academicJournals

Vol. 11(32), pp. 513-517, 25 August, 2017
DOI: 10.5897/JMPR2015.5874
Article Number: 58D1EE065745
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

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

Deepak Chandra[1*], Gunjan Kohli[2], Kundan Prasad[2], G. Bisht[2], Vinay Deep Punetha[2] and H. K. Pandey[2]

[1]Department of Chemistry, D.S.B. Campus Nainital 263002, Uttarakhand India.
[2]Defence Institute of Bio-Energy Research (DIBER) Pithoragarh, Uttrakhand-262501, India.

Received 22 June, 2015; Accepted 27 August, 2015

The families Violaceae (alternatively known as Alsodeiace or Leoniaceae 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, 4, 6-Tetramethyl-2-heptene (11.52%), Hexen-3-ol (6.56%), and Cis Verbeno (l 4.77%). The chemical constituents in the essential oil from *V. serpens* were identified in the following classes or groups of chemical compounds, such as monoterpens, 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.

INRTODUCTION

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".

*Corresponding author. E-mail: deepakk006@gmail.com.

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 Wadhawa, 1984). The whole plant is medicinally useful. It is aperients, 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, voiline 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, Uttarakhand, 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 Na_2SO_4 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 N_2 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 used was 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 C_8-C_{28}, 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).

$$KI = 100\ [\ n + (N-n)\ \times\ \frac{\log t^1_R\ (\text{unknown}) - \log t^1_R\ (C_n)}{\log t^1_R\ (CN) - \log t^1_R\ (Cn)}\ ]$$

where t^1_R is the net retention time ($t_R - t_0$); t_0 is the retention time of solvent (dead time); t_R 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 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%.

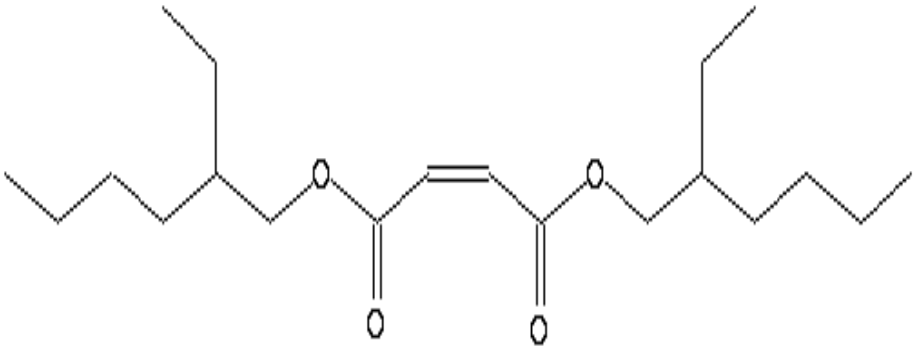
Essential oils are found in various parts of the plants, such as leaf, flower, root and are stored in special oil cells and gates. The essential oils extracted from plants are indispensable materials in the pharmaceutical, food, and cosmetics sectors, because of the increasing concern with harmful synthetic 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;

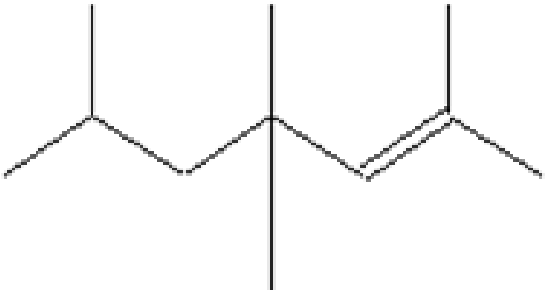
Table 1. Essential oil composition of *Viola serpens*

Compound	Area (%)	Molecular formula	Molecular weight	RI	Mode of identification
Hexen-3-ol	6.56	$C_6H_{12}O$	100	778	a,b
Ethyl Lactate	0.22	$C_5H_{10}O_3$	118	814	a,b
3-Methylene-1,7-octadiene	1.4	C_9H_{14}	122	863	a,b
2,4,4,6-Tetramethyl-2-heptene	11.52	$C_{11}H_{22}$	154	951	a,b
2,5-Heptanedione	0.73	$C_7H_{12}O_2$	128	989	a,b
2-Isopropyl-5-oxohexanal	2.55	$C_9H_{16}O_2$	156	1112	a,b
Cis Verbenol	4.77	$C_{10}H_{16}O$	152	1141	a,b
2-Hexyltetrahydrofuran	0.89	$C_{10}H_{20}O$	156	1147	a,b
Isogeranial	0.5	$C_{10}H_{16}O$	152	1179	a,b
2,3-Dimethylundecane	1.8	$C_{13}H_{28}$	184	1185	a,b
Methyl Salicylate	0.8	$C_8H_8O_3$	152	1192	a,b
Verbenyl acetate	1.42	$C_{12}H_{18}O_2$	194	1282	a,b
Methyl Myrtenate	0.35	$C_{11}H_{16}O_2$	180	1296	a,b
n-Tridecane	1.28	$C_{13}H_{28}$	184	1313	a,b
0-methoxy 3-Decanone	1.57	$C_{11}H_{22}O_2$	186	1327	a,b
α-Copaene	0.38	$C_{15}H_{24}$	204	1375	a,b
4,8-Dimethyltridecane	0.47	$C_{15}H_{32}$	212	1384	a,b
β-Elemene	0.52	$C_{15}H_{24}$	204	1390	a,b
Caryophyllene	0.42	$C_{15}H_{24}$	204	1424	a,b
Aromadendrene	1.31	$C_{15}H_{24}$	204	1438	a,b
β -Farnesene	1.11	$C_{15}H_{24}$	204	1452	a,b
6-Methyl-2-tridecanone	0.47	$C_{14}H_{28}O$	212	1485	a,b
Valencene	0.38	$C_{15}H_{24}$	204	1492	a,b
Germacrene B	0.21	$C_{15}H_{24}$	204	1544	a,b
1-Iodo-2-methylundecane	0.32	$C_{12}H_{25}I$	296	1564	a,b
Myrcenol	0.88	$C_{10}H_{18}O$	154	1586	a,b
Longiborneol	1.41	$C_{15}H_{26}O$	222	1601	a,b
(5-Iodopentyl)benzene	0.32	$C_{11}H_{15}I$	274	1606	a,b
Cetane	0.27	$C_{16}H_{34}$	226	1612	a,b
Epicubeno	1.62	$C_{15}H_{26}O$	222	1631	a,b
Cadinene	0.44	$C_{15}H_{24}$	204	1676	a,b
Heptadecane	2.88	$C_{17}H_{36}$	240	1700	a,b
Pentadecanal	1.21	$C_{15}H_{30}O$	226	1701	a,b
(1-Ethylnonyl)benzene	0.91	$C_{17}H_{28}$	232	1724	a,b
Phytone	2.29	$C_{18}H_{36}O$	268	1841	a,b
Nonadecane	1.62	$C_{19}H_{40}$	268	1900	a,b
(1-Ethylundecyl)benzene	0.94	$C_{19}H_{32}$	260	1922	a,b
Tridecane, 3-phenyl	0.26	$C_{19}H_{32}$	138	1924	a,b
n-Hexadecanoic acid	1	$C_{16}H_{32}O_2$	256	1968	a,b
Eicosane	0.84	$C_{20}H_{42}$	282	2009	a,b
Z-2-Octadecen-1-ol	1.13	$C_{18}H_{36}O$	268	2061	a,b
n-Heneicosane	0.35	$C_{21}H_{44}$	296	2109	a,b
6-phenyl Pentadecane	0.77	$C_{21}H_{36}$	288	2121	a,b
Geranylgeraniol	1.19	$C_{20}H_{34}O$	290	2192	a,b
n-Docosane	0.3	$C_{22}H_{46}$	310	2200	a,b
Phytol acetate	0.08	$C_{22}H_{42}O_2$	338	2212	a,b
Octadecanoic acid, 2-oxo-, methyl ester	1.54	$C_{19}H_{36}O_3$	312	2213	a,b
Bis(2-ethylhexyl) maleate	15.62	$C_{20}H_{36}O_4$	340	2224	a,b
Tetracosane	0.16	$C_{24}H_{50}$	338	2407	a,b
Oxalic acid, hexyl tetradecyl ester	1.4	$C_{22}H_{42}O_4$	370	2543	a,b
Total Identified	81.38%	-	-	-	-

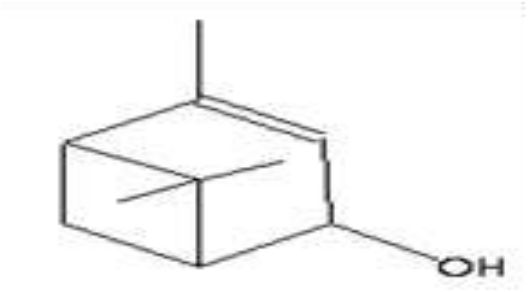
a=Retention index (RI), b=MS (GC-MS).



Bis(2-ethylhexyl) maleate



2, 4, 4, 6-Tetramethyl-2-heptene



Cis Verbenol

Figure 1. Structure of major isolated compound

Teixeira da Silva, 2004). 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.

ACKNOWLEDGEMENTS

The authors are grateful to AIRF, Jawaharlal Nehru University, New Delhi for the G.C coupled with Mass Spectrometry (GC-MS), and G.C with FID analysis facilities.

REFERENCES

Adams RP (2001). Identification of Essential oils by Gas Chromatography Quadruple Mass Spectrometry. Allured Publishing Corporation, Carol Stream, USA.

Anwar F, Hussain AI, Sherazi STH, Bhanger MI (2009). Changes in composition and antioxidant and antimicrobial activities essential oil of fennel (*Foeniculum vulgare* Mill.) fruit at different stages of maturity. J. Herbs Spices Med. Plants 15:1-16.

Bal SN (1932). The Industrial Section of the Indian Museum. Calcutta: Botanical Survey of India, Govt. of India, Central Publication Branch; Catalogue of Medicinal Plant Exhibits. pp. 118-119.

Balakumbahan R, Rajamani K, Kumanan K (2010). *Acorus calamus*: An overview. J. Med. Plants Res. 4(25):27-40.

Bhuvaneswari R, Balasundaram C (2009). Anti-bacterial activity of Acorus calamus and some of its derivates against fish pathogen Aeromonas hydrophila. J. Med. Plants Res. 3(7):538-547.

Buchbauer G (2000). The detailed analysis of essential oils leads to the understanding of their properties. Perfumer Flavorist. 25:64-67.

Buchbauer G, Jager W, Jirovetz L, Imberger J, Dietrich H (1993). Therapeutic properties of essential oils and fragrances. In ACS symposium series (USA).

Celiktas OY, Kocabas EEH, Bedir E, Sukan FV, Ozek T, Baser KHC (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on locate and seasonal variations. Food Chem. 100:553-559.

Chawdhary HJ, Wadhawa BM (1984). Calcutta: Botanical Survey of India, Dept. of Environment; 1984. Flora of India, Series 2, Flora of

Himachal Pradesh. P 80.
Dhar U, Kachroo P (1983). India: Scientific Publishers; Alpine Flora of Kashmir. Himalaya pp. 86-87.
Fatouma M, Abdoul-Latif, Nabil M, Prosper E, Adwa AA, Samatar OD, Louis-Clément O, Ismael HNB, Mamoudou HD (2011). Antimicrobial and antioxidant activities of essential oil and methanol extract of Matricaria chamomilla L. from Djibouti. J. Med. Plants Res. 5(9):1512-1517.
Hammer KA, Carson CF, Dunstan JA, Hale J, Lehmann H, Robinson CJ, Prescott SL, Riley TV (2008). Antimicrobial and anti-inflammatory activity of five Taxandria fragrans oils in vitro. Microbiol. Immunol. 52:522-530.
Hay RKM, Svoboda KP (1993). In Volatile oil crops: their biology, biochemistry and production. In Hay RKM, Waterman PG (eds.), Longman Scientific and Technical, Harlow. pp. 5-22.
Houghton PJ (2000). Use of small scale bioassays in the discovery of novel drugs from natural sources. Phytother. Res. 14:419-423.
Hussain AI, Anwar F, Sherazi STH, Przybylski R (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem. 108:986-995.
Jihua W, Liang X, Ling Y, Zhilong L, Ligang Z (2011). Composition, Antibacterial and Antioxidant Activities of Essential Oils from *Ligusticum sinense* and *L. jeholense* (Umbelliferae) from China. Rec. Nat. Prod. 5:314-318.
Kovats E (1958). Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone. Hel. Chim. Acta 41(7):1915-32.
Kumar P, Digvijay (2014). Assessment of Genetic Diversity of Viola Serpens Wall. In Himachal Pradesh Using Molecular Markers. World J. Pharm. Res. 3(2):2716-2726.
Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem. 91:621-632.
Teixeira da Silva JA (2004). Mining the essential oils of the Anthemideae. Afr. J. Biotechnol. 3:706-720.