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Aroma Profile of Clary Sage (*Salvia sclarea* L.): Influence of Harvesting Stage and Post Harvest Storage in Uttarakhand Hills

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ABSTRACT

An experiment was conducted to determine the impact of harvesting stage and postharvest storage on essential oil (EO) content and composition of clary sage (*Salvia sclarea* L.) cv. 'CIM-Chandni' in the Kumaon region of western Himalaya. Clary sage inflorescences were collected at five different stages viz., prior to seed setting, all seeds whitish green, half seeds brown, all seeds brown and seed shattering stages. The EO was isolated by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The EO content varied from 0.18 to 0.31% during these stages. The major components of these oils were linalool (25.38-34.32%), linalyl acetate (27.98-48.59%), α-terpineol (4.03-5.25%) and sclareol (1.02-1.59%). Storage of clary sage inflorescences under shade for 96 h prior to distillation significantly reduced the EO recovery (from 0.30 to 0.13%). Linalool increase after storage (28.86 to 37.47%) but linalyl acetate initially increased (from 39.68 to 43.30%) thereafter it decreased after storage.

Keywords: composition, essential oil yield, flower ontogeny, Lamiaceae, storage
Abbreviations: GC, gas chromatography; **GC-MS**, gas chromatography-mass spectrometry; **EO**, essential oil; **FID**, flame ionization detector; **RI**, retention index

INTRODUCTION

Clary sage (*Salvia sclarea* L.; family: Lamiaceae; **Fig. 1**), a plant of southern European and central Asian origin, is cultivated commercially in Russia, Bulgaria, France and Morocco (Asilew 1930; Mishra and Negi 2009). The plant is known for its high value essential oil (EO), concrete and absolute, which is widely used in perfumery industries as a source of fragrance with refreshing and long lasting notes (Furia and Bellanca 1975). The EO is used for clearing the eye vision since ancient times; hence, it is popularly referred to as 'clary' or 'clear eye' (Gunther 1949). Besides, the EO also has several medicinal properties and is used to treat stress, tension, depression, insomnia, sore throat, indigestion, menstruation, hysteria, wild colics and other disorders (Grieve 1974). The EO or extracts of the aerial part of *S. sclarea* have analgesic, anti-inflammatory (Moretti *et al.* 1997), antioxidant, antifungal (Pitarokili *et al*. 2002), antibacterial (Peana *et al.* 1999; Gulcin *et al*. 2004) and genotoxic activities (Zani *et al.* 1991).

Analysis of the EO of *S. sclarea* has been carried out in the past by a number of researchers (Lalande 1984; Mazza 1988a, 1988b; Lawrence 1990; Elnir *et al.* 1991; Dzumayev *et al*. 1995; Souleles and Argyriadou 1997; Torres *et al.* 1997; Peana *et al.* 1999; Shawl *et al.* 1999; Carruba *et al.* 2002; Pesic and Bankovic 2003; Farkas *et al.* 2005). The most important components in flower EOs were linalool, linalyl acetate, α-terpineol, α-terpinyl acetate, and geranyl acetate. Principle components of the leaf EO was germacrene D, bicyclogermacrene, spathulenol, caryophyllene oxide, and α-copaene (Farkas *et al.* 2005). However, the amount of these components varied widely. Linalool and linalyl acetate were found at 9-16 and 49-73.6%, 20.3-28.5 and 44.9-53.4% and 10.4-19.5 and 45.3-61.8% in French, American and Russian clary sage EOs, respectively (Dzumayev *et al*. 1995). On the other hand, Italian clary sage EO was rich in α-terpineol (47.4%) and α-terpinyl acetate (22.1%) (Peana *et al.* 1999).

Fig. 1 ***Salvia sclarea*** **growing in Uttarakhand.**

Although the EO composition of aromatic plants is significantly influenced by a number of factors, harvesting stage and postharvest storage are the most important (Dzumayev *et al*. 1995; Bagchi *et al.* 2003a, 2003b; Ram *et al.* 2005; Lattoo *et al.* 2006; Maric *et al.* 2006; Moghaddam and Omidbiagi 2007; Verma *et al.* 2010a, 2010b, 2010c). As *S. sclarea* is a new crop for Uttarakhand hills, information concerning optimum harvesting stage and postharvest storage for better yield and quality of EO are not available from this region. Therefore, in the present study, EOs derived from five different stages of plant maturity were investigated by GC and GC-MS. In addition to this, the postharvest storage effect on *S. sclarea* inflorescences was also carried out.

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MATERIALS AND METHODS

Plant material and isolation of EO

Fresh inflorescences of clary sage cv. 'CIM-Chandni' were collected at 5 different stages viz., pre-seed setting (Stage 1), all seeds whitish-green (Stage 2), half of the seeds brown (Stage 3), all seeds brown (Stage 4) and seed shattering (Stage 5) stages from an experimental field of the Central Institute of Medicinal and Aromatic Plants, Research Centre Purara, Uttarakhand in May-June and used to isolate the EO. The site is located at 1250 m and has a temperate climate. In the second part of the study, a total of 24 samples of clary sage inflorescences were collected and divided into 8 batches with 3 samples per batch. The first batch was immediately hydrodistilled and the remaining two were kept in the shade and distilled consecutively after 4, 8, 12, 24, 48, 72 and 96 h of storage. The EOs of clary sage inflorescences were isolated by hydro-distillation for 3 h using a Clevenger-type apparatus. The EO contents were estimated on a fresh weight basis. The EO samples obtained were dehydrated over anhydrous sodium sulphate and kept in a cool and dark place before analyses.

GC and GC-MS analysis

Gas chromatography (GC) analysis of the EO samples was carried out on a Nucon gas chromatograph model 5765 and Perkin Elmer Auto XL GC equipped with a flame ionization detector (FID) and two different stationary phases, BP-20 (30 m × 0.25 mm × 0.25 µm film thickness) and PE-5 (60 m × 0.32 mm; 0.25 µm film coating) fused silica capillary columns, respectively. Hydrogen was the carrier gas, fed at 1.0 ml/min. The temperature was programmed from 70-230°C at 4°C/min with an initial and final hold time of 2 min (for BP-20) and from 70-250°C at 3°C/min (for PE-5). The split ratio was 1: 30. The injector and detector temperatures were 200 and 230°C on BP-20 and 220 and 300°C on PE-5 column, respectively. Gas chromatography-mass spectrometry (GC-MS) was carried out on a Perkin Elmer Auto System XL GC and Turbo Mass Spectrometer fitted with a fused silica capillary column, PE-5 (50 m × 0.32 mm, film thickness 0.25 µm). The column temperature was programmed at 100-280°C at 3°C/min using helium as the carrier gas at a constant pressure of 10 psi. MS conditions were: EI mode 70 eV, ion source temperature 250°C.

Identification of components

Identification of EO constituents was done on the basis of retention index (RI, average of all five stages, determined with reference to homologous series of *n*-alkanes, C_9-C_{24}, under identical experimental conditions), co-injection with standards or known essential oil constituents, MS Library search (NIST and WILEY), by comparing with MS literature data (Davies 1990; Adams 1995). The relative amounts of individual components were calculated based on GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

Ontogenic changes in EO content and composition

The EO content in fresh inflorescences of *S. sclarea* harvested at different stages of flowering viz., Stage 1, Stage 2, Stage 3, Stage 4 and Stage 5 was 0.18, 0.30, 0.31, 0.27 and 0.25%, respectively. The GC and GC-MS analysis results of the EOs are presented in **Table 1**. A typical chromatogram of the EO of *S. sclarea* grown in Uttarakhand hills is shown in **Fig. 2**. The major compounds of the EOs were linalool, linalyl acetate, α-terpineol, sclareol, geraniol, and geranyl acetate. Linalyl acetate was lowest during the Stage 1 (27.98%) but increased as the crop aged and became highest at the Stage 4 (48.59%). However, towards the Stage 5, linalyl acetate started to decrease (43.12%). On the other hand, linalool was higher during the Stage 1 (34.32%) followed by Stage 5 (29.39%), and varied from 25.38 to 27.32% during the remainder of the stages. A similar trend was also noted earlier by Dzumayev *et al.* (1995) where linalool was 34.0% at full bloom and reduced towards the full seed ripening stage (20.0%), while linalyl acetate increased from the full bloom stage (25.0%) to the full seed ripening stage (40.0%). In addition to this, it was also reported that the linalool content increased with an advancement in flower maturity and reached a maximum when 60% of

Table 1 Changes in the essential oil content and composition of *Salvia sclarea* in North Indian hills during flower ontogeny.

Compound	RI[a]	RI[b]	Content (%) / Harvesting stage				
			A	B	C	D	E
β-Myrcene	1158	989	2.89	2.99	1.72	1.78	1.60
Limonene	1194	1030	0.73	0.81	0.44	0.49	0.36
(*Z*)-*β*-Ocimene	1234	1041	0.90	0.90	0.61	0.66	0.56
(*E*)-*β*-Ocimene	1251	1053	1.60	1.71	1.15	1.31	1.13
(*Z*)-Linalool oxide	1435	1072	-	-	0.29	0.06	t
Camphor	1507	1141	0.20	0.15	0.11	t	t
β-Bourbonene	1515	1383	t	t	t	t	-
Linalool	1550	1101	34.32	26.02	25.38	27.32	29.39
Linalyl acetate	1561	1257	27.98	40.69	48.48	48.59	43.12
Terpinen-4-ol	1606	1175	0.75	1.50	0.78	0.20	0.84
α-Humulene	1660	1450	t	t	t	t	-
Neral	1681	1239	2.94	2.05	0.86	0.76	0.65
α-Terpineol	1685	1187	5.01	4.70	5.25	5.25	4.03
Geranyl formate	1695	1298	-	t	t	t	t
Germacrene-D	1708	1477	0.35	0.22	0.10	t	t
Neryl acetate	1720	1356	1.68	1.54	1.28	1.26	1.23
β-Bisabolene	1727	1507	t	t	t	-	-
δ-Cadinene	1739	1527	0.38	0.26	0.22	0.22	0.20
Geranyl acetate	1760	1380	3.48	2.62	2.59	2.63	2.29
Nerol	1801	1224	1.23	1.43	1.03	1.07	0.77
Geraniol	1859	1251	6.68	3.76	2.60	2.78	3.42
Caryophyllene oxide	1995	1581	t	0.14	0.98	t	-
Spathulenol	2137	1574	t	t	t	t	t
β-Eudesmol	2252	1649	t	t	t	t	t
Sclareol	2385	2223	1.53	1.02	1.32	1.20	1.59
Total identified (%)			92.65	92.51	95.21	95.69	91.18
Essential oil (%)*			0.18	0.30	0.31	0.27	0.25

[a]RI: Retention Indices on polar column (BP-20); [b]RI: Retention Indices on non-polar column (PE-5); **A**: Pre- seed setting stage; **B**: All seeds whitish green stage; **C**: Half seeds brown stage; **D**: All seeds brown stage; **E**: Seed shattering stage: **t**: trace (<0.1%); *Fresh weight basis

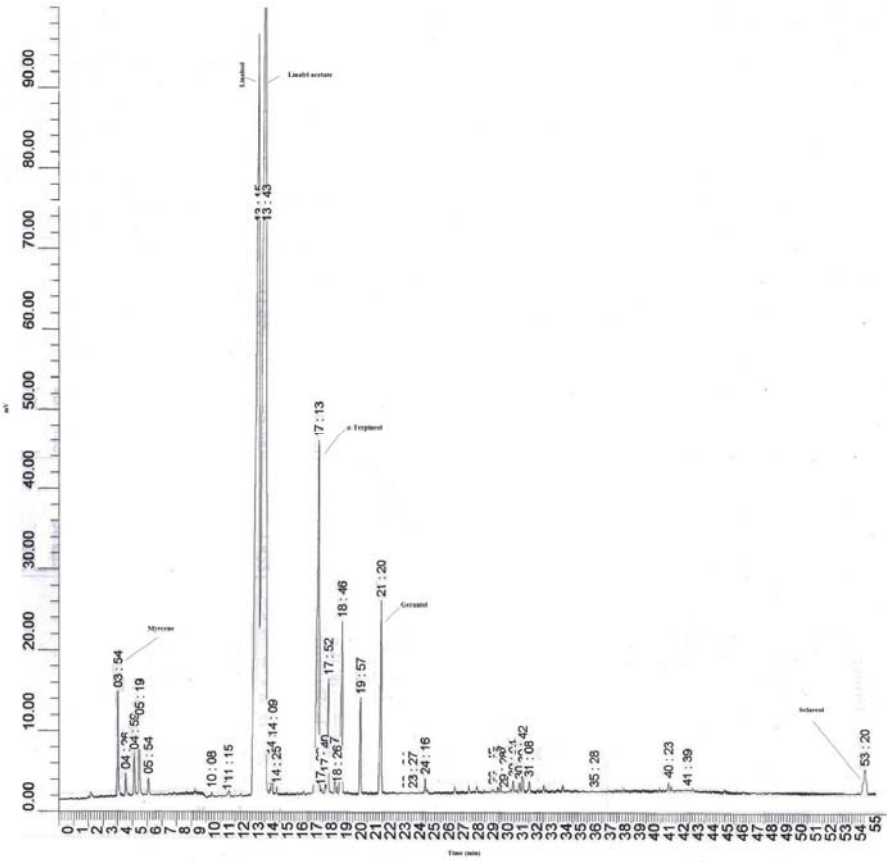

Fig. 2 Chromatogram (GC) of the essential oil of *Salvia sclarea* grown in Uttarakhand.

Table 2 Changes in the essential oil yield and composition of *Salvia sclarea* during post harvest storage in north Indian hills.

Compound (%)	RI[a]	RI[b]	Storage period (h)							
			0	**4**	**8**	**12**	**24**	**48**	**72**	**96**
β-Myrcene	1158	989	1.72	1.11	1.36	2.40	2.64	-	1.93	3.14
Limonene	1194	1030	1.09	0.73	1.02	-	-	-	-	-
(*Z*)-*β*-Ocimene	1234	1041	0.59	-	-	-	-	-	-	-
(*E*)-*β*-Ocimene	1251	1053	2.54	2.03	2.14	1.40	1.53	-	1.13	-
(*Z*)-Linalool oxide	1435	1072	0.12	0.11	-	-	-	-	-	-
Camphor	1507	1141	0.17	0.04	-	-	0.04	-	0.32	-
Linalool	1550	1101	28.86	29.16	35.22	37.47	36.32	29.81	33.51	33.33
Linalyl acetate	1561	1257	39.68	43.30	37.34	36.47	29.59	31.58	36.93	33.36
(*E*)-*β*-Terpineol	1572	-	0.69	0.55	0.45	0.31	0.08	0.11	0.48	1.17
Terpinen-4-ol	1606	1175	0.64	0.09	0.19	0.40	0.54	0.12	0.10	0.19
α-Terpineol	1685	1187	5.61	5.51	4.78	6.40	7.38	4.89	6.29	5.98
Geranyl formate	1695	1298	0.11	0.09	0.10	-	0.11	-	-	-
Germacrene-D	1708	1477	0.08	0.06	-	-	0.09	0.06	0.06	-
Neryl acetate	1720	1365	1.12	1.07	1.17	1.46	1.48	0.95	1.30	1.23
Geranial	1728	1265	0.12	0.12	0.26	0.12	0.11	0.11	0.16	0.20
δ-Cadinene	1739	1527	0.22	0.19	-	0.14	0.03	0.17	0.18	0.18
Geranyl acetate	1760	1380	2.39	2.27	2.31	2.84	2.94	2.11	2.48	2.35
Nerol	1801	1224	0.84	0.64	0.68	0.85	1.40	0.58	0.84	1.00
Geraniol	1859	1251	2.10	2.10	2.19	2.94	3.30	2.48	2.75	2.31
Caryophyllene oxide	1995	1581	0.42	0.39	0.48	0.29	0.30	0.63	0.47	0.59
Sclareol	2387	2223	2.43	2.16	1.95	0.66	2.33	2.83	1.84	2.26
Total identified			91.54	91.72	91.64	94.15	90.21	76.43	90.77	87.29
Essential oil (%)[*]			0.30	0.22	0.20	0.18	0.17	0.15	0.13	0.13
Moisture loss (%)			-	10.86	23.53	31.50	48.00	63.50	69.75	73.29
Essential oil loss (%)			-	26.7	33.3	40.0	43.3	50.0	56.7	56.7

[*] Fresh weight basis

seeds were milky white (Lattoo *et al.* 2006), which was not observed in the present study. This could be due to variation of climatic and soil conditions (Chippine and Falchi 1996). Further, geraniol (6.68%), geranyl acetate (3.48%), neryl acetate (1.68%), *δ*-cadinene (0.38%) and camphor (0.20%) were higher during the Stage 1, whereas *β*-myrcene (2.99%), (*E*)-*β*-ocimene (1.71%), terpinen-4-ol (1.50%), nerol (1.43%) and limonene (0.81%) were maximum during Stage 2. However, *α*-terpineol reached higher values during Stage 3 and Stage 4 (5.25%) whilst sclareol percentage was higher at Stage 5 (1.59%).

Post harvest storage effect on EO content and composition

The EO content of *S. sclarea* inflorescences was decreased on storage (from 0.30% to 0.13%). Loss in the EO recovery was 26.7% to 56.7% after 4 to 96 h of storage. Therefore, it is quite obvious that storage of *S. sclarea* inflorescences cannot be recommended. Variation in the EO content, EO loss, moisture loss, and EO composition with respect to period of storage prior to distillation is given in **Table 2**. The major constituents in the EO of fresh inflorescences were linalyl acetate (39.68%), linalool (28. 86%), α-terpineol (5.61%), sclareol (2.43%), geraniol (2.10%), geranyl acetate (2.39%), (*E*)-β-ocimene (2.54%), β-myrcene (1.72%), neryl acetate (1.12%) and limonene (1.09%). Notable minor constituents of the EO were camphor (0.17%), (*Z*)-linalool oxide (0.12%) and terpinen-4-ol (0.64%). The chemical composition of the EO changed with the period of storage. Linalyl acetate increased up to 4 h (43.30%); thereafter it started to decrease and reached the lowest value after 24 h (29.59%). On the other hand, linalool increased during storage and reached a higher percentage after 12 h (37.47%). α-Terpineol, neryl acetate, geraniol and geranyl acetate were higher in 24 h stored inflorescences followed by 12 h. Sclareol (2.83%) and caryophyllene oxide (0.63%) were also higher in 48 h stored material.

CONCLUSIONS

The results of present study showed that harvesting stages (Stages 1-5) and post harvest storage (0-96 h) had significant effect on EO yield and quantitative composition of *S. sclarea* grown in Uttarakhand hills. Therefore, it is concluded that harvesting the inflorescences during Stage 3 and effecting the distillation immediately after harvesting can achieve best results in terms of quantity as well as quality of EO.

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