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Full Length Research Paper

Variability in essential oil composition of sage (*Salvia officinalis* L.) grown under North Western Himalayan Region of India

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Common sage (*Salvia officinalis* L.; Lamiaceae) is one of the most important herb known for its essential oil richness and extensive use in folk medicine. The essential oil from aerial parts of *S. officinalis* L. collections grown under temperate climate of Uttarakhand, India, was analyzed by gas chromatography and gas chromatography mass spectroscopy (GC/MS). The essential oil content of *S. officinalis* was found to vary between 1.11 to 2.76% on dry weight basis. A total of 35 compounds were identified, representing 94.21 to 99.36% of the total oils. The range of major constituents present among six sage collections were: α-thujone (21.43 to 40.10%), β-thujone (2.06 to 7.41%), camphor (11.31 to 37.67%), 1,8-cineole (4.47 to 9.17%), α-humulene (4.58 to 9.51%), camphene (1.89 to 7.04%), viridiflorol (2.14 to 5.56%), α-pinene (1.55 to 6.17%), β-pinene (1.68 to 3.49%) and β-caryophyllene (1.06 to 5.59%). The essential oil composition of sage collections showed presence of larger quantities of the oxygenated terpenes (59.43 to 70.68%) as compared to monoterpene hydrocarbon (13.41 to 17.01%), sesquiterpene hydrocarbon (7.78 to 15.36%) and oxygenated sesquiterpenes (2.14 to 7.73%). The presence of comparatively high concentration of oxygenated compounds mainly thujones, 1,8-cineole and camphor in sage oils may be attributing its carminative, antispasmodic, antiseptic, and astringent properties. Hence, these sage collections may be exploited for various biological and therapeutic applications.

Key words: *Salvia officinalis,* essential oil, composition, Lamiaceae, terpenes, thujones.

INTRODUCTION

Sage is one of the most valued herb known for its essential oil richness and its plethora of biologically active compounds extensively used in folk medicine (Guenther, 1949). The garden sage (*Salvia officinalis* L.) is native to Southern Europe and Turkey. Presently, it is grown in Canada, USA, Spain, Italy, Yugoslavia, Greece, Albania, Germany, France, Turkey, England (Anonymous, 1972). The genus exhibits a very high variability in both morphological and genetic characters according to their geographical origin (Hedge, 1984). Many studies have focused on the chemical composition of *S. officinalis* and large variations have been reported from different countries (Boelens and Boelens 1997; Chalchat et al., 1998; Lawrence 2001). The essential oil composition differs significantly, depending on the individual genetic variability, different plant parts and development stages (Perry et al., 1999).

The presence and concentration of certain chemical constituents also fluctuates according to the season, climatic condition, and site of plant growth (Hossein et al., 2006). Sage leaves and essential oils are stated to possess carminative, antispasmodic, antiseptic, and astringent properties (Lawrence, 2005). The biological properties of essential oil of *S. officinalis* are attributed

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mainly to α- and β-thujone, camphor and 1,8-cineole (Raal et al., 2007).

Keeping in view importance of this species, National Bureau of Plant Genetic Resources, Regional Station, Bhowali, Nainital, Uttarakhand, India made a successful attempt for the first time by establishing and multiplying it in hilly areas of Uttarakhand state (Negi et al.,1998; 2004). Presently, six strains of sage (chemotypes) procured from different countries are being maintained at this station. In the present study, the chemical composition of essential oils of these collections of *S. officinalis* L. grown in Northwestern Himalayan region of Uttarakhand, India were investigated to study variability in chemical composition of essential oils.

MATERIALS AND METHODS

Plant

Salvia officinalis accessions were grown in temperate climatic conditions of National Bureau of Plant Genetic Resources, Regional Station, Bhowali (1600 m altitude, 79° 30′ N latitude and 29° 20′ E longitude) situated in rain fed sub-temperate hills of Uttarakhand. The aerial parts of these plants (500 g) were harvested during the flowering period in April to May, 2007 and 2008, which is the best time to obtain maximum oil yield. Harvested aerial plant parts were dried in the shade at room temperature (20 to 25°C). These dried plant samples were ground and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus (Guenther, 1949). The essential oils extracted were dried over anhydrous sodium sulfate. The oils were stored in a sealed glass vial in the dark at 4°C until further analysis. The oils had a light yellow color with a distinct sharp odor.

Gas chromatography and gas chromatography mass spectroscopy (GC and GC/MS) analysis

Oil sample analyses were performed on Agilent Gas Chromatograph 7890 equipped with a Flame Ionization Detector and a non-polar HP-5MS capillary column made up of 5% Phenyl methyl silicone, 95% dimethylpolysiloxane (30 m × 0.25 mm i.d., 0.25 µm film thickness). Chromatographic conditions were as follows: Helium as carrier gas at a flow rate of 1 mL/min; injection volume was 0.1 µl; column temperature was 60°C for 10 min, and programmed at the rate of 3°C/min to 200°C, and finally held isothermally for 10 min. The detector and injector temperatures were 250 and 220°C, respectively. The percentage composition of the essential oil was computed from Gas chromatography/Flame ionization detection (GC/FID) peak areas without correction factors. Samples were injected by splitting, and the split ratio was 1:20. Analysis was performed on GC/MS (Agilent Model 7890 MSD) equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm coating) and temperature programming was done as described above. The carrier gas used was Helium at a flow rate of 1.0 mL/min and the split mode had a ratio of 1:20. The injection port was set at 220°C. Significant quadrupole MS operating parameters: interface temperature 240°C; electron impact ionization at 70 eV with scan mass range of 40 to 400 *m/z* at a sampling rate of 1.0 scan/s. The components were identified by comparing their relative retentions times with the retention times of authentic standards, and mass spectra with National Institute of Standards and Technology (NIST), Wiley library data of the GC/MS system and literature data (Adams, 2009).

RESULTS

The essential oil content of six accessions of *S. officinalis* was found to vary between 1.11 to 2.76% on dry weight basis. The essential oil composition of *S. officinalis* collections analyzed by GC/MS is given in Table 1. Compounds are listed in order of the retention indices with area percentage. Thirty-five compounds were characterized in this oil, representing 94.21 to 99.36% of the total oil. GC/MS chromatograph showing oil composition of *S. officinalis* is presented in Figure 1. The classification of the identified compounds based on functional groups (Table 2) showed that oxygenated monoterpenes represented the most abundant fraction in the sage oil (59.43 to 70.68%), which consisted mainly 1,8-cineole, α-thujone, β-thujone, camphor and borneol. The monoterpene hydrocarbon fraction ranged from 13.41 to 17.01%, α-pinene, camphene, β-pinene and myrcene being its main constituent.

In contrast, the sesquiterpene fraction (7.78 to 15.36%) represented a lower percentage in the oil, represented by β-caryophyllene (1.06 to 5.59%), α-humulene (4.48 to 9.51%), whereas oxygenated sesquiterpenes were dominated by viridiflorol (2.14 to 5.56%) as the major component. Comparative chemical composition (%) of *S. officinalis* L. accessions based on compound group classification is depicted in Figure 2. The range of main constituents identified and their percentage ranges in the sage oil were as follows: α-pinene (1.55 to 6.17%), camphene (1.89 to 7.04%), β-pinene (1.68 to 3.49%), 1,8-cineole (4.47 to 9.17%), α-thujone (21.43 to 40.10%), β-thujone (2.06 to 7.41%), camphor (11.31 to 37.67%), β-caryophyllene (1.06 to 5.59%), α-humulene (4.58 to 9.51%), and viridiflorol (2.14 to 5.56%).

DISCUSSION

The oil composition of *S. officinalis* was found to be rich in oxygenated monoterpenes, with its range varying from 59.43 to 70.68%. Main constituents identified were α-thujone, β-thujone, 1,8-cineole, camphor and borneol. The oil composition was found to meet the standards given by ISO 9909 for use of *S. officinalis* for medicinal purposes (Berotiene et al., 2007). Good quality sage oils contain a high percentage (> 50%) of epimeric α- and β-thujones and a low proportion (< 20%) of camphor (Raal et al., 2007). Results showed that C3 and C4 collections of sage had oil rich in thujone content which ranged from 41.31 to 47.51%. The α-thujone constituted the major constituent, varying from 36.06 to 40.10% in it.

α-Thujone is known to be more toxic than β-thujone, and is reported to attribute biological property to the sage oil. The collection C4 showed maximum thujone as well

Table 1. Chemical composition (%) of the essential oils isolated from aerial parts of *S. officinalis* L. collections grown in Northwestern Himalayan region of Uttarakhand, India.

RI[a]	Compound[b]	Collection numbers					
		C1	C2	C3	C4	C5	C6
847	(Z)- Salvene	0.47	0.49	1.46	1.75	0.71	0.32
858	(E)- Salvene	0.07	0.08	0.23	0.28	-	-
921	Tricyclene	0.22	-	0.16	0.17	0.68	0.11
931	α -Thujene	0.24	0.19	-	0.46	-	0.18
939	α -Pinene	2.79	6.17	4.09	2.60	2.51	1.55
954	Camphene	6.44	1.89	3.84	3.85	7.04	5.31
977	Sabinene	0.17	-	0.34	0.30	-	0.18
980	β- Pinene	1.71	1.79	3.49	2.57	2.64	1.68
992	Myrcene	0.66	0.59	0.71	0.62	0.83	0.69
1017	α -Terpinene	0.19	0.26	0.14	0.22	-	0.13
1026	p- Cymene	0.46	0.46	0.8	0.83	-	0.34
1031	Limonene	1.76	0.94	0.93	0.89	2.30	2.11
1032	**1,8-Cineole**	**5.55**	**8.47**	**9.17**	**4.47**	**8.13**	**5.97**
1053	(E)- β-Ocimene	-	0.23	0.29	0.16	-	-
1063	γ-Terpinene	0.39	0.57	0.35	0.52	-	0.31
1082	Terpinolene	0.39	0.31	0.18	0.19	-	0.50
1101	**α-Thujone**	**25.16**	**32.61**	**36.06**	**40.10**	**28.28**	**21.43**
1112	β-Thujone	2.06	3.51	4.25	7.41	3.17	2.06
1149	**Camphor**	**25.55**	**11.62**	**11.31**	**12.18**	**29.45**	**37.67**
1158	trans-Pinocamphone	0.13	0.16	0.08	0.26	-	-
1170	Borneol	4.30	1.70	2.29	2.77	1.65	2.79
1253	Sabinene hydrate acetate	0.58	1.03	0.72	0.89	-	0.48
1324	Myrtenyl acetate	0.29	0.33	0.12	0.44	-	-
1419	β-Caryophyllene	4.08	5.59	4.44	3.20	2.75	1.06
1432	(E)-α-Bergamotene	-	-	0.65	-	-	0.58
1455	**α-Humulene**	**8.76**	**9.51**	**6.89**	**4.58**	**5.70**	**7.80**
1458	allo-Aromadendrene	0.06	0.26	0.08	-	-	-
1475	γ- Gurjunene	-	-	-	-	-	0.24
1489	β-Selenene	-	-	-	0.48	-	-
1505	α- Farnesene E,E	-	0.26		-	-	0.10
1506	α-Bisabolene	0.11	0.16	0.16		-	0.09
1582	Caryophyllene oxide	0.76	-	-	0.67	-	-
1596	**Viridiflorol**	**4.28**	**5.56**	**4.67**	**4.14**	**2.14**	**4.95**
	Unidentified	0.59	-	0.72	1.79	-	0.54
	Total (%)	**98.25**	**94.74**	**98.62**	**94.21**	**97.98**	**99.36**

[a]Retention indices on non-polar HP-5MS column. [b]Main compounds in bold letters

as essential oil content in aerial plant parts. Sage collections C5 and C6 were found to be camphor-rich (37.67 and 29.45%, respectively), with thujone as the second major compound (> 10%). Sage collections C1 and C2 were found to contain comparatively higher sesquiterpenes. A perusal of earlier results on oil composition of *S. officinalis* from various countries suggested that Italian, French, Romanian, Czech, Portuguese and Turkish sage oils are characterized by camphor (22.0 to 31.79%) as the most important component (Dob et al., 2007). In contrast, samples from many countries are represented by α-thujone (21.5 to 31.5%) as the major compound in their oils. The chemical composition of sage oil from Uttarakhand, India was found to be closely similar to that reported from Italy, Yugoslavia, Bulgaria and Iran. It was characterized by its large amount of oxygenated monoterpenes, with α-thujone, 1,8-cineole and camphor as the principal compounds which are known to exhibit antimicrobial, anti-inflammatory and antioxidant features (Radulescu et al., 2004). Sage

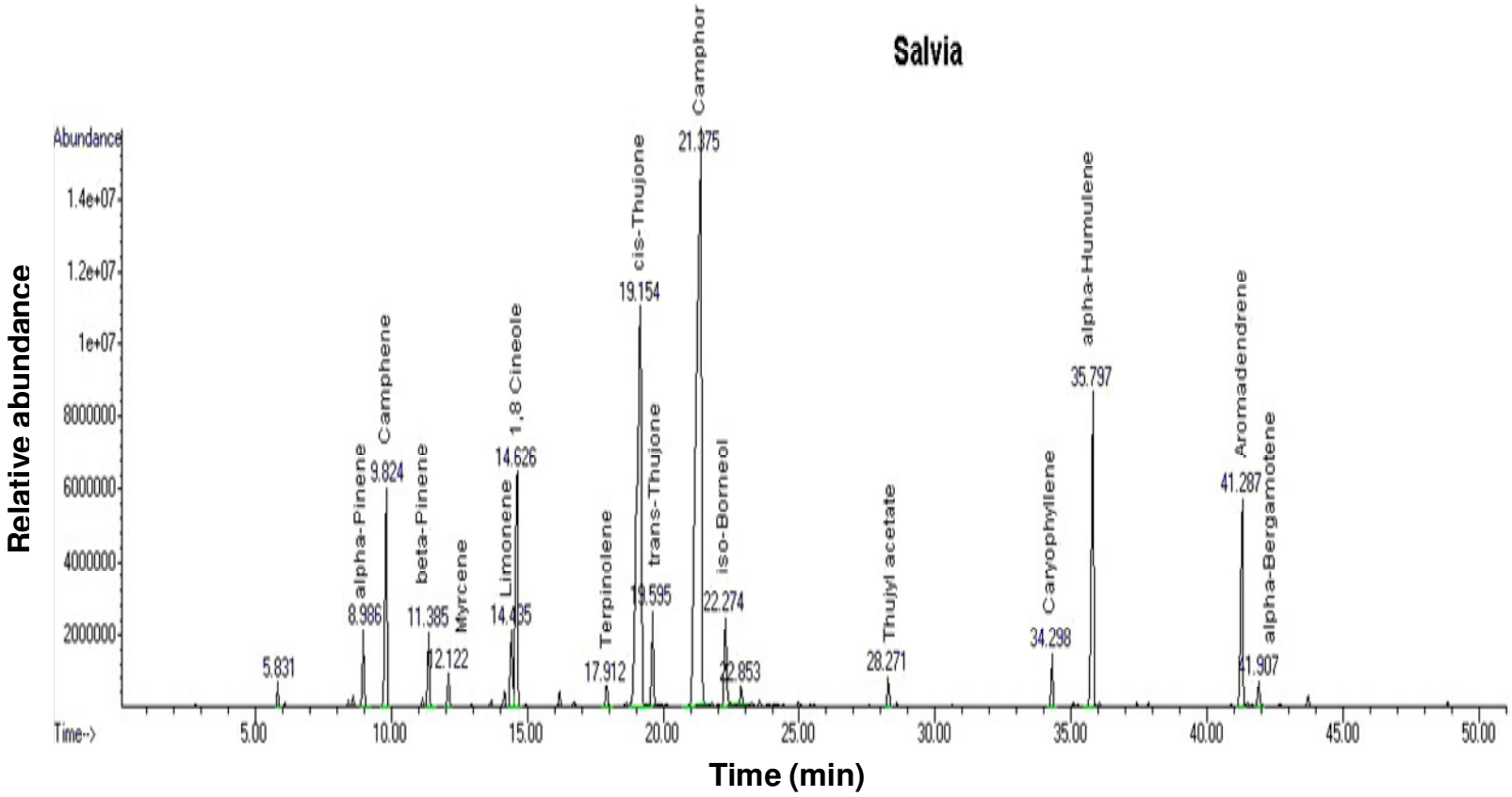

Figure 1. GC/MS Chromatogram of *Salvia offficinalis* essential oil.

Table 2. Group composition (%) of essential oil of *Salvia officinalis*.

Grouped compounds	Relative area (%)
Monoterpene hydrocarbon	13.41-17.01
Oxygenated monoterpenes	59.43-70.68
Sesquiterpene hydrocarbon	7.78-15.36
Oxygenated sesquiterpene	2.14-7.73
Others	0.59-1.79
Total	94.21-99.36

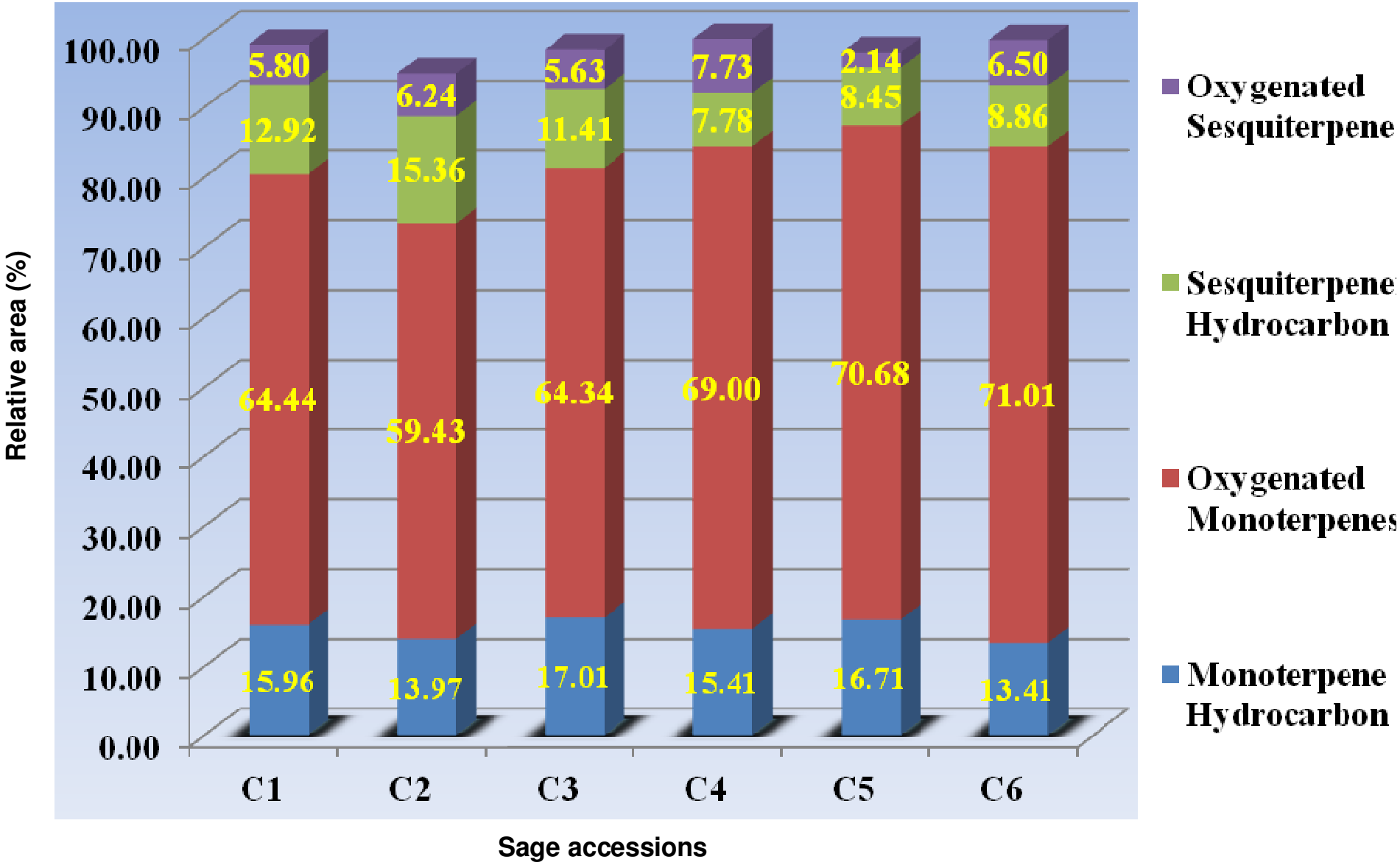

Figure 2. Comparative chemical composition (%) of *Salvia officinalis* L. oil based on compound group classification.

leaves and essential oils are known to possess carminative, antispasmodic, antiseptic, and astringent properties. Hence, the essential oils from these sage collections can be used for these applications by the medical industry.

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REFERENCES

Adams RP (2009). Identification of Essential Oil Components by gas Chromatography/Mass Spectroscopy, 4th Edition, Allured Publishing Corporation Co., Carol Stream IL.

Anonymous (1972). The Wealth of India. A Dictionary of Indian Raw materials Industrial products. Vol. 9, CSIR, New Delhi pp. 196.

Berotiene G, Nivinskiene O, Butkiene R, Mockute D (2007). Essential oil composition variability in sage (*Salvia officinalis* L.). Chemija 18:38-43.

Boelens MH, Boelens H (1997). Chemical and sensory evaluation of three sage oils. Perfum. Flavor. 22:19-40.

Chalchat JC, Michet, A, Pasquier B (1998). Study of clones of *Salvia officinalis* L. Yields and composition of essential oil. Flavour Fragr. J. 13:68-70.

Dob T, Berramdane T, Dahmane D, Benabdelkader T, Chelghoum C (2007). Chemical composition of the essential oil of *Salvia officinalis* from Algeria. Chem. Nat. Compd. 43:491-494.

Guenther E (1949). The Essential Oils, Vol. 3, 710-717.

Hedge C (1984). A Global Survey of the Biogeography of Labiatae, Harley RM, Reynolds (eds) Royal Botanical Gardens, Kew, pp 7.

Hossein MM, Salehi P, Ali Sonboli A, Mohammadi MV (2006). Essential oil variation of *Salvia officinalis* aerial parts during its phenological cycle. Chem. Nat. Compd. 42:19 -23.

Lawrence BM (2001). Progress in essential oils -sage oils. Perfum. Flavor. 26: 66-72.

Lawrence BM (2005). The Antimicrobial /Biological Activity of Essential Oils, Allured Publishing Corp. Carol Stream, IL, USA.

Negi KS, Muneem, KC, Pant VK (1998). Status of medicinal and aromatic plants in *ex-situ* field gene bank of U.P. Himalaya. J. Non-Timber Prod. 5:85-95.

Negi KS, Srivastava KS, Muneem KC, Pant VK (2004). Prospects of the garden sage as a new essential oil yielding crop for Uttaranchal. J. Econ. Taxon. Bot. 28:13-17.

Perry NB, Anderson RE, Brennan NJ, Dougles MH, Heaney AJ, McGimpsey JA, Smallfield BM (1999). Essential oils from Dalmatian sage (*Salvia officinalis* L.): Variation among individuals, plant parts, seasons and sites. J. of Agric. Food Chem. 47:2048-2054.

Raal A, Orav A, Arak E (2007). Composition of the essential oil of *Salvia officinalis* L. from various European countries. Nat. Prod. Res. 21: 406-411.

Radulescu V, Chiliment S, Opera E (2004). Capillary gas chromatography-mass spectrometry of volatile and semi-volatile compounds of *Salvia officinalis*. J. Chromatogr. A, 1027:121-126.