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VARIATIONS IN VOLATILE OIL COMPOSITIONS OF DIFFERENT WILD COLLECTIONS OF *Hyptis suaveolens* (L) Poit FROM WESTERN GHATS OF INDIA

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Abstract- *Hyptis suaveolens*, belonging to Labiatae family is common aromatic weed and a potent invader in Deccan peninsular region. Samples collected from ten locations of four provinces from Western Ghats, one of the 34 biodiversity hot spots of the world were examined for differences in their essential oil profiles and yields. Forty eight components accounting for 72.94-99.63% of the essentials were identified by GC-FID-LRI and GC-MS analysis. The essential oil content of the leaf obtained by hydro distillation varied from 0.09% to 0.41%. β-Caryophyllene (7.31-36.57%), sabinene (1.50-20.76%), 1:8 cineole (1.02-37.4%), terpinolene (0.11-16.19%), bergamotol (0.83-7.70%), abietatriene (0.18-7.24%), and β-pinene (0.52-6.40%) were identified as the major components. Sesquiterpenes along with diterpenods were predominant in all of the studied accessions and β-caryophyllene was more than 15.77%, except the samples collected from Bangalore and Hyderabad plains. The essential oils showed significant differences in their compositions.
Keywords- *Hyptis suaveolens*, Labiatae, essential oil composition, Western Ghats, leaf oil, β-caryophyllene, 1, 8-cineole.

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Introduction

Hyptis, genus belongs to Lamiaceae family with about 400 species [40]. It is a common weed native to Tropical America, and is now spread all over the world, especially in the tropical area. *Hyptis suaveolens* Poit, commonly known as wyali tulasi, is naturalised in India. It has now established in Deccan Peninsula, North East India, Vindhyan Highland, and Andaman and Nicobar Islands [38,41]. *H. suaveolens* is of common occurrence along the rail tracks, roadsides [39], foothills of open forests, forest clearings [31] and can heavily infest wastelands particularly arid and rocky substrates. It is a ruderal weed and is capable of heavy infestations displacing native flora and is said to be a potent invader of Vindhyan Highlands [34]. *H. suaveolens* is aromatic weed and reported to have significant medicinal activities. It is frequently referred in the treatment of gastrointestinal infections, cramps and pain as well as in the treatment of skin infections. *H. suaveolens* is widely used for fevers, headaches, catarrh, cold, indigestion, stomach pain, nausea, flatulence, as a stimulant to milk secretion, affections of gallbladder and muscle contractions [2]. Previous investigations showed its antiseptic [36], anticarcinogenic [24], antibacterial [5,15,36], antifungal [22,26,30,43], anticonvulsant [3], nematicidal [8] and larvicidal properties [33]. The diterpenes suaveolol and methyl suaveolate showed anti-inflammatory activity [17], and dehydroabietinol and 13α-epidioxiabiet-8(14)-en-18-ol abietane-type diterpenoids from this plant revealed the antiplasmodial activity [10,42]. The mosquito repellent activity of this plant is also reported [21]. The leaf has an appearance like patchouli leaf, and generally used in the adulteration of the patchouli leaf and its essential oil in some food preparations for flavoring [13]. This plant is also known as chia has attracted commercial interest because of the presence of polyunsaturated fatty linoleic acid representing nearly 80% of the total fatty acids of the seeds [19]. The essential oil of this species is composed of mono-, sesqui-, and di-terpenes and has been the subject and attracted investiga-

tors and several chemo variations of the species were reported. Variation in essential oil compositions can occur as a result of differing soil conditions, altitude, climatic conditions, seasonal factors and other environmental features, in some cases leading to the evolution of different chemical variants or chemotypes [20].

Earlier literature indicates seven chemo variations of *H. suaveolens* with 1,8-cineole [2,6,7,13,15,27,29] sabinene [4,6,15,27, 29,32,37], β-caryophyllene [4,13,29,32,37], trans- α-bergamotene [4,13,37], terpinene-4-ol [4,13], fenchone [14], Eugenol [18] and menthol [9] as one of the major component in the chemo varieties.

Recently, the influence of photoperiod, plant age and, nutrients, nitrogen, phosphorus, and potassium availability on the essential oil composition of *H. suaveolens* from Alfenas (MG), Brazil were studied [28]. In continuation of our work on the screening of wild aromatic flora for potential source of aromatic plants from the Western Ghats, one of the world biodiversity hot spot, the importance of the *H. suaveolens* and large volume of the work, wide variations, and several chemo types attracted us to investigate the essential oil from the leaf for commercial utilization of the leaf along with its edible seeds. Western Ghats with a wide variety of ecological habitats can certainly provides several ecotypes and chemo types in some of the aromatic plants. We have collected *H. suaveolens* aerial parts from ten locations of four provinces from in Western Ghats area during January-February 2008. The essential oils obtained by hydro distillation was analysed for its detailed chemical composition by using GC-FID-LRI and GC-MS.

Results and discussion

The aerial parts of *H. suaveolens* samples collected from Western Ghats, were analysed and compared for differences in their essential oil profiles and yields. The oils obtained were analysed by GC using methyl polysiloxane and wax columns. The analysis led to the identification of forty eight constituents accounting for 72.94-99.63% of the total oil compositions in the present investigation. The details of the hydro distilled essential oil yields (v/w) and time of collections, the identified peaks along with the Leaner Retention Indices and respective concentration of each component is listed in Table 1 in order of their elution on dimethylpolysiloxane column.

In the present investigation the samples collected have yielded the oil content in the range of 0.12 to 0.41% as earlier reported in literature, except higher yields of the essential oil from fresh leaves by Ahmed et al (1994) (2.4%) and Malele et al (2003) (1.2%). 1-octen-3-ol (0.61-8.12%) and 3-octanol (0.14-0.69%) was observed and reported first time from the collected samples examined from India in the range of 0.37-8.12%. The samples collected from Bangalore and Hyderabad predominantly dominated by the monoterpene hydrocarbons (63.31% & 59.02%) and accounting total sesquiterpenes (20.04% & 16.35%). It appear that the variety belong to a chemotype having lesser β-caryophyllene (7.31% & 7.53%) and more of sabinene (20.40% & 20.76%), 1:8-cineloe (14.81% & 22.38%) and terpinolene (16.19% & 0.11%). Bangalore sample resembles the terpinolene chemotype reported from the south east provinces of El Salvador (Grassi, Nunez, Varmuza & Franz, 2005) having more of terpinolene (13.8%). The sample collected from Madurai is also rich in the monoterpene hydrocarbons (58.09%) and with more of sesquiterpenes (29.92%). It closely resembles the variety belong to a chemotype having considerable amount of β-caryophyllene (15.77%) with more of 1:8-cineloe (37.40%) and others sabinene(7.51%), β-pinene(5.61%) being less. β-caryophyllene was noticed as one the major sesquiterpenoid in the range of 36.57-15.77% in all samples except the samples collected from Bangalore (7.31%) and Hyderabad (7.53%). Previously β-caryophyllene, 43.7% was reported as highest in a sample from Benin from Nigeria [25].

The next higher compounds reported from *Hyptis* are sabinene (20.76-1.52%) or 1:8-cineole (37.4-1.02%). 1:8-cineole was normally the highest reported compound from *Hyptis* oil and it is 47.64% highest from a sample from Brazil [30]. Very low percentage of monoterpene hydrocarbons were noticed in samples from Mandya and Karkala, in which the sesquiterpene hydrocarbons and diterpenes were predominated. Terpinolene (16.19%) is observed highest in sample from Bangalore and lowest (0.24%) in sample collected from Mandya.

The other important compounds hydrocarbons α-pinene (0.3-3.33%), 1-Octene-3-ol (0.61-3.17%), β-pinene (0.52-6.4%), terpinen-4-ol (0.56-3.31%), *trans*-α-bergamotene (0.82-8.27%), bicyclogermacrene (0.30-4.47%) and bergamotol (0.83-7.70%) were observed. In the diterpenods compounds abietatriene (0.2-7.24%) was the highest noticed.

Earlier Malele et al (2003) has reported a sample from Tanzania having high concentrations of sesquiterpenes and vary less amounts of sabinene (0.5%), β-pinene (0.2%), terpinolene (2.5%) and nil percentages of 1:8-cineloe. There is a considerable increase in sabinene (18.90%), 1:8-cineloe (7.92%) and terpinolene (9.42%) and, reduction in β-caryophyllene (19.50%) in the sample which was collected from Karkala in Aug 2006 and re-grown in CIMAP, RC, Bangalore when compared with the Karkala sample. This can be explained due to genetic diversity of the plant in two years of time due to climate changes. On the basis of observations in this study, and earlier reports, it can be concluded that there is considerable intrapopulation variation in the genetic resources of *H suaveolens* and chemical diversity and thereby biological activity, and the essential oil can be better utilised in perfumes and in flavouring, cosmetic and pharmaceutical purposes.

Experimental

Plant materials

The aerial parts of the *H. suaveolens* samples were collected from ten locations of four provinces from Western Ghats of India and nearby plains. Western Ghats is situated along the western coastal zone of India. Western Ghats constitutes the Malabar province of the Oriental realm, running parallel to the west coast of India (8°N to 21°N latitudes) for 1600 km. The hills reach up to a height of 2800 m before they merge to the east with the Deccan Plateau at an altitude 550 to 750 m. The voucher specimens were identified by Dr. R.R. Rao CSIR Emeritus Scientist, and deposited (Nos. DST-505, 284, 187, 266, 386, 488, 462, 467, 477, 503) at the herbarium in CIMAP, Research Centre, Bangalore. The sample DST-187, collected in Aug. 2006 at Karkala, Karnataka state, is re-grown in CIMAP RC campus and sample was taken in Jan. 2008.

Extraction of essential oil

The leaf samples were hydro-distilled in triplicate in Clevenger type [11] apparatus for 5 hrs. The average of essential oil yields obtained from the samples is shown in the Table 1. The oil sam-

ples after drying over anhydrous Na_2SO_4 were stored at 5°C till the samples were subjected to GC and GC/MS analyses.

Analysis of essential oils

GC: Gas Chromatographic analysis of the essential oil samples was done on a Varian CP-3800 gas chromatograph (GC) fitted with two Flame Ionization Detectors (FID), split/split less capillary injectors, and Star workstation software. Dimethylpolysiloxane (100%) column (CP Sil-5 CB: 50m x 0.25mm i.d. film thickness 0.4µ) and CP-Wax column: (60m x 0.25mm i.d. film thickness 0.4µ) were used with Nitrogen as the carrier gas with pressures of 16 and 17 *psi,* respectively. Samples 0.2µL were injected in split mode with a ratio 1:100. The column was initially held at 60º C for 5 min., then heated to 220º C at 5º C per min., ramp rate, and was held for 3 min. It was further raised to 250º C at 5º C per min ramp rate and was held at this temperature for 4 min. Injector and detector temperatures were kept at 250º C and 300º C, respectively.
GC/MS: Analysis was carried out on a Perkin Elmer Turbo mass Auto XL Instrument at 70eV ionization energy, employing PE-5 column (5% phenyl 95% dimethylpolysiloxane) of 50m length, 0.32 mm i.d with a film thickness of 0.33 mm. Oven temperature was programmed from 100-280º C at 3º C /min rising rate with an initial hold time of 2 min. Helium was used as the carrier gas at 10 *psi* inlet pressure.

Identification of compounds

Linear retention indices (LRI) were obtained from the gas chromatograms by comparing with natural homologous series of hydrocarbons. Compounds were identified by comparison of retention indices of the peaks on CP Sil-5 CB and CP-Wax columns with literature values (Davies, 1990; Jennings & Shibamoto, 1989; Ramaswamy, Briscese, Gargiullo & Von Geldern, 1988), computer matching against library spectra built up using pure substances and components of known essential oil, and finally confirmed by matching their mass spectra with those recorded in the MS library data already available in NIST library and literature (Adams, 2007). Relative amounts of individual components are based on peak areas obtained without FID response factor correction. Identified compounds are listed in Table 1.

Essential oil yields

Essential oil yields varied from 0.09-0.41% of biomass. The yields corroborate the findings of earlier researchers. The samples collected from different elevations from of Malebenur Ghats, in Karnataka State had markedly higher essential oil yields (0.41%) relative to other locations.

Conclusions

The quantitative analysis of the samples from Bangalore, Hyderabad, Madurai and Karkala for aroma compounds, 1:8-cineole, sabinene and β-caryophyllene will be an interesting area for further study for considering the *H. suaveolens* as another alternative source for these compounds. Since occurrence of the aromatic weed is abundant, and it can be cultivated or collected wild very easily, along with the potentially important seed oil and proteins, the rich mono- and sesquiterpenes can be better utilised in the aroma industry as co-products of *H. suaveolens.*

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Table 1- Percentage composition of volatile components of essential oils of Hyptis suaveolens collected (n = 3)

	Place of Collection					Banga-lore	Hydera-bad	Madurai, TN	Karkala sample grown in Banga-lore	Anewadi, Maharas-tra	Malebe-nur Ghats, Bellary	Srisail-am	Mandya Karna-taka	Karkala	Shimoga
						DST-505	DST-285	DST-266	DST-187	DST-386	DST-448	DST462	DST-467	DST-477	DST-503
	Percentage yield of oil					**0.12**	**0.12**	**0.11**	**0.15**	**0.09**	**0.41**	**0.3**	**0.14**	**0.17**	**0.12**
S.No	Collection time	CP Sil-5 CB LRI	Lit LRI	CP-Wax LRI	Lit LRI	Jan. 2008	Jan. 2008	Aug. 2006	Jan. 2008	Nov. 2008	Jan. 2008	Jan. 2008	Feb. 2008	Feb. 2008	Feb. 2008
1	Tricyclene	926	927	1008	1010	0.36	0.52	0.13	0.26	0.26	0.49	0.4	0.06	0.11	0.22
2	α- Pinene	935	936	1025	1018	1.82	3.33	1.57	1.31	1.96	1.53	2.16	0.3	0.42	0.82
3	Camphene	952	950	1070	1062	-	0.1	-	-	-	-	-	-	-	-
4	1-Octen-3-ol	965	962	1417	1430	3.17	1.21	0.61	1.12	0.89	2.25	2.87	0.37	0.96	1.44
5	Sabinene	970	973	1112	1102	20.4	20.76	7.51	18.9	8.7	12.99	9.11	1.52	3.09	8.82
6	β-Pinene	975	978	1124	1088	6.4	5.29	5.6	4.88	5.26	5.37	3.23	0.52	1.99	4.06
7	3-Octanol	981	981	1520	1520	0.15	-	-	0.2	-	0.14	0.28	0.14	0.24	0.24
8	Myrcene	983	987	1161	1156	0.58	0.79	0.53	0.38	0.73	0.24	0.22	0.06	0.08	0.04
9	α-Phellandrene	997	1002	1070	1177	0.16	2.99	-	-	1.97	0.13	3.18	0.88	0.1	0.04
10	α- Terpinene	1010	1013	1168	1178	0.84	0.1	0.22	0.38	0.38	1.08	0.52	0.31	0.38	0.14
11	p-Cymene	1013	1015	1274	1252	0.23	1.79	1.08		0.35	0.31	0.62	0.26	0.08	2.18
12	1:8-Cineole	1024	1024	1224	1206	14.81	22.38	37.74	7.92	17.02	4.15	10.77	1.02	1.19	3.63
13	γ- Terpinene	1052	1049	1249	1224	1.34	0.24	0.43	0.61	0.68	1.64	0.51	0.74	0.68	0.39
14	t- Sabinene hydrate	1057	1053	1461	1458	-	0.54	-	0.42	-	0.47	0.44	0.34	0.17	0.29
15	Fenchone	1072	1069	1405	1410	0.79	2.55	0.16	0.79	0.64	0.15	1.36	1.01	2.59	0.18
16	Terpinolene	1081	1082	1285	1287	16.19	0.11	0.33	9.42	5.06	9.4	0.3	0.24	2.59	3.39
17	Linalool	1088	1086	1547	1527	0.24	0.18	0.36	0.27	0.33	0.37	0.37	0.08	0.12	0.26
18	cis-Sabinene hydrate	1100	1082	1537	1538	0.18	0.08	0.13	0.42	0.63	-	-	0.11	0.08	0.11
19	Camphor	1125	1023	1518	1518	0.35	0.12	0.26	0.25	-	0.24	0.29	0.34	0.04	0.31
20	p-Cymene-8-ol	1164	1169	1840	1846	-	0.14	-	-	-	-	-	-	0.24	1.66
21	Terpinen-4-ol	1165	1164	1604	1628	2.68	0.69	1.24	1.05	0.56	2.97	1.52	1.61	1.4	3.31
22	α-Terpineol	1174	1176	1731	1731	0.17	0.08	0.19	-		0.19	0.14	0.24	0.12	0.33
23	α- Copaene	1365	1379	1488	1493	-	0.11	0.11	-	0.36	-	-	0.07	0.04	0.13
24	β-Bourbonene	1381	1386	1546	1406	0.6	0.14	0.12	-	1.3	-	-	0.08	0.05	0.05
25	β-Elemene	1391	1389	1596	1596	0.48	0.62	0.49	0.42	1.29	0.53	0.55	0.66	1.07	0.68
26	β- Caryophyllene	1428	1421	1618	1618	7.31	7.53	15.77	19.5	19.75	28.07	25.36	22.98	36.57	18.63
27	t-α-Bergamotene	1436	1434	1586	1590	1.81	0.91	0.82	3.11	2.53	2.74	3.71	8.27	1.58	2.83
28	α-Humulene	1459	1455	1690	1707	0.67	0.55	1.38	1.48	1.58	1.93	1.51	1.6	2.75	1.4

Table 1- Continue

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	Percentage yield of oil					**0.12**	**0.12**	**0.11**	**0.15**	**0.09**	**0.41**	**0.3**	**0.14**	**0.17**	**0.12**
S.No	Collection time	CP Sil-5 CB LRI	Lit LRI	CP-Wax LRI	Lit LRI	Jan. 2008	Jan. 2008	Aug. 2006	Jan. 2008	Nov. 2008	Jan. 2008	Jan. 2008	Feb. 2008	Feb. 2008	Feb. 2008
29	Germacrene- D	1477	1479	1712	1712	-	0.83	-	-	0.27	-	0.16	0.16	0.08	0.15
30	**β**- Selinene	1483	1486	1727	1727	0.54	0.37	-	-	1.47	-	0.12	0.12	0.07	0.07
31	**α**- Muurolene	1490	1496	1730	1730	-	0.3	--	1.09	1.08	1.24	1.47	1.43	1.47	1.28
32	**α**-Selinene	1494	1492	1712	1729	0.22	0.08	0.89	-	0.34	-	-	0.16	0.25	-
33	Bicyclogermacrene	1498	1494	1740	1744	3.71	0.3	0.51	2.88	4.73	2.67	4.74	1.17	4.1	2.5
34	**γ**-Cadinene	1508	1507	1768	1766	0.1	0.37		-	0.33	-	-		0.04	0.1
35	**δ**-Cadinene	1519	1520	1762	1761	0.18	0.17	0.21	-	0.25	-	-	0.08		0.09
36	Spathulenol	1571	1572	2089	2084	-	1	0.18	-		-	-	0.09	0.07	0.35
37	Caryophyllene oxide	1576	1578	2020	2023	1.26	0.08	0.19	0.86	1.53	0.84	1.33	0.28	1.62	4.16
38	Globulol	1584	1589	2108	2104	0.18	0.5	7.11	1.8	2.38	2.08	0.22	5.4	4.01	4.86
39	1,10-di-epi-Cubenol	1613	1615	2118	2122	0.23	0.13	-	-	0.36	0.29	0.29	-	0.42	0.54
40	β-Eudesmol	1635	1641	2230	2233	0.29	0.29	0.32	0.26	0.72	0.48	0.53	0.22	1.08	1.31
41	**α**-Cadinol	1652	1643	2240	2211	-	0.76	0.62	-	1.09	0.73	0.72	0.17	-	1.33
42	Intermedeol	1658	1654	2240	2247	-	-	0.37	-	2.08	-	0.31	0.79	-	-
43	Bergamotol	1683	1680			2.46	1.31	0.83	7.68	3.22	5.07	4.01	4.94	4.89	7.7
44	Abietatriene	2039	2019	2420	2446	2.23	0.18	0.2	3.47	3.15	2.98	5.83	7.24	7.08	1.35
45	Abietadiene	2072				0.37	0.1	3.04	-	0.85	0.79	1.31	3.25	1.64	-
46	Phytol	2094	2066		2571	0.18	-	-	-	0.34	0.29	0.61	0.78	0.88	2.78
47	dehydroabietal	2121				-	-	-	-	0.76		1.21	2.07	1.75	0.39
48	Abietol	2415				-	1.17	-	-	2.21		-	-	-	-
	Aliphatic alcohols					3.32	1.21	0.61	1.32	0.89	2.39	3.15	0.51	1.2	1.68
	Aliphatic ketones					1.14	2.67	0.42	1.04	0.64	0.39	1.65	1.35	2.63	0.49
	Monoterpene hydrocarbons					63.31	59.02	55.27	44.9	43	37.8	31.46	6.36	10.96	24.13
	Oxygenated monoterpenes					3.09	1.09	1.79	1.32	0.89	3.53	2.03	1.93	1.88	5.56
	Sesquiterpene hydrocarbons					15.62	12.28	20.3	28.48	35.28	37.18	37.62	36.78	48.07	27.91
	Oxygenated sesquiterpenes					4.42	4.07	9.62	10.6	11.38	9.49	7.41	11.89	12.09	20.25
	Diterpene hydrocarbons					2.78	0.28	3.24	3.47	4.34	4.06	7.75	11.27	9.6	4.13
	Oxygenated diterpenes					0.99	1.17	0	0.3	3.21	0.92	1.21	8.03	1.75	0.39
	Total % of identified compounds					93.68	81.79	91.25	91.13	99.73	95.13	92.89	72.94	89.06	87.32