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

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**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 Uttarakhand 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-hexadecatetralin (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*

**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.[5] The essential oil of *S. costus* was also studied for food repellency.[6] The total plant extract of *S. costus* as well as its Ayurvedic preparations, ‘Asava’ and ‘Arishta’, were screened for antimicrobial activity.[6] Costus oil, used in perfumery, being rich in sesquiterpene lactones, is responsible for numerous cases of allergic contact dermatitis.[7] 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.

**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. 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 x 0.32 mm x 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 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.

Gas chromatography-mass spectrometry
The gas chromatography-mass spectroscopy (GC-MS) analysis of the oil was performed on a Perkin Elmer mass spectrometer (Model Claurus 500) coupled to a Perkin Elmer Claurus 500 GC with a 60 m x 0.32 mm x 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. 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 component.
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 (+)-g-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.\textsuperscript{14} Elemol (5.84%) has been reported to possess antimicrobial activities and is demonstrated in various growth inhibitions,\textsuperscript{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.\textsuperscript{16} Among the hydrocarbon, β-elemene (2.1%), which

| S.N | Components                        | RI* | Korea\textsuperscript{(1)} | China\textsuperscript{(1)} | **India** |
|-----|-----------------------------------|-----|-----------------------------|-----------------------------|-----------|
| 26  | Estragole                         | 1207| –                           | 0.90                        | –         |
| 27  | Pregnanol                         | 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-Cedrene                         | 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-Humulenene                      | 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)-γ-Farnesene                 | 1618| –                           | 3.28                        | –         |
| 55  | β-Eudesmol                        | 1623| –                           | 4.62                        | 0.48      |
| 56  | α-Eudesmol                        | 1631| –                           | 0.28                        | –         |
| 57  | (+)-5-epi-Neointermedoel          | 1635| –                           | 0.96                        | –         |
| 58  | (T2, 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  | Isocitronilide                    | 1850| –                           | 1.65                        | –         |
| 65  | Dehydrocostuslactone              | 1866| 10.97                       | 10.30                       | 16.7      |
| 66  | Dehydrocostuslactone              | 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-Pregnanol                    | 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\textsubscript{9}-C\textsubscript{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
belongs to the sesquiterpenes group, is reported to inhibit mouse pancreatic cancer and neoplastic metasis, and have antitumor effect[17] and (E)-β-oicimene (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-himachalene, 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 S. costus. Inhibitory effects of oriental herbal medicines on IL-8 (Interleukin-8) was found to be

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