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Effect of Natural Drying Methods on Flavour Profile of Camphor Rich *Ocimum americanum* L. from North India

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In the present study, fresh aerial parts of *Ocimum americanum* L. were collected from Ramnagar, India and sun and shade drying was applied until constant weights. The fresh and dried plant materials were hydrodistilled by Clevenger apparatus and the extracted oils were analyzed by GC and GC/MS. One-way ANOVA and correlation were performed to evaluate the difference and correlation between drying treatments using SPSS 16.0. The major compounds of the fresh oil were camphor (33.41 %), maillol (11.96 %), β-selinene (8.34 %), α-selinene (6.92 %), β-gurjunene (5.43 %) and (*E*)-caryophyllene (5.07 %). In the dried samples, significant increase ($p < 0.05$) in the percentage of camphor and maillol was observed while the percentage of (*E*)-cayophyllene, β-gurjunene, β-selinene and α-selinene significantly ($p < 0.05$) decreased during drying process. Drying caused loss of fourteen constituents with appearance of four compounds. The oil yield was the highest under sun drying condition.

Keywords: *Ocimum americanum* L., Camphor, Maillol, β-Selinene, α-Selinene, β-Gurjunene, (*E*)-Caryophyllene.

INTRODUCTION

Ocimum americanum L., commonly known as African basil, is a wild aromatic herb with hairy leaves and scented flowers (family Lamiaceae) and is native to tropical Africa [1]. The leaves are used to add flavour in the preparation of soup, tea and salad [2]. The leaf oil of the plant has been traditionally used for the treatment of constipation, diabetes, dysentery, diarrhea and piles [3,4]. Furthermore, the essential oil is a potential source of novel compounds such as *trans*-β-ocimene, 1,8-cineole, citral, linalool, methyl chavicol, (*E*)-methyl cinnamate, camphor and bisabolenes which are responsible for strong biological activities including antibacterial, antifungal, antioxidant, mosquito repellent, larvicidal and hepatoprotective activities [3-10].

The need for high quality raw material has increased due to the high demand of processed and preserved food products. Drying is one of the common methods of herbs and spices preservation which inhibits enzymatic degradation, reduces moisture content of plants and increases the shelf life of product [11]. Drying also affects the essential oil composition of aromatic and medicinal plants and consequently affects their flavour profile [12]. Although, sun and shade drying are the most economical and widely used methods, they depend on the weather conditions and take longer times [13]. According to Hossain *et al*. [14] drying may improve the quality of the aroma profile of the plants either due to the formation of new volatile components through oxidation or esterification reactions or the loss of volatile compounds [14]. The effect of different drying methods on the essential oil composition of bisabolene rich *O. americanum* collected from Ranikhet (Inida) was studied by Bhatt *et al*. [15,16]. Baritaux *et al*. [17] and Di Cesare *et al*. [18] have reported the effect of different drying methods on the essential oil composition of *Ocimum basilicum*. There are several reports on the effect of drying conditions on the essential oil composition of some other Lamiaceae plants such as *Plectranthus glandulosus*, *Mentha piperita*, *Leonotis leonurus* and *Mentha spicata* [19-23]. Different drying conditions also affect the essential oil yield. The essential oil yield of shade dried *Ocimum basilicum*, *Mentha piperita*, *Origanum vulgare* and *Origanum onites* was the highest [24-26]. However, according to a study from United States, neither drying duration nor drying condition had a significant effect on oil yield of *Mentha spicata* [27]. These drying studies have revealed that the essential oil composition of Lamiaceae plants varied with varying temperature and humidity

conditions. Selection of optimized drying method could assist in maintaining the quality of the oil and minimizing the loss of volatiles.

After surveying the existing literature, it was found that no work has been reported on the impact of shade and sun drying on the flavour profile of camphor rich *Ocimum americanum* from India. Therefore, the aim of the present work was to compare the effect of different natural drying methods on the flavour profile of *Ocimum americanum* collected from Ramnagar, India.

EXPERIMENTAL

Collection and identification of plant material: Fresh aerial parts of *Ocimum americanum* L. (6 kg) at flowering stage were collected from the wild field of Ramnagar (India) in the month of October. A fraction of plant material was sun (25 ± 5 ºC) and shade (20 ± 5 ºC) dried until constant weight was obtained. The identification of the plant was done at Botanical Survey of India (BSI), Dehradun, India (Acc No. 116348).

Isolation of essential oils: Fresh, shade and sun dried plant materials were sliced into small parts and 1000 g, 500 g and 500 g of each sample was extracted by using hydrodistillation method in a Clevenger apparatus for 5 h [28] and 1 mL, 1 mL and 1.5 mL oils were obtained, respectively. The oils were dried over anhydrous sodium sulphate and stored in glass vials. The sealed glass vials were stored in BOD incubator prior to the analysis [28]. All the experiments were conducted in three replicates.

Analysis of essential oils: Shimadzu 2010 GC fitted with Rtx-5 capillary column (30 m × 0.25 mm with film thickness 0.25 μm) and FID was used for oil analysis. For shade dried oil analysis, the column temperature was programmed at 50 ºC (hold time: 2 min) to 210 ºC (hold time: 2 min) at 3 ºC min^{-1} and then 210-280 ºC at 10 ºC min^{-1} with final hold time of 14 min. For fresh and sun dried oil, the column temperature was programmed at 50 ºC (hold time: 2 min) to 210 ºC (hold time: 2 min) at 3 ºC min^{-1} and then 210-280 ºC at 8 ºC min^{-1} with final hold time of 14 min. Nitrogen at 30 mL/min column head pressure was used as carrier gas. The injector and FID temperature for both the programme was 260 and 270 ºC, respectively. The injection volume was 0.1μL neat oil with split mode (split ratio 1:40) [16].

The GC/MS used was 2010 GC coupled with Shimadzu QP 2010 plus with thermal desorption system TD 20 having Rtx-5 capillary column (30 m × 0.25 mm with film thickness 0.25 μm). The GC-MS was programmed in similar conditions to those of GC. Helium was used as carrier gas and the injector temperature was 230 ºC. The neat oil (0.1 μL) was taken for analysis with split ratio of 1:40. MS were taken at 70 eV with mass range of 40-650 amu [16].

Identification of the components: Identification of the oil components was done on the basis of their retention index (RI) which was calculated with respect to C_9-C_{33} *n*-alkane series (Polyscience Corp., Niles IL) under same GC conditions, MS Library search (NIST:NIH version 2.1 and WILEY: 7th edition), comparison with the MS literature data [29] and co-injection with standards (α-pinene and limonene). The relative concentration of individual volatile component was calculated on the basis of GC peak area without using any correction factor [16].

Statistical analysis: The mean and standard deviation of triplicate values were calculated using MS-Excel and the analyzed data was presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to compare mean values of percentage of constituents under different drying methods at a probability level of $p < 0.05$ using SPSS 16.0 statistical software (Duncan Multiple Range Test) [28]. Correlation was also applied using SPSS 16.0 software to correlate the major constituents at probability levels of $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSION

Essential oil yield: From the GC (Figs. 1-3) and GC/MS data, it was clear that there was variation in the chemical composition of *Ocimum americanum* after drying. In sun dried sample, the oil yield was maximum. The oil yields of the fresh, shade and sun dried samples were 0.1, 0.2 and 0.3 % (v/w), respectively. In a previous report, essential oil yield of sun dried *Ocimum americanum* (0.7 %) was higher as compared to the oil (0.1 %) of fresh plant [16]. Asekun *et al.* [21] found that the oils obtained

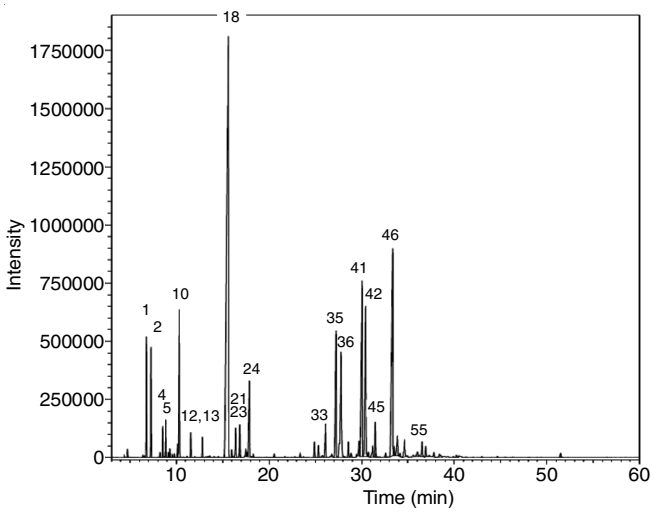

Fig. 1. Gas chromatogram of fresh *Ocimum americanum* L. oil

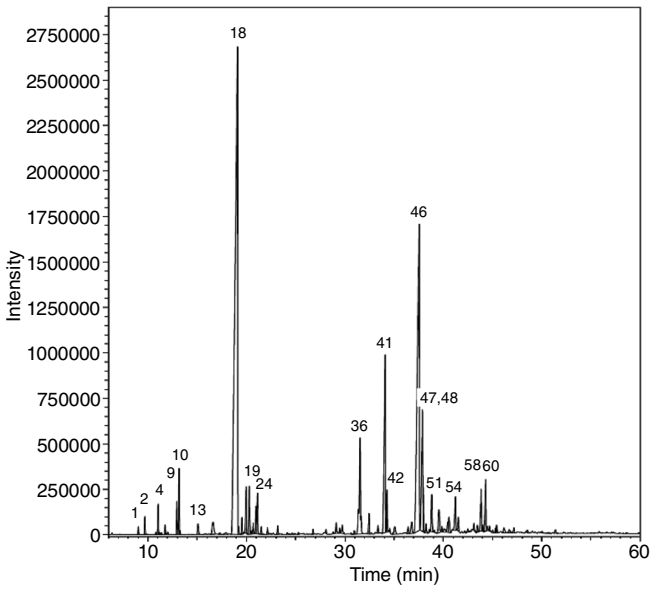

Fig. 2. Gas chromatogram of shade dried *Ocimum americanum* L. oil

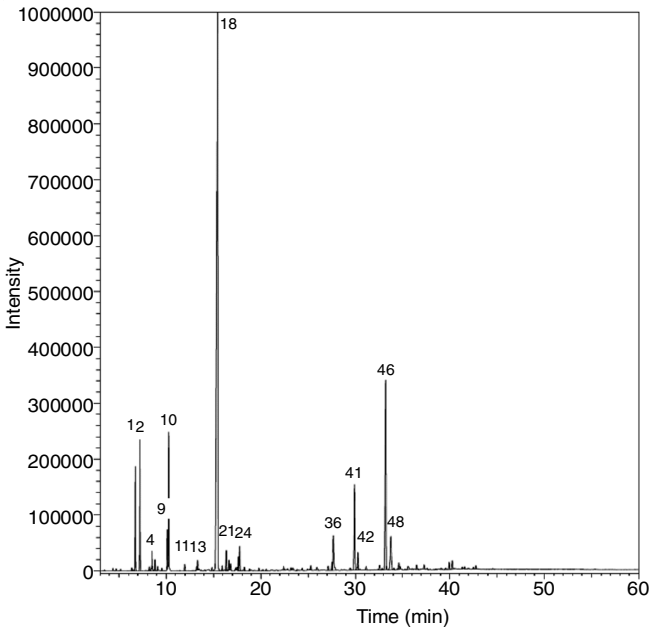

Fig. 3. Gas chromatogram of sun dried *Ocimum americanum* L. oil

from the aerial parts of sun-dried *Leonotis leonurus* had better yield as compared to those from the air and oven-dried materials. However, according to Halva [30], the oil yield can be decreased from 2.55 to 1.94 % during drying processes. On the contrary, the highest oil yield was acquired by shade drying treatment in *Ocimum basilicum* (0.9 %), *Mentha piperita* (3.68 %), *Origanum vulgare* (2.53 %) and *Origanum onite* (1.96 %) [24-26].

Essential oil composition: Forty four compounds out of 87 compounds for fresh, 36 out of 80 compounds for shade dried and 35 out of 65 compounds for sun dried *O. americanum* have been identified representing 96.04, 91.93 and 94.09 % of the total oil, respectively (Table-1). Shade drying resulted in loss of 8 % volatile components while sun drying caused the loss of 25 % major volatile constituents of *O. americanum*. The major constituents in the fresh oil were camphor (33.41%), maaliol (11.96 %), β-selinene (8.35 %), α-selinene (6.92 %), β-gurjunene (5.43 %) and (*E*)-caryophyllene (5.07 %) along with limonene (4.09 %), myrtenol (2.83 %), α-pinene (2.44 %), camphene (2.33 %), β-elemene (1.18 %) and δ-cadinene (1.11 %) as minor constituents. *Trans*-β-ocimene (29 %) and 1,8-cineole (41.3 %) were the major components in the essential oil of *O. americanum* from Africa [6] and eugenol (28.46 %) and methyl eugenol (17.34 %) from Egypt [31]. Six chemotypes of *Ocimum americanum* were observed from India including 1,8-cineole (4.5-16.8 %), methyl chavicol (1.7-12.9 %), eugenol (27.6-38.2 %), β-bisabolene (9.9-17.8 %), (*E*)-γ-bisabolene (9.8-15.8 %) rich (Nainital, Banbasa, Rushi and Champawat), methyl chavicol (28.9 %), eugenol (14.7 %), β-bisabolene (14.7 %), (*E*)-γ-bisabolene (7.1%), aliphatic hydrocarbons (8.4 %) rich (Dhoulchina), eugenol (45.2 %), methyl eugenol (14.8 %), (*E*)-caryophyllene (30.2 %) rich (Rudrapur), linalool (14.2 %), methyl chavicol (78.3 %) rich (Dharchula), 1,8-cineole (11.5-28.4 %), camphor (49.4-42.0 %), eugenol (0-8.6 %) and aliphatic hydrocarbons rich (1.1-11.4 %) (Almora and Kilbury) and camphor (23.0 %), aliphatic hydrocarbons (44.1%) rich from Mussoorie [9].

TABLE-1
EFFECT OF DRYING ON THE ESSENTIAL OIL COMPOSITION OF *O. americanum* COLLECTED FROM RAMNAGAR

S. No.	Name of compound	$RI_{Calculated}$	RI [Ref. 27]	RT_{Fresh} (min)	Percent of oil (fresh sample) (mean± SD)	$RT_{Shade\ dried}$ (min)	Percent of oil (shade dried sample) (mean± SD)	$RT_{Sun\ dried}$ (min)	Percent of oil (sun dried sample) (mean± SD)
1	α-Pinene	933	932	6.8	2.44[a]±0.51	9.0	0.16[b]	6.7	3.37[c]±0.35
2	Camphene	948	946	7.3	2.33[a]±0.57	9.7	0.37[b]	7.2	4.39[c]±0.34
3	β-Pinene	976	974	8.2	0.13	10.8	0.05	8.2	0.16
4	1-Octen-3-ol	980	974	8.5	0.73	11.0	0.62	8.5	0.79
5	Myrcene	991	988	8.8	0.85	11.2	0.06	8.8	0.45
6	α-Phellandrene	1004	1002	9.3	0.20	–	ND	–	ND
7	(3*Z*)-Hexenyl acetate	1008	1004	9.6	0.08	11.7	0.20	9.5	0.11
8	α-Terpinene	1016	1014	9.8	0.10	–	ND	–	ND
9	*p*-Cymene	1024	1020	10.1	0.35	12.9	0.64	10.1	1.40
10	Limonene	1029	1024	10.3	4.09[a]±0.14	13.2	1.27[b]±0.38	10.3	4.99[c]±0.18
11	1,8-Cineole	1031	1026	10.7	0.02	13.3	0.07	11.9	0.30
12	γ-Terpinene	1059	1054	11.6	0.62	–	ND	–	ND
13	*cis*-Sabinene hydrate	1070	1065	–	ND	15.1	0.33	13.2	0.18
14	Terpinolene	1089	1086	12.8	0.55	–	ND	–	ND
15	*trans*-Sabinene hydrate	1105	1098	–	ND	17.3	0.07	13.3	0.48
16	*cis*-Limonene oxide	1134	1132	–	ND	–	ND	14.8	0.18
17	3-*iso*-Thujanol	1127	1134	–	ND	17.9	0.12	–	ND
18	Camphor	1151	1141	15.6	33.41[a]±0.52	19.1	40.45[b]±0.18	15.4	49.74[c]±0.39
19	Isomenthol	1188	1179	–	ND	20.3	1.29	–	ND
20	Isoborneol	1158	1155	16.0	0.20	–	ND	15.9	0.24
21	Borneol	1168	1165	16.4	0.90	–	ND	16.3	0.98
22	Menthol	1175	1167	–	–	–	ND	16.6	0.45
23	Terpinen-4-ol	1179	1174	16.8	0.99	–	ND	16.8	0.30
24	Myrtenol	1200	1194	17.9	2.83	21.1	1.07	17.8	1.25
25	Verbenone	1211	1204	18.3	0.09	21.5	0.17	18.3	0.16

26	*trans*-Carveol	1221	1215	–	ND	–	ND	18.8	0.08
27	Carvone	1244	1239	–	ND	23.2	0.21	19.8	0.13
28	*trans*-Myrtanol	1262	1258	19.8	0.02	–	ND	–	ND
29	Myrtenyl acetate	1327	1324	20.6	0.12	26.8	0.12	–	ND
30	Cyclosativene	1369	1369	23.4	0.15	–	ND	–	ND
31	α-Copaene	1379	1374	24.9	0.50	29.1	0.30	24.9	0.07
32	β-Bourbornene	1388	1387	25.3	0.39	–	ND	25.3	0.25
33	β-Elemene	1395	1389	26.1	1.18	29.7	0.29	26.0	0.27
34	α-Gurjunene	1413	1409	26.8	0.12	–	ND	–	ND
35	(*E*)-Caryophyllene	1425	1417	27.2	5.07^{a}±0.59	–	ND	27.1	0.25^{b}
36	β-Gurjunene	1438	1431	27.8	5.43^{a}±0.12	31.6	3.84^{b}±0.21	27.7	2.27^{c}±0.29
37	α-Humulene	1458	1452	28.6	0.50	–	ND	–	ND
38	9-*epi*-(*E*)-Caryophyllene	1464	1464	28.9	0.17	–	ND	–	ND
39	α-Amorphene	1482	1483	29.5	0.08	–	ND	–	ND
40	Germacrene D	1486	1484	29.7	0.77	–	ND	–	ND
41	β-Selinene	1494	1489	30.1	8.34^{a}±0.29	34.1	6.60^{b}±0.10	30.0	4.56^{c}±0.18
42	α-Selinene	1502	1498	30.4	6.92^{a}±0.30	34.3	0.93^{b}	30.3	0.93^{b}
43	Germacrene A	1510	1508	30.7	0.14	–	ND	–	ND
44	7-*epi*-α-Selinene	1520	1520	31.2	0.47	35.1	0.38	31.2	0.22
45	δ-Cadinene	1528	1522	31.5	1.11	–	ND	–	ND
46	Maaliol	1577	1566	33.4	11.96^{a}±0.44	37.5	20.17^{b}±0.13	33.2	11.92^{a}±0.07
47	Globulol	1598	1590	33.5	0.25	37.9	4.38	–	ND
48	Viridiflorol	1600	1592	–	ND	38.0	0.77	33.8	2.69
49	Juneol	1617	1618	–	ND	38.8	1.05	–	ND
50	1-*epi*-Cubenol	1634	1627	34.1	0.09	–	ND	34.1	0.09
51	Muurola-4,10(14)-dien-β-ol	1634	1630	–	ND	39.6	0.97	–	ND
52	Caryophylla-4(12),8(13)-dien-5α-ol	1639	1639	–	ND	40.4	0.33	–	ND
53	α-Muurolol	1648	1644	36.0	0.27	40.6	0.51	36.0	0.08
54	Himachalol	1659	1652	–	ND	41.2	1.11	–	ND
55	α-Cadinol	1660	1652	36.5	0.57	–	ND	36.5	0.30
56	Caryophyllene (14-hydroxy-9-*epi*-*E*)	1673	1668	36.9	0.35	–	ND	–	ND
57	Eudesma-4(15),7-dien-1β-ol	1698	1687	–	ND	42.5	0.11	–	ND
58	Cedroxyde	1723	1713	–	ND	43.5	0.19	–	ND
59	Oplopanone	1742	1739	–	ND	43.8	1.20	–	ND
60	Aristolone	1767	1762	38.4	0.16	44.3	1.58	38.5	0.06
	Total				96.04		91.93		94.09

SD = Standard deviation; ND = Not detected (< 0.01 %).

Ocimum americanum collected from Ranikhet was dominated by the presence of β-bisabolene (29.06 %), (*E*)-γ-bisabolene (17.49 %), 1,8-cineole (9.14 %), methyl chavicol (7.56 %) and (*Z*)-β-ocimene (7.18 %) [15]. The major components in the shade dried plant were camphor (40.45 %), maaliol (20.17 %) and β-selinene (6.60 %) while in the sun dried plant, the major components were camphor (49.74 %) and maalliol (11.92 %) (Fig. 4). Fourteen components such as α-phellandrene, α-terpinene, γ-terpinene, terpinolene, *trans*-myrtanol, cyclosativene, α-gurjunene, α-humulene, 9-*epi*-(*E*)-caryophyllene,

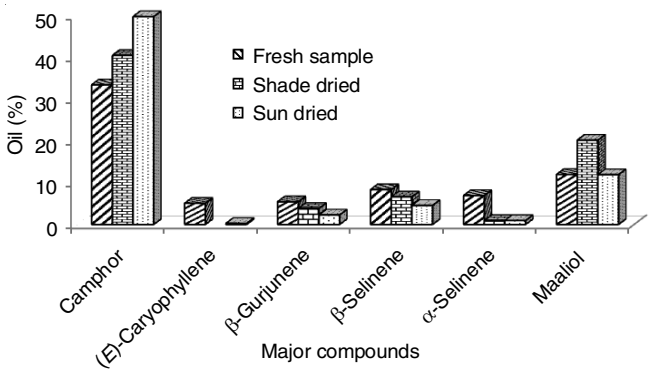

Fig. 4. Variation in the major constituents of *O. americanum* L. after drying

α-amorphene, germacrene D, germacrene A, δ-cadinene and caryophyllene (14-hydroxy-9-*epi*-*E*) were disappeared during drying while four compounds (*cis*-sabinene hydrate, *trans*-sabinene hydrate, carvone and viridiflorol) appeared in dried plant material which were totally absent in the fresh plant.

Pirbalouti *et al*. [13], Hamrouni-Sellami *et al*. [32] and Rahimmalek and Goli [33] have reported the presence of essential oil components in dried samples that were not present in essential oil of fresh samples. Compounds such as 3-iso-thujanol, isomenthol, juneol, muurola-4,10(14)-dien-β-ol, caryophylla-4(12), 8(13)-dien-5α-ol, eudesma-4(15),7-dien-1β-ol, cedroxyde and oplopanone were present only in the shade dried *Ocimum americanum*. After sun drying, only three components namely *cis*-limonene oxide, menthol and *trans*-carveol appeared. Percentage of camphor (33.41-49.74 %) and maaliol (11.92-20.17 %) increased while that of (*E*)-cayophyllene (5.07-0.25 %), β-gurjunene (5.43-2.27 %), β-selinene (8.34-4.56 %) and α-selinene (6.92-0.93 %) decreased on drying *O. americanum*. Content of camphor was also found to increase in *O. americanum* oil collected from Ranikhet on sun drying [16]. Microwave drying was observed to retain high percentage of marker compounds (eugenol, methyleugenol, eucalyptol and linalool) in *Ocimum basilicum* as compared to the air and freeze drying

TABLE-2
CORRELATION AMONG MAJOR CONSTITUENTS OF *O. americanum*

Compounds	α-Pinene	Camphene	Limonene	Camphor	Endoborneol	Myrtenol	Calarene	β-Selinene	α-Selinene	Maillol
α-Pinene	1	0.968	0.999*	0.370	-0.909	0.493	-0.280	-0.326	0.236	-0.961
Camphene		1	0.955	0.590	-0.777	0.259	-0.511	-0.552	-0.014	-0.861
Limonene			1	0.324	-0.929	0.534	-0.233	-0.280	0.283	-0.973
Camphor				1	0.050	-0.627	-0.995	-0.999*	-0.816	-0.097
Endoborneol					1	-0.810	-0.145	-0.096	-0.619	0.989
Myrtenol						1	0.698	0.662	0.962	-0.715
Calarene							1	0.999*	0.867	0.002
β-Selinene								1	0.841	0.051
α-Selinene									1	-0.496
Maillol										1

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

[18]. It was observed by Baritaux *et al.* [17] that the contents of methyl chavicol and eugenol decreased along with the significant increase in the content of *trans*-bergamotene, linalool and 1,8-cineole in basil (*Ocimum basilicum* L.) [17]. The percentage of oxygenated monoterpenes and oxygenated sesquiterpenes increased in *O. americanum*. On the other hand, the percentage of sesquiterpene hydrocarbons decreased on shade and sun drying of *Ocimum americanum*. Increase in the percentage of monoterpene hydrocarbons was noted after sun drying and decrease in the percentage of monoterpene hydrocarbons was observed after shade drying (Fig. 5).

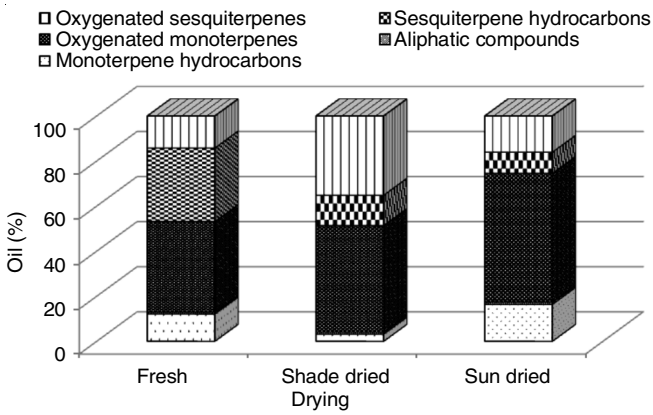

Fig. 5. Comparative class composition of essential oils of *O. americanum* affected by drying

Correlation among major constituents: Correlation analysis of 10 major constituents was done to evaluate relationship between drying methods. α-Pinene was significantly and positively correlated with limonene ($r = 0.999$; $p < 0.05$) while calarene was positively correlated ($r = 0.999$; $p < 0.05$) with β-selinene. Camphor was found to be negatively correlated ($r = -0.999$; $p < 0.05$) to β-selinene (Table-2).

Conclusion

In the present study, the essential oil composition of aerial parts of *Ocimum americanum* subjected to natural drying methods were compared with fresh oil using One-way ANOVA. Camphor and maaliol were the common major constituents in fresh and dried materials. Oil content and percentage of camphor and maaliol were increased significantly ($p < 0.05$) in sun dried material. Therefore, sun drying could be used a significant drying method for *Ocimum americanum*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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