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

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**Purpose:** Two plant essential oils (EOs), including those from *Heracleum transcaucasicum* and *Heracleum anisactis* (Umbelliferae) 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* (Apiaceae) were obtained by hydrodistillation from aerial parts of the plants. The chemical analyses of the EOs were performed by GC/MS spectrometry. 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 *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.

**Keywords:** *Heracleum transcaucasicum*; *Heracleum anisactis*; Umbelliferae; Essential oil composition; GC/MS Spectrometry; Myristicin

**Introduction**

The genus *Heracleum* is one of the largest genera of Umbelliferae (Apiaceae) and there are almost 125 *Heracleum* species in the world. This genus is widely distributed in Asia and represented by 10 species in the flora of Iran. 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. Consequently, phytochemical analysis in much *Heracleum* species has been focused on EOs of their various parts and a diversity of compounds have been isolated so far. Aliphatic esters such as hexyl butyrate, octyl acetate, hexyl 2-methylbutanoate, hexyl hexanoate, octyl-2-methyl butanoate and monoterpenes including limonene and γ-

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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 Azerbaijan 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 overnight 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 10⁸ CFU/ml. Then the final concentration of inoculum was adjusted to approximately 10⁶ CFU/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 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. 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.
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