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Chemical composition of the essential oil of *Viola serpens* from Bageshwar (Shama), Uttarakhad, India

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The families Violaceae (alternatively known as Alsodeiace or Leoniiaceae or Retrosepalaceae) comprise twenty genera and about 800 species. *Viola serpens* belongs to family Violaceae and commonly known as “Banafsa”. It is a small glabrous, perennial herb, which is found throughout India in moist woods and hilly districts. The essential oil of aerial parts of *V. serpens*, were extracted by steam distillation. The quantitative and qualitative analysis of volatile essential oil constituents of the plant was done by Gas Chromatography (GC) and GC-Mass Spectrometry. A total of 50 components of the essential oil of *V. serpens* were identified, accounting for 81.38% of the total oil. The main compounds found were Bis (2-ethylhexyl) maleate (15.62%), 2, 4, 6-Tetramethyl-2-heptene (11.52%), Hexen-3-ol (6.56%), and Cis Verbeno (1.47%). The chemical constituents in the essential oil from *V. serpens* were identified in the following classes or groups of chemical compounds, such as monoterpenes, sesquiterpenes volatile organic compounds and their oxygenated hydrocarbons. Therefore, the essential constituents could be used as antioxidant, antifungal or antimicrobial agent in new drugs preparation for therapy of infectious diseases.

**Key words:** *Viola serpens*, essential oil, gas chromatography, mass spectrometry.

**INTRODUCTION**

Mother earth has gifted the mankind with lots of plants which has the ability for curing the health disorders of human being. These feature has been identified in the pre-historic times (Balakumbahan et al., 2010), and the world wide use of herbal therapies and health care preparations that are prescribed in ancient books like vEDAS and the bibles pave way for the discovering of natural products with medicinal values (Bhuvaneswari and Balasundaram, 2009). 80% of the world’s population meets their primary health care through traditional medicines, as estimated by WHO. Medicinal plants possess secondary metabolites which are the main sources of medicinal drugs having curative nature. 7500 species are being used as medicinal plants in India (Balakumbahan et al., 2010). *Viola serpens* Wall belongs to family Violaceae and commonly known as “Banafsha”.

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It is a small glabrous, perennial herb, which is found throughout India in moist woods and hilly districts. It is also found in China, Java, Ceylon, Philippines, and Thailand up to an altitude of 2000 m in India. It is distributed in the Himalayan region, hills of Meghalaya, Nagaland, and Manipur (Bal, 1932; Dhar and Kachroo, 1983). It is also found in Ganjam Hills of Orissa, Himachal Pradesh, Uttarakhand, Karnataka and Tamilnadu (Chawdhary and Wadhwa, 1984). The whole plant is medicinally useful. It is aperient, antiseptic, antipyretic, cooling, demulcent, diaphoretic, diuretic, emetic, emollient, expectorant, febrifuge, and purgative in action. It is one of the most useful medicinal plants and used as antipyretic, demulcent, diaphoretic and diuretic drug. It is useful in asthma, bleeding piles, cancer of the throat, constipation, cough, fever, skin diseases and headache (Kumar and Digvijay, 2014). Some workers reported glycoside methyl salicylate, quercitrin, alkaloid, volatile gum, mucilage, sugar and saponin, saponins, tannins, amino acids, terpenoids, reducing sugars, glycosides, and flavonoids were isolated form whole plants of V. serpens.

MATERIALS AND METHODS

Plant material

The plant V. serpens was collected in the month of October, 2013 from Shama (Kapkote) 52 km away from Bageshwar, Uttarakhnad, India. The plant was authenticated by Botanical Survey of India (BSI), Dehradun. A voucher specimen (No.114835) was deposited in the Herbarium Section at BSI, Dehradun, India.

Essential oil extraction

The fresh aerial parts of V. serpens (5 kg) were chopped and steam-distilled using copper still fitted with spiral glass condensers. The distillate was saturated with NaCl and extracted with n-hexane. Anhydrous Na2SO4 was then added to dry the organic phase which was separated using separating funnel and finally the solvent was evaporated under reduced pressure. The percentage content of the oil was calculated on the basis of dry weight of plant material. The oil was then stored in screw-capped vials, under refrigeration until needed.

Gas chromatographic analysis (GC)

The oil was analyzed by using a Shimadzu 2010 (Phenomenex, Inc., Torrance CA, USA) auto system GC. The column temperature was programmed at 80°C (holding time for 2 min) to 210°C (holding time 5 min) at 3°C min⁻¹ and then 210 to 300°C at 20°C min⁻¹ with final hold time of 15 min, using N2 at 30.0 ml/min column head pressure as carrier gas, the injector temperature was 270°C and detector (FID, Flame ionization detector) temperature 280°C.

GC-MS analysis and identification

The GC-MS was used Autosystem 2010 GC (Rtx-5, 30 m × 0.25 mm, I.D. FID 0.25 μm) coupled with Shimadzu QP 2010 plus with thermal desorption system TD 20 with (Rtx-5) fused silica capillary column (30 m × 0.25 mm with film thickness 0.25 μm). The column temperature was 80°C (holding time for 2 min) to 210°C (holding time 5 min) at 3°C min⁻¹ and then 210 to 300°C at 20°C min⁻¹ with final hold time of 21 min, using helium as carrier gas. The injector temperature was 230°C 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 to 650 amu.

Identification of the compounds

Identification of constituents were done on the basis of Retention Index (RI), determined with reference to homologous series of n-alkanes Cn-C28, under identical experimental condition), MS library search (NIST and WILEY), and by comparison with MS literature data (Adams, 2007). The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor. Retention indices (RI) were determined with reference to a homologous series of normal alkanes, by using the following formula (Kovats, 1958).

\[ KI = 100 \left( \frac{n+ (N-n)}{n} \right) \left( \frac{\log t_R^{1} (\text{unknown}) - \log t_R^{1} (\text{Cn})}{\log t_R^{1} (\text{CN}) - \log t_R^{1} (\text{Cn})} \right) \]

where \( t_R^{1} \) is the net retention time (\( t_b - t_t \)); \( t_t \) is the retention time of solvent (dead time); \( t_b \) is the retention time of the compound; \( C_n \) is number of carbons in longer chain of alkane; \( C_n \) is number of carbons in shorter chain of alkane; \( n \) is the number of carbon atoms in the smaller alkane; \( N \) is the number of carbon atoms in the larger alkane.

RESULTS AND DISCUSSION

The GC and GC-MS analysis of leaf oil of V. serpens resulted in the identification of 50 constituents in Table 1. The identified constituents of the oil are listed in Table 1 in the order of their elution in Rtx-5 column. The main compounds found were Bis(2-ethylhexyl) maleate 15.62%, 2,4,4,6-Tetramethyl-2-heptene 11.52%, Hexen-3-ol 6.56%, and Cis Verbenol 4.77% (Figure 1). The minor chemical constituents were found to be Phytol acetate 0.08%, Tetracosane 0.16%, Germacrene B 0.21%, Ethyl Lactate 0.22%.

Essential oils are found in various parts of the plants, such as leaf, flower, root and are stored in special oil cells and gates. The essential oils extracted from plants are indispensable materials in the pharmaceutical, food, and cosmetics sectors, because of the increasing concern with harmful synthetic additives (Sacchetti et al., 2005). A great majority of the essential oils are used as fragrance in perfumes and aromas in food industry. The essential oils have a number of biological activities, including antibacterial, antifungal and antioxidant properties (Fatouma et al., 2011 and Jihua et al., 2011).

Essential oils constitute a major group of agro-based industrial products and they find applications in various types of industries, such as food products, drinks, perfumes, pharmaceuticals and cosmetics (Anwar et al., 2009a, b; Burt, 2004; Celiktas et al., 2007; Hammer et al., 2008; Hay and Svoboda, 1993; Hussain et al., 2008;
### Table 1. Essential oil composition of *Viola serpens*

| Compound                              | Area (%) | Molecular formula | Molecular weight | RI   | Mode of identification |
|---------------------------------------|----------|-------------------|------------------|------|------------------------|
| Hexen-3-ol                            | 6.56     | C₆H₁₀O             | 100              | 778  | a,b                    |
| Ethyl Lactate                         | 0.22     | C₆H₁₀O₂            | 118              | 814  | a,b                    |
| 3-Methylene-1,7-octadiene             | 1.4      | C₆H₁₂               | 122              | 863  | a,b                    |
| 2,4,6-Tetramethyl-2-heptene           | 11.52    | C₇H₁₄O₂            | 154              | 951  | a,b                    |
| 2,5-Heptanedione                      | 0.73     | C₈H₁₄O₂            | 128              | 989  | a,b                    |
| 2-Isopropyl-5-oxohexanal              | 2.55     | C₄H₈O              | 156              | 1112 | a,b                    |
| cis Verbenol                          | 4.77     | C₁₀H₁₆O            | 152              | 1141 | a,b                    |
| 2-Hexyltetrahydrofuran                | 0.89     | C₁₀H₁₆O            | 156              | 1147 | a,b                    |
| Isogeranial                           | 0.5      | C₁₀H₁₆O            | 152              | 1179 | a,b                    |
| 2,3-Dimethylundecane                  | 1.8      | C₁₀H₂₈            | 184              | 1185 | a,b                    |
| Methyl Salicylate                     | 0.8      | C₁₀H₁₇O₃           | 152              | 1192 | a,b                    |
| Verbenyl acetate                      | 1.42     | C₁₀H₁₇O₂           | 194              | 1282 | a,b                    |
| Methyl Myrtenate                      | 0.35     | C₁₀H₁₇O₂           | 180              | 1296 | a,b                    |
| n-Tridecanol                          | 1.28     | C₁₁H₂₄             | 184              | 1313 | a,b                    |
| 3-Methoxy 3-Decanone                  | 1.57     | C₁₁H₂₀O₂           | 186              | 1327 | a,b                    |
| α-Copaene                             | 0.38     | C₁₀H₂₄             | 204              | 1375 | a,b                    |
| 4,8-Dimethyltridecane                 | 0.47     | C₁₁H₂₄             | 212              | 1384 | a,b                    |
| α-Elemene                             | 0.52     | C₁₁H₂₄             | 204              | 1390 | a,b                    |
| Caryophyllene                         | 0.42     | C₁₁H₂₄             | 204              | 1424 | a,b                    |
| Aromadendrene                         | 1.31     | C₁₁H₂₄             | 204              | 1438 | a,b                    |
| β-Farnesene                           | 1.11     | C₁₁H₂₄             | 204              | 1452 | a,b                    |
| 6-Methyl-2-tridecane                  | 0.47     | C₁₂H₂₈O            | 212              | 1485 | a,b                    |
| Valencene                             | 0.38     | C₁₀H₂₄             | 204              | 1492 | a,b                    |
| Germanene B                           | 0.21     | C₁₀H₂₄             | 204              | 1544 | a,b                    |
| 1-Iodo-2-methylundecane               | 0.32     | C₁₁H₂₆I           | 296              | 1564 | a,b                    |
| Myrcenol                              | 0.88     | C₁₀H₁₈O            | 154              | 1586 | a,b                    |
| Longiborneol                          | 1.41     | C₁₀H₁₈O            | 222              | 1601 | a,b                    |
| (5-Iodopentyl)benzene                 | 0.32     | C₁₀H₁₇I           | 274              | 1606 | a,b                    |
| Cetane                                | 0.27     | C₁₀H₁₆             | 226              | 1612 | a,b                    |
| Epicubeno                             | 1.62     | C₁₀H₂₈O            | 222              | 1631 | a,b                    |
| Cadinene                              | 0.44     | C₁₁H₂₄             | 204              | 1676 | a,b                    |
| Heptadecane                           | 2.88     | C₁₁H₂₆             | 240              | 1700 | a,b                    |
| Pentadecane                           | 1.21     | C₁₁H₂ₐO           | 226              | 1701 | a,b                    |
| (1-Ethynyl)benzene                    | 0.91     | C₁₁H₂₈             | 232              | 1724 | a,b                    |
| Phytone                               | 2.29     | C₁₁H₁₈O           | 268              | 1841 | a,b                    |
| Nonadecane                            | 1.62     | C₁₁H₂₆             | 268              | 1900 | a,b                    |
| (1-Ethylundecyl)benzene               | 0.94     | C₁₁H₂₆             | 260              | 1922 | a,b                    |
| Tridecanol, 3-phenyl                   | 0.26     | C₁₁H₂₆             | 138              | 1924 | a,b                    |
| n-Hexadecanoic acid                   | 1        | C₁₁H₁₂₂₃₄O₂         | 256              | 1968 | a,b                    |
| Eicosane                              | 0.84     | C₁₂H₂₄             | 282              | 2009 | a,b                    |
| Z-2-Octadecen-1-ol                    | 1.13     | C₁₁H₁₇O₃           | 268              | 2061 | a,b                    |
| n-Heneicosane                         | 0.35     | C₁₁H₂₄             | 296              | 2109 | a,b                    |
| 6-phenyl Pentadecane                  | 0.77     | C₁₁H₁₆             | 288              | 2121 | a,b                    |
| Geranylgeraniol                       | 1.19     | C₁₁H₁₈O            | 290              | 2192 | a,b                    |
| n-Docosane                            | 0.3      | C₁₂H₂₄             | 310              | 2200 | a,b                    |
| Phytol acetate                        | 0.08     | C₁₂H₂₄O₂           | 338              | 2212 | a,b                    |
| Octadecanoic acid, 2-oxo-, methyl ester| 1.54   | C₁₁H₁₇O₃           | 312              | 2213 | a,b                    |
| Bis(2-ethylhexyl) maleate             | 15.62    | C₁₀H₁₇O₄           | 340              | 2224 | a,b                    |
| Tetracosane                           | 0.16     | C₁₂H₂₀             | 338              | 2407 | a,b                    |
| Oxalic acid, hexyl tetradecyl ester   | 1.4      | C₁₂H₂₄O₄           | 370              | 2543 | a,b                    |
| Total Identified                      |          |                   |                  | 81.38| a,b                    |

a=Retention index (RI), b=MS (GC-MS).
The compounds from the plant based essential oil are useful as an alternative therapy, either directly or as models for new synthetic products (Houghton, 2000). Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure diseases, infections and indispositions by means of inhalation (Buchbauer, 2000; Buchbauer et al., 1993). This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects. Hopefully, this will lead to new information on plant applications and new perspective on the potential use of these natural products.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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