Composition And Antimicrobial Activity Of The Essential Oil Of Heracleum Lanatum Michx. From Uttarakhand Himalaya

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Abstract: The essential oil from the flowering aerial parts of Heracleum lanatum Michx. family Apiaceae was obtained by hydrodistillation and analysed by combination of GC (RI) and GC-MS. The chemical composition of the isolated oil is reported for the first time. Sixty five compounds were identified representing 96.95% of the total oil. The chemical composition of the isolated oil was characterised by a high proportion of monoterpene hydrocarbons (39.42%), among which β-phellandrene (14.01%), sabinene (6.75%) and (E)-β-ocimene (6.45%) were the major compounds. The sesquiterpene hydrocarbons (22.50%) represented the second major fraction of the oil followed by the oxygenated sesquiterpenes (21.42%). Furthermore, antimicrobial activity of the essential oil was evaluated using agar well diffusion method.

Index terms: Antimicrobial activity, Apiaceae, essential oil composition, Heracleum lanatum, β-phellandrene, sabinene,

1 INTRODUCTION

The genus Heracleum belongs to the family Apiaceae. There are almost 125 Heracleum species in the world, of which 70 species are distributed in Europe and Asia in temperate region [1], [2]. In India, 15 species have been reported to be distributed in the Himalayas, of which 8 species are found in North West Himalayas (J and K), 4 in Western Himalayas (H.P. and Uttarakhand) while 3 in the Eastern Himalayas [3]. Heracleum lanatum Michx. (H. candicans Wall. ex DC., H. maximum Bartr.) is an aromatic herb with rootstock stout, perennial odourous, stem upto 1.75cm tall and densely pubescent. It is wide spread from Pakistan to south west China 1800-5000m in open slope and drier area [4], [5]. Roots of H. lanatum are useful in the treatment of leukoderma and psoriasis. It is also used in the preparation of sun-tan lotion. Flower and leaves of H. lanatum are used to make paste and applied on forehead to cure headache [6]. As far as our literature survey could ascertain, although the essential oil composition of different members of Heracleum have been reported [7], [8], [9], [10] there have been no attempts to study the chemical composition and antimicrobial activity of H. lanatum grown in India.

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2 EXPERIMENTAL

2.1. Plant collection and identification

H. lanatum was collected in July 2011 from Kedartal, Gangotri region (Uttarakhand, India). The plant was identified by Prof. Y.P.S. Pangtey, Botany Department, Kumaun University, Nainital, which has been deposited in the herbarium repository of Botanical Survey of India, Dehradun for future reference (Acc. No. 113646).

2.2. Isolation of essential oil

Fresh aerial parts (2 kg) were subjected to hydrodistillation using Clevenger apparatus for 3h. The essential oil was dried over anhydrous sodium sulphate (Merck) until the last traces of water were removed and then stored in a dark glass bottle at 4 ºC prior to GC-MS analysis. The yield of the essential oil obtained from flowering aerial parts of H. lanatum was 0.10% (v/w).

2.3. Analysis of the essential oil

The oil was analysed by using a Shimadzu 2010 auto system GC fitted with Rtx -5 column and FID detector. The column temperature was programmed at 80ºC (hold time for 2 minute) to 210ºC (hold time 5 minute) at 3ºC min^{-1} and than 210º-300ºC at 20ºC min^{-1} with final hold time of 15 minute, using N_2 at 30.0 mL/min column head pressure as carrier gas, the injector temperature was 270ºC and detector (FID, Flame ionisation detector) temperature 280ºC. The GC-MS used was Autosystem 2010 GC (Rtx- 5, 30m x 0.25 mm, i.d. FID 0.25µm) coupled with Shimadzu QP 2010 plus with thermal desorption system TD 20 with (Rtx-5) fused silica capillary column (30 m x 0.25mm with film thickness 0.25µm). The column temperature was 80ºC (hold time for 2 minute) to 210ºC (hold time 5 minute) at 3ºC min^{-1} and than 210º-300ºC at 20ºC min^{-1} with final hold time of 21 minute, using helium as carrier gas. The injector temperature was 230ºC and 0.2 µL in n-hexane, with split ratio of 1:30 MS were taken at 70 eV with a mass range of 40- 650amu.

2.4. Identification of the components

Identification of constituents was done on the basis of Retention Index, MS Library search (NIST and WILLY), and also comparing with the MS literature data [11].

2.5 Antimicrobial activity

The essential oil was screened for antimicrobial activity against bacteria Klebsiella pneumoniae (MTCC3384), Staphylococcus aureus (MTCC3103), Salmonella enterica (MTCC3224), Escherichia coli (MTCC443), Pseudomonas aeruginosa (MTCC424), Proteus vulgaris (MTCC1771) and fungus Pichia guilliermondii (MTCC 4052) and Candida albicans (MTCC227) were prepared by emulsifying single colony in 5 ml of sterilized required nutrient broth. Tubes with require nutrient broth and inoculated bacterial cultures were incubated overnight at 37º C for 24 hr. Turbid cultures were then used as stock inoculums. The bacterial strains were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India, as Microbial Type Culture Collection (MTCC). All bacterial strains were cultured and maintained in microbiology laboratory, Department of Biotechnology, Bhimtal campus, Kumaun University, Nainital by regular sub culturing and preserved as glycerol stock at -20ºC for further use.

2.5.1. Zone of inhibition (ZI) and minimum inhibitory concentration (MIC)

Antimicrobial activities of the essential oil against above microorganism and a few reference materials were determined by Agar Well Diffusion method [12], [13]. The organisms were cultured in nutrient broth (bacterial strains) and malt yeast broth (fungal strains) and the tests were carried out on Mueller Hinton agar and Potato Dextrose agar plates respectively. The inoculums of the microbial strains were prepared from 24 h broth cultures, the cultures were adjusted to 10^6 CFU/ml with sterile water. The different concentration range from 5 to 1000 µL/mL of essential oils was prepared by dissolving them in hexane. Muller Hinton agar and Potato Dextrose agar were poured into petridishes. After solidification, 100µL of test strains were spread on the media plates separately. Care was to taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. After the inoculation, a well was made in the plates with sterile borer (3 mm). The oil samples (30µL/well) of different concentrations (5µL to 1000 µL) were introduced into the well and plates were incubated at 37°C for 24 hrs. All samples were tested in triplicate. Microbial growth was determined by measuring the zone of inhibition. Gentamicin and Kanamycin for bacterial strain and Nystatin for fungal strain were used as positive control. Hexane was used as negative control. The MIC value was defined as the lowest concentration of the volatile oil required for inhibiting the growth of each microorganism.

3. RESULTS AND DISCUSSION

The yield of the essential oil obtained from leaves of H. lanatum was 0.10% (v/w). The constituents of the essential oil of H. lanatum were identified in order of their elution in Rtx-5 column in GC (Figure 1) and GC-MS. Sixty five compounds were characterised in the oil, accounting for 96.95 % of the oil (Table 1). The oil of H. lanatum is characterised by a high content of β-phellandrene (14.01%) and lavandulyl acetate (8.87%), the major constituents, followed by bicyclogermacrene (7.94%), germacrene D (6.75%), sabinene (6.75%), (E)-β-ocimene (6.45%) and γ-eudesmol (5.60%). The oil of H. lanatum leaves contained major amount of monoterpene hydrocarbons (39.42%). Major constituents reported in H. sphondylium ssp. ternatum were aliphatic esters, 1-octnol (50.3%), octyl butyrate (24.6%) and octyl acetate (7.3%) constituting 92.6% of the total oil [7]. Analysis of the essential oil of leaves, seeds and rhizomes of H. sprengelianum in India by [14] and the oil was found to be rich in monoterpene hydrocarbons, accounting 91.5%, 87.7%, 90.0% respectively. In the essential oil of H. rechingeri, 37 compounds, representing 94.26% of the total oil, were characterized. Octyl acetate (29.49%) and elemicine (23.06%) were found to be the main constituents [2].

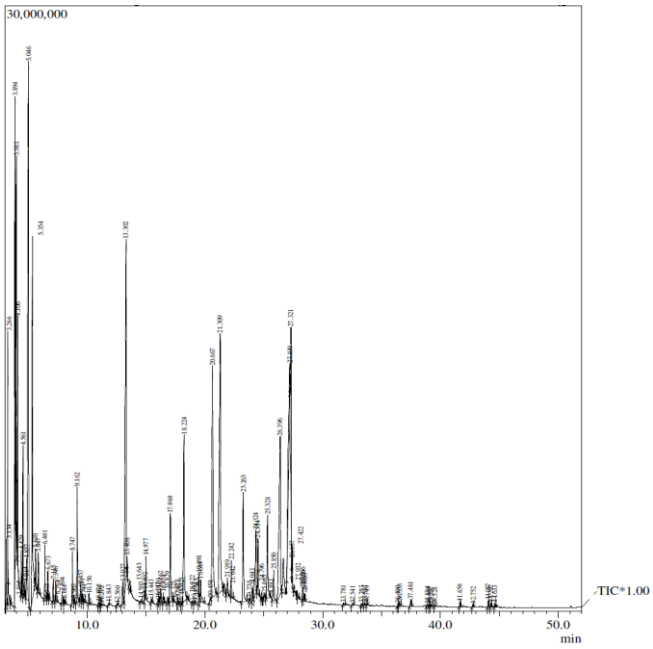

Figure 1. *Gas chromatogram of Heracleum lanatum Michx.*

TABLE 1. Essential oil composition of flowering aerial parts of H. lanatum Michx.

Compounds[a]	RI_{Exp}[b]	RI[c]	(%)
α-Thujene	929	930	0.56
α-Pinene	937	932	2.24
Camphene	953	954	0.10
Sabinene	979	975	**6.75**
β-Pinene	985	979	3.79
Myrcene	993	990	2.20
α-Phellandrene	1009	1002	0.44
δ-2-Carene	1014	1002	1.51
α-Terpinene	1019	1017	0.12
p-Cymene	1027	1024	0.34
β-Phellandrene	1035	1029	**14.01**
(E)-β-Ocimene	1048	1050	**6.45**
γ-Terpinene	1059	1059	0.35
cis-Sabinene hydrate	1068	1070	0.40
Terpinolene	1091	1088	0.56
Linalool	1102	1096	t
n-Nonanal	1105	1100	t
cis-p-Menth-2-en-1-ol	1123	1121	0.25
trans-p-Menth-2-en-1-ol	1140	1140	0.17
trans-Verbenol	1146	1144	0.10
Lavandulol	1167	1169	0.71
Terpinen-4-ol	1180	1177	1.62
Cryptone	1189	1185	0.25
α-Terpineol	1193	1188	0.11
cis-Piperitol	1198	1196	0.10
trans-Piperitol	1209	1208	0.14

(Z)-Hexenyl-2-Methyl butanoate	1231	1232	t
Thymol methyl ether	1235	1235	t
Piperitone	1255	1252	t
Bornyl acetate	1287	1288	0.35
Lavandulyl acetate	1294	1290	**8.87**
Terpin-4-ol acetate	1297	1299	0.18
cis-α-Necrodol acetate	1303	1299	0.16
δ-Elemene	1338	1338	0.55
α-Cubebene	1350	1348	0.10
Cyclosativene	1367	1371	0.10
α-Copaene	1376	1376	0.16
(E)-Myrtanol acetate	1385	1386	0.10
β-Cubebene	1391	1388	1.63
Methyl eugenol	1407	1403	0.10
α-Gurjunene	1410	1409	t
β-Caryophyllene	1421	1419	3.55
β-Copaene	1429	1432	t
β-Gurjunene	1438	1433	t
α-Humulene	1453	1454	0.46
(E)-β-Farnesene	1457	1456	0.49
γ-Muurolene	1478	1479	t
Germacrene D	1483	1485	**6.75**
Bicyclogermacrene	1500	1500	**7.94**
(E,E)-α-Farnesene	1509	1505	0.15
Cubebol	1516	1515	0.14
δ-Cadinene	1524	1523	0.62
Elemol	1551	1549	2.36
(E)-Nerolidol	1565	1563	t
Germacrene D 4-ol	1572	1575	0.26
Spathulenol	1579	1578	1.51
Caryophyllene oxide	1584	1583	1.21
Viridiflorol	1592	1592	t
Humulene epoxide II	1606	1608	1.93
10-epi-γ-Eudesmol	1620	1623	0.63
γ-Eudesmol	1635	1632	**5.60**
Cubenol	1643	1646	1.45
β-Eudesmol	1657	1650	2.92
α-Eudesmol	1660	1653	2.93
α-Cadinol	1663	1654	0.48
Total			**96.95**

Grouped components	
Monoterpene hydrocarbons	39.42 %
Oxygenated monoterpenes	13.61%
Sesquiterpene hydrocarbons	22.50%
Oxygenated sesquiterpenes	21.42%
Total identified	**96.95 %**

Mode of identification: Retention Index, on Equity-5 column, MS (GC-MS), [b]Retent ion indices determined on the Equity-5 column using an n-alkane homologous series (C_9–C_{33}); [c]retention indices from the literature (Adams, 2007), t = trace (<0.01%), Bold numbers indicate major constituents\, t = trace (<0.10%) The essential oil of flowering aerial parts of H. lanatum was screened for antimicrobial activity against six standard strains of bacteria and two standard fungal strains. The results of in vitro test (Table 2) showed that the oil had moderate activity against the tested pathogens on the basis of zone of inhibition and MIC values as compared with the standard antibiotics. The essential oil of the plant showed mean zone of inhibition for the bacterial strains varied from 12.33±0.58 (Klebsiella pneumoniae) to 8.67±0.58 mm (Proteus vulgaris) with the MIC values 75 µl/ml and 125 µl/ml respectively. The oil exhibited maximum activity against Klebsiella pneumoniae with inhibition zone of 12.33±0 mm diameter and MIC value of 100 µl/ml followed by Staphylococcus aureus (ZI, 11.67±0.58 mm, MIC, 75 µl/m), Escherichia coli (ZI;10.33±0.58 mm, MIC;125 µl/ml), Salmonella enterica (ZI; 9.67±0.58 mm, MIC; 100 µl/ml), Pseudomonas aeruginosa, (ZI; 9.33±0.58mm, MIC; 125 µL/mL) and Proteus vulgaris (ZI; 8.67±0 mm, MIC; 125 µL/mL). The plant oil showed good activity against Pichia guilliermondii (ZI; 11.33±0.58 mm, MIC; 150 µg/mL) and Candida albicans (ZI; 10.67±0.58 mm, MIC; 175 µg/mL) as compared to the standard (MIC; 250 µg/mL). To the best of our knowledge, this is the first report on the essential oil composition and antimicrobial activity of H. lanatum from India.

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Table 2. Antimicrobial activity of Heracleum lanatum flowering aerial parts essential oil

Microorganism		*Heracleum lanatum* (**HL**)		**Standard antibiotics**			
				Gentamicin		**Kanamycin**	
Bacteria	**MTCC**	**ZI[a] (mm)**	**MIC[b] µl/ml**	**ZI[a] (mm)**	**MIC µg/ml**	**ZI[a] (mm)**	**MIC[b] µg/ml**
Klebsiella pneumoniae	3384	12.33±0.15	100	10.67±0.20	50	10.67±0.22	50
Staphylococcus aureus	3103	11.67±0.25	75	17.33±0.02	50	16.33±0.03	50
Salmonella enterica	3224	9.33±0.02	100	14.67±0.18	50	13.33±0.02	75
Escherichia coli	443	10.33±0.01	125	10.67±0.22	50	9.67±0.22	100
Pseudomonas aeruginosa	424	9.33±0.04	125	13.33±0.04	50	12.67±0.33	50
Proteus vulgaris	1771	8.67±0.22	125	15.67±0.17	50	13.33±0.04	75
Fungus				ZI[a] (mm) **Standard Nystatin** MIC[b] (µg/ml)			
Pichia guilliermondii	4052	11.33±0.03	150	15		250	
Candida albicans	227	10.67±0.23	175	13		250	

(a) Inhibition zone diameter (mm), Size of well 3mm not included in ZI
(b) Minimum inhibitory concentration values in 1000 µl/ml to 5µl/ml

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