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Chemical Composition and Antibacterial Activities of the Essential Oil and Extract of *Cirsium Congestum*

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ABSTRACT: Medicinal plants are considered as valuable natural sources. In this study, the antibacterial properties of *Cirsium congestum* essential oil and methanolic extract were investigated against *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Candida albicans* and *Klebsiella pneumonia.* The results of agar disc-diffusion method showed that essential oil and extract exhibited maximum and minimum inhibition zone diameter against *Candida albicans* and *Escherichia coli* respectively. Minimum inhibitory concentration (MIC) of essential oil and methanolic extract observed for *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Candida albicans* and *Klebsiella pneumoniae* respectively. Lowest MIC related to *Candida albicans. Cirsium congestum* essential oil had higher antibacterial activity in comparison with the extract. The chemical composition of the *Cirsium congestum* essential oil was analysed by gas chromatography mass spectrometry (GC–MS). Twenty-six compounds representing 99.98% of the total essential oil was identified. The Results showed sescoe turpen hydrocarbons and imidazole 4- propionic acid were the major compounds in *Cirsium congestum* essential oil.

Keywords: Antibacterial, *Cirsium congestum*, Essential Oil, Extract, GC-MS.

Introduction

Medicinal plants are considered as valuable natural sources; their secondary metabolites such as essential oils and herbal extracts were evaluated in terms of containing antibacterial properties. Most of the herbal essential oils have antifungal, antiparasitic, antibacterial and antiviral properties (Hanumantappa *et al*., 2014). During the past two decades, it was demonstrated that antibacterial activities of different parts of plant like roots, stems, leaves, flowers, fruit and seeds are considered as the medicinal plants (Hossain *et al*., 2012).

Essential oils obtained from medicinal plants are naturally aromatic because of a mixture of multifarious chemical substances belonging to various chemical families, including terpenes, aldehydes, alcohols, esters, phenolic, ethers, and ketones (Akhtar *et al*., 2014).

Essential oils are considered as non-phytotoxic compounds and potentially effective against microorganisms (Hossain *et al*., 2012). Plant essential oils and

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extracts are sometimes applied as antimicrobial agents, food flavoring, enhance shelf life of processed foods and reduce the risk of food poisoning (Kazemizadeh, 2010).

Nowadays there is a growing interest regarding the use of edible medicinal plants, herbs, and spices which have long been applied as natural agents for food preservation. The antimicrobial impacts of herbal essential oils and extracts and their chemical components have been recognized by several researchers including different plants as: clove (Siddiqua *et al.,* 2014), black pepper (Kapoor *et al.,* 2014), eucalyptus essential oil (Tyagi *et al.*, 2013), lemongrass, cinnamon leaf, and basil (Duan & Zhao, 2009), merremia borneensis (Hossain *et al*., 2012), cassia sophera L. (Rahman *et al*., 2017), cordia verbenacea (Meccia *et al*., 2009).

Cirsium is a genus of perennial and biennial flowering plants and one of several genera known commonly as thistles. They are more precisely referred to as plume thistles. They are native to Eurasia and northern Africa, with about 60 species from North America., although, several species have been introduced outside their native ranges. Thistles are known for their effusive flower heads, in purple, rose or pink, yellow or white colors too. They have erect stems and prickly leaves, with features enlarged base of the flower which is commonly spiny. Certain species of *Cirsium* like *Cirsium monspessulanum, Cirsium pyrenaicum and Cirsium vulgare, have been traditionally applied as food in country sides of southern Europe. Cirsium oleraceum is cultivated as a food source in Japan and India (Van Der Kooi et al., 2015).*

Some *Cirsium* species grows in mountainous areas of Iran and have been used in soups, stews and even in salads. *Cirsium congestum* dried leaves contain about 6 to 11 percent water and 10 to 12 percent minerals and are rich in potassium and magnesium salts. Cirsium congestum is one of the plants with very low essential oil and it is not known as aromatic plant because of its less essential oil (Zargari, 1992).

Kozyra *et al.* (2015) investigated the chemical composition of the essential oils of inflorescences *Cirsium* species (*Cirsium pannonicum, Cirsium ligulare Boiss, Cirsium heterophyllum, Cirsium acaule, Cirsium oleraceum, Cirsium dissectum, Cirsium decussatum* and *Cirsium eriophorum* by GC/MS method. Their study showed the differences in chemical composition of volatile oils in the inflorescences of *Cirsium* species. The essential oil is mainly consisted of ketones and aldehydes. Volatile oils also contained small amounts of terpenes: thymol, β-linalool, eugenol, carvacrol and fatty acids (Kozyra *et al.*, 2015).

In this study, the antibacterial properties and chemical composition of *Cirsium congestum* essential oil and methanolic extract were investigated.

Materials and Methods

- *Materials*

The fresh stems and leaves of *Cirsium congestum* were collected in May 2017, in Sabzevar city, Iran. Stems and leaves were dried in the oven at 40˚C grounded using a Black & Decker grinder (Model no. JBG60, USA), and sealed in a brown bottle. Then it was kept at 4-5˚C until the extraction process. All chemicals in analytical grade were purchased from Merck Company.

The bacterial strain cultures were obtained from the *Iranian Research Organization for Science and Technology* (IROST). The 5 bacterial species used in the experiment were *Escherichia coli*, *Bacillus cereus, Staphylococcus aureus, Candida albicans* and *Klebsiella pneumoniae*

- *Methods*

- *Preparation of the essential oil*

About 500 g stems and leaves powder of *Cirsium congestum* were subjected to Clevenger-type apparatus hydrodistillation for 3 h. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4˚C until further analysis.

- *Preparation of extract*

The extraction process was carried out through using a reflux system set up in a water bath. 200 g of *Cirsium congestum* was placed into a flask with 500 ml of 80% methanol. The flask was put in a water bath (Model LAUDA E200, Germany) which was kept at 60±1^{o}C, for 2 hours (Shahidi *et al*., 2020). The *Cirsium congestum* extract was filtered using filter paper Wattman 40 and concentrated at a low temperature (<50^{o}C) using a vacuum rotary evaporator (BUCHI- water bath B-480, Flawil, Switzerland). The concentrated extract was stored in air tight dark glass bottles and kept refrigerated (4^{o}C) for further treatments after solvent separation (Sharifi *et al*., 2015).

- *Gas chromatography–mass spectrometry (GC–MS) analysis*

The chemical analysis of the essential oils was carried out by an Agilent 7890N gas chromatograph coupled with a mass spectroscopic detection 9575C; moreover, it was equipped with a Capillary column (HP-PLOT Al2O3, 30 m length, 0.25 mm I.D, 0.25 µm film thickness). The ionization voltage in the mass spectrometer was 7V. Helium was applied as the carrier gas at a flow rate of 0.8 mL/min in a split ratio of 1:10. Temperature program was as follows: oven temperature was 80°C, held for 5 min; increased at a rate of 3°C/min from 80 to 240°C, held for 1 min; increased at a rate of 25°C/min from 240 to 280°C, and held for 3 min to be kept at temperature of 280°C. Injector and detector were held at 240 and 290°C, respectively, and 2 µL of essential oil was always injected. The identification of compounds and their concentration were determined and calculated by standard and peak area respectively (Hossain *et al*., 2012).

- *Determination of antibacterial activity of essential oils and crude extracts*

- *Preparation of bacterial culture medium*

For preparation of bacterial culture medium (nutrient *agar,* muller-hinton *agar,* LB broth *and* nutrient *broth*) and bacterial suspension, the methods of Iranian national standard number (9899) were applied.

- *Disc diffusion method*

Antibacterial activity of essential oil and methanolic extract were evaluated with agar disc diffusion assay. Muller-hinton *agar for* essential oil and nutrient agar medium for extract were prepared, autoclaved and transferred aseptically to sterilize petri plates. 100 µl of bacterial suspension (10^8 cfu/mL) was spread on plates and then filter paper disks were impregnated with 10 µl of the essential oil prepared by dissolving into 10 µl of dimethyl sulfoxide and incubated at 37°C for 24 hours for all bacteria. Chloramphenicol (30 µg/disc) was applied as a positive control to determine the sensitivity of one strain in each microbial species tested. The zones of inhibition around each of the discs were calculated through measuring the diameter in mm as a measure of the antimicrobial activity after incubation time. Each test was taken in triplicate (Azhdarzadeh and Hojjati, 2016).

- ***Minimum inhibitory concentration (MIC)***

Active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks and inoculated in LB medium at 37˚C for 24 h. The tested samples were incorporated into LB broth medium to get the final concentration ranging from 0 to 1000 µg/ml. 20 µl inoculums of each bacterial strain (10^7CFU/ml) were transferred to each tube, and the tests were performed in a volume of 2 ml. The control tube contained only organisms, and the tested samples were not included in. The culture tubes were incubated at 37 ˚C for 24 h. After macroscopic evaluation, the lowest concentration of the test samples, which did not show any visual growth of tested organisms, was determined as MIC expressed in µg/ml (Van *et al*., 2022; Rahman *et al*., 2017).

- ***Statistical Analysis***

All experiments were conducted in triplicate and an analysis of variance was performed. The least significant difference at $p< 0.05$ was calculated using the Duncan Multiple Range Test on SAS software version 9.3.

Results and Discussion

- ***Chemical composition of the Cirsium congestum essential oil***

The yield of the hydrodistillation essential oil of *Cirsium congestum* was 0.12% (v/w). Based on the results of Table 1, the chemical composition of the *Cirsium congestum* essential oil analyzed by GC–MS, was mainly constituted of twenty-six compounds representing 99.98% of all compounds detected in essential oil. Linoleic acid ethyl ester (13.98%), Hexadecanoic acid ethyl ester (13.5%), Imidazole 4- propionic acid (13.11%), 1-Limonene cyclohexane (10.58), and other ingredients (48.83%) were the major compounds in *Cirsium congestum* essential oil.

Essential oil of *Cirsium congestum* is being considered as a leading compound for new classes of natural bactericides, resulted from the presence of oxygenated mono-and sesquiterpene hydrocarbons and these findings are in agreement with the previous studies (Rahman *et al*., 2017; Shunying *et al*., 2005).

Joshi *et al.* (2013) reported the GC-MS profile of the essential of large cardamom (*Amomum subulatum Roxb*.) growing in India. The major compounds of the collected essential oil were oxygenated monoterpenes (65.31–75.54%), monoterpene hydrocarbons (10.53–17.12%), terpene alcohols (15.32–18.80%), and sesquiterpene hydrocarbons (5.02–9.19%). That oxygenated monoterpene 1,8-cineole (50.55–60.46%) was the dominant substance.

- ***Antibacterial activity of essential oil and extract of Cirsium congestum***
- ***Effect of essential oil and extract of Cirsium congestum on inhibition zone (mm)***

The results of data variance analysis showed all of microorganisms studied had significant effect on inhibition zone ($P<0.05$). Essential oil and extract exhibited maximum and minimum zone of inhibition against *Candida albicans* and *Escherichia coli* respectively (Figure 1). *Cirsium congestum* essential oil had larger inhibition zone in comparison with extract ($P<0.05$). These results are similar to previous researchers' results.

Table 1. Chemical composition of the *Cirsium congestum* essential oil

Number	Ingredients	%	Abundance	R.T
1	Linoleic Acid Ethyl Ester	13.98	3200000	13.84
2	Hexadecanoic acid ethyl ester	13.5	3600000	12.57
3	Imidazole 4- propionic acid	13.11	2110000	13.66
4	1-Limonene cyclohexane	10.58	3000000	5.93
5	n-Penta dekan	8.9	250000	8.94
6	Palmitic acid	8.68	290000	12.51
7	Dibotrophenyl hydrazine	8.01	2450000	13.78
8	Eicosan	7.98	2180000	7.98
9	Phenol	2.64	790000	13.90
10	Normal honey kouzan	2.29	945000	12.91
11	Hexanic Acid 2- propanyl ester	2.27	930000	7.43
12	Propandivic acid	1.89	710000	8.09
13	Hexadecane	1.70	750000	12.61
14	Normal heptadecane	1.52	490000	12.56
15	Gamma trypin cyclohexane	1.23	500000	6.73
16	10-Methyl nanodekan	1.17	460000	13.07
17	Normal dekan	1.15	595000	3.90
18	Tetra chlorethylene	1.06	67000	2.46
19	Normal tetradekan	1.05	653000	10.62
20	Pentadcane	1	700050	11.05
21	Normal eoscane	0.96	530000	9.57
22	Normal hexanol	0.85	530000	3.01
23	Bicyclone 2-2-1- heptane	0.83	430000	9.23
24	Normal Terry dekan	0.81	565000	9.45
25	Benzene methyl toluene	0.79	580000	2.25
26	Normal two Dekan	0.62	403000	3.09

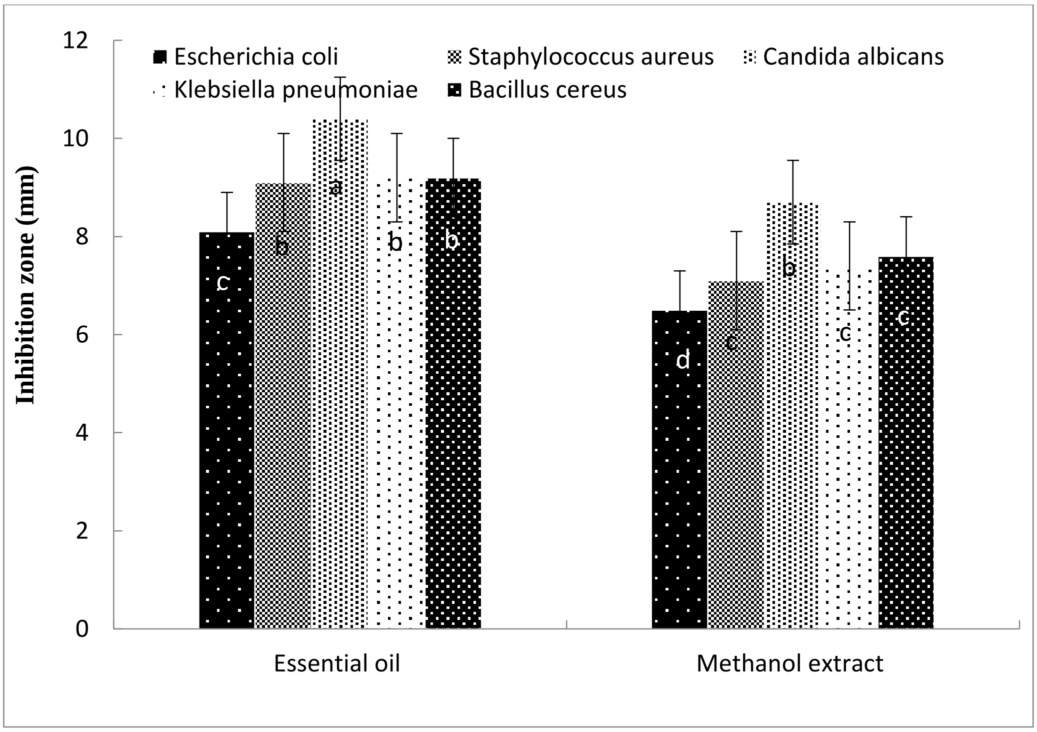

Fig. 1. Effect of essential oil and extract of *Cirsium congestum* on inhibition zone (mm).

Adiguzel *et al.* (2009) examined antimicrobial and antioxidant activities of *Nepeta cataria* essential oil and methanolic extract. It was revealed the essential oil of N. *Cataria* had antimicrobial activity against 11 bacteria, and 12 fungi and 1 yeast species. The methanolic extract showed antimicrobial activity on only 5 bacteria and 7 fungi. Hojjati and Barzegar (2017) put chemical composition and biological activities of lemon leaf essential oil under the investigation. Their results obtained in this study showed the moderate antimicrobial activities of lemon leaf essential oil. Siddiqua *et al.* (2014) and Kapoor *et al.* (2014) investigated the antibacterial activity of clove oil and black pepper respectively. They reported essential oils are rich in phenolic compounds like thymol and carvacrol, and have high antibacterial activities.

- *Minimum inhibitory concentration (MIC)*

Minimum inhibitory concentration of essential oil and methanolic extract observed the most to the least for *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans* and *Klebsiella pneumoniae* respectively. Lowest minimum inhibitory concentration related to *Candida albicans* (Tables 2 and 3). Highest inhibition zone (mm) and lowest minimum inhibitory concentration related to *Candida albicans* (Figure 1). This finding was in line with Hojjati and Barzegar (2017) study.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *Cirsium congestum* essential oil

	Microorganism	**Exame**	**100 %**	**50 %**	**25 %**	**12.5 %**	**6.25 %**	**3.12 %**	**1.56 %**	**0.78 %**	**0.39 %**	**0.19 %**	**0.09 %**
1	*Staphylococcus aureus*	MIC	-	-	-	-	-	+	+	+	+	+	+
		MBC	-	-	-	-	-	+	+	+	+	+	+
2	*E. coli*	MIC	-	-	-	-	-	-	-	+	+	+	+
		MBC	-	-	-	-	-	-	-	+	+	+	+
3	*Bacillus cereus*	MIC	-	-	-	-	-	-	-	-	+	+	+
		MBC	-	-	-	-	-	-	-	-	+	+	+
4	*Candida albicans*	MIC	-	-	-	-	-	-	-	-	-	+	+
		MBC	-	-	-	-	-	-	-	-	-	-	+
5	*Klebsiella pneumoniae*	MIC	-	-	-	-	-	-	-	-	+	+	+
		MBC	-	-	-	-	-	-	-	-	+	+	+

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *Cirsium congestum* extract

	Microorganism	**Exame**	**100%**	**50%**	**25%**	**12.5%**	**6.25%**	**3.12%**	**1.56%**	**0.78%**	**0.39%**	**0.19%**	**0.09%**
1	*Staphylococcus aureus*	MIC	-	-	-	-	+	+	+	+	+	+	+
		MBC	-	-	-	-	+	+	+	+	+	+	+
2	*E. coli*	MIC	-	-	-	-	-	+	+	+	+	+	+
		MBC	-	-	-	-	-	+	+	+	+	+	+
3	*Bacillus cereus*	MIC	-	-	-	-	-	-	-	+	+	+	+
		MBC	-	-	-	-	-	-	-	+	+	+	+
4	*Candida albicans*	MIC	-	-	-	-	-	-	-	+	+	+	+
		MBC	-	-	-	-	-	-	-	+	+	+	+
5	*Klebsiella pneumoniae*	MIC	-	-	-	-	-	-	-	+	+	+	+
		MBC	-	-	-	-	-	-	-	+	+	+	+

One of the important sescoe turpen hydrocarbons types found in *Cirsium congestum* essential oil was linoleic acid ethyl ester which would have antibacterial effects.

Alim *et al*. (2009) demonstrated *Nepeta nuda* essential oil had antibacterial activity towards *Klebsiella pneumoniae* and *Salmonella typhi*. The oil showed weak antimicrobial activity against *S. aureus*, *E. coli, C. diphteriae, P. vulgaris, B. subtilis* and *C. albicans.*

In study of Rahman *et al.* (2017), the highest activity against *Bacillus megaterium* with a MIC value of 62.5 µg/ml was elaborated by the ethanolic extract. They reported the essential oil and organic extracts exhibited higher antibacterial activity in comparison with common drug.

Conclusion

The researchers are struggling with finding new herbal medicine because of the damage to human health and drug resistance of chemical medicine and antibiotics. In this study, chemical composition and antibacterial activities of the *Cirsium congestum* essential oil and extract were investigated. Twenty-six compounds representing 99.98% of the total *Cirsium congestum* essential oil was identified. Sescoe turpen hydrocarbons and imidazole 4- propionic acid were the major compounds in *Cirsium congestum* essential oil. One of the important sescoe turpen hydrocarbons that found in *Cirsium Congestum* essential oil was linoleic acid ethyl ester. The results of agar disc-diffusion method showed that essential oil and extract exhibited maximum and minimum zone of inhibition against *Candida albicans* and *Escherichia coli* respectively. Minimum inhibitory concentration of essential oil and methanol extract obtained the most to the least for *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Candida albicans* and *Klebsiella pneumoniae* respectively. Lowest minimum inhibitory concentration related to *Candida albicans. Cirsium congestum* essential oil had higher antibacterial activity in comparison with extract. Findings of this study would suggest that the essential oil and extract of *Cirsium congestum* can be a source of natural antimicrobial agents with potential of application as a natural preservative in foods but their effect on sensory quality of various foods need to be studied.

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