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Chemical constituents of leaf essential oils of *Heracleum candicans* Wall. ex DC from western Himalaya of Uttarakhand, India

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Abstract
The chemical constituents from leaf essential oil of *Heracleum candicans* Wall. ex DC was analyzed GC and GC-MS. The chemical composition of the isolated oil was characterized by 45 components having germacrene D (18.5%), 1, 8-cineole (11.8%), sabinene (2.87%), α-pinene (2.77%) (*Z*)-β-ocimene (2.50%), γ-terpinene (2.74%) *cis*-sabinene hydrate (2.04%) *trans*-sabinene hydrate (2.06%), linalool (4.50%), terpinen-4-ol (2.54%), germacrene D-4-ol (2.05%), caryophyllene oxide (5.16%), pimaradiene (2.65%), sandaracopimara-8-15-diene (2.02%) present as major compounds.

Keywords: Apiaceae, essential oils, *Heracleum candicans,* germacrene D, 1, 8-cineole

1. Introduction
The genus *Heracleum* is one of the largest genera of Umbelliferae (Apiaceae) and there are 125 *Heracleum* species in the world [1]. In India, 15 species have been reported to be distributed in the Himalayas; of which eight species are found in northwestern Himalayas (Jammu and Kashmir) four in western Himalayas (Himachal Pradesh and Uttarakhand) while three in the eastern Himalayas [2]. *Heracleum candicans* Wall. ex DC. (syn. *H. lanatum* Michx, *H. nepalense* D. Don.; family Apiaceae), commonly known as "Gandharajan" or "Patrala," is a large herb found in temperate forests of Himalayan regions [3]. In Indian system of medicines, it is used as an aphrodisiac, nerve tonic and also used in the treatment of skin diseases [4]. The plant has been shown to possess potent stimulatory effect on melanogenesis with significant enhancement of cell proliferation [5]. The fruits and leaves of this genus are used as antiseptic, carminative, digestive and analgesic in Iranian traditional medicine [6]. Literature survey revealed that *Heracleum* species growing in Siberia, such as *Heracleum dissectum* L. is an edible plant. *H. dissectum* L. grows in broken forests, in forest glades and in the meadows of Siberia, Central Asia and Far East. It is spread in all areas of forest zone and the neighboring forest-steppe and steppe zones. In the north of Krasnoyarsk region of Russia areal reaches 70º north latitude. In the west, areal border within forest zone lies along Irtysh and Ob rivers [7-9]. 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 [6].As evident from the literature, the essential oils of *Heracleum* species have been extensively studied for their anti-bacterial [10, 11] anti-fungal [12, 13] anti-dermatophytic [10] and insecticidal activities [14, 15].

2. Experimental
2.1 Plant collection and identification
The fresh aerial parts with fruits were collected from the Milam glacier (Uttarakhand, India) at an altitude of 3400 m in the month of September in 2008 at mature stage. The identification was done from Botany Department, Kumaun University, Nainital and Botanical Survey of India, Dehradun. The voucher specimens (Chem. /DST/08/01) have been banked in the Phytochemistry lab in the Chemistry Department, Kumaun University, Nainital.

2.2 Isolation of essential oil
The fresh plant materials (1 kg) were subjected to steam distillation using a copper electric still, fitted with spiral glass condensers. The distillates were saturated with NaCl and extracted with n-hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulfate and the solvents were distilled off in a rotary vacuum evaporator at 30 ºC and the percentage oil content was computed along the basis of fresh weight of plant materials.

2.3 GC and GC-MS analysis

The oils were analyzed by using a Nucon 5765 gas chromatograph (Rtx-5 column, 30 m × 0.32 mm, FID), split ratio 1: 48, N_2 flow of 4 kg/cm2 and on Thermo Quest Trace GC 2000 interfaced with MAT Polaris Q Ion Trap Mass spectrometer fitted with a Rtx-5 (Restek Corp.) fused silica capillary column (30 m × 0.25 mm; 0.25 µm film coating). The column temperature was programmed 60^0-210 ^{0}C at 3 ^{0}C/min using He as carrier gas at 1.0 mL/min. The injector temperature was 210 ^{0}C, injection size 0.1µL prepared in hexane, split ratio 1:40. MS were taken at 70 eV with a mass range of 40-450 amu.

2.4 Identification of the components

Identification of constituents was done on the basis of Retention Index (RI, determined with reference to a homologous series of *n*-alkanes (C9-C24, Polyscience Corp., Niles, IL) under identical experimental condition), co-injunction with standards (Sigma and known essential oil constituents (standard isolates), MS Library search (NIST and WILEY), by comparing with the MS literature data [16]. The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

Chemical components of leaf essential oil of *H. candicans* from Uttarakhand Himalaya

Sr. No.	Compounds	RI	% Composition (FID)	Mode of identification
1.	tricyclene	926	0.54	a,b
2.	α–thujene	932	1.11	a,b
3.	α–pinene	939	2.77	a,b
4.	camphene	954	1.15	a,b
5.	sabinene	978	2.87	a,b
6.	β–pinene	981	0.54	a,b
7.	β–myrcene	994	0.23	a,b
8.	α–phellandrene	1006	0.14	a,b
9.	β-phellandrene	1037	1.2	a,b
10.	1, 8-cineole	1038	11.08	a,b
11.	(*Z*)-β-ocimene	1041	2.50	a,b
12.	(*E*)-β-ocimene	1050	1.1	a,b
13.	γ-terpinene	1065	2.74	a,b
14.	*cis*-sabinene hydrate	1069	2.04	a,b
15.	*trans*-sabinene hydrate	1069	2.06	a,b
16.	linalool	1101	4.50	a,b
17.	*cis*-*p*-menth-2-en-1-ol	1120	0.1	a,b
18.	dihydrolinalool	1130	1.3	a,b
19.	*trans*-*p*-menth-2-en-1-ol	1145	1.1	a,b
20.	benzyl acetate	1157	0.71	a,b
21.	pinocarvone	1160	0.25	a,b
22.	terpinen-4-ol	1175	2.54	a,b
23.	*p*-cymen-8-ol	1177	0.23	a,b
24.	thymol methyl ether	1192	0.21	a,b
25.	carvacrol methyl ether	1195	0.26	a,b
26.	β-elemene	1389	0.1	a,b
27.	(*Z*)-β-farnesene	1440	0.21	a,b
28.	(*E*)-β-farnesene	1459	1.50	a,b
29.	germacrene D	1482	18.5	a,b
30.	α-selinene	1498	0.6	a,b
31.	α-muurolene	1499	0.21	a,b
32.	γ-cadinene	1524	0.44	a,b
33.	germacrene D-4-ol	1574	2.05	a,b
34.	caryophyllene oxide	1581	5.16	a,b,
35.	humulene epoxide II	1606	0.14	a,b
36.	10-*epi*-γ-eudesmol	1619	0.36	a,b
37.	γ-eudesmol	1630	4.0	a,b
38.	*epi*-α-cadinol	1640	1.81	a,b
39.	cubenol	1645	0.3	a,b
40.	*epi*-β-bisabolol	1674	0.16	a,b
41.	laurenene	1876	0.22	a,b
42.	*epi*-laurenene	1901	0.19	a,b
43.	isopimara-9 (11),15-diene	1903	0.62	a,b
44.	pimaradiene	1945	2.65	a,b
45.	sandaracopimara-8 (14)15-diene	1965	2.02	a,b
Total			84.51	

*Mode of identification: Retention Index (LRI, Based on homologous series of n-alkenes; C_8-C_{24}), co injection with Standards/Peak enrichment with known oil constituents, MS (GC-MS), t= trace (<0.1%); (-) = not detected, RI: Literature value (Adams, 2007)

3. Results & Discussion

The oil yield obtained from leaves of *H. candicans* was 0.30% (v/w). The chemical constituents present in the essential oil of *H. candicans* were identified by GC and GC-MS. Forty five (45) compounds were characterized in the oil, accounting for 84.51% of the oil (Table 1). The major compounds were germacrene D (18.5%), 1, 8-cineole (11.8%), sabinene (2.87%), α-pinene (2.77%), (*Z*)-β-ocimene (2.50%), γ-terpinene (2.74%), *cis*-sabinene hydrate (2.04%), *trans*-sabinene hydrate (2.06%), linalool (4.50%), terpinen-4-ol (2.54%), germacrene D-4-ol (2.05%), caryophyllene oxide (5.16%), pimaradiene (2.65%), sandaracopimara-8 (14)-15-diene (2.02%) as major compounds. Literature survey revealed that the vinylcyclohexane, octyl acetate, 2-methyloctyl propanoate, and β-alanine were present in essential oil from fruits of *H. dissectum* L. growing in Krasnoyarsk Russia [17]. *n*-Octyl acetate, *o*-cymene, limonene, *δ*-2-carene, *cis*-thujone, isobornyl acetate, *n*-octanol, 1, 8-cineol, *n*-tridecanol, and safrole were reported from *H. siamicum* essential oil from Thiland [18]. Myristicin, as a major component, was characterized by high amounts in both essential oils in aerial parts of *H. transcaucasicum* and *H. anisactis* as 70% and 93.5% respectively [19]. Saraswathy and Sasikala reported 1, 8-Cineole was the dominant component in rhizome (23.10%) and leaves (21.20%) of *H. sprengelianum* essential oil, and *β*-phellandrene (11.35%) *β*-Pinene (16.18%), 1,8-cineole (21.20%), sabinene (8.74%), *p*-cymene-8ol (6.64%), and *β*-Caryophyllene (2.62%) dominated components in leaves of *Heracleum sprengelianum* essential oil [20]. Vridiflorol (23.05%), elemol (3.63%), spathulenol (3.34%) and 2-tetradecanol (3.38%) were found as major constituents from root essential oil of *Heracleum persicum* [21]. Previously furocoumarins was reported from *H. candicans* and *β*-phellandrene, sabinene, and (*E*)-β-ocimene were the major compounds and antimicrobial activity reported from *H. lanatum* from Kumaun Himalaya [22, 23].

4. Conclusions

Leaf essential oils of *H. candicans* from western Himalaya of Uttarakhand was dominated by germacrene D (18.5%), 1, 8-cineole (11.8%), sabinene (2.87%), α-pinene (2.77%), (*Z*)-β-ocimene (2.50%), γ-terpinene (2.74%), *cis*-sabinene hydrate (2.04%), *trans*-sabinene hydrate (2.06%), linalool (4.50%), terpinen-4-ol (2.54%), germacrene D-4-ol (2.05%), caryophyllene oxide (5.16%), pimaradiene (2.65%), sandaracopimara-8 (14)-15-diene (2.02%) as major compounds. 1,8-Cineole and germacrene D, though present most of the Indian *Heracleum* sp. Other common components were *β*-phellandrene, *β*-pinene, sabinene, *p*-cymen-8-ol, and *β*-caryophyllene present in most of *Heracleum* sp. The composition from Milam glacier becomes a good source of these compounds as pharmaceutical utilizations.

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