Volatile constituents of *Saussurea costus* roots cultivated in Uttarakhand Himalayas, India

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Submitted: 25-08-2012 Revised: 22-09-2012 Published: 22-05-2013

ABSTRACT

Background: *Saussurea costus* (Falc.) Lipschitz, syn *Saussurea lappa* C.B. Clarke, one of the best-known species within this genus, is commonly known as costus. Due to the remarkable biological activity of *S. costus* and its constituents it will have an appropriate place in various systems of medicines all over the globe. **Objective:** The main aim is to study the volatile constituents of *S costus* cultivated in Uttarakhand Himalayas. **Material and Methods:** The volatile constituents were isolated from the root of *S costus* cultivated in Chamoli district of Uttrarakhand by hydro distillation and were analyzed by gas chromatography-mass spectroscopy (GC-MS). **Results:** A total 35 aroma compounds representing about 92.81% of the total composition were identified. Aldehyde like (7Z, 10Z, 13Z)-7, 10, 13-hexadecaterinal (25.5%) was found as a major compound including other ketones like dehydrocostus lactone (16.7%), alcohols like elemol (5.84%), γ-costol (1.80%), vulgarol B (3.14%), valerenol (4.20%), and terpinen-4-ol (1.60%), etc. Esters and acids were found to be completely absent in our samples. **Conclusion:** *S. costus* volatile oil constituents are superior in terms of total identified constituents. Where relative area quantum is higher in Uttarakhand Himalayas samples, when compared with those originated to China and Korea.

Key words: C.B. Clarke, essential oil, GC-MS, *Saussurea costus*

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.112424

Quick Response Code:

INTRODUCTION

The genus *Saussurea* DC. of the family Asteraceae comprises about 300 species in the world of which about 61 species exist in India. *Saussurea costus* has become an important drug in the international market as well as Indian systems of medicine.[1] It is used either as a single drug or in combination with other drugs. In India, this is endemic in the sub alpine region of Jammu and Kashmir, Himachal Pradesh and Uttarakhand from altitudes of 3200–3800 m. This is a critically endangered species. Government of India has prohibited exports of 29 medicinal and aromatic species, including *S. costus*, either in crude form or in processed products. Its roots are used mainly as an antispasmodic in asthma, cough, and also in treatment of cholera, chronic skin diseases, and rheumatism.[2] The essential oil of *S. costus* was also studied for food repellency.[3] The total plant extract of *S. costus* as well as its Ayurvedic preparations, 'Asava' and 'Arishta', were screened for antimicrobial activity.[4] Costus oil, used in perfumery, being rich in sesquiterpene lactones, is responsible for numerous cases of allergic contact dermatitis.[5] Roots essential oil is studied by various workers.[6-9] Oil of *S. costus* is very costly and used in high grade perfumes, it has a very strong odor. The oil blends well with vetiver, patchouli, rose, violet, and sandal wood used in preparation of hair oil, insecticide and insect repellent, and making incense.[10] According to planning commission of India, *S. costus* requirement is 0.43 tons/annum. In view of the above importance, cultivated species ingredient need to be explored. However, no systematic study has been carried out in essential oil compositions of the species from Uttarakhand Himalayas.

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MATERIAL AND METHODS

Collection of plant material

Roots of *S. costus* were collected from Ghat region District Chamoli Uttarakhand India in a manner of three samples (different biological replicates) in the month of March 2010. The species was identified by Dr. Sunil Sah,

Taxonomist, at our Center. Voucher specimen has been kept in the herbarium (CAP-94) of Centre for Aromatic Plants, Selaqui Dehradun, India. The roots were air dried and were grounded to uniform powder using grinder (Philips, India), and stored in air-tight bottles till further analysis.

Isolation of the Essential oils

The essential oil of the grounded powder was extracted by hydro-distillation for 6 hours using Clevenger apparatus.[11] The oil content (v/w%) was estimated. The oil dehydrated over anhydrous sodium sulfate and kept in cool place before analysis.

Gas chromatography

The gas chromatograph (GC) analyses of the oil samples was carried out by using HP6890 GC manufactured by Agilent equipped with a flame ionization detector (FID) detector and a HP-5 fused silica column (30 m × 0.32 mm × 0.2 μm film thickness). The sample was injected directly into the column. Nitrogen was used as a carrier gas during analysis. The injector and detector temperature were maintained at 210°C and 230°C, respectively. The column oven temperature was programmed from 60°C to 220°C with an increase in rate of 3°C/min. The injection volume was 0.02 μl neat.

Gas chromatography-mass spectrometry

The gas chromatography-mass spectroscopy (GC-MS) analysis of the oil was performed out on a Perkin Elmer mass spectrometer (Model Claurus 500) coupled to a Perkin Elmer Clarus 500 GC with a 60 m × 0.32 mm × 0.2 μm film thickness column (RtX5). The sample was injected directly into the column. Helium was used as the carrier gas (flow rate 1 ml/min). The oven temperature was programmed from 60°C to 220°C at 3°C/min. Other conditions were the same as described under GC. The mass spectrum was taken with a mass range of 40–600 Daltons.

Identification of components

The identification of constituents was performed on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes,C_9-C_{24} under experimental conditions, co injection with standards (Aldrich and Fluka), MS library search (2.0 version, NIST 2002 and WILEY-7th edition, May 2003), and by comparing with the MS literature data.[12,13] The relative amounts of the individual components were calculated based on the GC peak area (FID response) without correction factors.

RESULTS AND DISCUSSION

The essential oil was found to be 0.02% (v/w).GC and GC-MS analysis of the essential oil of *S. costus* led to the identification of 41 components representing 92.81% of the total oil composition. Results of present study are summarized in Table 1. Among the aldehydes (7Z, 10Z, 13Z)-7, 10, 13-hexadecatrienal was identified as a major

Table 1: Comparison between constituents of Korean and Chinese *S. costus* root oil and that from Uttarakhand

S.N	Components	RI*	Korea[17]	China[17]	**India
1.	Caproaldehyde	–	0.07	–	tr
2.	Furfural	849	–	tr	–
3.	α-Thujene	924	0.02	–	0.23
4.	α-Pinene	938	0.07	tr	0.06
5.	Camphene	955	tr	tr	tr
6.	β-Pinene	965	0.24	–	0.17
7.	Myrcene	989	tr	tr	tr
8.	Sabinene	1001	0.19	–	0.06
9.	α-Terpinene	1003	0.04	–	0.59
10.	*p*-Cymene	1030	–	0.03	1.42
11.	Limonene	1032	tr	–	0.14
12.	β-Phellandrene	1034	0.10	0.03	tr
13.	1,8 cineol	1040	tr	–	0.99
14.	(E)-β-Ocimene	1048	tr	–	2.30
15.	γ-Terpinene	1064	0.10	tr	–
16.	α-Terpinolene	1092	0.040	tr	–
17.	Linalool	1107	0.05	tr	0.56
18.	Chrysanthenone	1125	tr	–	0.82
19.	Camphor	1133	tr	–	0.30
20.	Menthone	1141	tr	–	–
21.	α-Fenchene	1150	–	3.53	–
22.	Citronellal	1163	–	0.02	0.50
23.	Terpinen-4-ol	1184	0.62	0.14	1.6
24.	Cryptone	1190	–	tr	tr
25.	α-Terpineol	1193	0.13	0.14	–

Table 1: Contd...

S.N	Components	RI*	Korea[17]	China[17]	**India
26.	Estragole	1207	–	0.90	–
27.	Pregnane	1246	tr	0.03	–
28.	Carvotan acetone	1250	0.04	0.06	–
29.	Phellandrene	1272	–	0.02	0.60
30.	Anethol	1286	–	0.09	0.20
31.	Thymol	1302	–	0.23	0.11
32.	Citronellyl propionate	1349	–	0.03	–
33.	α-Copaene	1353	0.05	tr	–
34.	β-Elemene	1373	4.69	4.07	2.10
35.	α-Cederene	1390	0.26	0.17	–
36.	α-Ionone	1416	2.41	2.90	3.91
37.	Dehydro β-ionone	1422	–	0.21	–
38.	(E)-Caryophyllene	1460	–	0.08	2.31
39.	α-Humulene	1475	0.65	0.36	–
40.	Geranyl acetone	1476	1.15	1.35	3.82
41.	allo-Aromadendrene	1479	0.20	0.18	–
42.	β-Selinene	1488	0.63	2.86	0.56
43.	α-Curcumene	1495	4.94	2.87	–
44.	β-Ionone	1499	1.10	1.95	2.30
45.	α-Selinene	1508	2.67	1.69	1.20
46.	β-Guaiene	1515	2.67	1.32	–
47.	β-Himachalene	1525	0.90	0.67	–
48.	δ-Cadinene	1530	0.31	0.21	–
49.	cis-α-Bisabolene	1545	0.44	0.18	–
50.	Elemol	1550	1.78	2.98	5.84
51.	Elimicin	1556	–	0.08	–
52.	Nerolidol	1566	–	0.28	0.29
53.	Caryophyllene oxide	1575	0.83	0.52	1.97
54.	(E,Z)-α-Farnesene	1618	–	3.28	–
55.	β-Eudesmol	1623	–	4.62	0.48
56.	α-Eudesmol	1631	–	0.28	–
57.	(+)-5-epi -Neointermedeol	1635	–	0.96	–
58.	(7Z, 10Z, 13Z)-7,10,13-Hexadecatrienal	1654	23.32	21.20	25.5
59.	Heneicosane	1665	–	0.02	-
60.	(+) –γ-Costol	1724	2.19	2.50	1.8
61.	Vulgarol B	1744	–	5.06	3.14
62.	Valerenol	1756	4.15	5.30	4.20
63.	Cyercene-4	1832	–	0.52	-
64.	Isocritonilide	1850	–	1.65	-
65.	Dehydrocostuslactone	1866	10.97	10.30	16.7
66.	Dehydrosaussurea lactone	1878	–	1.11	3.24
67.	Costuslactone	1888	0.17	0.13	2.61
68.	Methyl linoleate	2080	0.04	0.11	–
69.	14 β-Pregnane	2105	tr	tr	–
70.	Ethyl linoleate	2185	0.03	0.02	–
71.	Linoleic acid	–	–	t	–
72.	Unidentified	–	–	–	0.20
73.	Unidentified	–	–	–	0.99
TOTAL			65.26	87.24	92.81

*Retention index relative to n-alkanes C_9-C_{24} calculated on nonpolar Rtx-5 Capillary column** Percentage of components (Average of three different analyzed biological replicates in same place). tr- In trace amount (< 0.10%), - Absent

component (25.5%), this is with the tune of reports from China and Korea. As the molecular weight, the odor profile gradually tends to give more pleasant fruity character. Among the alcoholic compounds (+) –γ-costol (1.80%), vulgarol B (3.14%), valerenol (4.20%), elemol, (5.84%), and terpinen-4-ol (1.60%) and other minor alcohols such as linalool, 1,8-cineol, etc., were found.1,8-cineole is widely used in the flavor industry and is an important ingredient in pharmaceuticals.[14] Elemol (5.84%) has been reported to possess antimicrobial activities and is demonstrated in various growth inhibitions,[15] which is found in higher quantum as compared with China and Korea originated oils. Dehydrocostus lactone (16.7%) detected as second major components, which found greater quantity as compared with China and Korea, showed various biological activities.[16] Among the hydrocarbon, β-elemene (2.1%), which

belongs to the sesquiterpenes group, is reported to inhibit mouse pancreatic cancer and neoplastic metasis, and have antitumor effect[17] and (E)-β-ocimene (2.3%) possessed higher quantum as compared with other hydrocarbon like α-thujene, α-pinene, camphene, β-pinene, myrcene, sabinene, α-terpinene, p-cymene, limonene, β-phellandrene. Where α-terpinolene, α-terpineol, estragole, pregnane, carvotan acetone, dehydro β-ionone were completely absent in our sample. However, compounds like β-guaiene, β-himanchalene, δ-cadinene, and cis-α-bisabolene were found abundant in the samples of China and Korea but not detected in present investigation.

CONCLUSION

The investigated species (*S. costus*) was found to be rich in dehydrocostus lactone, which is a sesquiterpene compound, that is reported to act as selective inhibitor and can be potentially used as an ingredient of new anticancer drug.[17] Yield of *S. costus* root oil was 0.02% (v/w) in Indian Himalayan region, which is similar to the reports from Korean origin root oil. However, Uttarakhand's essential oil is more volatile in flavor compounds and higher percentage of total peak area as compared with Korea and China. The potential of this species in food repellency[3] and in Ayurveda medicines[4] strengthens the possibility of its commercial cultivation in the hilly tracks of Uttarakhand Himalayas for industrial utilization.

ACKNOWLEDGMENT

The authors wish to thank colleagues of Centre for Aromatic Plants, Selaqui for the support and help. The authors are also thankful to anonymous reviewers for their valuable suggestions in improving earlier version of manuscript.

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Cite this article as: Gwari G, Bhandari U, Andola HC, Lohani H, Chauhan N. Volatile constituents of *Saussurea costus* roots cultivated in Uttarakhand Himalayas, India. Phcog Res 2013;5:179-82.

Source of Support: The authors thankfully to Ministry of Micro, Small, and Medium Enterprises (Formerly known as Ministry of Small Scale Industry), Govt. of India, New Delhi to provide support service facilities for quality assessment laboratory., **Conflict of Interest:** We declared we do not have Conflict of Interest.