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Evaluation of antioxidant potential and quality of volatile constituents of fresh and sun dried *Ocimum gratissimum*

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Ocimum gratissimum (Family: Lamiaceae), is an aromatic herbaceous plant which is native to tropical countries especially India and West Africa. It has been traditionally used for medicinal, condiment and culinary purpose. Fresh leaves of *Ocimum gratissimum* were collected from the cultivated field of Haldwani (Uttarakhand), India at full blooming stage. The leaves of *Ocimum gratissimum* were hydrodistilled and analysed immediately after collection (fresh) and after sun drying to evaluate the quality of volatile constituents in terms of composition by GC and GC-MS and antioxidant activity by 2,2´-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Duncan and paired t-test were performed to evaluate the difference between drying treatments using SPSS 16.0 and MS excel respectively. The fresh oil had a very high amount of eugenol (72.70%) and sun drying resulted in significant decrease ($p < 0.01$) in the percentage of this constituent (17.31%). Drying caused complete loss of three volatile constituents with appearance of sixteen compounds. Fresh oil showed better DPPH radical scavenging activity as compared to the sun dried oil.

Keywords: *Ocimum gratissimum*, Lamiaceae, eugenol, (*E*)-β-ocimene, drying, antioxidant activity.

Introduction

Fresh aromatic and medicinal plants require more space and are more susceptible to microorganisms attack and thus, pose difficulties in transportation and storage. Drying is one of the most common methods to preserve quality of aromatic and medicinal plants as it reduces the water content, preserve the plant material from microorganism and the material becomes easier to handle[1]. The drying processes include air-drying, shade-drying, sun-drying, oven-drying, microwave-drying and freeze-drying. The drying method and temperature conditions used for drying may have a considerable influence on the flavour quality of the medicinal and aromatic plant materials. The selection of drying method depends on the desired product. Shade drying is usually preferred to maintain colour of leaves and flowers[2]. Oven drying at very high temperature can cause thermal damage and can critically alter the colour and volatile composition of herbs[3]. Volatile flavour components present in the aromatic plants are sensitive towards the drying methods[4]. There are some reports on the effect of different drying methods on the essential oil profile of *Mentha piperita*[5,6], *Mentha*[7], *Plectranthus glandulosus*[8], *Ocimum gratissimum* from Brazil[9], *Mentha spicata*[10], *Ocimum americanum*[11], *Murraya koenigii*[12] and *Ocimum gratissimum* from Nigiria[13]. The oil content of shade-dried leaves of *Ocimum basilicum*, *Mentha piperita*, *Ocimum gratissimum, Origanum vulgare* and *Origanum onites* was higher than fresh material[13–16].

Antioxidants are the natural compounds present in our food. These agents protect our bodies from certain diseases by removing the deleterious effects of free radicals by retarding the oxidation of organic matter caused by these free radicals[17]. Phenolic compounds, diterpenoids and flavoniods are the classes of compounds which have been observed to possess antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation[18]. Fruits, vegetables, spices and essential oils are considered as primary sources of natural antioxidants for humans[19]. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been used as synthetic antioxidants in food processing industries. However, due to their instability and carcinogenic capacity, their use in

food products started to decrease drastically[20]. Therefore, there has been an increasing interest to discover and develop natural and safe additives as potential antioxidants[21].

Ocimum gratissimum belonging to the family Lamiaceae, is commonly known as Ram tulsi and is distributed and cultivated in India, Chittagong, Ceylon, Deccan, Nepal, Nigeria and West Africa[22]. It is an erect herbaceous aromatic plant with heart shaped leaves and attains a height not more than 12 cm[23]. The grinded leaves juice of *Ocimum gratissimum* have been traditionally used for the treatment of bronchitis, headaches, fever, influenza, conjunctivitis, skin infections and sunstroke[24,25]. A number of studies have been carried out on the essential oil composition of *Ocimum gratissimum*. The major compound in the essential oil of *Ocimum gratissimum* from Algeria, Brazil, Colombia, India, Kenya and Nigeria was eugenol while linalool was the major constituent in the essential oil from Romania. Thymol was the major component in the *Ocimum gratissimum* oil from Portugal and Republic of Guinea[26–39].

Antioxidant activity of *Ocimum gratissimum* oil has been reported from Western Ghats region of North Karnataka[37]. Numerous reports are present on the antioxidant activity of extracts of *Ocimum gratissimum*[40–42]. Antioxidant capacity of *Ocimum gratissimum* extracts has been reported to be affected by drying methods due to change in chemical composition of the plant[40,43,44]. To the best of our knowledge, no report on the effect of drying on the essential oil composition and antioxidant activity of *Ocimum gratissimum* from North India has been found in available literature. Therefore, the present study was aimed to find out the effect of sun drying on the quality of *Ocimum gratissimum* oil collected from North India.

Experimental

Collection and identification of plant material:

Fresh *Ocimum gratissimum* plants at flowering stage were collected from the cultivated field of Haldwani (Uttarakhand) in April 2017. A fraction of plant material was sun dried at an average temperature of 30±1°C for five days until constant weight. The identification of the plant was done at Botanical Survey of India (BSI), Dehradun (Acc. no. 117788).

Isolation of essential oil:

Fresh and sun dried plant materials were sliced into small parts and 1000 g and 500 g of each sample in triplicate was extracted by using hydrodistillation technique in a Clevenger apparatus moisture for 5 h[12] and 2 mL and 1 mL oils were obtained respectively. The extracted oil was stored in glass vials and excess moisture was dried over anhydrous sodium sulphate (Merck). The sealed glass vials were stored in BOD incubator prior to GC and GC-MS analysis[12].

Analysis of essential oil:

The oil was analyzed using Shimadzu 2010 GC equipped with Rtx-5 column (30 m×0.25 mm with film thickness 0.25 μm) and Flame Ionisation Detector (FID). The column temperature was programmed with initial temperature of 50°C for 2 min hold time to 210°C (hold time 2 min) at a rate of 3°C min^{-1} and then 210° to 280°C at 10°C min^{-1} with final hold time of 12 min. Nitrogen at a rate of 30.0 mL/min column head pressure was used as carrier gas. The injector and FID temperature was programmed at 260°C and 270°C respectively[12].

The GC-MS used was 2010 GC coupled with Shimadzu QP 2010 plus with thermal desorption system TD 20 with (Rtx-5) fused silica capillary column 30 m×0.25 mm with film thickness 0.25 μm). The GC-MS was programmed under similar conditions to those of GC. The injector temperature was 230°C and helium was used as carrier gas. For analysis of components, 0.2 μL neat oil was taken. The split ratio was 1:30 and MS was taken at 70 eV with mass range of 40–650 amu.

Identification of the components:

The components of the oil were identified on the basis of their Retention Index (RI; calculated with respect to C_9-C_{33} *n*-alkane series) under similar GC conditions, MS Library search (NIST: NIH version 2.1 and WILEY: 7th edition), comparison with the MS in literature data[45] and co-injection with standard (*E*-caryophyllene). The relative percentage of individual constituent in the oil was calculated on the basis of GC peak area without using any correction factor[12].

Antioxidant activity:

DPPH radical scavenging activity was determined using the method given by Quiroga *et al*. (2013)[46] with some modifications. According to Pyrzynska and Pekal (2013)[47], DPPH radical scavenging activity usually shows complete antioxidant capacity of the sample. The 1.0 mL solution of samples and standards comprising of different concentrations (0.05, 0.10, 0.15, 0.20, 0.40, 0.80, 1.00 μg/mL) in methanol were

mixed with 5.0 mL of methanolic solution of DPPH (0.2 mM). After vigorous shaking, the mixture was allowed to stand at room temperature for 30 min. The absorbance of the solutions was measured at 517 nm against methanol as the blank in a spectrophotometer (Thermo scientific Evolution 201 UV-Visible Spectrophotometer, India). The DPPH radical scavenging activity was calculated as a percentage of DPPH discoloration (percentage inhibition) using the following equation:

$$\% \text{ Percentage inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control containing all reagents except the test sample and A_{sample} is the absorbance of the oils/standards. The experiments were performed in triplicate. The oil concentration (mg/mL) giving 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against oil concentration. The results were compared with BHT and catechin which were used as the standards.

Statistical analysis:

All the analyses in the present study were done in triplicate and the results were presented as mean ± standard deviation (SD). A paired t-test (2 tailed) was performed to compare means of major essential oil components between fresh and dried *Ocimum gratissimum* using data analysis in MS excel at probability level of $p < 0.01$ and $p < 0.05$. The data was also subjected to One-way analysis of variance (ANOVA) at a probability level of $p < 0.05$[48] to compare mean values of percentage inhibition and IC_{50}. The Duncan's multiple range test was performed to compare the differences between mean values using SPSS 16.0 statistical software.

Results and discussion

Essential oil yield:

The extracted oils were clear colourless liquid. The yield of essential oil from fresh and dried plant was similar i.e. 0.2% (v/w). However, according to Njoku *et al*. (2017), the oil yield of *Ocimum gratissimum* increased in air dried (0.61–1.33%) and decreased in sun and oven dried plant material (0.61% to 0.58% and 0.59% respectively)[13]. The oil yield of aromatic plant can be decreased from 2.55% to 1.94% during drying processes[49].

Essential oil composition:

Gas Chromatography (Fig. 1; Fig. 2) revealed the pres-

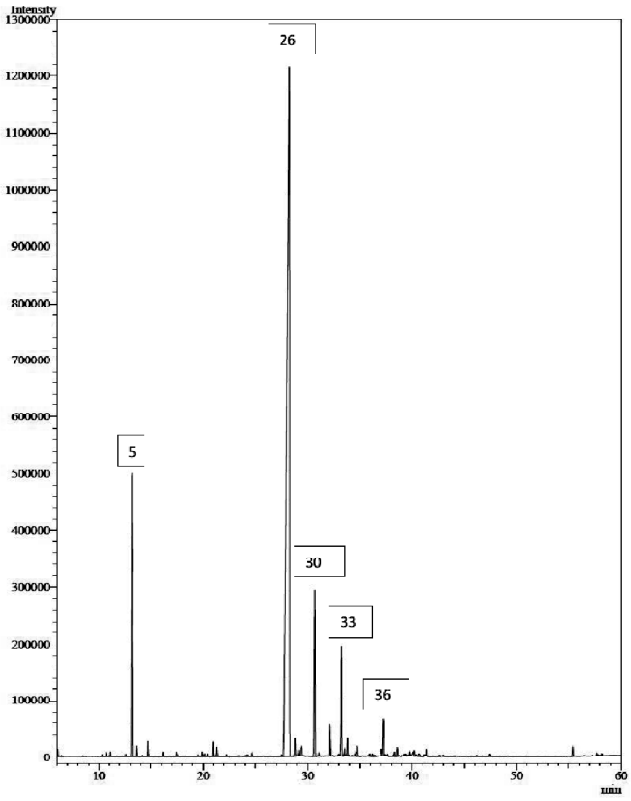

Fig. 1. Gas chromatogram of *Ocimum gratissimum* fresh oil.

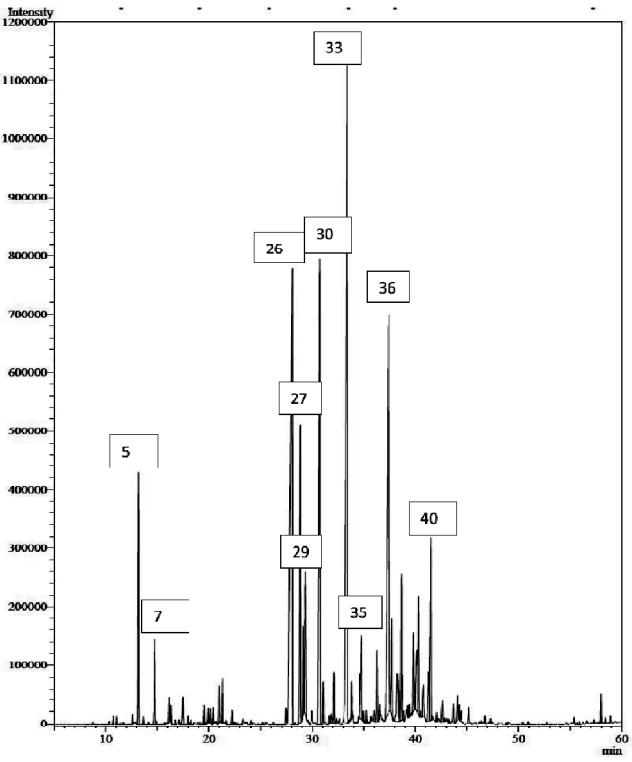

Fig. 2. Gas chromatogram of *Ocimum gratissimum* sun dried oil.

ence of 71 and 100 compounds of which 29 compounds for fresh and 42 compounds for sun dried *Ocimum gratissimum* oil have been identified representing 95.48% and 83.99% of the total oil respectively (Table 1). The main components in the fresh oil were eugenol (72.70%) and (*E*)-β-ocimene (7.74%). The major compound in the essential oil of *Ocimum gratissimum* from India[28,36,37,39], Brazil[29], South Africa[30], Kenya[31,32], Algeria[33], Nigeria[34] and Benin[35] was eugenol while linalool was the major constituent in the essential oil from Romania[38]. Thymol was the major component in the *Ocimum gratissimum* oil from Portugal[26] and Republic of Guinea[27].

Germacrene D (18.89%), eugenol (17.31%) and caryophyllene oxide (11.39%) were the major constituents of the sun dried oil (Table 1). The results revealed that sun drying significantly ($p < 0.05$) changed the chemical composition of the essential oil. The variations in the oil components may be due to the formation of new compounds by oxidation, esterification, glycoside hydrolysis, and/or other processes[50]. The mean percentage of oil constituents, especially (*E*)-β-ocimene and eugenol decreased significantly ($p < 0.01$) from 7.74% to 3.06% and 72.70% to 17.31% respectively when *Ocimum gratissimum* was sun dried (Table 1; Fig. 3). According to Díaz-Maroto *et al.* (2004)[50], drying

Table 1. Effect of drying on the essential oil composition of *Ocimum gratissimum* collected from Haldwani

Sr. No.	$RI_{Calculated}$	RI [45]	Name of compound[a]	RT_{Fresh} (min)	Mean percent ± SD (Fresh sample)	RT_{Dried} (min)	Mean percent ± SD (Dried sample)
1.	978	974	1-Octen-3-ol	10.4	0.06	10.3	0.04
2.	987	988	Myrcene	10.7	0.10	10.7	0.08
3.	1022	1020	p-Cymene	11.7	0.02	11.7	0.03
4.	1026	1024	Limonene	12.6	0.07	12.6	0.11
5.	1044	1044	(*E*)-β-Ocimene	13.2	7.74±0.65	13.1	3.06**±0.12
6.	1055	1054	γ-Terpinene	14.1	0.04	14.1	0.03
7.	1069	1065	*cis*-Sabinene hydrate	14.7	0.05	14.7	1.09
8.	1178	1174	Terpinen-4-ol	19.9	0.14	–	ND
9.	1189	1190	Methyl salicylate	20.4	0.08	–	ND
10.	1099	1095	Linalool	–	ND	16.1	0.29
11.	1104	1100	n-Nonanol	–	ND	16.3	0.18
12.	1122	1118	p-*cis*-Menth-2-en-1-ol	–	ND	17.3	0.02
13.	1138	1131	(*Z*)-Myroxide	–	ND	18.0	0.10
14.	1143	1141	Camphor	–	ND	18.2	0.05
15.	1158	1157	(2*E*)-Nonenal-1	–	ND	18.9	0.02
16.	1163	1165	Lavandulol	–	ND	19.2	0.03
17.	1170	1166	p-Mentha-1,5-dien-8-ol	–	ND	19.5	0.29
18.	1178	1174	Terpinen-4-ol	–	ND	19.9	0.23
19.	1189	1190	Methyl salicylate	–	ND	20.4	0.20
20.	1195	1198	Shisofuran	–	ND	20.7	0.02
21.	1216	1217	β-Cyclocitral	–	ND	21.7	0.05
22.	1230	1229	(3*Z*)- Hexenyl-2-methylbutanoate	22.2	0.07	22.2	0.18
23.	1235	1233	n-Hexyl-2-methylbutanoate	–	ND	22.5	0.05
24.	1281	1284	Bornyl acetate	24.7	0.11	-	ND
25.	1345	1345	α-Cubebene	27.5	0.06	27.5	0.25
26.	1363	1356	Eugenol	28.2	72.70±2.94	28.3	17.31**±0.73
27.	1378	1374	α-Copaene	28.8	0.57	28.8	5.06**±0.59
28.	1384	1387	β-Bourbornene	29.1	0.17	29.1	1.18
29.	1388	1387	β-Cubebene	29.4	0.44	29.3	3.13**±0.33

Table-1 (contd.)

30.	1421	1417	(*E*)-Caryophyllene	30.7	5.45±0.54	30.7	9.80**±0.72
31.	1428	1430	β-Copaene	31.1	0.11	31.1	0.56
32.	1452	1452	α-Humulene	32.1	0.96	32.1	0.72
33.	1484	1484	Germacrene D	33.5	3.75±0.90	33.4	18.89**±0.98
34.	1494	1493	*Epi*-Cubebol	33.8	0.56	33.8	0.47
35.	1517	1522	δ-Cadinene	34.7	0.35	34.8	1.91
36.	1584	1584	Caryophyllene oxide	37.4	1.27±0.25	37.4	11.39**±1.30
37.	1593	1594	Salvol-4(14)-en-1-one	37.6	0.06	37.7	1.54*±0.56
38.	1607	1608	Humulene epoxide II	38.3	0.19	38.3	1.45
39.	1648	1639	Aromadendrane (epoxide-allo)	39.7	0.11	39.8	1.37
40.	1673	1676	Mustakone	40.2	0.17	40.3	1.74**±0.22
41.	1763	1759	Benzyl benzoate	44.1	0.03	44.1	0.42
42.	1766	1766	β-Costal	–	ND	44.3	0.16
43.	1771	1779	14-Hydroxy-α-muurolene	–	ND	44.4	0.14
44.	1792	1803	14-Hydroxy-δ-cadinene	–	ND	45.2	0.30
45.	1824	1828	Isopropyl tetradecanoate	46.2	0.05	46.3	0.05
					95.48		83.99

[a]Mode of identification: Retention Index on RTx-5 column; ND = Not Detected; SD = Standard Deviation; Mean values ±SD followed by ** and * indicate significance difference between pairs (fresh and sun dried) at $p < 0.01$ and $p < 0.05$, respectively.

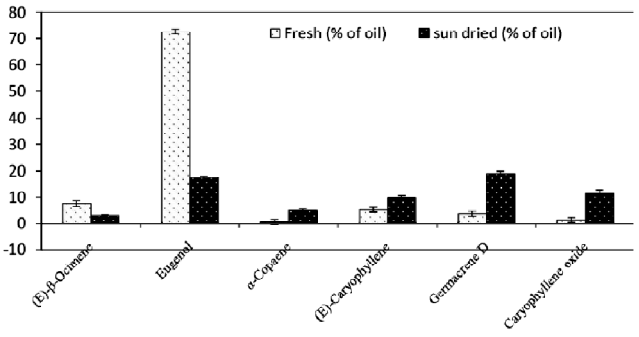

Fig. 3. Variation in the major constituents of fresh and sun dried *Ocimum gratissimum* oil. All pairs of major constituents between fresh and dried plant materials were significantly different as per paired t-test at $p < 0.01$.

causes a slight expansion in the cell structure which could release volatile components into the atmosphere, resulting in the loss of volatile compounds. On the other hand, the mean percentage of some of the components including (*E*)-caryophyllene, germacrene D and caryophyllene oxide increased significantly ($p < 0.01$) from 5.45% to 9.80%, 3.75 to 18.89% and 1.27% to 11.39% respectively on sun drying (Table 1; Fig. 3). Constituents such as terpinen-4-ol, methyl salicylate and bornyl acetate were present only in fresh oil while 16 constituents such as linalool, n-nonanol, p-*cis*-menth-2-en-1-ol, (*Z*)-myroxide, camphor, (2*E*)-nonenal-1, lavandulol, p-mentha-1,5-dien-8-ol, terpinen-4-ol, methyl salicylate, shisofuran, β-cyclocitral, n-hexyl-2-methyl butanoate, β-costal, 14-hydroxy-α-muurolene and 14-hydroxy-δ-cadinene which were totally absent in the fresh sample, appeared in the sun dried sample. The fresh leaves of *O. gratissimum* from Nigeria contained umbellulone (14.68%) as the major component; while after drying, the main components were changed to caryophyllene oxide (11.84%), terpinene-4-ol (17.98%) and β-myrcene (12.11%) in air, sun and oven dried material respectively[13]. Twelve compounds were found to be common and present in appreciable amounts in both oils, these compounds included (*E*)-β-ocimene (3.06–7.74%), eugenol (17.31–72.70%), α-copaene (0.57–5.06%), β-bourbornene (0.17–1.18%), β-cubebene (0.44–3.13%), (*E*)-caryophyllene (5.45–9.80%), germacrene D (3.75–18.89%), δ-cadinene (0.35–1.91%), caryophyllene oxide (1.27–11.39%), humulene epoxide II (0.19–1.45%), aromadendrane (epoxide-allo) (0.11–1.37%) and mustakone (0.17–1.74%). The study also demonstrated that the percentage of monoterpenes decreased significantly ($p < 0.01$) in samples dried under solar radiations. In contrast, significant increase ($p < 0.01$) in the percentage of sesquiterpenes was observed in sun dried plant material (Fig. 4). Due to lower molecular weight

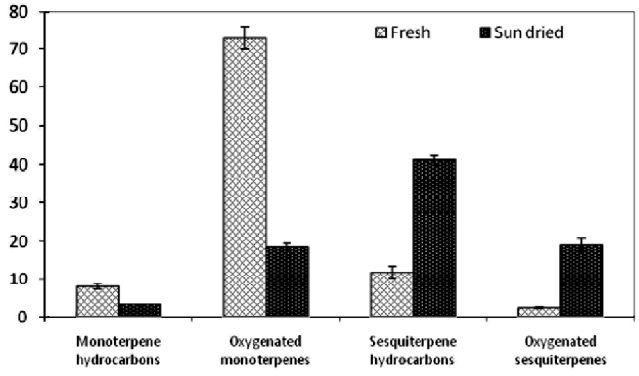

Fig. 4. Variation in the class of compounds of fresh and sun dried *Ocimum gratissimum* oil. All pairs of compound classes between fresh and dried plant materials were significantly different as per paired t-test at $p < 0.01$.

of monoterpenes, evaporation is easier as compared to squiterpenes[51].

Monoterpene compounds such p-cymene, sabinene, β-myrcene and β-phellandrene generally lost during the drying process as they have more affinity towards water present in the leaves[52]. High temperature influences the biological structure of oil glands present in aromatic and medicinal plants which in turn causes cell expansion and release of volatile compounds into the atmosphere. A study by Santanaa *et al*. (2014) demonstrated that drying in a forced ventilation oven at 60°C caused damage of trichomes in the leaves of *Ocimum gratissimum*[53]. Oregano (*Origanum vulgare* subsp*. hirtum*) and ginger *(Zingiber officinale*) showed increase in the percentage of sesquiterpenes, especially β-caryophyllene after drying[54,55].

Antioxidant activity:

The DPPH radical scavenging activity (DRSA) of the oils is given in Table 2. Significant differences ($p < 0.05$) in the antioxidant activities of essential oils of fresh and dried treatments were obtained. It was observed that the mean DRSA value was significantly higher ($p < 0.05$) for fresh *Ocimum gratissimum* (81.49%) and lower for sun dried *Ocimum gratissimum* (49.23%) at 1.00 mg/mL. BHT showed the lowest mean inhibition activity (41.08%) while catechin had moderate inhibition activity (61.74%) at concentration of 1.00 μg/mL. The results of IC_{50} calculated using DPPH assay showed that fresh *Ocimum gratissimum* oil had lowest mean value of 0.11 μg/mL while sun dried *Ocimum gratissimum* oil had the highest mean value (IC_{50}: 1.03 μg/mL) (Table 2).

The better DPPH radical scavenging activity of fresh *Ocimum* oil may be due to the high percentage of oxygenated monoterpenes present in the fresh essential oil. Oxygenated monoterpenes are reported to show good antioxidant activity because they undergo antioxidation with very fast termination process and thus, reduce overall rate of oxidation[56]. High antioxidant activity was also reported for *cis*-verbenol, eugenol, nerol, geraniol and perillyl alcohol[21,57]. Fresh *Ocimum gratissimum* had the highest percentage of eugenol and (*E*)-β-ocimene. According to Riachi *et al.* (2015), the reactivity of DPPH radical is due to its reaction with reactive oxygen species and unsaturated hydrocarbons[57]. Therefore, DPPH discolouration with fresh *Ocimum gratissimum* could be attributed to eugenol and some other unsaturated terpenes present in it. Furthermore, by comparing the IC_{50} values of oils with standards, it was observed that antioxidant activity of fresh oil (0.64 μg/mL) was significantly higher ($p < 0.05$) than BHT 1.58 μg/mL) and catechin (0.99 μg/mL). Thus, the results indicated that fresh *Ocimum gratissimum* had better potential as radical scavenger than sun dried oil and both the standards. Olatunya and Akintayo (2017) also reported highest DPPH radical scavenging activity of fresh lime oil as compared to the dried lime oil due to the presence of geraniol and citral[58].

Table 2. Antioxidant activity of *Ocimum gratissimum* oils

Samples	% Mean inhibition ±SD							IC_{50}
	0.05 μg/mL	0.10 μg/mL	0.15 μg/mL	0.20 μg/mL	0.40 μg/mL	0.80 μg/mL	1.00 μg/mL	(μg/mL)
Fresh oil	48.00^{k}±0.08	$49.77^{o,p}$±0.03	51.53^{q}±0.11	53.29^{r}±0.20	60.34^{u}±0.57	74.44^{w}±1.30	81.49^{x}±1.67	0.64^{a}
Dried oil	24.01^{a}±0.12	25.34^{b}±0.10	26.66^{c}±0.11	$27.99^{d,e}$±0.15	33.30^{g}±0.38	43.92^{j}±0.89	$49.23^{n,o}$±1.14	1.03^{b}±0.05
BHT	26.49^{c}±0.09	$27.26^{c,d}$±0.09	$28.03^{d,e}$±0.08	28.80^{e}±0.08	31.87^{f}±0.08	38.01^{h}±0.07	41.08^{i}±0.07	1.58^{c}
Catechin	$48.39^{l,m}$±0.02	$49.09^{n,o}$±0.02	$49.79^{o,p}$±0.02	50.49^{p}±0.02	53.30^{s}±0.02	58.92^{t}±0.02	61.74^{v}±0.02	0.99^{d}±0.01

Mean values ±SD (standard deviation) followed by alphabets (a-x) for % inhibition and (a-d) for IC_{50} values are significantly different at $p < 0.05$ according to Duncan test.

Conclusion

The chemical composition and antioxidant potential of essential oil of *Ocimum gratissimum* leaves subjected to sun drying was investigated and compared with the fresh oil using One-way ANOVA and t-test in this study. Although there was no significant difference in the essential oil yield, drying caused a significant loss ($p < 0.01$) of eugenol from 72.70% to 17.31%, (*E*)-β-ocimene from 7.74% to 3.06% and antioxidant potential ($p < 0.05$) of *Ocimum gratissimum* (IC_{50} value increased from 0.64 to 1.03 μg/mL). Thus, sun drying may not be an effective method for drying *Ocimum gratissimum*.

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