Journal of Natural Products and Resources 1(1) (2015) 25–30

ISSN: 2455-0299

Contents List available at JACS Directory

Journal of Natural Products and Resources

journal homepage: http://www.jacsdirectory.com/jnpr

Comparison of Chemical Composition and Antioxidant Potential of Hydrodistilled Oil and Supercritical Fluid CO_2 Extract of *Valeriana wallichi* DC

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ARTICLE DETAILS

Article history:
Received 01 October 2015
Accepted 12 October 2015
Available online 23 October 2015

Keywords:
Valeriana wallichi DC
Hydrodistillation
Supercritical Fluid CO_2 Extraction
Antioxidant Activities
GC-MS

ABSTRACT

In the present study, chemical constituents of the essential oil and supercritical fluid extracts of *Valeriana wallichi* DC obtained by hydrodistillation and supercritical fluid CO_2 extraction have been studied by GC-MS technique. Seventy six compounds were obtained from hydro distillation, representing 98.6% of the total oil. The major component of hydro distillation was isovaleric acid (37.69%), methylvaleric acid (17.24%) and seychellene (6.49%) along with many other components in minor amounts. Ninty eight and seventy six components were identified by GC-MS in the supercritical CO_2 fluid extracts extracted at 100 and 200 bar respectively, where the major components were cis-adamantane-2-carboxylic acid, 4-hydroxy (36.88%, 40.26%), isovaleric acid (5.91%, 6.75%), β-bisabolol (6.48%, 5.71%), bornyl isovalerate (5.08%, 5.54%), of the *Nardostachys jatamansi* extract at 200 and 100 bar respectively. Other major components present in the supercritical fluid extracts were β-methasone valerate, valeranone, benzyl isovalerate , nerolidol, methylvaleric acid and geranyl isovalerate which were usually absent or trace in the hydrodistilled essential oil. The antioxidant activity of essential oil and supercritical fluid extracts was evaluated in DPPH radical, superoxide radical, hydroxyl scavenging assays. Reducing power of those oil and extracts were also studied. Essential oil and Supercritical fluid extracts gave comparable DPPH, superoxide radical scavenging and reducing power activity and there is no much deviation among various activities was observed. However, when we consider overall antioxidant assays, 200 bar extract of valerain is the best among essential oil and extracts.

1. Introduction

Indian valerian roots of *Valeriana wallichi* de Candolle, family *Valerianaceae*, which grows wild in the temperate Himalayas on the borders of Kashmir at altitude of 1500-3000 m and Afghanistan [1]. It is commonly known as 'tagar' in India and also called as Valerian, Indian valerian, Valerian jatamansi [2]. It is an ingredient of herbal medicines in Indian systems of medicine [3]. It is a small perennial herb of 14-45 cm height, with root stock, thick branching stem, sharply pointed leaves, white or pink flowers in clusters and hairy fruit [4]. The rhizome is solid also tough internally, it is greenish-brown in colour along with the odour is effectively valerianaceous [5].

Roots of *V. wallichi* are used as an aphrodisiac, insecticide and in mental disorders [6]. In India, Valeriana has long been used in Ayurveda and Unani systems of medicine, which describes its uses in skin diseases, Insanity, epilepsy and snake bite and considered to have remarkable sedative effects in nervous unrest, stress and neuralgia [7]. Valerian is the top ten selling retail herb for herbal supplements in North America and Australia [8]. It has also been prescribed as the perfect herbal tranquillizer, and was used for this purpose in the First World War to treat soldiers suffering from shell shock [9]. Antiinflammatory [4], antispasmodic [10], antioxidant [11], larvicidal [12], antianxiety [13], anti HIV [14], antidiarrhoeal, and bronchodilatory activities [15] of Valeriana have been scientifically reported. The plant is also used as cytotoxic [16]. Its essential oil exhibited antimicrobial activity against pathogenic bacteria and also exhibited potent antifungal activity against different human and plant fungal pathogens [17].

Literature survey revealed the presence of flavone glycosides [18-19] iridoids and lignans [20-22] in *V. wallichi.* The essential oil from root contain calarene, α-santalene, α-curumene, xanthorrhizol, valeranone, α, β and γ -patchoulene, α-fenchene, patchouli alcohol, maalilol, β-sitosterol, maali-oxide, valerenic acid, isovaleric and β-methylvaleric acid, formic, propionic, butyric, palmitic acid and stearic acids, and isovaleryl ester of D-α-hydroxyisovaleric acid. Patchouli alcohol (40%) as the major constituents followed by the presence of δ-guaiene (10%), seychellene (8%), acetoxyl patchouli alcohol (5%). The presence of valepotriates like valtrate, isovaleroxyhydroxy-dihydrovaltrate, 1α-acevaltrate, and didrovaltrate [23]. The root rhizome parts are highly aromatic and contain valepotriates and essential oils. The essential oil showed maaliol (36.8%) as the major constituent followed by the presence of β-gurjunene (21.3%), acoradiene (9.9%), guiaiol (8.6%) and α-santalene (5.5%) [24].

There are few studies published about the chemical composition and pharmacological aspects of *Valeriana wallichi* and almost all of these published essential oil composition were different from each other. Therefore, the aim of study is to determine the chemical composition of the essential oil and compare with chemical composition of supercritical fluid extracts of *Valeriana wallichi*. However, to the best of our knowledge, this is the first report on the gas chromatography- mass spectrometry (GC-MS) to study the chemical composition of supercritical fluid extracts of *Valeriana wallichi*. Hence, in the present investigation was made to study the antioxidant potential of *Valeriana wallichi* essential oil and supercritical fluid extracts and chemical constituents of the same were analysed by GC-MS.

2. Experimental

2.1 Plant Material

The dried rhizomes of *Valeriana wallichi* DC were purchased from the commercial market and the plant was authenticated by Dr. R. Kumuthakalavalli (Department of Biology, Gandhigram Rural Institute, Deemed University, Gandhigram, Tamil Nadu, India) and a voucher specimen is kept in the department of chemistry of the University for future reference.

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Cite this Article as: D. Sugumar Pandian, N.S. Nagarajan, Comparison of chemical composition and antioxidant potential of hydrodistilled oil and supercritical fluid CO_2 extract of *Valeriana wallichi* DC, J. Nat. Prod. Resour. 1(1) (2015) 25–30.

2.2 Chemicals

1-Diphenyl-2-picrylhydrazyl (DPPH·) radical, ascorbic acid, potassium ferricyanide,, trichloroaceticacid (TCA), hydrogen peroxide and phosphate buffer (pH 7.4), methanol, distilled Water, CO_2, α-tocopherol, tris-HCL buffer, nitroblue tetrazolium chloride (NBT), butylated hydroxyl anisole (BHA), ethylene diamine tetraacetic acid (EDTA), thiobarbituric acid (TBA), nicotinamide adenine dinucleotide (NADH), deoxyribose, ferric chloride. All chemicals used including solvents were of analytical grade.

2.3 Isolation of Oil and Extract

2.3.1 Hydrodistillation Method

The classical method of hydrodistillation using the Clevenger type apparatus for 4 h was used for the isolation of the essential oil from *Valeriana wallichi*. The essential oil was collected and stored at 4 °C until their analysis by gas chromatography/mass spectrometry (GC-MS) and other studies. The essential oil yield was 4 %.

2.3.2 Supercritical Fluid CO_2 Extraction Method

Supercritical CO_2 extraction was performed on a supercritical fluid extractor (ATI technology, Bangalore, India) with the extractor volume 5 kg. The flow rate of CO_2, the extraction temperature and pressure were adjusted by the control panel, and the extraction time was measured by the stopwatch. The CO_2 gas supplied from storage tank to super cooler, where gas CO_2 converted into liquid CO_2 and it went to the extractor. Before liquid CO_2 passed into the extraction vessel, filled with the samples, by the means of a pump, it was pressurized to the desired pressure and heated to the specified temperature in order to reach the supercritical state. In this study, extractions were performed at temperatures 55 °C, two different pressure levels (100 and 200 bar) and dynamic extraction time 1.5 hours each. The supercritical CO_2 flow rate was maintained at 2.5 mL/min and the duration of static extraction time was fixed to 55 °C min. The introduction of some rigid materials such as glass beads with the ground sample, contributed to maintaining a proper flow rate of CO_2 in the extractor vessel as well as in maintaining the desired permissibility of the particle during extraction process [25-26]. The powdered plant material (2 kg) placed into the extractor vessel. The extractions were performed, during the dynamic extraction time, CO_2 carrying the crude extract flowed out of the extraction vessel unit and into a collection vessel. The extracts for each parameter were stored in amber coloured tubes in a refrigerator (4 °C) until further analysis and the yields were 0.41 kg at 100 bar and 0.45 kg at 200 bar. The identification of extracted constituents was carried out by GC-MS.

2.4 GC-MS Analysis

The oil and CO_2 extract was analyzed by GC-MS using on an Agilent GC 6890N model gas chromatograph-5973N model mass spectrometer equipped with a 7683B series auto-sampler injector (Agilent, USA). The GC was equipped with a HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm film thickness). The GC oven temperature was kept at 40 °C for 3 min and programmed to 100 °C at a rate of 25 °C for 5 min, 180 °C at a rate of 25 °C for 5 min, 220 °C at a rate of 25 °C for 5 min and 280 °C at a rate of 25 °C for 12 min . Both oil and extract was analyzed by dissolving in $CHCl_3$. Injection volume was 1.0 µL, inlet pressure was 7.06 psi and the injector temperature was 250 °C. Helium was used as carrier gas and linear velocity (u) was 36 cm/sec. Injection mode was split (30:1) and MS interface temperature was 250 °C. Mass spectra were recorded in the scan mode at energy was 70 eV and MS spectra were scanned from 50 to 550 m/z at 2.2 scan s^{-1}. Compound identification was based on the comparison of estimation retention indices using a MS library. The NIST 05a and Wiley7a spectrometer data bank was used to determine the percentage composition of the compounds [27].

2.5 DPPH Radical Scavenging Activity

Various concentration of hydrodistillated and supercritical fluid CO_2 extracted samples of *Valeriana wallichi* roots individually (0.3 mL of hydro distilled oil, CO_2 extract at 100 bar and 200 bar) mixed with 2.7 mL of methanol solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and allowed to stand for 60 minutes in the dark. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. Ascorbic acid and α-tocopherol were used as standards [28]. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration, using the formula;

$$\% \text{ RSA} = [(Abs_{DPPH} - Abs_s) / (Abs_{DPPH})]x\ 100$$

Where Abs_s is the absorbance of the extract sample and Abs_{DPPH} is the absorbance of the DPPH solution.

2.6 Superoxide Radical Scavenging Activity

The superoxide radical scavenging activity of the various concentrations of hydrodistillated and supercritical fluid CO_2 extracted samples of *Valeriana wallichi* was studied using the method [29]. Superoxide radicals are generated by 1 mL of Tris-HCl buffer (16 mM, pH=8), 1 mL of NBT (50 µM), 1 mL NADH (78 µM). The reaction mixture was incubated at 25 °C for 5 min and the absorbance was measured at 560 nm (Jenway 6100, Dunmow, Essex, UK). A control tube containing Tris-HCl buffer was also processed in the same way without test sample. Different concentration of ascorbic acid and BHA were used as standards. The radical scavenging activity (RSA) was calculated as a percentage of super oxide discoloration, using the equation: $\% \text{ RSA} = [(A_{control}-A_{Sample})/A_{control}] \times 100$.

2.7 Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the various concentrations of hydro distillated and supercritical fluid CO_2 extracted samples of *Valeriana wallichi* was estimated by following the method [30]. The hydroxyl radical was generated by a fenton-type reaction. The reaction mixture contained 0.2 mL of sample in varied concentrations to which, 0.1 mL EDTA (1 mM)-$FeCl_3$ (10 mM) mixture, 0.1 mL H_2O_2 (10 mM), 0.36 mL deoxyribose (10 mM), 0.33 mL phosphate buffer (50 mM, pH 7.4) and 0.1 mL of ascorbic acid (1 mM) was added in sequence. The mixture was incubated at 37 °C for 1 h. To this mixture was added 1.0 mL each of TCA (10%) and TBA (0.67%) and kept in boiling water bath for 20 minutes. The colour developed was read at 532 nm. The control tube contains phosphate buffer, instead of sample.

2.8 Reducing Power

The reducing power of *Valeriana wallichi* was determined [31]. Various concentration of hydrodistillated and supercritical fluid CO_2 extracted samples of *Nardostachys jatamansi* DC of (1 mL), phosphate buffer (1 mL, 0.2M, pH=6.6) and potassium ferricyanide (1 mL, 10 mg/mL) were mixed together and incubated at 50 °C for 20 min. TCA (1 mL, 100 mg/mL) was added to mixture and centrifuged at 8,000 rpm for 5 min. The supernatant (1 mL) was mixed with distilled water (1 mL) and ferric chloride (0.1 mL, 1 mg/mL) and then the absorbance was measured at 700 nm.

2.9 Statistical Analysis

All the results are expressed as mean values standard deviation (SD), n = 3. Data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison *post hoc* test using SPSS software 16.0 versions. Values of $p < 0.05$ were considered statistically significant [32].

3. Results and Discussion

3.1 Chemical Composition of the Extract and Essential Oil

The *Valeriana wallichi* DC extract were extracted in supercritical fluid CO_2 extraction method. Ninety eight (100 bar) and seventy six (200 bar) compounds were identified, representing, 98.6% and 98.76% respectively of the total extract as shown in Table 1 by GC-MS analysis. Their main compounds were cis-adamantane-2-carboxylic acid, 4-hydroxy (36.88%, 40.26%), isovaleric acid (5.91%, 6.75%), β-bisabolol (6.48%, 5.71%), bornyl isovalerate (5.08%, 5.54%), isoquinolin-6,7-diol-1-carboxylic acid, N-acetyl-1-methyl- (1.74%,3.87%), patchouli alcohol (2.19%, 2.22%), β -methasone valerate (1.33%, 2.34%), 1H-indole, 1-acetyl-2,3-dihydro-6-nitro (1.50%, 1.49%), valtrate (0.92%, 1.80%), benzyl isovalerate (0.96%, 1.45%), nerolidol (1.64%, 0.83%), valeranone (1.64%, 0.63%), methylvaleric acid (1.15%, 1.19%), geranyl isovalerate (0.91%, 0.80%) at 100 and 200 bar respectively. Some of the compound only found in CO_2 extract at 100 bar which were propyl valerate, p-cresol, citronellol, phenyl ethyl alcohol and bornyl butyrate. At the same time some of the compound only found in CO_2 extract at 200 bar which were 2,6-Diamino-4-cyclohexyl-4H-thiopyran-3,5-dicarbonitrile and cholestan-3-one, cyclic 1,2-ethanediyl aetal. The phenolic compound of such as 4- terpineol, α-terpineol, carvacrol, p-cresol, patchouli alcohol and α-tocopherol were present in both extracts. The fatty compounds present in 100 and 200 bar extract were palmitic acid, eicosane, tricosane, tetracosane and methyl linoleate etc.

The essential oil of *Valeriana wallichi* was extracted for hydrodistllation method. The results obtained by GC-MS analysis of the oil are presented in Table-1. Seventy six compound of the total oil were identified, representing 98.6%. The major compounds were isovaleric acid (37.69%), methylvaleric acid (17.24%) and seychellene (6.49%). Other compounds

were present considerable amount such as calarene (3.24%), γ-gurjunene (3.40%), patchouli alcohol (3%), aromadendrene (1.62%), α-patchoulene (2.03%), β-patchoulane (1.50%).

Table 1 Chemical constituents and retention indices of the *V. wallichii* oil, SFE extract 100 and 200 bar

Componenets	CO_2 Extract 100 bar	CO_2 Extract 200 bar	H.oil	RI
I-Propyl 6,9,12-hexadecatrienoate	0.21	--	--	203
Methyl isovalerate	--	0.01	0.13	721
Isovaleric acid	5.91	6.75	37.69	811
Furfural	--	--	0.21	831
2-Butenoic acid, 3-methyl-	--	--	0.15	860
Ethyl valerate	--	--	0.03	884
Methylvaleric acid	1.15	1.19	17.24	910
(-)-β-Pinene	0.10	0.19	--	943
Camphene	0.64	0.77	0.12	943
α-Pinene	0.13	0.24	--	948
Propyl valerate	0.03	--	--	984
cis-2,6-Dimethyl-2,6-octadiene	0.56	0.21	--	985
γ-Terpinene	0.01	0.01	--	998
Limonene	0.06	0.08	0.06	1018
Cycloheptane, 1,3,5-tris(methylene)-	0.10	--	--	1039
O-Cymene	0.02	0.03	0.06	1042
Eucalyptol	--	--	0.01	1059
Linalool	0.06	0.02	0.27	1082
Phenylethyl Alcohol	0.01	--	--	1136
4-Terpineol	0.11	0.13	--	1137
Borneol	0.34	0.17	0.04	1138
α-Terpineol	0.05	0.03	0.04	1143
cis-Linalool oxide	--	--	0.05	1164
1,2-Diisopropylbenzene	--	--	0.38	1176
Citronellol	0.03	--	--	1179
Amyl valerate	--	--	0.01	1183
P-Creosol	0.04	--	--	1203
Cyclolongifolene oxide, dehydro-	--	--	0.19	1208
α-Santalene	0.51	0.20	0.69	1211
Hexyl isovalerate	0.06	--	0.05	1218
α-Copaene	0.04	--	0.03	1221
Geraniol	0.08	0.08	--	1228
Thymol methyl ether	0.15	0.14	0.08	1231
Furfuryl 3-methylbutanoate	--	--	0.12	1243
Carvacrol	0.66	0.02	--	1262
1,2,6-Hexanetriol	--	--	0.02	1265
Seychellene	0.73	0.35	6.49	1275
Bornyl acetate	0.92	0.35	0.10	1277
Isobornyl acetate	0.20	0.24	--	1277
2α, 4aβ, 8aβ-Decahydro-2-naphthalenol	--	0.12	--	1289
Ledene oxide-(ii)	--	--	0.15	1293
Neoclovene oxide	--	--		1305
Megastigma-4,6(e),8(z)-triene	--	--	0.64	1317
Geranyl acetate	0.47	0.18	--	1352
Ethanone, 1-(2,4,5-trimethylphenyl)-	--	--	0.23	1369
α-Copaen-11-ol	--	--	0.30	1377
Ledane	--	--	0.17	1380
7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl	--	--	2.55	1385
3-Iodomethyl-3,6,6-trimethyl-cyclohexene	--	0.12	--	1385
Alloaromadendrene	--	--	0.64	1386
Aromadendrene	0.12	--	1.62	1386
Benzene, 2-(1,1-dimethylethyl)-1,4-dimethoxy-	0.15	--	--	1386
Patchoulane	--	--	0.21	1393
β-Patchoulane	--	--	1.50	1393
Benzyl isovalerate	0.96	1.45	--	1394
Aromadendrene, dehydro-	--	--	0.92	1396
Isolongifolene, 9,10-dehydro-	--	--	0.45	1398
Neoisolongifolene, 8,9-dehydro-	--	--	0.50	1398
α-Patchoulene	0.21	0.10	2.03	1403
Calarene	--	--	3.24	1403
(-)-α-Panasinsen	0.19	--	0.71	1416
Widdrene	--	--	0.08	1416
Patchouli alcohol	2.19	2.22	3.00	1420
α-Bergamotene	0.36	0.15	0.27	1430
Cyperene	0.17	--	--	1432
1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene	--	--	0.10	1443
α-Farnesene	0.11	0.15	--	1458
γ-Gurjunene	--	--	3.40	1461
Aromadendrene oxide-(2)	--	--	0.97	1462
γ-Elemene	0.10	--	0.08	1465
β-Selinene	--	--	1.00	1469
Eudesma-4(14),11-diene	--	--	0.12	1469
Eremophilene	--	--	0.10	1474
Valencene	--	--	0.17	1474
α-Selinene	--	--	0.21	1474
Bornyl butyrate	0.61	--	0.16	1476
cis-Adamantane-2-carboxylic acid, 4-hydroxy-	36.88	40.26	--	1486
α-Guaiene	0.41	0.15	0.13	1490
δ-Guaiene	1.39	0.45	1.02	1490
Phenethyl isovalerate	0.80	0.46	--	1493
Caryophyllene	0.07		0.05	1494
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	1.42	0.42	--	1494
Germacrene A	0.78	0.45	0.29	1505
Caryophyllene oxide	0.18	0.18	0.27	1507
Eudesma-3,7(11)-diene	--	0.09	--	1507
Bornyl Isovalerate	5.08	5.54	--	1512
cis-α-Bisabolene	0.55	0.19	--	1518
B-Guaiene	0.92	--	--	1523
α-Curcumene	0.98	0.35	--	1524
Caryophyllene-(i1)	--	--	1.03	1528
Epiglobulol	0.17	0.10	0.49	1530
Spathulenol	-	--	0.70	1536
1-Isopropenyl-3,3-dimethyl-5-(3-methyl-1-oxo-2-butenyl)cyclopentane	0.38	--	--	1541
Limonen-6-ol, pivalate	0.40	0.37	--	1560
Nerolidol	1.64	0.83	--	1564
β-Humulene	--	--	0.09	1574
Bornyl valerate	--	--	0.75	1576
Geranyl isovalerate	0.91	0.80		1586
2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	--	--	0.56	1586
Isolongifolen-5-one	0.19	--	--	1587
Ethanone, 1-[2-(5-hydroxy-1,1-dimethylhexyl)-3-methyl-2-cyclopropen-1-yl]-	0.73	0.57	--	1591
Valeranone	1.64	0.63	--	1615
β-Bisabolol	6.48	5.71	--	1619
1(2h)-Naphthalenone, octahydro-4,8a-dimethyl-6-(1-methylethenyl)-, (4α,4aβ,6α,8aβ)-	0.38	0.48	0.43	1634
Cedren-13-ol, 8-	--	--	0.17	1646
Baldrinal	0.69	0.90	--	1689
6,7-Dimethoxy-2-tetralone	0.64	0.63	--	1716
Murolan-3,9(11)-diene-10-peroxy	--	--	0.21	1729
Khusilic acid	--	--	0.20	1747
Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (s)-	0.31	0.27	--	1776
1H-Indole, 1-acetyl-2,3-dihydro-6-nitro-	1.50	1.49	--	1791
Ethyl 9,9-Diformylnona-2,4,6,8-tetraenoate	0.19	0.71	--	1867
Methyl palmitate	--	--	0.11	1878
Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	0.06	0.52	0.98	1904
6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1h-naphthalen-2-one	--	--	0.77	1916
Cyclohexadecane	0.12	--	--	1918
Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-	--	--	0.30	1933
Estra-1,3,5(10)-trien-17β-ol	0.59	0.43	--	1949
Coumarin-6-ol, 3,4-dihydro-5,7,8-trimethyl-	0.95	0.97	--	1952
2,6-Dimethyl-8-(tetrahydropyran-2-yloxy)-octa-2,6-dien-1-ol	0.46	0.85	--	1953
Palmitic acid	--	0.40	--	1968
Methyl (10E)-10-heptadecen-8-ynoate	0.06	--	--	2003
Eicosane	1	0.14	--	2009
Deoxysericealactone	0.19	0.09	--	2025
9-Methyl-Z,Z-10,12-hexadecadien-1-ol acetate	--	0.07	--	2029
Methyl linoleate	0.13	--	--	2093
Methyl 9,12,15-octadecatrienoate	0.21	--	--	2101
Methyl 10,13-octadecadiynoate	0.04	--	--	2112
Valeric acid, 4-pentadecyl ester	--	--	0.02	2112
Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)-	0.12	--	--	2251
Tricosane	0.23	--	--	2307

Cite this Article as: D. Sugumar Pandian, N.S. Nagarajan, Comparison of chemical composition and antioxidant potential of hydrodistilled oil and supercritical fluid CO_2 extract of *Valeriana wallichi* DC, J. Nat. Prod. Resour. 1(1) (2015) 25–30.

9-Tricosene, (z)-	0.35	--	--	2315
14-Oxononadec-10-enoic acid, methyl ester	0.80	--	--	2321
2-(5-Hydroxypent-2-ynyl)-3-oxocyclopentyl]thioacetic acid, s-t-butyl ester	0.58	0.66	--	2350
Tetracosane	0.16	--	--	2407
Isoquinolin-6,7-diol-1-carboxylic acid, N-Acetyl-1-methyl-	1.74	3.87	--	2447
2h-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	0.19	0.06	--	2453
1,3,5-Cycloheptatriene, 2,5-bis(Tetrahydropyranyloxymethyl)-7,7-dimethyl-	0.84	0.92	--	2549
2,6-Diamino-4-cyclohexyl-4H-thiopyran-3,5-dicarbonitrile	--	1.69	--	2582
Chiapin b	--	--	0.13	2612
Cholestan-3-ol, 2-methylene-, (3β,5α)-	0.37	0.99	0.12	2652
Valtrate	0.92	1.80	--	2686
Glyceryl linolenate	0.21	0.69	--	2705
γ-Sitosterol	0.12	0.37	--	2731
Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5β)-	--	0.56	--	2770
Nonacosane	0.38	--	--	2904
Squalene	0.28	0.32	--	2914
Clocortolone Pivalate	0.50	1.14	--	3124
α-Tocopherol	0.05	0.27	--	3149
β - Methasone valerate	1.33	2.34	--	3307
1-Heptatriacotanol	0.32	0.27	--	3942
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	1.04	1.61	--	4187

It was reported hydrodistilled oil contains valerianian, α-pinene, camphene and terpineol [33-34]. Other constituents present in roots were α-valene, β-bisabolene, β-elemene, β-phellandrene, β-pinene, β-valene, borneol, bornyl acetate, bornylformate, camphene, limonene, myrcene, caryophyllene. A study revealed that the chemical compositions of the oil show two chemotypes within *V. wallichii*. The type1st was characterized by presence of maaliol (64.3%), viridiflorol (7.2%) and sesquiterpene hydrocarbons (19.2%). The type 2nd contained patchouli alcohol (40.2%), viridiflorol (5.2%), acetoxy-patchouli alcohol (4.5%) and sesquiterpene hydrocarbons (34.5%) [35-36]. Two new flavone glycosides, acecetin 7-0-β-sophorosideand acacetin 7-0-(6-0-α-l-rhamnopyranosyl)-β-sophoroside were isolated from the rhizomes and roots of *V. wallichii* [37]. Rhizomes and roots contain cyclopentapyrans, acacetin-7-0-rutinosides, linarin-iso-valerinate, 4-methoxy-8-pentyl-1-naphthoic acid [38], lignin prinsepiol-4-omicron-β-d-glucoside, coniferin, hexacosanic acid, limonene, choline, chatinine, valerianine, actinidine, tannins and resins 4-methoxy, 8-pentyl-l-napthoic acid, methyleicosanoate, cubenol, caryophyllene oxide, cadinol and aristolene are other constituents isolated from this plant [39-40].

The aroma compounds were present tiny amount in CO_2 extract as well as oil such as furfural, ethyl valerate, α and β pinene, limonene, cymenecitronellol, geraniol, phenyl ethyl alcohol, linalool, bornyl acetate, linalool oxide, hexyl isovalerate, amyl valerate etc.

To our knowledge, this is the first report on the composition of supercritical fluid CO_2 extract of *Valeriana wallichi* DC. The new compound identified from the extract of *Valeriana wallichi* DC were chiapin b and clocortolone pivalate in very small amount.

3.2 DPPH Radical Scavenging Activity

Percent DPPH radical scavenging capability of hydrodistilled *Valeriana wallichi* essential oil along with supercritical fluid CO_2 against the concentration was illustrated in Fig. 1. The scavenging effect of the essential oil (40.25% - 60.54%), supercritical fluid extract at 100 bar (30.27 – 52.57%) and supercritical fluid extract at 200 bar (39.57% – 63.05%) on DPPH radical linearly increased with increasing concentration from 0.2 to 1.0 mg. All the essential oil and supercritical fluid extract showed good to excellent percent scavenging activity in comparison with BHA (47.38% –89.39%) and Ascorbic acid (56.96% – 90.67%). At the higher concentration (1 mg) *V. wallichi* supercritical fluid extracts are able to give equally good radical scavenging activity in comparison with positive control ascorbic acid. At lower concentration (0.2 mg) *Valeriana wallichi* essential oil gave better DPPH radical activity than supercritical fluid extracts. Based on IC_{50} value, the percent radical scavenging activity can be ranked in the following order, Hydrodistilled *V. wallichi* essential oil (0.51 mg/mL)> SFE of *V. wallichi* 200 bar (0.53 mg/mL)> SFE of *V. wallichi* 100 bar (0.92 mg/mL). Sudhanshu et al. (2012) reported methanolic extract of *V. wallichi* showed appreciable DPPH scavenging activity which is comparable to standard ascorbic acid. Our results are well supported with the above literature. It has been reported that presence of phenolic compounds plays an important role in antioxidant activity of natural products [41]. From GC-MS studies (Table 1), we can see supercritical fluid extract at 100 bar contains 0.04% p-creosol, 0.66% of carvacrol and 0.05% of α-tocopherol. Also, supercritical fluid extract at 200 bar contains 0.02% of carvacrol and 0.27% of α-tocopherol. These antioxidant compounds might play vital role in contributing antioxidant properties of *Valeriana wallichi supercritical* fluid extracts. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defence system against free radical attack [41].

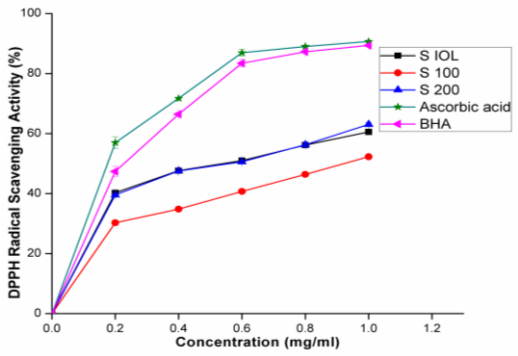

Fig. 1 Effect of *Valeriana wallichi* by DPPH radical scavenging activity in different concentration. Each value represents a mean ± SD ($p< 0.05$)

3.3 Superoxide Radical Scavenging Activity

Percent superoxide radical scavenging capability of hydrodistilled *Valeriana wallichi* essential oil along with supercritical fluid CO_2 against the concentration was illustrated in Fig. 2. The superoxide scavenging effect of the essential oil (16.87% - 67.01%) supercritical fluid extract at 100 bar (23.60% – 70.64%) and supercritical fluid extract at 200 bar (18.17% – 64.05%) on superoxide radical linearly increased with increasing concentration from 0.05 to 1.0 mg. Supercritical fluid extracts and *V. wallichi* essential oil showed comparatively less percent scavenging activity in comparison with Ascorbic acid (46.53% –80.51%) and BHA (42.37% –80.33%). At the lower concentration (0.2 mg), all the extracts exhibited lesser radical scavenging effect than positive controls, however at 1 mg level, which exhibited more than 60% radical scavenging effect. Based on IC_{50} value, the superoxide radical scavenging activity can be ranked in the following order, SFE of *V. wallichi* 100 bar (0.19 mg/mL) > SFE of *V. wallichi* 200 bar (0.30 mg/mL) > Hydrodistilled *V. wallichi* essential oil (0.33 mg/mL).

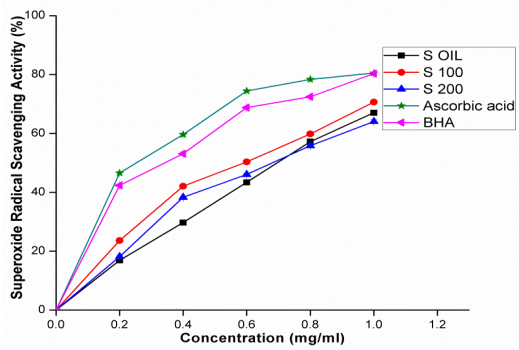

Fig. 2 Effect of *Valeriana wallichi* by superoxide radical scavenging activity in different concentration. Each value represents a mean ± SD ($p< 0.05$)

3.4 Hydroxyl Radical Scavenging Activity

Percent hydroxide radical scavenging capability of hydrodistilled *Valeriana wallichi* essential oil along with supercritical fluid CO_2 against the concentration was illustrated in Fig. 3. The scavenging effect of the essential oil (70.80% - 84.64%) supercritical fluid extract at 100 bar (75.03% – 84.83%) and supercritical fluid extract at 200 bar (57.40% – 78.95%) on hydroxide radical linearly increased with increasing concentration from 0.2 to 1.0 mg. All the essential oil and supercritical fluid extract showed good to excellent percent scavenging activity in comparison with BHA (68.65 –88.69%) and ascorbic acid (72.52 – 90.89). At the lower concentration (0.2 mg) *V. wallichi* supercritical fluid extract

at 100 bar and essential oil are able to comparatively similar hydroxide radical scavenging activity in comparison with ascorbic acid. All the tested extracts gave higher hydroxide radical scavenging activity at 1.0 mg level which is relatively similar to positive controls. Based on IC_{50} value, the percent hydroxyl radical scavenging activity can be ranked in the following order, SFE of *V. wallichi* 100 bar (0.13 mg/mL)> Hydrodistilled *V. wallichi* essential oil (0.14 mg/mL)> SFE of *V. wallichi* 200 bar (0.17 mg/mL). In the hydroxyl radical scavenging system, the concentration of hydrogen peroxide in water depends upon the phenolic compounds. Phenolic compounds present in the *V. wallichi* essential oil and supercritical fluid extract may be good electron donors and they may accelerate the conversion of H_2O_2 to H_2O [42].

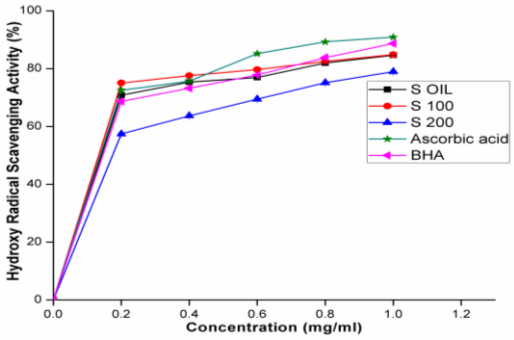

Fig. 3 Effect of *Valeriana wallichi* by hydroxy radical scavenging activity in different concentration. Each value represents a mean ± SD (p< 0.05)

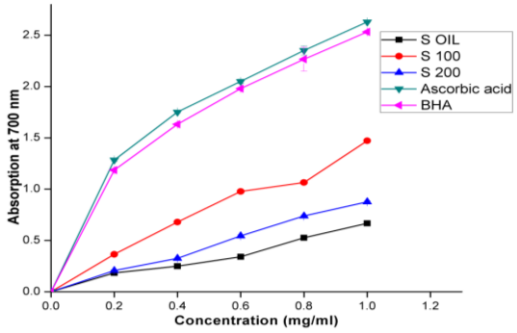

Fig. 4 Effect of *Valeriana wallichi* by reducing power method in different concentration. Each value represents a mean ± SD (p< 0.05)

3.5 Reducing Power

Reducing power of hydrodistilled *Valeriana wallichi* essential oil along with supercritical fluid CO_2 against the concentration was illustrated in Fig. 4. The increase of absorbance value at 700 nm denotes the increase of reducing power of particular sample. The reducing power of the essential oil (0.18 – 0.67) supercritical fluid extract at 100 bar (0.36 – 1.47) and supercritical fluid extract at 200 bar (0.21 – 0.88) linearly increased with increasing concentration from 0.2 to 1.0 mg. Supercritical fluid extracts comparatively showed good percent scavenging activity than essential oil. However, effects of extract as well as oil were lesser than ascorbic acid (1.2 –2.6) and BHA (1.18-2.53). At the higher concentration (2 mg) *V. wallichi* supercritical fluid extract at 100 and 200 bar are able to similar reducing power as like lower concentration of positive controls. Based on IC_{50} value, the percent hydroxyl radical scavenging activity can be ranked in the following order, SFE of *V. wallichi* 100 bar (0.28 mg/mL)> SFE of *V. wallichi* 200 bar (0.55 mg/mL)> Hydrodistilled *V. wallichi* essential oil (0.77 mg/mL). The reducing powers of essential oil might be due to their hydrogen-donating ability [43] and is generally associated with presence of reductones [44].

The components present in essential oil and supercritical fluid extracts of *V. wallichi* could act as good reductants, which could react with free radicals to stabilize and terminate radical chain reactions.

4. Conclusion

Summarizing these results, it can be concluded that the *Valeriana wallichi* DC essential oil and its supercritical fluid extracts exhibited broad spectrum of antioxidant activity against the tested various antioxidant assays. More studies are needed to clarify the antioxidant mechanisms of the antioxidant activity of essential oil and supercritical fluid extracts. On the basis of above reults, it was observerd that *Valeriana wallichi* essential oil and supercritical fluid extracts provided comparatively equivalent antioxidative activity as compared to synthetic antioxidants, which provides a way to screen antioxidants for foods, cosmetics and medicine.

Acknowledgement

We are thankful to Head, Chemistry Department, Gandhigram Rural University, Gandhigram for providing laboratory facilities and MR. P.C. Jain, Pragathi Aroma Oil Distillers Pvt. Ltd. for providing instrumentation facility for GC-MS analysis.

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Cite this Article as: D. Sugumar Pandian, N.S. Nagarajan, Comparison of chemical composition and antioxidant potential of hydrodistilled oil and supercritical fluid CO_2 extract of *Valeriana wallichi* DC, J. Nat. Prod. Resour. 1(1) (2015) 25–30.