Torbati *et al.*, **BioImpacts,** 2014, 4(2), 69-74
doi: 10.5681/bi.2014.004
http://bi.tbzmed.ac.ir/

Research Article

Chemical composition and *in vitro* antioxidant and antibacterial activity of *Heracleum transcaucasicum* and *Heracleum anisactis* roots essential oil

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Article info

Article History:
Received: 02 Sep. 2013
Revised: 18 Nov. 2013
Accepted: 24 Nov. 2013
ePublished: 29 May 2014

Keywords:
TLC-bioautography
Heracleum transcaucasicum
Heracleum anisactis
GC/MS spectrometry
Myristicin
Eugenol

Abstract

Introduction: *In vitro* antioxidant and antibacterial activity and volatile compositions of two Heracleum species (Apiaceae) including *Heracleum transcaucasicum* and *Heracleum anisactis* roots Essential Oil (EO) were investigated.
Methods: The volatile compositions of EOs were analyzed by GC/Mass spectroscopy. To detect the antioxidant activity of essential oils TLC-bioautography and DPPH radical scavenging assay by spectrophotometry was performed. Additionally, the antibacterial activity of two essential oils were studied and compared against four pathogenic bacteria by agar disc diffusion method and MIC values of the EOs were determined using the broth dilution method.
Results: Myristicin was the dominant component in both EOs. It was identified as 96.87% and 95.15% of the essential oil composition of *H. transcaucasicum* and *H. anisactis* roots, respectively. The TLC-bioautography showed antioxidant spots in both EOs and IC_{50} of *H. anisactis* and *H. transcaucasicum* EO was found to be 54 µg × ml (-1) and 77 µg × ml (-1), respectively. Regarding the antimicrobial assay, *H. anisactis EO* exhibited weak to moderate antibacterial activity against gram-positive bacteria and also *Escherichia coli*, whereas the essential oil from *H. transcaucasicum* was inactive.
Conclusion: Based on the results from this study, both tested EOs mainly consist of myristicin. Despite the presence of myristicin with known antibacterial property, the EO from *H. transcacausicum* showed no antibacterial activity. Thus it is supposed that the biological activity of plants is remarkably linked to the extracts' chemical profile and intercomponents' synergistic or antagonistic effect could play a crucial role in bioactivity of EOs and other plant extracts.

Introduction

Essential oils of various species of edible and medicinal plants have been extensively used in folk medicine, food flavoring, and perfumery, cosmetic and pharmaceutical industries. Variation in chemical composition of essential oils in particular and extracts of medicinal plants may be observed due to the origin, the environmental conditions, and the developmental stage of collected plant materials. The genus *Heracleum* is one of the largest genera of *Umbelliferae (Apiaceae)*. This genus is widely distributed in Asia[1] and represented by 10 species in the flora of Iran[2] from which *H. transcaucasicum* and *H.anisactis* are two abundant species in Azerbaijan region.[3,4] *Heracleum* species are well known as essential oil rich plants and medicinal plants as well. In Iranian folk medicine, fruits, stems and leaves of *Heracleum* species are used as spices, carminative and antiseptic.[4] The ripe fruits, leaves and young stems of *H. persicum (Golpar)* are also used as antiseptic, carminative, digestive and analgesic in the Iranian folk medicine.[3]

A great number of studies refer to the compositions of *Heracleum* species essential oils. Various studies on *Heracleum* species have reported variations in the essential oil composition according to the stage of growing and different parts of the plant[5-7] and biological activities.

The main volatile substances in the majority of EOs from *Heracleum* species are aliphatic esters such as octyl acetate and hexyl butyrate, hexyl-2-methylbutanoate and hexyl isobutyrate. (E)-anethole β-pinene, ρ-cymene, terpinolene, α-caryophylene, α-bergamotene, α-farnesene,

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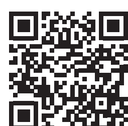

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zingiberene, spathulenol, cis-anethole, stragole, 2,5-dimethyl styrene and β-springene were reported in leaves EO.[8,9] The major constituents of *H. persicum* roots essential oil were identified as viridiflorol (23.05%), elemol (3.63%), β-maliene (3.07%), spathulenol (3.34%) and 2-tetradecanol (3.38%).[10] The main components of stem oil before flowering were (e)-anethole (47.0%), terpinolene (20.0%), [gamma]-terpinene (11.6%) and limonene (11.5%), while stem oil at the full flowering stage contained (e) -anethole (60.2%), terpinolene (11.3%) and [gamma]-terpinene (7.1%). The unripe and ripe seeds oil of *H. persicum* contained aliphatic esters, aliphatic alcohols and monoterpenes esters and monoterpenes such as hexyl butyrate, octyl acetate and hexyl isobutyrate.[11,12] The antimicrobial activity and other biological activities of an essential oil are mainly attributed to its major components, although sometimes the synergistic or antagonistic effect of one compound in minor percentage of mixture plays a key role in activity. Therefore, EOs exhibit various biological activities based on variations in their individual components.[13]
In the framework of our investigation on the chemical compositions of *H. transcaucasicum* and *H. anisactis*, we reported the composition and antibacterial activity of EOs of aerial parts.[14] In the present study, we examined the essential oils of their roots as well as their antioxidant and antimicrobial activities against four pathogenic bacteria, which have not been the subject of previous investigations.

Materials and methods

Plant material

Roots of *H. transcaucasicum* (in full fruiting stage) were collected from Varzeghan in East Azerbaijan province, Iran (38° 43, 49, N latitude, 46°, 38, 46, E longitude and 2271 m above sea level), in June 2011. *H. anisactis* roots (in full fruiting stage) were collected from Sahand mountain in East Azerbaijan province, Iran (37°, 45, 39 N latitude, 46°, 25, 25 E longitude, and 2648 m above sea level), in June 2011. Voucher specimens of the plants (Tbzmed-FPh-740 and Tbzmed-FPh-741) were deposited at the Herbarium of the Faculty of Pharmacy, Tabriz University of Medical sciences, Iran.

Essential oil extraction

Air-dried roots of *H. transcaucasicum* and *H. anisactis* were subjected to hydro-distillation using a Clevenger-type apparatus. The yields of essential oils were respectively 1.7% v/w and 1.3% in terms of dried starting material. The essential oils were preserved in sealed vials in a refrigerator prior to analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)

Essential oils were analysed on an Agilent 6890 GC-quadrupole MS system (Agilent Technologies, Palo Alto, USA) with fused capillary column HP-5 (phenyl methyl siloxane, 30 m × 0.25 mm; 0.25 µm film thickness, Agilent Technologies) and a flame ionization detector (FID) which was operated in EI mode at 70 eV. Injector was set at 210 °C. Essential oils were dissolved in n-hexane (1:4) and 1 µl of each essential oil solution was injected with a split ratio of 1:25. Analyses were performed with the column held initially at 60 °C for 2 min and then increased by 4 °C/min up to 240 °C. Helium was employed as carrier gas (0.8 ml/min). The MS operating parameters were as follows: ionization potential, 70 eV; transfer line temperature 270 °C; ion source temperature 230 °C; quadrupole 150 °C; solvent delay 2 min; scan speed 2000 amu/s; and scan range 30-600 amu. 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.[15]

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.[15] For quantification purpose, relative area percentages were obtained by FID without the use of correction factors.

Determination of *in vitro* antioxidant activity

To detect the activity of individual components of each EOs, TLC- bioautography with 1, 1-Diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich Quımica S.A., Madrid, Spain) radical as a detection reagent was carried out. The components of EOs were separated on pre-coated Silica gel aluminum plate F_{254} with 0.2 mm thickness (Merck- Germany) using Toluene-Ethylacetate in the ratio of 93:7 as the mobile phase[16] and DPPH solution (0.04% in MeOH) for detection. Then the plate was allowed to dry at room temperature. The antioxidant components were visualized by spraying the plate with DPPH reagent and were left 15 min at room temperature. The separated antioxidant spots were appeared as yellow on a purple background.[17-19] The TLC plate was observed with visible light (Fig. 1).

Fig. 1. TLC photography of essential oil of *H. anisactis* and *H. trascaucasicum* seeds colorized with 0.08% DPPH

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The free radical scavenging capacities of the EOs were measured from the bleaching of the purple-colored methanol solution of DPPH (0.008 mg%). The stock concentration 1 mg/ml of the EOs was prepared followed by dilution in order to obtain concentrations of 5×10^{-1}, 2.5×10^{-1}, 1.25×10^{-1}, 6.25×10^{-2}, 3.13×10^{-2} and 1.56×10^{-2} mg/ml. The obtained concentrations in equal volumes of 2 ml were added to 2 ml of a 0.008% of DPPH solution. After a 30 min incubation period at 25 °C, the absorbance at 517 nm was determined against a blank. Tests were carried out in triplicate where the average absorption was noted for each concentration.[20]

Test organism and antibacterial assay

Two strains of gram-negative bacteria [*Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853)], and two strains of gram-positive bacteria [*Staphylococcus epidermidis* (ATCC 12228) and *Staphylococcus aureus* (ATCC 25923)] were used. The bacterial strains in lyophilized form were purchased from Pasteur institute, 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 Ringer 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 10^6 CFU/ml with sterile saline solution.

Antibacterial activity of essential oils was firstly screened by the disc diffusion method. One hundred µl of the bacterial suspensions were spread over the plates containing Mueller-Hinton agar plus 4% polysorbate 20 using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. The bacterial suspension was spread on the surface of the plates in a way that nothing remained afloat. The essential oils were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) and sterilized by filtration through a 0.45 µm membrane filter. Sterilized discs (Whatman no.1, 6 mm diameters) were impregnated with 50 µL of different concentrations (1:1, 1:5, 1:10) of the respective EOs in two steps; firstly the dry disks were placed on the surface of the agar plate. Then 30 µL of each sample was poured directly on the center of the disk using sampler and after 15 min, the remaining 20 µL was poured on the same disk. Paper disc moistened with 10% aqueous DMSO in the same way as test samples was placed on the seeded plate as a vehicle control. A standard disc containing Amikacin (30 mg) was used as reference control. The plates were incubated for 30 min at refrigerator and then they were incubated at 37 °C for 18 h. The refrigeration was needed for slowing down the growth of the bacteria and consequently for giving the oil enough time to diffuse before bacterial growth. After the incubation period, the zone of inhibition was measured with a calliper. All experiments were performed in triplicate, and mean values were calculated.

Determinations of minimum inhibitory concentration

The agar dilution method was employed to determine minimum inhibitory concentration (MIC) of the EOs according to CLSI guidelines. The prepared inoculum was added to Muller Hinton broth plus 4% polysorbate 20 medium to reach the final concentration of 106 CFU/ml. One milliliter of the inoculated medium was added to 8 sterile tubes including seven tests containing serially diluted EOs in 10% and a control tube with 10% aqueous DMSO without essential oil respectively. The tubes were incubated at 37 °C for 24 h. After that, from the content of each tube streak culture was performed onto Mueller Hinton Agar plates. The first concentration with no sign of bacterial growth on plates was considered as MIC. All experiments were performed in three separate occasions.

Results

The Pale yellow EOs were obtained in the yields of 1.7% and 1.3% (v/w) on a dry weight basis from *H. transcaucasicum* and *H. anisactis roots*, respectively. EOs were analyzed by GC and GC-MS and the chemical constituents of both essential oils were identified qualitatively and quantitatively. Eleven components (which accounted for 99.18% of total constituents in oil) were identified as constituents of *H. transcaucasicum* roots essential oil. The major components of this essential oil were myristicin (which accounted for 96.87% of total constituents in oil), followed by apiol (1.87%) and eugenol (0.12%). In a smaller percent, Eugenol methyl ether (0.05%) and Cis-isomyristicin (0.05%) were identified as constituents of the oil (Table 1). Analysis of *H. anisactis* roots essential oil revealed 17 components accounting for 96.89% of the total essential oil composition. The main component was found to be myristicin (95.15%) followed by isoelemicin (0.8%), nerolidol (0.44%), osthole (0.13%) and eugenol (0.08%) (Table 2). Among components identified in EOs, eugenol was not reported, previously. Moreover, myristicin, an alkenylbenzene derivative, prevalent in

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

No.	Compounds	Rt	Area (%)
1	Octyl acetate	14.16	0.02
2	Eugenol	18.99	0.12
3	β- Elemene	20.06	0.01
4	Eugenol methyl ether	20.48	0.05
5	γ- Elemene	21.34	0.03
6	β- Cubebene	22.84	0.02
7	Bicyclogermacrene	23.30	0.03
8	β- Bisabolene	23.62	0.01
9	Myristicin	25.49	96.87
10	Apiol	28.98	1.87
11	Cis-isomyristicin	29.95	0.05
Total			**99.18%**

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

No.	Compounds	Rt	Area (%)
1	Hexyl 2-ethylacetate	14.16	0.07
2	Hexyl butanoate	14.6	0.01
3	Decenal	15.89	0.01
4	Eugenol	19.03	0.08
5	α-Copaene	19.55	0.01
6	β- Elemene	20.06	0.04
7	Eugenol methyl ether	20.50	0.05
8	caryophyllene	20.95	0.02
9	β- Selinene	21.99	0.01
10	2- Tridecanone	23.27	0.03
11	α- Muurolene	23.41	0.03
12	β- Bisabolene	23.65	0.03
13	Myristicin	25.44	95.15
14	Cis-isoelemicin	26.35	0.82
15	nerolidol	26.35	0.44
16	Isomyristicin	28.18	0.09
17	Osthole	39.99	0.13
Total			**96.89%**

both of the oils was evident as a major component, while most components were only found as a trace (≤1%).
According to the result of TLC-bioautography both EOs showed three distinctive yellowish antioxidant spots with RF values of 0.35, 0.6, 0.85 and 0.9, although antioxidant spots of *H. anisactis* were almost larger than those of *H. transcaucasicum* EO (Fig. 1).
DPPH radical scavenging test on the two species of *Heracleum* with ultraviolet-visible spectrophotometry revealed that the concentration of *H. anisactis* after scavenging 50% of DPPH radicals (IC_{50}) was 54 μg × ml(-1) whereas the figure for *H. transcaucasicum* was 77 μg × ml(-1).

Assessment of antimicrobial activity

Firstly the EOs of the plants were screened for antimicrobial activity against the selected strains using the agar disc diffusion method. The results are presented in Table 3. According to the results, the EOs of *H. anisactis* exhibited weak to moderate antimicrobial activity against the gram positive strains and also *E. coli* as our gram negative indicator. As it is shown in Table 3, the maximum inhibition zone diameter was obtained from *H. anisactis* roots when tested against *S. epidermidis* [13 ± 2.2 mm,1/5(v/v)] (Fig. 2). *S. aureus* was slightly less susceptible than *S. epidermidis* with the inhibition zone diameter of [12± 0.8 mm, 1/5 (v/v)] followed by [11 ± 1.3mm, 1/1 (v/v)] for *E. coli* (Fig. 3 and Fig. 4). No sign of grown inhibition was observed in the case of all the bacterial strains when examined with the EO of *H. transcacausicum*.
MIC values of the essential oil of *H. anisactis* were determined using the broth dilution method and the results are depicted in Table 3 as well. As can be seen in Table 3, the lower MIC values were related to *S. aureus* and *S. epidermidis* (1/5 v/v). The highest MIC value was obtained from testing of the oil of *H. anisactis* against *E. coli*.

Fig. 2. Photograph of *Staphylococcus epidermidis* culture plate

Fig. 3. Photograph of *Staphylococcus aureus* culture plate

Table 3. Inhibition Zone Diameters and MIC values for amikacin and *H. anisactis* roots essential oil against the selected strains

Microorganism	Inhibition Zone Diameter ± SD (mm) Amikacin	Inhibition Zone Diameter ± SD (mm) *H. anisactis*	MIC (V/V in 10% DMSO)
Staphylococcus aureus	16±0.4	12 ± 0.8	1.5
Staphylococcus epidermidis	23±0.5	13 ± 1.2	1.5
Escherichia coli	18±0.6	11 ± 1.3	1.1
Pseudomonas aeruginosa	21±0.5	-	

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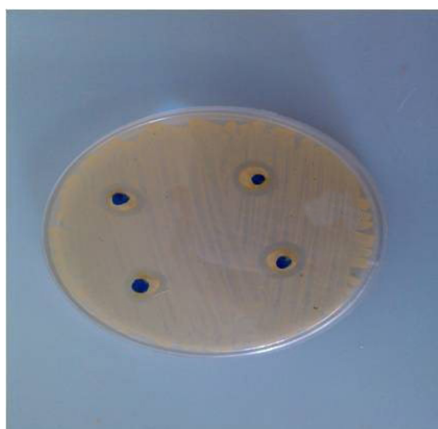

Fig. 4. Photograph of *Escherichia coli* culture plate

Discussion

Essential oil bearing plants have been known for a very long time and owing to the variety of their aromatic components they have been regarded for different medical purposes. They have also great importance as flavoring and antiseptic agents in food, perfumery and pharmaceutical industries. Plants belonging to the genus *Heracleum* are aromatic and based on their ethnomedicinal uses as antiseptic all over the world, the essential oil compositions of different *Heracleum* species have repeatedly been studied. Major constituents reported in *Heracleum* species are aliphatic esters (e.g. octyl acetate, octyl butanoate, hexyl butanoate, octyl hexanoate), monoterpene hydrocarbons (e.g. ρ-cymene, γ–terpinene, α- and β–pinene, limonene), oxygenated monoterpenes (e.g. iso-bornyl acetate, linalool, n-octanol, terpineneol), sesquiterpene (caryophyllene oxide), alkenylbenzenes (e.g. myristicin, apiol) and coumarins (e.g. osthole). The presence of myristicin and (E)- anethole in stems and flowers as major components has repeatedly been reported.[3,21,22] In our previous study, myriticin was found to be the major component of the EOs from aerial parts of *H. anisactis* and *H. transcaucasicum* (70.12% and 93.54%, respectively). Similar to our results, myristicin was reported to be the main compound (53%) in the essential oil of *H. pastinacifolium* aerial parts.[3] However most of the reports on EOs from different parts of *Heracleum* species indicated that hydrocarbon esters, especially octyl acetate, was the major constituent.[3,23] The differences between our results and others could be justified by the fact that the composition profile of plants is correlated to their growing geographic area, growth stage and collection season.

Assessment of antioxidant activity of EOs indicated that volatile oil from two species of *Heracleum* has almost the same number of antioxidant active spots in TLC-bioautography, while the antioxidant spots of *H. anisactis* were larger than those of *H. transcaucasicum*. The antioxidant activity determination results of EOs with UV-Visible spectroscopy were consistent with the result of TLC-bioautography, showing that EO from *H. anisactis* roots is more active antioxidant than EO from *H. transcaucasicum*. This could be attributed to the presence of a variety of antioxidant components and concentration of individual antioxidant components of EOs as well.

The findings of the study on antibacterial activity of EOs indicated that the EO from *H. anisactis* roots showed moderate activity, whereas the *H. transcaucasicum* EO showed no activity. The essential oil of *H. anisactis* was found to be more active against gram positive bacteria. *S. epidermidis* was the most sensitive strain with the strongest inhibition zone of 13 ± 1.2 mm. This finding is in line with previous studies in that they indicated the higher sensitivity of the gram positive bacteria to the natural herbal extracts in comparison with their gram negative counterparts. It can be justified with the intrinsic resistance of gram negative bacteria with lipopolysaccharide outer layer.[24] These results also could be accounted for the synergism or antagonism effect of various components of EOs.

Conclusion

To our knowledge, this is the first report on the EO from *H. anisactis* and *H. transcaucasicum* roots. The present results demonstrate high antioxidant activity for both EOs and moderate antibacterial activity only for *H. anisactis.* Various compounds were detected in both EOs, from which myrisitin, anethole, elemicin, osthole, eugenol, nerolidol and hydrocarbon esters could be predominant contributors to the free radical scavenging and antibacterial activity of the EOs. Considering all results, it is supposed that particular bioactive functional properties of plants are highly affected by their chemical contents profile.

Acknowledgments

The authors would like to thank Drug Applied Research Center of Tabriz University of Medical Sciences for recording the mass spectra. Financial support of this work by the Research Vice-Chancellor of Tabriz University of Medical Sciences is faithfully acknowledged. This article was written based on a data set of Ph.D. thesis, registered in Tabriz University of Medical Sciences (5/52/5691).

Ethical issues

Not applicable in this study.

Competing interests

Authors declare no competing interests.

References

1. Pimenov MG, Leonov MV. The Asian Umbelliferae Biodiversity Database (ASIUM) with Particular Reference to South-West Asian Taxa. *Turkish Journal of Botany* **2004**;28:139-145.
2. Mozaffarian VA. Dictionary of Iranian Plant Names [In Persian]. 6th edition. Tehran: Farhang Moaser Press; 2010. p .272.
3. Firuzi O, Asadollahi M, Gholami M, Javidnia K. Composition and biological activities of essential oils from four Heracleum species. *Food Chem* **2010**;122:117-122
4. Amin G. Popular medicinal plants of Iran [in Persian].

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Tehran: Tehran University of Medical Sciences Press; 2008.
5. Asgarpanah J, Dadashzadeh Mehrabani G, Ahmadi M, Ranjbar R, Safi-Aldin Ardebily M. Chemistry, pharmacology and medicinal properties of Heracleum persicum Desf. Ex Fischer: A review. *Journal of Medicinal Plants Research* **2012**;6:1813-1820.
6. Kuljanabhagavad T, Sriubolmas N, Ruangrungsi N. Chemical composition and antimicrobial activity of the essential oil from Heracleum siamicum. *J Health Res* **2010**;24:55-60.
7. Mahboubi M, Iranian medicinal plants as antimicrobial agents. *Journal of Microbiology, Biotechnology and Food Sciences* **2013**;2:2388-2405.
8. Mojab F, Rustaiyan A, Jasbi AR. Essential oils of Heracleum persicum Desf. Fiscer leaves, ujm. *DARU* **2002**;10:6-8.
9. Guleria S, Saini R, Jaitak V, Kaul VK, Lal B, Rahi P, *et al.* Composition and antimicrobial activity of the essential oil of Heracleum thomsonii (Clarke) from the cold desert of the western Himalayas. *Nat Prod Res* **2011**; 25:1250–1260.
10. Mojab F, Nikavar B. Composition of the Essential Oil of the Root of Heracleum persicum from Iran. *Iran J Pharm Res* **2003**;2:245-247.
11. Sefidkon F, Dabiri M, Mohammad N. Analysis of the oil of Heracleum persicum L. (Leaves and Flowers). *Journal of Essenial oil Research* **2002**;14:295-297.
12. Sefidkon F, Dabiri M, Mohammad N. Analysis of the Oil of Heracleum persicum L. (Seeds and Stems). *Journal of Essenial oil Research* **2004**;16:296-298.
13. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol* **2004**;94:223–253.
14. Torbati M, Nazemiyeh H, Lotfipour F, Asnaashari S, Nemati M, Fathiazad F. Composition and Antibacterial Activity of Heracleum transcaucasicum and Heracleum anisactis Aerial Parts Essential Oil. *Adv Pharm Bull* **2013**;3:415-418.
15. Adams RP. Identification of Essential oil Components by Gas Chromatography/ Quadrupole Mass Spectroscopy. Carol Stream, IL: Allured Pub Corp.; 2004.
16. Wagner H, Bladt S. Plant drug analysis. 2nd edition. Springer; 1996. p.174.
17. El-Baroty GS, Abd El-Baky HH, Farag RS, Saleh MA. Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. *African Journal of Biochemistry Research* **2010**;4:167-174.
18. Rajauria G, Abu-Ghannam N. Isolation and Partial Characterization of Bioactive Fucoxanthin from Himanthalia elongata Brown Seaweed: A TLC-Based Approach. *International Journalof Analytical Chemistry* **2013**; 2013:1-6.
19. Jaime L, Mendiola JA, Herrero M. Separation and characterization of antioxidants from Spirulina platensis microalga combining pressurized liquid extraction, TLC, and HPLC-DAD. *J Sep Sci* **2005**;28:2111–2119.
20. Nahar L, Russell WR, Middleton M, Shoeb M, Sarker SD. Antioxidant phenylacetic acid derivatives from the seeds of Ilex aquifolium. *Acta Pharmaceutica* **2005**;55;187-193
21. Chu SS, Cao J, Liu QZ, Du SS, Deng ZW, Liu ZL. Chemical composition and insecticidal activity of Heracleum moellendorffii Hance essential oil. *Chemija* **2012**;23:108-112.
22. Scheffer JJC, Hiltunen R, Aynehchi Y, von Schantz M, Svendsen AB. Composition of essential oil of Heracleum persicumfruits. *Planta Medica* **1984**;50:56–60.
23. Kuljanabhagavad T, Sriubolmas N, Ruangrungsi N. Chemical composition and antimicrobial activity of the essential oil from heracleum siamicum. *Journal of Health Research* **2010**;24:55-60.
24. Lotfipour F, Nazemiyeh H, Fathi-Azad F, Garaei N, Arami S, Talat S, *et al.* Evaluation of antibacterial activity of some medicinal plants from north-west of Iran. *Iranian Journal of Basic Medical Science* **2008**;11:80-85.

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