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Composition and Antibacterial Activity of *Heracleum Transcaucasicum* and *Heracleum Anisactis* Aerial Parts Essential Oil

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ABSTRACT

Purpose: Two plant essential oils (EOs), including those from *Heracleum transcaucasicum and Heracleum anisactiss* (Umbeliferae) were studied to detect the chemical constituents and evaluated for their antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli and Pseudomonas aeruginosa.* ***Methods:*** The EOs of *H. transcaucasicum and H.anisactis* (Apiacae) were obtained by hydrodistillation from aerial parts of the plants. The chemical analyses of the EOs were performed by GC/Mass spectrometry (GC/MS). Myristicin was found to be the principal constituent in both EOs. The susceptibility tests of EOs were performed by agar disc diffusion technique against Gram-positive and Gram-negative bacterial strains. ***Results:*** Eight components comprising 99.97% of the total essential oil of *H. transcaucasicum* and a total of three compounds accounting for 98.5% of the total oil composition of aerial parts of *H. anisactis* were identified, of which myristicin was the main compound in both EOs. The EOs of *H. transcaucasicum and H. anisactis* showed weak antibacterial property against Gram-positive strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* with no measurable effect on *Escherichia coli and Pseudomonas aeruginosa.* ***Conclusion:*** Our GC-MS study revealed myristicin to be the major constituent of *H. transcaucasicum and H.anisactis* aerial parts. In spite of all the information available on the antibacterial properties of plants essential oils, we were not able to find significant antibacterial activity for both EOs.

Introduction

The genus *Heracleum* is one of the largest genera of Umbellifereae (Apiaceae) and there are almost 125 Heracleum species in the world. This genus is widely distributed in Asia[1] and represented by 10 species in the flora of Iran.[2] Umbelliferous plants have been used not only as food-stuff and spice, but also as traditional folk medicine. In Iran *H. persicum* (Golpar) fruits are used commonly as spices, while the fruits and stems are used as a flavoring agent for making pickles. The fruits and leaves of this genus are also used as antiseptic, carminative, digestive and analgesic in Iranian traditional medicine.[3] Consequently, phytochemical analysis in much Heracleum species has been focused on EOs of their various parts[4] and a diversity of compounds have been isolated so far. Aliphatic esters such as hexyl butylate, octyl acetate, hexyl 2-methylbutanoate, hexyl hexanoate, octyl-2-methyl butanoate and monoterpenes including limonene and γ-terpinene have been reported as the major components of *H. persicum* fruits essential oil,[5] while, (E)-anethole, octyl acetate, n- octanol and hexyl butanoate were reported in leaves of *H. persicum.*[6]

As evident from the literature, the essential oils of Heracleum species have been extensively studied for their antibacterial,[7,8] antifungal,[9,10] anti-dermatophytic[11] and insecticidal activity.[12]

It has been found that medicinal plants, spice and EOs bearing plants possess a diversity of pharmacological activities. In particular, for EOs inhibition a wide range of microorganisms have been described.[13]

To the best of our knowledge, according to the literature there is no report on the essential oil composition and antibacterial activity of *Heracleum transcaucasicum* and *Heracleum anisactis* (from Azarbyjan) aerial parts. In this study we report the

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essential oil constituents and antibacterial activities of the plants.

Materials and Methods

Plant material

Aerial parts of *H. transcaucasicum and H. anisactis* (in full fruiting stage) were collected from Varzeghan in East Azarbaijan province, Iran, in June 2011. A voucher specimen of the plants has been deposited at the Herbarium of the Faculty of Pharmacy, Tabriz University of Medical science, Iran.

Essential oil extraction

Air-dried plants material of aerial parts of *H. transcaucasicum and H. anisactis* were subjected to hydrodistillation using a Clevenger-type apparatus. The obtained essential oils were stored in sealed glass vials at 4-5 °C prior to analysis.

Test organism and Antibacterial assay

Two strains of Gram-negative bacteria [*Escherichia coli* ATCC (8739), *Pseudomonas aeruginosa ATCC* (9027)], and two strains of Gram-positive bacteria [*Staphylococcus epidermidis* ATCC (12228) and *Staphylococcus aureus* (ATCC 6538)] were used. The bacterial strains in lyophilized form were purchased from institute of pasture, Iran. After activating, the cultures of bacteria were maintained in their appropriate agar media at 4 °C throughout the study and used as stock cultures. A single colony from the stock plate was transferred into Mueller Hinton Broth and incubated over night at 37 °C. After incubation time the cells were harvested by centrifugation at 3000 rpm for 15 min and washed twice and re-suspended in Saline solution to provide an optical density equal to 0.5 McFarland or bacterial concentration around 10^8 CFU/ml. Then the final concentration of inocolum was adjusted to approximately 10^6CFU/ml with sterile Saline solution.

Antibacterial activity of essential oils was evaluated by the agar disc diffusion method. One hundred microliters of the suspensions were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. The essential oils were dissolved in 10% aqueous dimethylsulfoxide (DMSO) and sterilized by filtration through a 0.45 µm membrane filter. Sterilized discs (Whatman no.1, 6 mm diameter) were impregnated with 50 µL of different concentrations (1:1, 1:5, 1:10) of the respective essential oils and placed on the agar surface. A paper disc moistened with aqueous DMSO was placed on the seeded plate as a vehicle control. A standard disc containing Amikacin (30mg) was used as reference control. The plates were incubated for 30 min in refrigerator to allow the diffusion of oil, and then they were incubated at 37°C for 18 h. After the incubation period, the zone of inhibition was measured with a calliper. All experiments were performed in triplicate, and mean value was calculated.

Gas Chromatography-Mass Spectrometry (GC-MS)

Essential oils were analysed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5050A) with capillary column DB-1 (60 m, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) which was operated in EI mode at 70 eV. Injector and detector temperatures were set at 210°C and 240°C, respectively. One microliter essential oils were injected and analyzed with the column held initially at 60 °C for 2 min and then increased by 3°C/min up to 240 °C. Helium was employed as carrier gas (1.3 ml/min). The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature 270 °C; quadrupole 100 °C; Solvent delay 2 min; scan speed 2000 amu/s; scan range 30-600 amu and EV voltage 3000 volts. The relative amount of individual components of the total oil is expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds, or by mass spectra.

Identification of the compounds

The identification of compounds was based on direct comparison of the retention times and mass spectral data with those for the standards and by computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparing the fragmentation patterns of the mass spectra with those reported in the literature.[14] For quantification purpose, relative area percentages were obtained by FID without the use of correction factors.

Results

The Pale yellow EOs were obtained in the yields of 0.2% and 0.3% (V/W) on a dry weight basis respectively. EOs were analyzed by GC-FID and GC-MS and the compositions of both essential oils were identified qualitatively and quantitatively. Analysis of *H. transcaucasicum* aerial parts essential oil revealed seven components accounting for 99.95% of the essential oil (Table 1). The aerial parts of *H. anisactis* were investigated for their essential oil and three ingredients were found representing 99.98% of the essential oil (Table 2). Myristicin, as a major component, was characterized by high amounts in both EOs. It was identified as 70% and 93.5% of the essential oil composition of *H. transcaucasicum* and *H. anisactis* aerial parts, respectively.

The EOs were tested against 4 microorganisms in order to estimate their antimicrobial potentials. Both EOs were almost inactive against the tested microbial strains as compared with Amikacin.

Table 1. Chemical constituent of the essential oil from aerial parts of *H. transcaucasicum*.

No.	Compounds	Rt*	Area (%)
1	n- octanol	19.47	14.285
2	octyl acetate	30.619	7.79
3	isobutyl 2-methylpropanoate	46.23	1.29
4	myristicin	56.04	70.12
5	3,4-dimethyl-1-pentanol	62.45	1.29
6	geranyl nitrile	77.36	3.89
7	alpha.-methylpentenal	82.38	1.29
Total			**99.95%**

*Rt: Retention time

Table 2. Chemical constituent of the essential oil from aerial parts of *H. anisactis*

No.	Compounds	Rt*	Area (%)
1	3-Methylpentanol	19.24	1.61
2	Myristicin	56.04	93.54
3	5-cyano-2,2,3-rimethyl-2H-pyrrole 1-oxide	77.43	4.83
Total			**99.98%**

*Rt: Retention time

Discussion

Plant essential oils have been used for many thousands of years. It is necessary to scientifically investigate those plants which have been used in traditional medicine to improve the quality of healthcare. Some biological activities, to mention a few, such as antimicrobial, antioxidant, anti-inflammatory, antispasmodic and relaxing properties have been described for Eos.[14] Since the biological activities of medicinal plants are linked to their complex chemical components, analysis of the EOs begins to be considered an important goal for researchers in order to justify their bioactivities.

The constituents of essential oils of Heracleum species have been isolated by many researchers.[15,6,16] Characteristic constituents Heracleum species have frequently been reported as octyl acetate,n- octanol, myristicin and elemicin. Moreover, myristicin was reported as a major compound (53%) in *H. pastinacifolium*[6] and n- octanol was the main component of *H. Sphondylium.*[17] Octyl acetate was detected as a major compound (29%) followed by elemicin (23%) in *H. Rechingeri.*[18] In the present study, the volatile pattern of *H. transcaucasicum*, was characterized by myristicin (77%), n-octanol (14.2%) and octyl acetate (7.7%) and myristicin were the major components (93%) of *H. anisactis*.

Our results are somewhat similar to those of the previous reports. The presence of myristicin as a predominant compound is in accordance with many other studies but in terms of the number of components both EOs contain a small number of compounds.

Furthermore, in the present study both *H. transcaucasicum* and *H. anisactis* aerial parts EOs exhibited no activity against the selected bacterial strains.

These results are consistent with the previous study that proved myristicin was ineffective against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa.*[19] This report also showed that myristicin induced antiproliferative activity against K-562 (human chronic myelogenous leukemia), NCI-H460 (human lung tumor), and MCF-7 (human breast adenocarcinoma) cell lines.

Conclusion

In conclusion, the high amounts of myristicin in these plants EOs suggest some limitations for using these two Heracleum species as spices in diet. Furthermore, these results suggest more in-depth studies aimed at defining the safety of these oils and elucidating their anti tumor and other biological activities. The results also concluded that the presence of myristicin as a main compound provides the rationale to take into account the key role of myristicin in biological activity of both EOs.

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Conflict of Interest

The authors report no conflict of interest in this study.

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