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Antimicrobial activities of *Caucalis platycarpos* L. and *Eryngium caucasicum* Trautv. essential oils

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Abstract

Caucalis platycarpos L. and *Eryngium caucasicum* belong to Umbelliferae and are well represented in the flora of Iran by about 120 genera, 3500 species, and 100 endemic plants. This family is really valuable from economic and medicinal points of view and some species of this family are well known either because they have medicinal properties or because of great essential oils that they contain. Additionally, the mentioned genus has been wildly used in traditional medicine in Iran as an antitumor, antioxidant, antibacterial, and anti-inflammatory agent. In this regard, this study was conducted to provide useful information about essential oils of these two species. Volatile constituents of aerial parts of *Caucalis platycarpos* L. and *Eryngium caucasicum* Trautv. were investigated using gas chromatography/mass spectrometry (GC–MS) and gas chromatography/flame ionization detection (GC–FID). Antimicrobial activities of the essential oils were tested against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Bacillus subtilis* using a disc diffusion method. The essential oils were mainly composed of *trans*-pinocarvyl acetate and caryophyllene oxide considered as the most significant constituents. According to a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC), the essential oils showed antimicrobial effects. Moreover, the oils extracted from *E. caucasicum* and *C. platycarpos* indicated antibacterial activities against *S. aureus* and *B. subtilis*.

Keywords: Bur parsley**,** Mass spectrometry, Sea holly, Umbelliferae, Volatile compounds

Introduction

Essential oils are composed of diverse volatile secondary metabolites which are results of various biosynthetic processes. They exhibit different biological activities and thus become good targets for pharmaceutical, agronomy, food, sanitary, cosmetic, and perfumery industries (Burt, 2004; Hamedi et al., 2014; Hamedi et al., 2017). In nature, the essential oils, because of their antibacterial, antifungal, and antioxidant activities, play a very important role in plant defense mechanisms against pathogens (Levin, 1976). The essential oils are growing in popularity in different societies as natural antimicrobial agents. This growing trend might be due to the ethnopharmacological background of their natural sources or the concerns about the growing pathogenic organism's antimicrobial resistance to mainstream antibiotics (Juteau et al., 2002; Ebrahimi et al., 2008; Wang et al., 2012; Velázquez-Nuñez et al., 2013). Among commonly used essential oil-bearing plants, Apiaceae family has a diverse valuable therapeutic and edible cases with antimicrobial properties (Helal et al., 2015). The Apiaceae family consists of annual or perennial herbs with minority shrubs or trees widely distributed in Europe, North America, Asia, and Africa.

Several genera of this family, such as Petroselinum (parsley), Foeniculum (fennel), Apium (celery), and Heracleum (hogweed), are widely cultivated and consumed as edible herbs in folk medicine or as foodstuff in various regions of the world, thereby indicating a high safety for their metabolites (Jeyabalan et al., 2015; Arai et al., 2001; Diao et al., 2014; Zhang et al., 2006).

On the other hand, many species in this family have been used as endemic vegetables or spices in different societies. Among these species, *Bunium persicum* (earth nut), *Eryngium foetidum* (long coriander), *Ligusticum scoticum* (Scottish licorice root), *Echinophora sibthorpiana* (Tarhana herb), *Chaerophyllum bulbosum* (bulbous chervil), and *Peucedanum ostruthium* (masterwort) can be mentioned, all of which come from the wide biodiversity of the species in this family (Ehsani et al., 2016; Akgul and Chialva, 1989; Guil-Guerrero and Rodriguez, 1999). Considering the secondary metabolites of this family, essential oils are the most diverse ones. About 760 different constituents with different chemical classes have been isolated from the essential oils of Apiaceae. There is some evidence to suggest antibacterial potentials of Apiaceae essential oils (Lo cantore et al., 2004; Oroojalian et al., 2010). Therefore, the antibacterial potentials of the essential oils of endemic plants with edible backgrounds have attracted a lot of attention for finding new active ingredients from these sources. One of the special Iranian floristic regions is Hyrcanian region. The Apiaceae family has a good distribution and growth condition in biodiversity in the Hyrcania region. The Hyrcania forest is an arc along the

Caspian Sea from Golestan province of Iran in the east to the Republic of Azerbaijan in the west. Despite the massive biodiversity of Hyrcania plants, no comprehensive investigation has been carried out on their territorial vegetables and their ethnomedical applications. Using spices made of endemic aromatic plants is very common amongst Hyrcanian people. Some of these popular plants are *C. platycarpos* and *E. caucasicum* (Apiaceae family) (Figure 1). Leaves of these plants, locally called Avieh (*E. caucasicum*) and Bur parsley (*C. platycarpos*), are mainly collected from wild forests and are sold in local markets as vegetables and spices. These plants are used as food additive and food flavoring agents. Furthermore, they are used as traditional antimicrobial remedies in the Hyrcanian region of Iran (Ehsani et al., 2016; Khoshbakht et al., 2007). But their chemical constituents and biological activity of them have not been explored much. Based on our knowledge about regional uses of these plants we conducted this investigation on *c.platycarpos,* and *E. caucasicum* volatile oils antibacterial potential on *S.aureus*, *E. coli*, *P.aeruginosa,* and *B. subtilis.*

Results and Discussion

Constituents of the essential oils of E.Caucasicum and C.platycarpos

Chemical compositions of essential oils and antibacterial activities of *C. platycarpos* and *E. caucasicum* were investigated in the present study. Hydro-distillation of air-dried aerial parts yielded four yellowish oils (1.3 and 1.1 v/w, respectively, on the basis of dry weight). Major components of the essential oils of *E. caucasicum* and *C. platycarpos* were *trans*-pinocarvyl acetate and caryophyllene oxide. Compounds of the essential oils were listed according to their elution on the DB-5 column. The major volatile compounds are shown in Table 1 and Figure 2.

Antimicrobial effects of chemical constituents of E.Caucasicum and C.platycarpos

Many of the chemical constitutions of the essential oils, such as alcohols, terpenes, and aromatic compounds, could be active against various pathogenic bacteria (Kalemba and Kunicka, 2003). The essential oils and their chemical components could be valuable starting points for the development of future bacterial infections control or new antibiotic molecular targets identification (Langeveld et al, 2014; Yap et al., 2014). The antibacterial potencies of these compounds are directly correlated with their structural class, hydrogen-bound capacity, and water solubility (Sikkema et al., 1995; Naigre et al, 1996; Togashi et al., 2007). The antibacterial activities of the essential oils were determined by the disc diffusion assay with the essential oils loaded on filter paper discs. The antibacterial potentials of the essential oils, as indicated by their zones of inhibition, were compared to penicillin as a standard antibiotic at first. The results of the antibacterial assays for *S. aureus*, *E. coli*, *P. aeruginosa,* and *B. subtilis* are reported in Table 2. The inhibition zone in the paper disk assay method does not accurately reflect the essential oils' antimicrobial potential. Owing to this, the effectiveness of samples of the essential oils was measured by the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The investigated essential oils showed better activities against Gram-positive than Gram-negative bacteria based on the MIC, MBC, and zone of inhibition antibacterial activity assays. Although the observed effects were not very distinctive, the antibacterial potentials of the tested essential oils were in this order: *E. caucasicum*>*C. platycarpus* (Table 3). Obviously, it has been found that mono and sesquiterpene oxygenated hydrocarbon show suitable antibacterial activities (Trombetta et al., 2005; Chakraborty et al., 2010; Christensen and Brandt, 2006). In this study, the compositions of the essential oils of *E. caucasicum* and *C. platycarpus* are marked by *trans*-pinocarvyl acetate and caryophyllene oxide in both species. It seems that there was a relationship between the high activities of *E. caucasicum* and *C. platycarpus* essential oils and the presence of *trans*-pinocarvyl acetate and caryophyllene oxide. A large number of reports exist on *trans*-pinocarvyl acetate and caryophyllene oxide antimicrobial potentials especially against Gram-positive bacteria (De feo et al., 2003; Khalighi-sigaroodi et al., 2005; Sabulal et al., 2006; Deba et al., 2008). Moreover, results obtained from the current study showed the similar evidence that the Gram-negative (*E. coli, P. aeruginosa*) bacteria were more resistant to oxygenated hydrocarbon compared to the Gram-positive (*S. aureus, B. subtilis*) ones. The potential differences between these essential oils could be attributed to their different chemical compositions and concentrations. The high mono and sesquiterpene oxygenated hydrocarbon percentage in *E. caucasicum* and *C. platycarpus* essential oils yielded better antibacterial potencies in contrast to other essential oils that contain more non-oxygenated -hydrocarbons such as (E)-β-Farnesene and β- Cedrene (Elgayyar et al., 2001).

Materials and methods

Plant materials

The aerial parts of wild growing flowered *C. platycarpos* and *E. caucasicum* were collected from Guilan province, Iran. Voucher specimens were authenticated by the Research and Development Centre of Plants and Medicinal Chemistry at Guilan University of Medical Sciences with voucher specimen No. 2587 and 2588 deposited in the Herbarium of Pharmacognosy, Department of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran. The aerial parts of the plants were collected during spring 2015 at Kacha village, Saravan jungle, situated at 37.0529, 49.3279, and 3,268 ft in Guilan province, Iran.

Extraction of the essential oils

Samples were dried in a laboratory for 6 days at 25 °C. Moisture contents of these samples were determined based on the Association of Official Agricultural Chemists (AOAC) method by calculating samples weight loss after drying using air-oven at 110 °C for 4 h (Mediani et al., 2014). Then, the air-dried aerial parts of the four species were finely grounded into a powder weighing 500 g and subjected to hydrodistillation (HD) for 3 h using a Clevenger type apparatus, yielding 1.9, 0.8, 1.3, and 1% v/w (based on dry plant materials). The samples were dehydrated with

Table 1. Major constituents of the volatile oils of *E. caucasicum* and *C. platycarpus*

	Chemical compounds	RI[a]	RI [b]	HD (%)[c]	
				E. caucasicum	*C. platycarpus*
1	2,4-Dimethyl-Hexane	729	736	-	1.3
2	1-Hexanol	864	869	-	7.4
3	β-Pinene	981	980	1.1	3.4
4	1-Octanol	1078	1070	2.0	0.5
5	n-Hexyl isobutyrate	1151	1150	11.9 *	0.6
6	Hexyl butyrate	1191	1190	2.3	-
7	Octyl acetate	1210	1196	-	0.6
8	Hexyl isovalerate	1241	1241	9.1 *	14.2 *
9	2-Decenal	1255	1261	5.9	0.8
10	Hexyl valerate	1298	1290	3.1	2.6
11	*trans*-pinocarvyl acetate	1298	1298	15.6 *	12.4 *
12	n-Octylisobutyrate	1348	1350	-	6.3
13	2-Decenoic acid, methyl ester	-	1352	-	-
14	α-Copaene	1376	1374	2.6	0.6
15	Hexyl hexanoate	1381	1380	2.9	6.1
16	Decyl acetate	1408	1407	2.8	0.9
17	(Z)- Caryophyllene	1407	1408	-	2.3
18	β- Cedrene	1419	1410	-	1.2
19	(E)-α- Bergamotene	1438	1411	-	0.6
20	Octyl 2-methylbutanoate	1430	1419	-	1.2
21	Octylisovalerate	1440	1438	-	-
22	α- Himachalene	1448	1449	-	1.5
23	(E)-β- Farnesene	1458	1454	0.7	2.1
24	(E)-β- Bisabolene	1509	1512	-	6.4
25	Hexyl octanoate	1580	1581	1.3	-
26	Caryophyllene oxide	1583	1584	13.2 *	10.5 *
27	(Z)- Nuciferol	1727	1728	-	1.5
28	3(10)-Caren-4-ol,acetoacetic acid ester	-	1740	3.1	-
29	Octyloctanoate	1779	1779	5.6	-
30	Hexahydrofarnesyl acetone	1845	1842	2.8	3.7
31	Z-falcarinol	2038	2035	5.5	-
	Total percentage			**91.5**	**86.9**

[a] The retention indices were given in the literature (NIST on non-polar HP-5 or DB-5 capillary columns).
[b] With respect to C5–C28 n-alkanes, the retention indices were calculated on non-polar DB-5 capillary column.
[c] The percentages were calculated by GC-FID on non-polar DB-5 capillary column.
* Major compositions in essential oils.

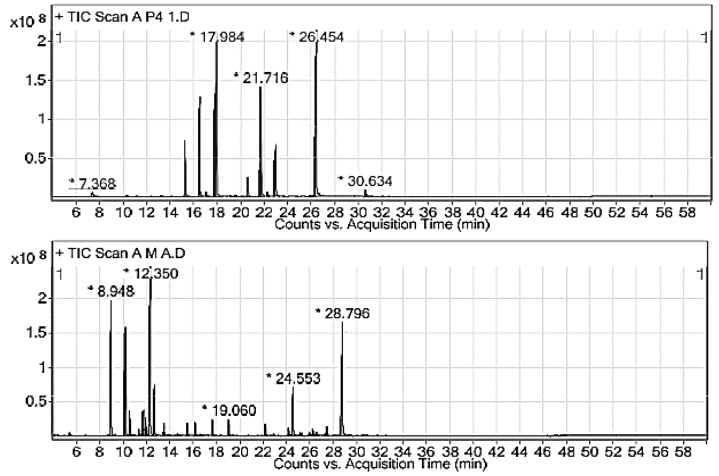
(a) (b)

Fig 1. The wild population of (***a***) *Eryngium caucasicum* Trautv. and (***b***) *Caucalis platycarpos* L. *in* Guilan province of Iran. Fig legends must be placed below each relevant Fig. All Figs along with their textboxes or tags must be prepared as a solid image with JPEG or TIF format.

Table 2. Inhibition zones of the essential oils of *E. caucasicum* and *C. platycarpus*.

Bacterial strain	*E. caucasicum*	*C. platycarpus*	Penicillin	10 µg/disk
E. coli	5-7 [a]	- [b]	-	
P. aeruginosa	7-9	5-8	12-18	
S. aureus	17-19	12-15	25-29	
B. subtilis	15-18	13-16	28-31	

[a] The inhibition zones are given as minimum and maximum inhibition zones in diameter (mm) around the disks impregnated at 1200 µg/disk doses. . [b] Not active.



(a)

(b)

What are a-1.a-2 etc.

Fig 2. Gas chromatography/mass spectrometry (GC–MS) chromatograms of (***a***) *E. caucasicum*, (***b***) *C. platycarpus* essential oils. The major compounds of *E. caucasicum* were (***a-1***) caryophyllene oxide, (***a-2***) *trans*-pinocarvyl acetate, (***a-3***) hexyl isovalerate, (***a-4***) n-hexyl isobutyrate. The major compounds of *C. platycarpus* were (***b-1***) caryophyllene oxide, (***b-2***) *(E)*-β- bisabolene, (***b-3***) hexyl isovalerate, (***b-4***) 1-hexanol.

a-1 0r a-2 were constituents of *E.caucasicum* that showed with (a word) because of that we showed all the constituents belonging to eryngium with a-1 a-2 etc. and for *C.platycarpos* we used (b word) and for its constituents we used b-1 b-2 etc

Table 3. MIC and MBC of the essential oils [a] of *E. caucasicum*and *C. platycarpus.*

Bacterial strain	*E. caucasicum*		*C. platycarpus*	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
E. coli	850	>1000	600	>1000
P. aeruginosa	>1000	>1000	1000	>1000
S. aureus	500	700	500	800
B. subtilis	400	1000	600	1000

[a] Essential oils were dissolved in 1% Tween 20 and then added in a liquid media with the test bacteria.

anhydrous sodium sulfate (Na_2SO_4) and stored at 4 °C in the dark until tested and analyzed using the gas chromatography (GC) and the gas chromatography-mass spectrometry (GC–MS).

Analysis of the essential oils

The essential oils were analyzed with Shimadzu GC-MS-QP5050A equipped with the fused methyl silicon DB-5 column (60 m × 0.25 mm i.d. and 0.25 µm film thickness). The carrier gas was helium at a flow rate of 1.3 mL/min. The injector temperature was 270 °C and the split ratio was adjusted at 1:33. The column temperature was kept at 50 °C for 3 min which increased to 300 °C at a rate of 5 °C/min and finally kept at 300 °C for 5 min. The injection volume was 1 µL. For the GC/MS detection, an electron impact (EI) quadrupolar system was used with ionization energy of 70 eV. Other GC/MS conditions include an ion source temperature of 200 °C, a quadrupolar temperature of 100 °C, a solvent delay of 2 min, EM voltage of 3,000 volts, resolution of 2,000 amu/s, and an amu scan range of 30–600 amu. The essential oil components were identified by comparing their relative retention times and mass spectra with Kovats indices, standard compounds and computer matching with the NIST NBS54K Library (Ashour et al., 2009; Senatore et al., 2000; Stahl-Biskup and Wichtmann, 1991). For quantization (area %), GC analyses were performed on an Agilent 6890 series apparatus fitted with an FID detector. The FID detector's temperature was 300 ºC. To obtain the same elution order as with GC–MS, a simultaneous auto-

injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Antimicrobial assay (Disk Diffusion Assay)

The bacterial cultures of *S. aureus* (ATCC 6538), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 9027), and *B. subtilis* (ATCC 6051) are standard strains routinely used for the evaluation of antibacterial properties. Micro-organisms that were grown on nutrient agar. *E. coli* and *P. aeruginosa* were incubated at 37 °C and *S. aureus* and *B. subtilis* were incubated at 30 °C. Whatman's No. 1 Filter paper disc (5 mm diameter) containing 600, 900, and 1,200 µg/disk doses of the essential oils were applied to the surface of the agar plates previously seeded by spreading 0.2 mL of a bacterial overnight culture (Pasdaran et al., 2012). Only plates with the 1200 µg/ paper disc after being incubated overnight at the appropriate temperature showed inhibition zones. These zones were measured in millimeters (Table 2).). Penicillin (10µg/disk) was used as the standard control treatment.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the essential oils was determined using a broth dilution method (Baydar et al., 2004; Kim et al., 1995). For each duplicate 50-mL Erlenmeyer flasks containing 19.6 mL of the sterile Mueller–Hinton broth (MHB), a 200 µL aliquot of the bacterial suspension was added at $l0^5$ CFU/mL and the essential oils were prepared to give a final concentration of 100, 250, 500, or 1000 µg/mL. The flasks were then incubated in a shaker incubator at 35 °C. An aliquot (0.1 mL) of each flask was taken at 0, 6, 12, 18, 24, and 36 h for measuring the turbidity at 540 nm. The lowest concentration at which no growth occurred in either flask was considered as the minimum inhibitory concentration (MIC). After determining the MICs, 1–2 µL of the bacterial suspensions were sub-cultured into 100 µL of MHB and incubated for 24 h at 35 °C. The lowest concentration of the subculture without any visible growth was recorded as the minimum bactericidal concentration (MBC). The experiments were conducted in quadruplicate for each essential oil at each test concentration (Table 3).

Data analysis

All experiments were conducted in triplicate and tests were duplicated. Statistical analyses of the data were done using $SPSS_{11}$. The results showed a significant difference at p <0.05 level.

Conclusion

Based on the obtained findings, it can be concluded that some major components in the essential oils of *E. caucasicum* and *C. platycarpus,* such as *trans*-pinocarvyl acetate and caryophyllene oxide, can play a key role in the antibacterial activities of the essential oils of these plants. However, it is very difficult to attribute these activities to one or a few active principles in these essential oils because the minor compounds may also be effective regarding these potentials. It is known that many compounds that are present in small amounts in a mixture can act as synergistic or antagonistic elements. Therefore, future research on the purified components of these essential oils can be useful for finding more active single compounds. Based on this research, the results indicated that the local use of these plants' essential oils as an anti-putrefaction additive in food correlates with our findings on their antibacterial effects.

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