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Volatile and Non-volatile Phytochemicals from Roots and Leaves of *Heracleum lasiopetalum* and their Radical Scavenging Ability

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Abstract: *Heracleum lasiopetalum* belongs to the Apiaceae family and is native to Iran. The fruits and seeds of the herb are used as spices and food additives. The genus has significant pharmacological activities. In order to study the phytochemicals of *H. lasiopetalum*, aerial parts of the plant were conducted to a separation process. Different chromatographic techniques were employed for the purification of its natural products. The chemical structure of the isolated metabolites was determined using spectroscopic methods such as NMR and IR as well as elemental analysis. Moreover, the essential oil composition of roots and aerial parts of the herb were characterized using GC-MS analysis. The purified compounds were elucidated as beta-sitosterol (plant steroid) and suberosin (coumarin). Identification of essential oil composition showed that 17 and 12 volatile compounds were present in the aerial parts and roots of the herb, respectively. Germacrene D, falcarinol, farnesol, and octanal were found as the major components. Findings showed that *H. lasiopetalum* contains important natural products such as steroids, coumarins, monoterpenoids, and sesquiterpenoids. According to its bioactive products, this herb could be considered for more applications in the cosmetics and pharmaceutical industries.

Keywords: *Heracleum*; hogweed; coumarin; steroids; essential oil.

1. Introduction

The genus *Heracleum* (Apiaceae) comprises more than 120 species in the world [1, 2]. Different species of this genus are distributed all around the world, especially in the Northern Hemisphere. Ten species of *Heracleum* members are representative in Iran, and three of them (*H. rechingeri*, *H. gorganicum*, *H. anisactis*) are endemic to the country [3].

Different parts of *Heracleum* members have a lot of uses in folk medicine. They are used for epilepsy, respiratory infections, skin disease, infected wound, gastric ulcers, diarrhea, and dyspepsia [4-6]. Some parts of *Heracleum* species such as seeds and leaves are used as a vegetable, flavoring agent, additive, and spice in many areas of the world [7, 8]. Modern scientific studies on *Heracleum* species showed a wide range of biological and

pharmacological activities. Essential oils of these plants showed anticonvulsant, analgesic, anti-inflammatory, and antifungal effects [9, 10]. Furthermore, significant pharmacological effects such as antioxidant, antibacterial, antiviral, immunostimulant, insecticidal, cytotoxic, gastroprotective, antifolliculogenesis, infertility, and hepatoprotective activity have been reported for extracts of this genus [11-16]. In total, more than 100 natural compounds have been isolated from various *Heracleum* species [1]. Aliphatic esters and monoterpenes are major compounds in the EOs of the genus [17]. The major isolated non-volatile compounds from *Heracleum* species are coumarins, anthraquinones, stilbene derivatives, and flavonoids [1, 18, 19]. Also, the most abundant compounds in this genus are coumarins such as bergapten, sphondin, xanthotoxin, pimpinellin, angelicin, candinoside A-D, imperatorin, heraclenin, 8-geranyloxypsoralen, and canditririn C-E [1].

Heracleum lasiopetalum (synonym: *Tetrataenium lasiopetalum*) is distributed in Turkey, Iraq, and Iran. In Iranian folk medicine, *H. lasiopetalum* is used as an antiseptic, spice, carminative, digestive, flavoring agent, and food additive. Several studies reported the antioxidant and antibacterial activity of this species. *H. lasiopetalum* Persian name is Golpar or Kersun, and its local name is Kashma in Kurdish regions, and its Turkish name is Baldirqan [20].

In the present study, *H. lasiopetalum* was subjected to phytochemical investigation. For this, EOs of the aerial parts and roots of the herb were isolated and characterized. Moreover, its dichloromethane extract was conducted to chromatographic separation process for purification of bioactive metabolites. Finally, isolated EOs, together with purified compounds, were evaluated for their radical scavenging activity.

2. Materials and Methods

2.1. Chemicals.

Column chromatography and preparative thin-layer chromatography experiments were performed using silica gel purchased from Merck (Germany). Solvents were obtained from DaeJung (South Korea) and Carlo Erba (Italy).

2.2. Plant material.

The aerial parts and roots of *Heracleum lasiopetalum* (3.5 kg) were collected in June 2016 from Kanikhoda Mountains, Piranshahr, West Azerbaijan province, Iran. It was identified taxonomically in the herbarium of faculty of pharmacy (UPSH), Urmia University of Medical Sciences, Urmia, Iran. Also, a voucher specimen (UPSH-347) was deposited for the collected plant sample.

2.3. Preparation of extracts.

Dried aerial parts of *H. lasiopetalum* were powdered and extracted by the maceration method. For this, 1000 g of powdered material was extracted with *n*-hexane, dichloromethane, and methanol sequentially on a magnetic shaker for 72 h at room temperature. After filtration, the solvents were evaporated under reduced pressure by a rotary vacuum evaporator at 40˚C to produce crude extracts.

2.4. Purification of compounds.

Dichloromethane extract (76 g) was chromatographed on a silica gel column (silica gel 0.063-0.200 mm, 600 g) and eluted with *n*-hexane-ethyl acetate (100:0 to 0:100), followed by increasing concentrations of methanol (up to 10%). The volume of each fraction was 200 mL, and 182 fractions were collected in total. Finally, on the basis of thin-layer chromatography (TLC) analysis, similar fractions were combined, and 20 fractions (F1-20) were obtained. TLC analysis of F6 (2.1 g) and F17 (0.6 g) showed clear spots under UV light and were selected for preparative thin-layer chromatography (PTLC) analysis. F6 and F17 were individually loaded on handmade PTLC sheets (20 mg on each 20×20 cm plates, 5 plates were used for each fraction), and observed fluorescent lines under UV light were scraped from the plates. Crushed lines were extracted by methanol and ethyl acetate. This procedure led to the purification of two compounds 1 (8 mg) and 2 (12 mg), from F6 and F17, respectively. The yield% for compound 1 was calculated 0.22% w/w and for compound 2 was 0.09% w/w in dichloromethane extract.

2.5. NMR experiments.

NMR experiments were carried out on Bruker Avance 400 MHz spectrometers (Bruker, Rheinstatten, Germany), operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C with TMS as inner standard. $CDCl_3$ was applied as the deuterated solvent.

2.6. FT-IR analysis.

IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer (Japan) by KBr pellets.

2.7. Melting point.

Melting points were determined in open glass capillaries by an Electrothermal melting point apparatus.

2.8. Elemental analysis.

Elemental analysis of C, H, and N atoms was followed out through the Costech elemental analyzer**.**

2.9. Isolation of essential oils.

Air-dried aerial parts (100 g) and roots (50 g) of the plant were crushed and hydrodistilled separately for 3 h by a Clevenger-type apparatus in one step, conforming to the method of the European Pharmacopoeia. The obtained EOs were dried over anhydrous Na_2SO_4 and stored in sealed vials at 4˚C until analysis [21].

2.10. GC-MS analysis.

The EOs were analyzed using a Thermoquest-Finnigan Trace MS instrument equipped with a DB-5 fused silica cap. Column (60 m×0.25 mm i.d., film thickness 0.25 µm). The oven temperature was kept at 60˚C initially, then raised at the rate of 5°/min until 250° and held for 10 min. The transfer line temperature was 250˚C. Helium was used as carrier gas at a flow rate of 1 mL/min with a split ratio of 1/50. Injector temperature was set at 250 °C. The quadrupole

mass spectrometer was used with a scan range between 35-465 amu. Measurements were performed with an ionizing voltage of 70 eV and an ionization current of 150 µA [22].

2.11. Identification of volatile compounds.

Identification of EOs components was accomplished based on a comparison of their spectra with those of the internal reference mass spectra library (NIST, Wiley, and Adams) or of authentic compounds [23]. Retention indices were calculated and compared with those of literature.

2.12. Antioxidant activity assessment.

2,2-Diphenyl-1-picrylhydrazyl (DPPH, molecular formula $C_{18}H_{12}N_5O_6$) was utilized for the investigation of the radical scavenging ability of isolated compounds and also EOs (Fluka Chemie AG, Bucks). Rutin was used as the standard drug. Different concentrations of each sample (dissolved in methanol) separately were provided (7.8-500 µg/mL). Eight mg DPPH was dissolved in methanol to provide a concentration of 80 µg/mL. One mL DPPH solution was mixed with one mL diluted sample solution and incubated for 30 min at room temperature for any reaction to occur. After incubation, UV absorbance of each compound was recorded at 517 nm. The experiments were performed in triplicates, and the mean absorption ± SD was noted for each sample. The same procedure was done by rutin as a positive control [24].

3. Results and Discussion

3.1. Structure elucidation of isolated compounds.

Chromatographic processes, together with the recrystallization technique, were employed to purify two natural compounds from *H. lasiopetalum*. Compound 1 was obtained as colorless needle crystals. According to the elemental analysis, compound 1 has an empirical formula of $C_{29}H_{50}O$. That was in accordance with plant steroids. The ^{1}HNMR and ^{13}CNMR results for this compound showed the presence of an olefinic bond (120.7 and 139.7 ppm) and characteristic methyl groups related to steroids (Pentacyclic ursane and oleane triterpenoids generally contain seven methyl groups, but steroids like beta-sitosterol contain six methyl groups of which four have appeared as doublets in ^{1}HNMR spectrum). FT-IR signals revealed the presence of –OH group in the structure. The comparison of obtained results with those of literature [25], showed the beta-sitosterol structure for compound 1 (Figure 1). Melting point results (138.5-140˚C) were also confirmed by the reported data in the literature [26]. This is the first report on the purification of β-sitosterol from *H. lasiopetalum*. *β*-Sitosterol is a bioactive steroid found in several plants. Different pharmacological properties have been reported for this compound, such as anticancer, anti-inflammatory, anticholinesterase, and hypolipidemic effects [25]. It was isolated previously from *H. sphondylium*, *H. pyrenaicum*, and *H. canescens* [27].

Compound 2 was obtained as yellowish crystals. Elemental analysis of compound 2 showed the $C_{15}H_{16}O_3$ empirical formula for this compound. This formula confirmed the presence of a coumarin backbone. Compound 2 showed aromatic signals and a clear methoxy group in both ^{13}CNMR (54.8 ppm) and ^{1}HNMR (3.9 ppm) spectra. Moreover, the presence of C-O, C=C, and C=O functional groups in FT-IR spectrum was observed. A pattern of coumarin

structure was clear from the spectroscopic data. Finally, compound 2 was elucidated as 7-methoxy-6-(3-methylbut-2-enyl)chromen-2-one. This compound is known as suberosin (Figure 2), a simple natural coumarin reported from the Apiaceae family. The recorded melting point (86-89˚C) was also in accordance with previously published data [28]. Suberosin has not been reported from *H. lasiopetalum* and other *Heracleum* species before. Some biological activities such as insecticidal, antibacterial, anticoagulant, and antifungal properties have been reported for this coumarin compound.

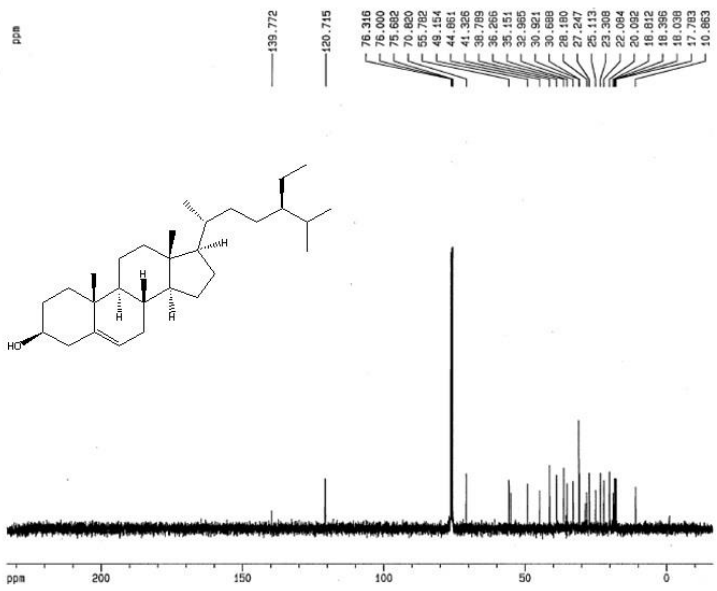

Figure 1. ^{13}CNMR of β-sitosterol at 100 MHz in $CDCl_3$ and its chemical structure.

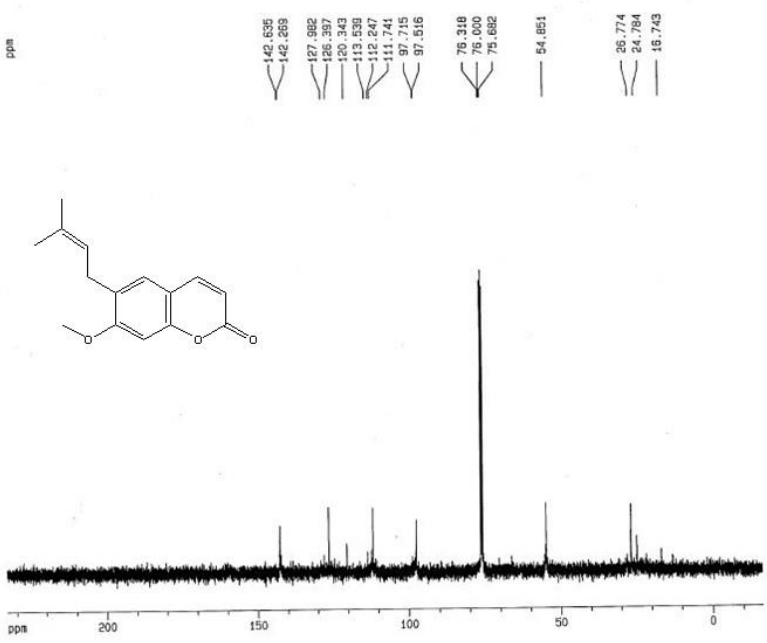

Figure 2. ^{13}CNMR of suberosin at 100 MHz in $CDCl_3$ and its chemical structure.

3.2. Essential oil composition.

Constituents of aerial parts and roots EOs of *H. lasiopetalum*, their retention indices, and percentages are depicted in Tables 1 and 2, respectively. The extraction yields of the EOs were 0.4% and 0.3% v/w for the aerial parts and roots, respectively. The EOs were subjected

to GC-MS analysis in order to determine their chemical composition. Due to the analysis of the aerial parts EO, 17 components were identified, making up 88.43% of the total composition. This EO was mainly represented by germacrene D (30.2%) and farnesol (20.5%). Generally, *Heracleum* species contain alkyl esters as main components of their EOs [1]. There is just one study that reported germacrene D (a hydrocarbon sesquiterpenoid) as a predominant compound in *H. candicans* [29], which has antimicrobial and insecticidal properties. Moreover, there is no study reporting farnesol as a major compound in the EOs of the genus *Heracleum*. Farnesol is acyclic sesquiterpene alcohol and is used in cosmeceutical products due to its flavor and antioxidant effects. The literature review showed that EO of aerial parts of *H. lasiopetalum* (*T. lasiopetalum*) from Lorestan province contains germacrene D, 2-ethyl hexyl acetate, α-zingiberene, and β-bisabolene as the most abundant volatiles [30]. In that study, similar to the present work, germacrene D is the dominant compound in aerial parts EO.

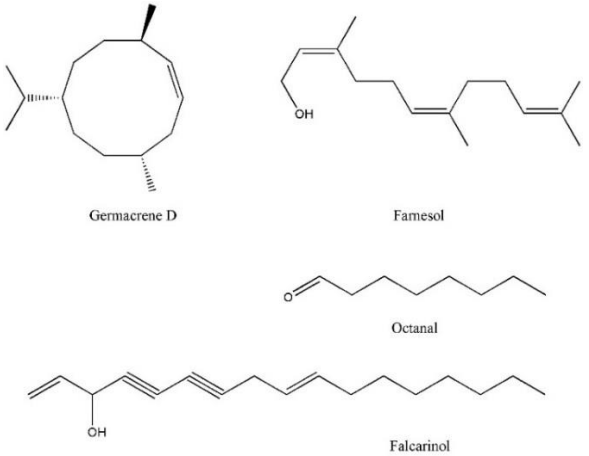

Figure 3. Major volatile components identified in *H. lasiopetalum.*

Table 1. Chemical composition of *H. lasiopetalum* aerial parts essential oil.

No.	Compound	Class	R.T. (Min)	RI-C	RI-L	Percentage (%)
1	1-Octanol	OT	7.72	1043	1046	0.2
2	*n*-Octyl acetate	ES	16.30	1190	1196	1.12
3	Citronellol acetate	MO	21.07	1321	1335	0.18
4	Caryophyllene	SH	24.95	1389	1394	0.21
5	*n*-Octyl 2-methyl butyrate	ES	26.4	1400	1413	2.02
6	Germacrene D	SH	28.15	1453	1459	30.2
7	Bicyclogermacrene	SH	28.83	1465	1470	3.56
8	Neryl isobutanoate	ES	28.97	1477	1475	1.23
9	δ-Cadinene	SH	29.72	1489	1486	0.53
10	Neryl isovalerate	ES	32.18	1540	1535	5.18
11	Spathulenol	SO	32.94	1546	1554	2.68
12	Viridiflorol	SO	33.24	1559	1560	0.15
13	Geranyl isovalerate	MO	33.46	1566	1582	6.55
14	α-Cadinol	SO	34.93	1622	1624	2.56
15	Farnesol	SO	36.68	1639	1665	20.57
16	Neophytadiene	D	42.33	1795	1830	6.96
17	Phytol	D	45.37	2085	2103	4.7
	Monoterpenoids (MO)					6.73
	Sesquiterpenoid hydrocarbons (SH)					34.32
	Oxygenated sesquiterpenoids (SO)					25.96
	Diterpenoids (D)					11.67
	Esters (ES)					9.55
	Other classes (OT)					0.2
	Total					88.43

RT: Retention time. RI-C: Calculated retention index. RI-L: Retention index from literature.

For the EO extracted from the roots, 12 volatile components were identified, representing 93.46% of the total composition (Table 2). The major component of roots EO was falcarinol, with 55.89% of the EO composition. Falcarinol is fatty alcohol known as a biopesticide. So, falcarinol rich EOs could be utilized as a natural pesticide. This is the first report of falcarinol as a major volatile compound in *Heracleum* members. The most abundant volatiles in aerial parts and roots of *H. lasiopetalum* could be seen in Figure 3.

Table 2. Chemical composition of *H. lasiopetalum* roots essential oil.

No.	Compound	Class	R.T. (Min)	RI-C	RI-L	Percentage (%)
1	Octanal	OT	7.72	973	968	19.6
2	Nonanal	OT	11.51	1076	1085	1.01
3	Octyl butanoate	ES	20.13	1357	1366	1.05
4	n-Octyl 2-methylbutanoate	ES	21.05	1400	1413	1.33
5	γ-Decalactone	OT	27.35	1437	1439	1.46
6	(2E)-Tridecenal	OT	31.55	1518	1537	3.87
7	Spathulenol	SO	32.91	1545	1548	2.75
8	Hexahydrofarnesyl acetone	OT	40.12	1800	1801	1.04
9	Methyl palmitate	ES	44.64	1880	1894	2.51
10	Falcarinol	PA	48.44	1994	2038	55.89
11	Tricosane	AL	56.48	2295	2300	1.1
12	Tetracosane	AL	59.33	2394	2400	1.85
	Oxygenated sesquiterpenoids (SO)					2.75
	Esters (ES)					4.89
	Polyacetylenes (PA)					55.89
	Other classes (OT)					26.98
	Alkanes (AL)					2.95
	Total					93.46

RT: Retention time. RT-C: Calculated retention index. RT-L: Retention index from literature.

3.3. Antioxidant activity.

In living systems, antioxidants play a significant role in the prevention of many chronic diseases like cancer, coronary heart disease, diabetes mellitus, Alzheimer's disease, Parkinson's diseases, hypertension, and other degenerative diseases [31]. In this study, the antiradical activity of the isolated compounds, together with EOs was evaluated. In this direction, DPPH radical scavenging activity of studied samples was demonstrated (Table 3). The EO of roots showed the highest radical scavenging effect (58.6 µg/mL), followed by EO of aerial parts (77.9 µg/mL). *H. sphondylium subsp. ternatum* (mainly composed of octyl acetate, octyl butanoate, and octyl hexanoate) exhibited week radical scavenging activity against DPPH and ABTS radicals [32]. In another study, EOs of *H. transcaucasicum* and *H. anisactis* roots, which were rich in myristicin, indicated antioxidant activity with IC_{50} values of 54 and 77 µg/mL, respectively [33]. Previous work on DPPH radical scavenging activity of *H. lasiopetalum* showed IC_{50} value of 170 µg/mL for the hydroalcoholic extract of its flowers [34].

Table 3. Radical scavenging activity of *H. lasiopetalum*.

Compound	IC_{50} (µg/mL)
β-Sitosterol	182.2 12.4
Suberosin	97.7 ± 9.2
Root EO	58.6 ± 4.9
Aerial parts EO	77.9 ± 6.8
Rutin	22.1 ± 1.6

4. Conclusions

The chemical composition of essential oils and also dichloromethane extract of *Heracleum lasiopetalum* from the Kanikhoda Mountains was investigated for the first time. Falcarinol, germacrene, farnesol, octanal, beta-sitosterol, and suberosin were identified as the major components of this herb. Moreover, radical scavenging activity of the EOs and pure compounds were evaluated, and strong antioxidant activity was observed. Findings showed that *H. lasiopetalum* has promising potential to be considered for possible uses as pharmaceuticals and cosmeceuticals based on its bioactive components.

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Conflicts of Interest

The authors declare no conflict of interest.

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