activities of the EO of eleven accessions of green cumin were assessed by the disc diffusion and serial dilution methods against bacteria strains, include *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Listeria monocytogenes*, *Proteus vulgaris* and *Salmonella typhimurium*. Antibacterial activities were expressed as DIZ, MIC and MBC values (Tables 4 and 5). The EOs exhibited varying levels of antibacterial activity against the investigated bacteria. The DIZ values of different concentrations of the EOs were between 8.0 ± 0.3 mm

Table 3. Correlation matrix showing relationship between of the percentage of main EO compounds, EO yield and environmental conditions.

Row/Column	Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	β-Pinene	1																				
2	*p*-Cymene	0.04	1																			
3	γ-Terpinene	0.41	**0.70***	1																		
4	Cunmindheyde	-0.36	0.28	-0.29	1																	
5	Safranal	-0.19	-0.57	-0.56	0.37	1																
6	γ-Terpinene-7-al	-0.15	-0.14	-0.26	-0.37	-0.61	1															
7	EO yield	-0.27	0.21	0.36	0.06	-0.09	-0.18	1														
8	Altitude (m a.s.l)	0.11	0.30	0.21	-0.29	-0.11	-0.06	-0.28	1													
9	Precipitation [a]	0.11	0.39	-0.07	0.31	-0.11	-0.01	-0.30	0.32	1												
10	Temperature [b]	0.09	-0.64	-0.16	-0.47	0.08	0.29	0.28	-0.44	-0.75*	1											
11	RH [c]	0.25	0.44	0.26	0.26	-0.29	-0.11	-0.22	-0.19	0.63	-0.61	1										
12	pH	0.25	0.17	-0.20	0.19	0.13	-0.03	-0.36	0.55	0.60	-0.34	-0.05	1.00									
13	E.C [d]	0.18	-0.19	0.08	-0.18	-0.26	0.32	0.34	-0.63*	-0.57	0.70	-0.21	-0.29	1.00								
14	O.C [e]	-0.13	0.05	0.03	0.32	0.11	-0.30	0.05	-0.32	0.13	-0.31	0.60	-0.53	-0.33	1.00							
15	N [f]	-0.12	-0.12	-0.22	0.34	0.13	-0.09	0.06	-0.45	0.22	-0.13	0.56	-0.33	-0.16	0.88**	1.00						
16	P [g]	0.35	-0.33	-0.37	0.32	0.50	-0.28	-0.58	-0.25	-0.07	0.06	0.10	0.27	0.12	0.03	0.14	1.00					
17	K [h]	-0.21	0.11	-0.08	0.16	0.02	0.01	-0.17	0.18	0.07	-0.17	0.15	-0.02	-0.46	0.51	0.56	0.12	1.00				
18	Zn [i]	0.01	-0.31	-0.27	0.23	0.26	-0.17	0.09	-0.44	0.18	-0.02	0.47	-0.27	-0.06	0.77*	0.94**	0.20	0.35	1.00			
19	Mn [j]	0.19	-0.46	-0.13	0.10	0.25	-0.19	0.26	-0.72	-0.30	0.39	0.14	-0.58	0.37	0.53	0.55	0.18	-0.17	0.66*	1.00		
20	Fe [k]	0.00	-0.09	-0.30	0.51	0.06	0.03	0.02	-0.50	0.16	-0.06	0.42	-0.20	0.05	0.70*	0.86*	0.28	0.42	0.79*	0.62*	1.00	
21	Cu [l]	0.22	0.54	**0.67***	0.41	0.10	**-0.77***	0.39	-0.15	0.21	-0.42	0.50	-0.03	-0.04	0.34	0.20	0.08	-0.11	0.23	0.17	0.10	1.00

[a] Average annual precipitation (mm); [b] Average annual temperature (°C); [c] Average annual relative humidity (%); [d] Electrical conductivity (dSm^{-1}); [e] Organic carbon (%); [f] Total nitrogen (%); [g] Available P ($mgkg^{-1}$); [h] Available K ($mgkg^{-1}$); [i] Zinc ($mgkg^{-1}$); [j] Manganese ($mgkg^{-1}$); [k] Iron ($mgkg^{-1}$); [l] Copper ($mgkg^{-1}$).

Table 4. Antibacterial activity of the EOs of different *C. cyminum* L. accessions by disc diffusion assay.

Bacteria	Concentration µg/mL	Growth inhibition (mm ± SD)											ANOVA
		Esfarayen	Ghayen	Boshroyeh	Sarayan	Ferdos	Nayshaboor	Bardeskan	Mahvalat	Sabzevar	Bajestan	Gonabad	
B. cereus	500	18.00 ± 1.00 *	19.78 ± 2.83	27.28 ± 5.11	26.33 ± 1.52	26.00 ± 0.01	28.50 ± 1.83	27.67 ± 5.67	26.33 ± 4.05	28.44 ± 1.50	21.89 ± 3.42	27.11 ± 3.47	$p \leq 0.01$
	250	17.50 ± 1.17	18.44 ± 3.01	19.67 ± 1.33	26.33 ± 3.50	23.00 ± 0.05	23.17 ± 3.17	26.00 ± 5.67	22.67 ± 3.52	25.00 ± 1.76	19.11 ± 2.52	24.67 ± 3.85	$p \leq 0.01$
	125	15.83 ± 0.50	17.89 ± 1.26	17.83 ± 2.50	25.22 ± 2.54	20.17 ± 0.17	21.50 ± 2.83	23.00 ± 5.00	19.67 ± 4.25	23.00 ± 2.90	17.56 ± 1.57	21.89 ± 3.79	$p \leq 0.01$
	62	15.00 ± 0.66	16.22 ± 0.77	16.33 ± 3.00	23.00 ± 2.52	18.33 ± 0.01	23.55 ± 5.64	21.83 ± 4.50	17.67 ± 3.46	20.11 ± 2.91	16.56 ± 1.07	20.11 ± 2.71	$p \leq 0.01$
	31	14.33 ± 0.33	14.67 ± 0.88	14.00 ± 3.33	20.44 ± 1.64	15.67 ± 0.67	15.17 ± 1.50	20.00 ± 5.33	16.11 ± 2.50	16.56 ± 0.19	15.33 ± 0.33	17.56 ± 0.69	$p \leq 0.05$
	16	13.17 ± 0.50	11.56 ± 1.26	13.00 ± 3.33	17.33 ± 0.67	13.83 ± 0.51	13.83 ± 1.83	16.50 ± 2.50	13.11 ± 3.25	14.44 ± 3.33	14.56 ± 0.19	15.89 ± 1.83	$p \leq 0.05$
	8	12.00 ± 0.33	8.56 ± 1.64	11.00 ± 3.00	14.33 ± 1.45	10.17 ± 1.17	12.83 ± 0.83	13.67 ± 0.67	11.56 ± 2.83	12.67 ± 1.52	13.11 ± 0.19	13.00 ± 0.88	$p \leq 0.05$
	Mean¶	15.11 ± 2.16b	15.30 ± 4.13b	17.02 ± 5.80ab	21.85 ± 4.88a	18.17 ± 5.18ab	19.79 ± 6.59a	21.23 ± 6.83a	18.16 ± 5.75ab	20.03 ± 5.82a	16.87 ± 3.18ab	20.3 ± 5.24a	$p \leq 0.05$
S. agalactiae	500	27.33 ± 4.81	29.00 ± 4.99	27.33 ± 5.19	25.44 ± 1.95	22.50 ± 1.83	27.67 ± 3.00	27.00 ± 0.58	25.67 ± 1.53	24.56 ± 4.62	18.44 ± 1.26	20.17 ± 4.17	$p \leq 0.01$
	250	24.44 ± 3.89	18.78 ± 1.68	24.78 ± 5.12	22.67 ± 1.45	20.67 ± 1.67	25.00 ± 2.33	23.50 ± 1.83	23.78 ± 4.52	19.00 ± 3.00	17.00 ± 1.45	18.00 ± 2.67	$p \leq 0.01$
	125	22.44 ± 4.09	17.33 ± 1.76	23.44 ± 3.89	19.11 ± 0.78	19.50 ± 0.83	22.33 ± 2.67	19.50 ± 0.83	22.11 ± 3.53	17.67 ± 2.00	15.67 ± 1.00	16.67 ± 1.67	$p \leq 0.01$
	62	18.56 ± 1.83	14.89 ± 0.51	17.33 ± 2.00	16.78 ± 1.18	17.00 ± 0.67	20.50 ± 2.51	16.83 ± 1.50	20.22 ± 3.56	17.17 ± 2.50	15.00 ± 1.53	16.17 ± 1.17	$p \leq 0.05$
	31	16.56 ± 0.84	14.89 ± 1.26	15.56 ± 1.83	15.67 ± 0.67	15.67 ± 0.01	17.00 ± 2.67	15.33 ± 2.00	17.56 ± 1.17	15.17 ± 2.83	13.44 ± 1.84	15.50 ± 1.17	$p \leq 0.05$
	16	14.67 ± 0.01	13.78 ± 0.51	13.67 ± 2.02	14.33 ± 0.67	15.50 ± 0.17	14.50 ± 1.17	12.67 ± 2.00	14.67 ± 0.88	13.67 ± 2.00	11.67 ± 3.21	15.00 ± 1.33	$p \leq 0.05$
	8	12.00 ± 2.33	12.11 ± 1.01	11.11 ± 1.07	11.44 ± 2.00	14.00 ± 0.67	13.17 ± 1.50	8.00 ± 0.33	13.78 ± 1.26	10.83 ± 2.83	10.22 ± 3.09	13.67 ± 1.00	$p \leq 0.05$
	Mean	16.45 ± 2.71ab	14.49 ± 3.27b	18.65 ± 4.81a	19.68 ± 4.87a	17.55 ± 6.23ab	20.03 ± 5.55a	17.83 ± 3.08ab	17.92 ± 4.75ab	19.03 ± 6.50a	17.25 ± 7.20ab	19.43 ± 5.82a	$p \leq 0.05$
S. aureus	500	26.89 ± 3.83	19.22 ± 2.16	27.11 ± 2.04	25.67 ± 3.12	23.67 ± 3.00	29.83 ± 5.17	24.22 ± 0.96	29.22 ± 1.07	24.61 ± 4.65	24.33 ± 2.08	17.67 ± 0.67	$p \leq 0.01$
	250	24.89 ± 2.91	17.44 ± 1.17	24.11 ± 1.68	22.81 ± 2.57	21.50 ± 1.83	26.50 ± 4.83	20.67 ± 1.00	25.78 ± 3.17	18.83 ± 2.50	21.22 ± 3.90	16.67 ± 0.00	$p \leq 0.01$
	125	23.00 ± 2.96	16.33 ± 0.88	21.33 ± 1.45	21.29 ± 3.72	19.67 ± 1.00	22.67 ± 4.67	18.17 ± 0.50	23.78 ± 4.07	17.83 ± 1.50	18.89 ± 2.55	15.50 ± 0.17	$p \leq 0.01$
	62	20.00 ± 2.52	16.00 ± 0.33	17.44 ± 1.01	20.22 ± 4.43	17.50 ± 0.17	19.00 ± 3.33	16.83 ± 0.83	20.89 ± 2.51	16.67 ± 2.33	18.00 ± 2.52	14.00 ± 0.67	$p \leq 0.05$
	31	18.78 ± 2.36	15.56 ± 0.84	15.89 ± 0.38	18.11 ± 3.69	15.83 ± 0.17	17.00 ± 1.67	16.33 ± 1.00	19.33 ± 1.33	15.33 ± 2.00	16.67 ± 2.40	13.83 ± 0.83	$p \leq 0.05$
	16	16.44 ± 1.84	14.22 ± 1.39	14.89 ± 0.38	17.67 ± 5.54	15.00 ± 0.33	15.50 ± 0.83	14.83 ± 0.83	16.56 ± 1.07	14.50 ± 1.83	15.00 ± 2.85	12.67 ± 1.67	$p \leq 0.05$
	8	12.89 ± 4.59	12.67 ± 0.88	13.33 ± 0.88	16.44 ± 4.69	13.33 ± 0.01	13.50 ± 0.83	9.67 ± 1.33	13.11 ± 2.69	12.00 ± 2.00	13.22 ± 3.09	10.33 ± 2.00	$p \leq 0.05$
	Mean	14.38 ± 2.53b	18.19 ± 4.28ab	17.11 ± 4.37ab	19.67 ± 4.88a	17.25 ± 4.41ab	20.57 ± 6.41a	18.07 ± 3.71ab	21.23 ± 4.71a	19.16 ± 4.99a	15.92 ± 0.26b	20.41 ± 5.33a	$p \leq 0.05$

¶Values in each row having similar letter are not statistically different at $p \leq 0.05$ by Duncan's multiple range test.

Table 4. *Continued*

Bacteria	Concentration µg/mL	Growth inhabitation (mm ± SD)											ANOVA
		Esfarayen	Ghayen	Boshroyeh	Sarayan	Ferdos	Nayshaboor	Bardeskan	Mahvalat	Sabzevar	Bajestan	Gonabad	
L. monocytogenes	500	27.00 ± 1.53	21.67 ± 2.03	22.89 ± 2.17	23.44 ± 2.26	23.00 ± 1.67	23.00 ± 0.67	24.83 ± 0.87	26.67 ± 0.88	24.72 ± 4.29	20.00 ± 0.67	18.33 ± 0.33	$p \leq 0.01$
	250	24.44 ± 1.02	18.11 ± 1.39	20.33 ± 2.19	20.78 ± 3.33	20.83 ± 1.50	20.83 ± 0.17	20.67 ± 1.33	23.67 ± 4.58	21.50 ± 0.17	17.67 ± 0.88	17.83 ± 0.50	$p \leq 0.05$
	125	22.67 ± 1.33	15.89 ± 0.38	18.22 ± 2.14	18.56 ± 2.59	19.50 ± 1.50	18.50 ± 0.17	19.33 ± 1.00	22.00 ± 5.23	18.33 ± 0.67	16.11 ± 0.96	16.50 ± 0.17	$p \leq 0.05$
	62	19.11 ± 1.35	15.56 ± 1.01	16.89 ± 2.41	17.78 ± 2.14	18.67 ± 1.33	17.00 ± 0.33	17.83 ± 0.17	20.00 ± 3.75	17.17 ± 0.50	14.78 ± 1.17	14.67 ± 0.67	$p \leq 0.05$
	31	17.22 ± 0.38	14.78 ± 0.51	15.67 ± 1.73	15.56 ± 1.64	17.67 ± 0.33	15.83 ± 0.17	16.83 ± 0.50	18.44 ± 2.46	15.00 ± 0.33	13.11 ± 2.41	14.17 ± 0.50	$p \leq 0.05$
	16	15.89 ± 0.69	14.22 ± 0.77	14.33 ± 1.33	14.00 ± 1.53	16.50 ± 0.17	14.50 ± 0.17	15.50 ± 0.17	17.67 ± 2.00	13.83 ± 0.50	11.78 ± 3.42	12.83 ± 1.17	$p \leq 0.05$
	8	13.89 ± 1.35	12.22 ± 2.79	11.89 ± 2.79	12.44 ± 2.71	14.83 ± 0.50	12.33 ± 1.00	10.67 ± 3.00	15.22 ± 0.77	11.17 ± 2.50	11.11 ± 4.29	11.00 ± 0.33	$p \leq 0.05$
	Mean¶	20.03 ± 4.66a	16.06 ± 3.15b	17.17 ± 3.94ab	17.51 ± 4.16ab	18.71 ± 2.76ab	17.42 ± 3.51ab	17.95 ± 4.34ab	20.52 ± 4.59a	17.39 ± 4.68ab	14.93 ± 3.64b	15.05 ± 2.59b	$p \leq 0.05$
S. typhimurium	500	28.78 ± 5.97	22.56 ± 1.71	29.89 ± 4.22	28.56 ± 7.69	24.00 ± 2.00	28.00 ± 2.33	33.17 ± 5.48	22.78 ± 4.85	23.17 ± 2.02	25.22 ± 2.14	18.00 ± 0.01	$p \leq 0.01$
	250	27.00 ± 5.57	19.33 ± 1.20	27.22 ± 3.37	25.67 ± 8.19	20.00 ± 0.67	25.17 ± 2.50	25.00 ± 8.77	22.22 ± 3.68	18.33 ± 1.67	22.33 ± 2.52	17.33 ± 0.02	$p \leq 0.01$
	125	24.33 ± 3.18	17.44 ± 0.96	25.11 ± 2.67	24.22 ± 8.00	17.50 ± 0.17	19.33 ± 4.00	22.67 ± 7.88	21.11 ± 3.23	17.00 ± 0.67	20.00 ± 3.06	16.50 ± 0.17	$p \leq 0.01$
	62	20.67 ± 1.76	16.67 ± 0.67	21.22 ± 1.84	23.44 ± 8.47	15.67 ± 0.33	19.33 ± 2.00	21.00 ± 6.89	20.11 ± 3.98	13.33 ± 3.33	17.89 ± 2.91	15.00 ± 1.00	$p \leq 0.01$
	31	19.11 ± 1.89	15.33 ± 0.88	18.22 ± 0.96	18.11 ± 3.09	15.17 ± 0.50	16.50 ± 2.17	17.67 ± 7.33	18.33 ± 3.21	11.50 ± 4.17	16.33 ± 3.46	14.67 ± 1.01	$p \leq 0.05$
	16	17.11 ± 1.68	14.33 ± 1.20	16.56 ± 1.26	15.89 ± 2.34	13.50 ± 0.17	15.17 ± 1.50	14.67 ± 7.33	15.67 ± 1.45	10.67 ± 3.67	14.33 ± 3.46	14.00 ± 1.67	$p \leq 0.05$
	8	13.67 ± 0.67	13.44 ± 1.17	14.56 ± 0.84	14.44 ± 3.00	11.17 ± 0.50	13.50 ± 1.17	11.67 ± 5.55	13.67 ± 1.33	10.00 ± 3.67	13.56 ± 3.72	12.83 ± 2.17	$p \leq 0.05$
	Mean	21.53 ± 5.95a	17.02 ± 3.13b	21.48 ± 5.84a	21.47 ± 7.36a	16.71 ± 4.11b	19.57 ± 5.40a	20.83 ± 4.28a	19.12 ± 4.28a	14.86 ± 5.22b	18.53 ± 4.79ab	15.48 ± 2.02 b	$p \leq 0.05$
P. vulgaris	*500*	28.11 ± 2.17	21.00 ± 2.65	26.22 ± 0.77	25.11 ± 3.20	25.00 ± 4.33	25.83 ± 1.50	23.33 ± 3.46	21.56 ± 7.19	24.72 ± 2.28	21.44 ± 2.12	16.33 ± 0.33	*$p \leq 0.01$*
	250	25.33 ± 2.09	18.44 ± 1.92	23.89 ± 1.35	22.00 ± 3.33	22.00 ± 4.67	24.67 ± 0.00	17.17 ± 5.50	23.67 ± 2.65	19.50 ± 1.17	19.22 ± 2.27	15.67 ± 0.33	$p \leq 0.01$
	125	23.56 ± 2.17	18.22 ± 2.01	21.56 ± 2.54	19.00 ± 2.96	20.00 ± 2.67	21.67 ± 0.67	14.50 ± 4.50	21.44 ± 3.01	17.00 ± 0.33	17.56 ± 1.35	14.83 ± 0.50	$p \leq 0.01$
	62	21.33 ± 1.86	17.22 ± 2.14	20.44 ± 2.59	17.67 ± 2.19	18.00 ± 2.33	18.00 ± 1.33	13.00 ± 4.67	20.00 ± 3.28	16.00 ± 0.67	16.67 ± 1.73	14.17 ± 0.50	$p \leq 0.05$
	31	19.00 ± 1.45	16.56 ± 2.41	18.56 ± 2.99	16.78 ± 1.84	16.67 ± 0.67	16.67 ± 1.00	11.17 ± 4.50	18.78 ± 3.95	13.67 ± 2.67	15.11 ± 1.54	13.33 ± 0.33	$p \leq 0.05$
	16	16.67 ± 0.67	15.00 ± 0.88	18.00 ± 2.89	14.89 ± 1.58	15.00 ± 0.33	14.83 ± 0.50	9.67 ± 4.67	16.22 ± 1.84	11.67 ± 3.00	13.00 ± 1.00	11.67 ± 0.67	$p \leq 0.05$
	8	15.33 ± 0.33	11.44 ± 2.54	14.11 ± 0.69	11.67 ± 3.84	13.50 ± 0.50	13.50 ± 0.83	8.17 ± 3.17	14.00 ± 0.58	10.67 ± 2.67	9.67 ± 2.91	10.67 ± 1.00	$p \leq 0.05$
	Mean	21.33 ± 4.64a	16.84 ± 3.37b	20.39 ± 4.21a	18.16 ± 4.83ab	18.59 ± 4.47ab	19.31 ± 4.64a	13.86 ± 6.12b	19.38 ± 4.45a	16.17 ± 4.93ab	16.09 ± 4.09ab	13.81 ± 2.02b	$p \leq 0.05$

¶Values in each row having similar letter are not statistically different at $p \leq 0.05$ by Duncan's multiple range test.

Table 5. MICs and MBCs (µg/mL) of the EOs of various *C. cyminum* L. accessions.

Bacteria	Esfarayen		Ghayen		Boshroyeh		Sarayan		Ferdos		Nayshaboor		Bardeskan		Mahvalat		Sabzevar		Bajestan		Gonabad		Am[a]	Cp[b]	Fl[c]
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIC	MIC
B. cereus	250	> 500	250	> 500	250	> 500	62	125	125	500	31	125	62	125	250	> 500	62	250	250	500	62	125	62	125	125
S. agalactiae	125	500	250	> 500	125	250	250	> 500	250	> 500	62	125	125	500	62	250	125	500	250	> 500	250	500	62	31	125
S. aureus	125	500	250	> 500	125	500	62	125	250	> 500	125	250	250	> 500	62	250	250	500	250	> 500	250	500	125	62	62
L. monocytogenes	125	250	250	> 500	250	500	250	500	125	250	125	> 500	125	500	62	250	250	> 500	250	> 500	250	> 500	62	125	62
S. typhimurium	62	125	250	> 500	62	125	62	125	250	500	125	250	62	250	250	> 500	125	500	250	> 500	250	500	62	125	62
P. vulgaris	62	250	250	> 500	62	250	250	> 500	125	250	62	125	250	> 500	125	500	250	> 500	250	> 500	250	500	31	31	125

[a] Am, ampicillin; [b] Cp, ciprofloxacin; [c] Fl, flumequine.

against *S. agalactiae* in the EO from the Bardeskan accession at 8 µg/mL and 29.9± 4.2 mm against *S. typhimurium* in the EO from the Boshroyeh accession at 500 µg/mL (Table 4). In general, all of the EOs investigated showed relatively high inhibitory activities against the six bacteria strains tested (Table 4). The MICs of the EOs were within concentration ranges 31 to 250 µg/mL, and the respective MBCs were 125 to >500 µg/mL (Table 5). Integrated results of the DIZ, MIC, and MBC values indicated the EOs of various accessions had high inhibitory activity against bacteria strains as follows:

- The Nayshaboor, Bardeskan, Sarayan, Gonabad, and Sabzevar accessions against *B. cereus*;
- The Nayshaboor accession against *S. agalactiae*;
- The Sarayan and Mahvalat accessions against *S. aureus*;
- The Esfarayen, Bardeskan, Boshroyeh and Sarayan accessions against *S. typhimurium*;
- The Esfarayen, Nayshaboor and Boshroyeh accessions against *P. vulgaris*;
- The Esfarayen and Mahvalat accessions against *L.*
- *monocytogenes.*

The antibacterial activity of green cumin EOs could be attributed to the high level of cuminaldehyde, a compound with known antimicrobial properties (Helander et al., 1998). Sekine et al. (2007) suggested that the main antifungal compound of the EOs from the spices was cuminaldehyde. In addition, results of a study by Scortichini et al. (2000) indicated ɣ-terpinene, ɣ-terpinene-7-al and β-pinene, as main components in the EOs of green cumin, inhibited the growth of bacteria strains. The mechanisms by which the EOs can inhibit microorganisms varies. In some cases it may be due to the hydrophobicity of the constituent in the EO which penetrates into the lipid bilayer of the cell membrane and makes the cells more permeable, leading to leakage of vital cell contents (Kim et al., 1995; Burt, 2004). The EO components dissolve in the membrane, causing swelling and reducing membrane function, and lead to cell death (Holly and Patel, 2005). Moreover, the antimicrobial activity of the EOs may be due to the presence of synergy between the major components and other constituents of the oils leading to various degrees of antimicrobial activity. This property could be resulted from the relatively high amount of terpinenes (ɣ-terpinene and *p*-cymene) and cuminaldehyde in the EOs of the Esfarayen, Nayshabor and Mahvalat accessions. Some researchers have reported a relationship between the chemical structures of the most abundant compounds in the EOs and their antibacterial activity. EOs containing phenolic compounds, e.g. thymol, carvacrol, ɣ-terpinene and *p*-cymene, are widely reported to possess high levels of antibacterial activity (Burt, 2004), which was confirmed and extended in the present study.

Material and methods

Plant material

Cumin fruits (0.5 kg) of cultivation accessions of *C. cyminum* L. were harvested at fully ripe during the harvest season in 20 May to 10 June 2011 from 33 farms (eleven accessions) in three provinces, Northeast of Iran. Selected geographic and characteristics of accessions are presented in Table 1 and Fig 1. The samples were cleaned manually before analysis. The voucher specimens were identified at the Herbarium, Agricultural and Natural Resources Research Center of Central (Razavi) Khorasan Province, Mashad, Iran. Each sample was labeled and its location was recorded using a Global Positioning System (GPS, Vista Garmin) receiver. Soil physical and chemical characteristics were determined and presented in Table 1. Climatic data of the locations were determined using the nearest meteorology station.

Essential oil extraction

The fruits were dried at room temperature for 10 days. Dried fruits were ground, and 100 g of plant matter was distillated with 1,000 mL water for 3 h using a Clevenger-type apparatus. The separated oil was dried over anhydrous sodium sulfate, and stored in dark glass bottles at 4 ± 2 °C prior to use.

Identification of the EO constituents

Composition of the EOs were determined by GC and GC/MS. GC analysis was done on a Younglin Acme 6000 gas chromatograph equipped with a Flame Ionization Detector (FID) and an HP-5 MS (30.00 m × 0.25 mm i.d., film thicknesses 0.25 µm). GC oven temperature was kept at 50 °C for 5 min and programmed to 240 °C at a rate of 3 °C/min, and then programmed to 300 °C at a rate of 15 °C/min. The 0.5 µL samples were injected manually in the split mode. GC/MS analysis was done on the mentioned an Agilent Technologies 5973 Mass Selective system with 6890 GC. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 50–550 (Adams, 2007). Compounds were identified by comparison of their KI (retention indices) relative to C_5-C_{24} *n*-alkanes obtained on a nonpolar DB-5MS column, with those provided in the literature, by comparison of their mass spectra with those recorded in NIST 08 (National Institute of Standards and Technology) and Willey (ChemStation data system). The individual components were identified by retention indices and compared with compounds known from the literature (Adams, 2007; McLafferty, 2009). The percentage composition of the essential oils was computed from GC–FID peak areas without correction factors.

Antibacterial activity

Clinical isolates of four Gram-positive bacterial strains, include *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Listeria monocytogenes* and two Gram-negative bacterial strains, include *Proteus vulgaris* and *Salmonella typhimurium* were obtained from the Food Microbiology Laboratory, Veterinary Medicine Faculty, (I.A.U.) Iran. The bacterial strains were identified using PCR–RFLP and conventional morphological as well as biochemical tests. Stock cultures of bacteria were kept in 20% glycerol PBS (phosphate buffered saline) at -70 °C. Active cultures were generated by inoculating 100 µL of the thawed microbial stock suspensions into 5 mL nutrient broth (Merck, Darmstadt, Germany) followed by overnight incubation at 37 °C. The density of bacterial culture required for the test was adjusted to 1.0 McFarland standards, (1.0×10^7 CFU/mL) measured using the spectrophotometer (Eppendorf AG, Hamburg, Germany). These experiments were performed by the disc diffusion method (NCCLS, 1999; NCCLS, 2006) with some modification. Sterile paper discs (6 mm in diameter) were impregnated with 60 µL of dilutions of known the EO concentrations (8 to 500 µg/mL) and incubated at 37 °C for 24 h. Bacterial growth inhibition was determined as DIZ around the discs (mm). The growth inhibition diameter was an average of three measurements, taken at three different directions. The MIC values were evaluated using the broth serial dilution method according to standard methods (NCCLS, 2003). Bacterial strains were cultured overnight at 37 °C in Muller Hinton Broth (MHB,

Oxoid). Stock solutions of the EOs and antimicrobial standards (ampicillin, ciprofloxacin, and flumequine) were prepared in 5.0% (v/v) DMSO. Dilution series, using MHB, were prepared from 4 to 500 µg/mL. After incubation at 37 °C for 24 h, the microorganism growth inhibition was evaluated by measuring absorbance at 630 nm, using a spectrophotometer. Experiments were performed in triplicate but at three different times. The MBC of the EOs determined according to the MIC values (NCCLS, 2006). First, 5 µL from MIC tubes were transferred to agar plates (MHA) and then incubated at 37 °C for 24 h. The MBC was referred to the minimum concentration of the EOs with no viable bacteria.

Statistical analysis

The data was statistically analyzed using one-way ANOVA by the program SPSS (19.0). Means of the main constituents of EOs were compared by Duncan's multiple range test at $p \leq 0.05$ level. Analytical data for Hierarchical cluster analysis were treated by means of the SPSS statistical software.

Conclusion

The results of the present study indicated that EOs components can vary with genetic (accession), environmental conditions and geographic origin (climate, edaphic, elevation and topography). The comparison of our results with other reports on green cumin EO components indicated that Khorasan accessions have less monoterpenes and sesquiterpenes while they are richer in safranal, cuminaldehyde and γ-terpinene-7-al. This is probably due to: (i) the arid and semiarid climate conditions in the regions and low irrigation level in farming systems used by farmers in Khorasan, and (ii) genetic potential of accessions which may produce secondary compounds. In almost all areas of Khorasan, water stress caused by drought and salinity is the most important abiotic factor limiting plant growth and crop productivity, may promotes the production of secondary metabolites which modify the essential oil chemotype. In general, Nayshaboor and Esfarayen accessions showed the highest cuminaldehyde content which is important in term of aromatic value. These two accessions along with Mahvalat also showed the highest antibacterial activity against foodborne pathogen. These three accessions can be used as a native alternative to synthetic antibacterial in the food industry.

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