

Isolation and Chemical Characterization of Essential oil of *Saussurea simpsoniana*

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Inflorescence of *Saussurea simpsoniana* (Asteraceae) grown in Himalayas were used traditionally in treatment of fever, snake bite etc. The present work was undertaken to extract essential oil from *Saussurea simpsoniana* and identifying its chemical composition. Essential oil was extracted by hydrodistillation. Chemical constituents of the essential oil were separated and identified by means of gas chromatography / Mass spectroscopy (GC-MS). The chromatographic analysis of oil showed the presence of totally 18 constituents. The main components were 5, 8, 11, 14, 17- eicosapentaenoic acid methyl ester (65.42%), 9, 12, 15- octadecatrien-1-ol (14.64%), caryophyllene oxide (2.19%), cedren-13-ol (2.12%) and verrucarol (1.96%). The remaining 13 compounds were found to be in minute quantities. The physicochemical properties of the oil were determined as Specific gravity 1.023g/cc, Specific rotation 21.02, Optical rotation 0.0214 and Refractive index 1.5216. The study indicates that sesquiterpenoids are major components accounting for 88% in the isolated oil. Physicochemical standards were determined for the isolated oil.

Keywords: *Saussurea simpsoniana*, Asteraceae, Essential oil composition, GC-MS, Sesquiterpenoids, Physicochemical properties.

INTRODUCTION

Plants are used medicinally all over the world both in crude and purified form. They are major source of many potent and powerful drugs¹. The Asteraceae family comprises approximately 1000 genera and 30,000 species distributed more or less throughout the globe of which approximately 177 genera and 1052 species are found in India². The genus *Saussurea* of the same family accounts for about 300 species in the world³, of which 61 species exist in India⁴. It grows in the sub alpine regions of Jammu & Kashmir, Himachal Pradesh and Uttranchal at an altitude of 3200- 3800m⁵. The major constituents of this genus includes flavonoidal glycosides, triterpenoids⁶, essential oil etc⁷⁻¹². *Saussurea* species are reported to have an anti-bacterial, hepatoprotective, anti-ulcer, immunomodulator, hypolipidemic and hypoglycemic activity¹³. It has been traditionally used for alleviating pain in abdominal distention and tenesmus, indigestion with anorexia, dysentery, nausea, and vomiting¹⁴ and also used for treatment of halitosis, dental caries, and periodontal disease^{15, 16}.

Hydrodistillation is a traditional method for extraction of essential oils¹⁷. It is a versatile process that can be employed both in small and large industries¹⁸. Hydrodistillation using clavenger's apparatus is the official method for the isolation of volatile oils and several researchers have used this technique to obtain volatile oil from different plant sources¹⁹.

MATERIALS AND METHODS

Aerial parts of *Saussurea simpsoniana* variety were collected from kinnaur district, Himalayas. The plant has been identified and authenticated at National Ayurveda Dietetics Research Institute, Bangalore.

Ref No. Drug Authentication /SMPU/NADR/BNG/2010-11/471. The voucher specimen of the plant was deposited in the Department of Pharmacognosy, Dayananda Sagar College of Pharmacy, Bangalore.

Extraction of essential oil

The cut pieces of aerial parts of *Saussurea simpsoniana* were subjected to hydrodistillation using Clavenger's apparatus²⁰.

Steam distillation

Known weight of aerial parts were taken in a reaction vessel and attached to a steam generator. A water cool condenser was also attached to the reaction vessel. Steam generator produced the steam which was passed through the sample. The essential oil was condensed and collected in the collecting tube. The oil was dried over anhydrous sodium sulphate and stored in a sealed vial at 4°C till GC-MS analysis was carried out²¹. The yield of the oil was calculated on the basis of fresh weight of sample.

GC-MS analysis

GC clarus 500 Perkin Elmer, equipped with mass detector Turbo mass Gold- Perkin Elmer- Turbomass 5.2 was used for the identification of different components of essential oil of *Saussurea simpsoniana*. Sample was injected on a Elite- 5 MS

(30x 0.25mm x 0.25 μ m df) column. Helium was used as a carrier gas in 1 ml per min and split ratio 101. The column temperature was maintained at 110 °C for 2 min up to 280 °C at the rate of 5 °C per min for a minute hold. Total GC running time was 36 minutes. Various components were identified by their retention time and peak enhancement with standard samples in gas chromatographic mode and NIST version 2005 Library search from the derived mass fragmentation pattern of various components of essential oil.

Estimation of Physical parameters²² of *Saussurea simpsoniana* oil

Solubility

The solubility of the volatile oil was determined by mixing increment volumes of volatile oil in specified volumes of following solvents acetone, chloroform, alcohol and petroleum ether.

Specific gravity

The actual weight or the tare of a vial was determined accurately. The vial was filled with water and weighed. The procedure was repeated using the volatile oil in place of water. The specific gravity of the oil is expressed as the ratio of the weight of the oil to that of an equal volume of pure water when both are determined at 25°C.

Specific rotation

Both the degree of rotation and its direction are important criteria of purity. The extent of optical activity of oil was determined by using a polarimeter which measures the degree of rotation. The zero on the polarimeter was adjusted. Previously cleaned and dried polarimeter tube was filled with 1% solution of volatile oil in acetone. The analyzer was rotated until equal illumination of light of the two halves of the visual field was achieved. The extent of rotation was read on scale, which denotes optical rotation of the sample.

Refractive Index

The test plate was attached to the refracting prism of Abbe's refractometer by moistening the plate with the liquid and pressing it against the refractive prism. The light was focused on the test plate. The instrument was adjusted until the borderline of the critical angle coincides with the cross hairs in the telescope, and the reading was taken. The test plate was removed, cleaned, and 2-3 drops of volatile oil was placed on the prism and the prism was clamped together firmly. The light source was fixed so that the light is reflected through the prisms and the instrument was adjusted until the borderline between the light and dark halves of the field of view exactly coincides with the cross hairs of the telescope. The reading was noted.

Thin Layer Chromatography

Stationary phase precoated silica Gel- G , mobile phase Toulene: Ethyl acetate (9.3 0.7) Detecting agent Vanillin in sulphuric acid.

Estimation of chemical parameters

Acid value

1.0g of the oil was accurately weighed and dissolved in 5 ml of a mixture of equal volumes of ethanol and ether, previously neutralized with 0.1M KOH to phenolphthalein solution and titrated with 0.1M KOH until the solution remains faintly pink after shaking for 30 secs.

Ester content

About 0.5g of the oil was accurately weighed and placed in a 250ml conical flask. 8ml of alcoholic KOH (0.5M) was added to it. Then it was fitted with a reflux condenser, a little pumice powder was added & boiled under reflux on a waterbath for 30 min. 1-2 drops of phenolphthalein solution was added and titrated immediately with 0.5 M HCl. The titration was repeated omitting the oil.

RESULTS & DISCUSSION

Extraction of essential oil

Essential oil from the whole plant of *Saussurea simpsoniana*, which was collected from Himalayas, was obtained by hydrodistillation technique. The yield of the oil was 0.06% (v/w).

Estimation of Physical parameters of *Saussurea simpsoniana* oil

The physicochemical properties like solubility, specific gravity, specific rotation, Refractive Index, acid value and ester content of the extracted oil was determined as per standard procedures. The observations are reported in Table 1 & 2. The Thin layer chromatography of the extracted oil was carried out using mobile phase, Toulene: Ethyl acetate (9.3 0.7). The spots were detected using vanillin sulphuric acid. Three prominent spots having the R_f value of 0.611 (blue black), 0.492 (light violet) and 0.417 (violet) were observed.

Table 1: Physical constants of the *Saussurea simpsoniana* oil

S.No.	Physical parameters	Values
1.	Specific Gravity	1.023 g/cc
2.	Specific rotation	21.02
3.	Optical rotation	0.0214
4.	Refractive index	1.5216

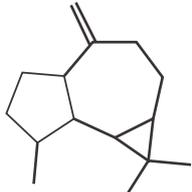
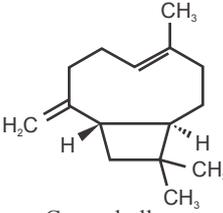
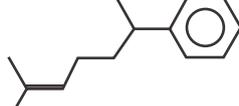
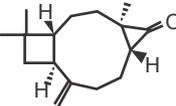
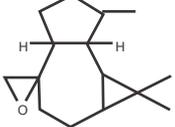
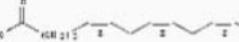
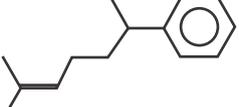
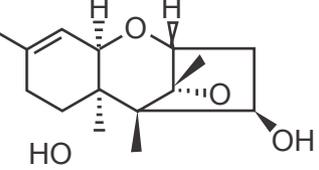
Table 2: Chemical Constants of the *Saussurea simpsoniana* oil

S.No.	Chemical parameter	Values
1.	Acid value	3.36
2.	Ester value	5.61

Table 3: Chemical composition of *Saussurea simpsoniana* oil.

S.No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1.	6.79	Cyclohexane, 1-ethenyl- 1-methyl-2,4-bis(1- methylethenyl)-, [1s- (1 α ,2 β ,4 β)]-	C ₁₅ H ₂₄	204	0.46
2	7.20	c-Elemene	C ₁₅ H ₂₄	204	0.12
3	7.23	Caryophyllene	C ₁₅ H ₂₄	204	0.29
4	7.85	Aramadenrene	C ₁₅ H ₂₄	204	0.09
5	7.90	Benzene, 1-(1,5- dimethyl-4-hexenyl)-4- methyl-	C ₁₅ H ₂₄	202	0.18
6	8.09	Naphthalene, 1,2,3,4,4a,5,6,8a- octahydro-4a,8- dimethyl-2-(1- metylethenyl)-,[2R- (2a,4,4aa,8aa)]-	C ₁₅ H ₂₄	204	0.19
7	10.10	9,12,15-Octadecatrien- 1-ol, (z,z,z)-	C ₁₈ H ₃₂ O	264	14.64
8	11.37	Cedren-13-ol, 8-	C ₁₅ H ₂₄ O	220	2.12
9	12.10	Caryophyllene Oxide	C ₁₅ H ₂₄ O	220	2.19
10	13.49	Oxacyclotetradeca-4,11- diyne	C ₁₅ H ₂₄ O	190	11.10
11	14.53	5,8,11,14,17- Eicosapentaenoic acid, methyl ester, (all-z)-	C ₁₅ H ₂₄ O ₂	316	65.42
12	14.73	Verrucarol C 15 H 24 O 4 266 1.96			
13	23.55	9,12-Octadecadienoic acid methyl ester, (E,E)-	C ₁₅ H ₂₄ O ₂	294	0.25
14	25.81	6aBicyclo[4,3,0]nonane, 5a-iodomethyl-1a- isopropenyl-4a,5a- dimethyl-	C ₁₅ H ₂₅ I	332	0.16
15	30.10	10-12-Pentacosadiynoic acid	C ₂₅ H ₂₄ O ₂	374	0.11
16	31.92	Aromadendrene oxide- (2)	C ₂₅ H ₂₄ O	220	0.15
17	33.42	(-)-Spathulenol C 25 H 24 O 220 0.19 18 34.05 Lanceol, cis	C ₂₅ H ₂₄ O	220	0.36

Table 4: Compounds identified in *Saussurea simpsoniana* oil by GC-MS Method.

 Aromadenrene	 Caryophyllene	 Cyclohexane 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1s-(1 α ,2 β ,4 β)]-
 Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl -	 9,12,15-Octadecatrien-1-ol, (z,z,z)-	 Caryophyllene Oxide
 (-)-Spathulenol	 Aromadendrene oxide-(2)	 5,8,11,14,17-Eicosapentaenoic acid methyl ester, (all-z)-
 Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl -	 Verrucarol	 9,12-Octadecadienoic acid, methyl ester, (E,E)-

GC-MS analysis

The oil obtained was analyzed by GC-MS to know its constituents. The results are shown in Table 3 and the structures are represented in Table 4.

Totally 18 compounds were identified out of that 1 mono, 15 sesqui and 2 diterpenes are present. The main compounds were found to be 5,8,11,14,17- Eicosapentaenoic acid methyl ester- 65.42%, followed by 9, 12, 15- Octa decatrien-1-ol- 14.64%, Oxacyclotetra deca-4,11-diene- 11.10%, Caryophyllene oxide- 2.19%, Cedren-13-ol- 2.12% and verrucarol- 1.96% . Similar type of sesquiterpenoids from Costus root oil (*Saussurea lappa*) are reported^{23, 24} . The difference in the relative proportion of the components of the oils can be attributed to different growing conditions perhaps to differing agronomic practices and different harvesting time.

CONCLUSION

The main focus of the work was to extract the volatile oil from *Saussurea simpsoniana*, as till now composition of the essential oil of *Saussurea simpsoniana* plant has not been reported in literature and to determine its physicochemical properties, characterize its chemical constituents. Chemically, volatile oil is a mixture of several constituents such as hydrocarbons, acids, alcohols, esters (Terpenes & Terpenoids) etc. These constituents can be determined in terms of saponification value, acid value etc., which serve to detect adulteration and to establish the quality and purity of volatile oils. Structures of oil constituents were characterized and confirmed with the help of analytical datas such as TLC, GC-MS with NIST 2005 Library.

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