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Effects of Different Drying Methods on the Chemical Composition of the Essential Oils of *Ocimum gratissimum* L. Leaves

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Abstract

The drying of the leaves of aromatic plants before extraction to reduce moisture content leads to the loss of many compounds which are dragged to the leaf surface by the evaporating water. In this research, the essential oil of *Ocimum gratissimum* leaves dried by different methods was obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry. The air-dried, sun-dried and oven-dried leaves yielded 1.33 %, 0.58 % and 0.59 % of the essential oils, respectively, whereas the percentage of oils was 0.61 % in the fresh plant material. The essential oils of *O. gratissimum* were composed mainly of monoterpenes and sesquiterpenes. The percentage composition of major constituents in the dried leaves also varied; air-dried-: caryophyllene oxide (11.84 %), sun-dried-: terpinene-4-ol (17.98 %), and oven-dried-: β -myrcene (12.11 %). The most abundant component in the fresh leaves oil umbellulone (14.68 %) was absent in the dried leaves oils. The yields and chemical compositions of the essential oils obtained from *O. gratissimum* were affected to varying degrees by the methods of drying used.

Keywords. *Ocimum gratissimum*, hydrodistillation, monoterpenes, caryophyllene oxide, β -humulene

Introduction

Drying is one of the most antique processes to preserve quality of aromatic and medicinal plants. It involves water removal from the raw material up to a level at which microbial spoilage and deterioration reactions are highly minimized (Rocha *et al.*, 2011). The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials. The leaves of aromatic plants are often dried before extraction to reduce moisture content. During this process, many compounds which are dragged to the leaf surface by the evaporating water are lost (Moyler, 1994). The technique used in drying plant materials includes air-drying, sun-drying, oven-drying, microwave drying and freeze-drying. The method used

depends on the desired product. Hot air drying method for instance can lead to thermal damage and can severely alter the volatile composition of herbs as well as the colour (Antal *et al.*, 2011). Shade drying is preferred to maintain or minimize loss of colour of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances (Mendonça *et al.*, 2006). Several workers have reported the effect of different drying methods on the chemical composition of essential oils. Asekun *et al.* (2007) reported that potentially harmful pulegone and menthone were not present in the oven-dried leaves of *Mentha longifolia*, whereas they were present in the fresh leaves. Fresh leaves essential oil of *Calendula officinalis* was dominated by sesquiterpenoids while monoterpenes predominated the dried leaves oils (Okoh *et al.*, 2008).

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The oil content of shade-dried leaves of *Mellisa officinalis* was higher than oven-dried (Shalaby *et al.*, 1995). In another research, the oil content of shade-dried flowers of *Tanacetum parthenium* cv. Zardband was found to be higher (0.48 % w/w) than those of oven dried at 40 °C (0.42 %) and sun-dried (0.27 %) (Omidbaigi *et al.*, 2007).

Khorshidi *et al.* (2009) observed that oven drying (45 °C) reduced the essential oil percentage of *Rosmarinus officinalis* L significantly compared to shade drying. The essential oil yields from fresh and dried leaves of *Ocimum gratissimum* increased with increase in days of drying except in the dried leaves for four days where the yield decreased. The chemotypic variations of these oils were attributed to changes in ambient temperature during drying which influenced the physiological condition in the leaves. This condition dictates the type of enzyme that mediates the biosynthesis of mono- and sesquiterpenoids from their respective precursors in the plant (Usman *et al.*, 2009). The variations noticed in the chemical composition of plants during moisture removal necessitated the need to study the effect of different drying methods on the chemical composition of the essential oil of *Ocimum gratissimum* leaves.

MATERIALS AND METHODS

Plant material

The healthy leaves of *Ocimum gratissimum* were collected from uncultivated farmland in Agbara in Ado Odo-Ota Local Government Area of Ogun State, Nigeria in June, 2016. The botanical identification and authentication was done in the Herbarium of the Department of Botany, University of Lagos, Nigeria. The voucher number for the plant was issued (LUH 7067) and the fresh plant submitted for future reference. The fresh leaves, air-dried, sun-dried and oven-dried (40 °C) were used for the extraction. Prior to extraction, the fresh leaves were cut into smaller pieces using table knife, while the dried leaves were pulverized using the Micron Glacis GC fine-grinding mill.

Extraction and analysis of the essential oils

The essential oils from the fresh leaves and each of the pulverized, dried samples (air-dried, sun-dried and oven-dried) were obtained by hydrodistillation of 300 g each of the plant material using the modified Clevenger-typed apparatus (Asekun and Ekundayo, 1999). The oils were dried over anhydrous sodium sulphate. The analysis of the oils was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass selective detector (VLMSSD, Agilent 5975C) equipped with a non-polar Agilent HP-5MS (5 %-phenyl methyl polysiloxane) capillary column (30 m × 0.32 mm i.d. and 0.25 µm film thickness) with Injector series (Agilent, 7683B). The carrier gas was helium with linear velocity of 1ml/min. Oven temperature was set at 80 °C for 2 minutes, then programmed until 120 °C at the rate of 5 °C/min with hold time of 2 minutes, and finally increased to 240 °C at the 10 °C/min rate, isothermal at the temperature for 6 min hold time. Injector and detector temperatures were 300 °C and 200 °C respectively. Injection mode, split less, volume injected, 1 µl of the oil. The MS operating parameters were as follows: Ionization potential, 70 eV; interface temperature, 200 °C and acquisition mass range; 50-800. Relative percentage amounts of the essential oil components were evaluated from the total peak area (TIC) by apparatus software. Identification of components in the volatile oil was based on the comparison of their mass spectra and retention time with literature data and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

RESULTS AND DISCUSSION

The leave essential oils of *Ocimum gratissimum* dried by different methods was obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry. A total of 18, 20, 26, and 20 components were obtained from the essential oils of the air-dried, sun-dried, oven-dried and fresh plant respectively. The air-dried, sun-dried and oven-dried leaf oils yielded 1.33 %, 0.58 % and 0.59 % respectively, while the yield of the fresh leaf oil was 0.61 % (Table 1).

The essential oil of *O. gratissimum* was composed of monoterpenes and sesquiterpenes. The major components of the **air** dried leaf oil were **caryophyllene oxide** (11.84 %), d-camphene (11.54 %), 4-carene (9.72 %), humulene (8.98 %) and caryophyllene (8.35 %). However, the major components of the **sun** dried leaf oil were **terpinene-4-ol** (17.98 %), β -humulene (11.82 %), 3-thujen-one (7.28 %), terpinen-4-ol (7.25 %), 4-terpineneol (6.80 %), caryophyllene (6.76 %), and 4-carene (6.50 %). The **oven** dried leaf oil had **β -myrcene** (12.11 %), d-camphene (7.79 %), 3-thujen-2-one (7.01 %), β -elemene (6.56 %), and terpinolene (5.09 %) as major components. The essential oil of the **fresh** leaves contained **umbellulone** (14.68 %), 4-carene (9.61 %), d-camphene (8.27 %), β -eudesmene (6.17 %) and γ -selinene (6.16 %) as major components. The different methods had significant effect on the chemical composition of the essential oil of *O. gratissimum* as reflected in the variation of major components in the essential oils from the various methods of drying; air-dried; caryophyllene oxide (11.84 %), sun-dried; terpinen-4-ol (17.28 %), oven-dried; β -myrcene (12.11 %)(**figure 1**). Eight compounds were common in appreciable amounts in all the oils obtained from the drying methods, these compounds include, terpinen-4-ol (1.26 – 17.98 %), caryophyllene oxide (3.63-11.84 %), 4-carene (5.53-9.61 %), caryophyllene (4.42-8.35 %), β -elemene (3.29-6.56 %), α -panasinsen (2.21-4.56 %), terpinolene (2.66-4.21 %) and jasmone (0.32-0.87 %) (**figure 2**). Eugenol was present in the oils from dried leaves of *O. gratissimum* but absent in the fresh plant. This could be due to the chemical transformation of some major components in the fresh leaves during the process of drying. The major compound in the fresh leaf oil umbellulone (14.68%) was absent in the dried leaf oils. According to earlier reports, the hydrodistilled volatile oils of leaves and seeds of *Ocimum gratissimum* consisted of γ -terpinene (52.86 %), Z-tert-butyl-4- hydroxy anisole (13.93 %), caryophyllene (10.37 %) and p-cymene (7.16 %) as the major compounds representing different chemotypes, while the essential oils of the seeds yielded α -pinene (48.19 %), caryophyllene (10.71 %), and 3-tert-butyl-4- hydroxyanisole (11.14 %) as the major compounds (Owokotomo *et al.*, 2012). The chemical composition of *Ocimum*

gratissimum essential oil was also reported to vary according to their chemotypes: tymol, eugenol or geraniol (Freira *et al.*, 2006). According to Ijeh *et al.*, (2005), the essential oil from the leaves of *Ocimum gratissimum* was dominated by monoterpenes which accounted for 92.48 %. These major components were eugenol (68.80 %), methyl eugenol (13.21 %), and cis-ocimene (7.47 %), germacrene D (4.25 %).

The major component that has been reported in unison is that *Ocimum gratissimum* oil predominantly contains monoterpenes and sesquiterpenes such as eugenol, caryophyllene (Matasyoh *et al.*, 2007; Owokotomo *et al.*, 2012; Freira *et al.*, 2006). However, it was observed that the essential oil of *O. gratissimum* investigated in this research did not fall into any of these chemotypes. Reports by other researchers have supported that changes can occur in the chemical composition of oils dried by different methods. The essential oil of *Mentha longifolia* underwent significant chemical transformation in its monoterpenoids when the leaves were dried by the three different methods (Asekun *et al.*, 2007). The same report opined that the yield and chemical composition of essential oils from medicinal plants are related to a variety of internal and external factors, for example, the drying process which lead to increasing essential oil losses with increasing temperature. The influence of the drying air temperature on *Ocimum selloi* essential oil composition has shown that the main components elimicin (69.80 %), trans-caryophyllene (6.00 %), germacrene D (3.70 %) and bicyclogermacrene (3.50 %), reduced when temperature was increased above 40 °C (David *et al.*, 2006). Sun-drying, shade-drying and oven-drying at 45 °C affected the yield and chemical composition of the essential oil of *Satureja hortensis* and drying of aerial parts of *S. hortensis* in the oven at 45 °C was reported to be the most suitable for high oil yield, as well as high-percentage of carvacrol (Sefidkon *et al.*, 2006). These variations in the chemical composition of the essential of *Ocimum gratissimum* by different drying methods could be due to the ease of conversion of the chemical component to other forms due to their isoprene linkage (Bauer *et al.*, 2001).

Table 1: Essential oil chemical composition of *Ocimum gratissimum* by different drying methods

S/N	Compound	Retention Indices	Air-dried (%)	Sun-dried (%)	Oven-dried (%)	Fresh (%)	
Page 3112	1	α -Fenchene	951	-	-	3.30	
	2	4-Carene	1001	9.72	6.50	5.53	9.61
	3	d-Sylvestrene	1025	-	-	4.58	-
	4	d-Limonene	1039	1.74	-	-	4.65
	5	2,5-Dimethyl Anisole	1091	-	-	-	1.63
	6	Terpinolene	1096	4.21	2.66	3.97	3.51
	7	d-Camphene	1097	11.54	-	7.79	8.27
	8	Cis-Sabinene hydrate	1101	2.63	-	-	2.86
	9	Thujone	1102	-	2.56	-	-
	10	1,3,8- ρ -Menthatriene	1110	5.80	-	-	-
	11	β -linalool	1112	2.48	-	3.41	3.60
	12	ρ -Menth-2-en-1-ol	1124	-	2.22	-	-
	13	Neo-allo-Ocimene	1142	-	5.70	-	-
	14	Cis- β -Terpineol	1144	-	-	2.36	-
	15	Umbellulone	1171	-	7.28	7.01	14.68
	16	Terpinen-4-ol	1179	5.71	17.98	1.26	3.16
	17	β -Myrcene	1180	-	-	12.11	-
	18	Trans-Piperitol	1205	-	1.60	0.79	-
	19	ρ -Mentha-1,4-dien-7-ol	1333	-	-	-	0.33
	20	Eugenol	1356	1.63	0.65	0.68	-
	21	Copaene	1391	3.46	7.63	2.65	-
	22	β -Elemene	1393	5.24	4.08	6.56	3.29
	23	Jasmone	1394		0.52	0.32	0.54
				0.87			
	24	Eugenol - methyl ether	1410	-	-	0.23	-
	25	β -Panasinsen	1413	-	1.06	1.64	-
	26	Anisole-2,3,6-trimethyl	1417	-	-	3.95	-
	27	Trans- α -Bergamotene	1434	-	3.99	2.17	-
	28	β -Humulene	1440	-	7.51	-	-
	29	Caryophyllene	1454	8.35	6.76	5.44	4.42
	30	γ -Selinene	1484		5.59	-	6.16
				4.82			
	31	Elixene	1492	-	-	3.32	-
	32	β -Eudesmene	1509	5.26	-	5.16	6.17
	33	α -Panasinsen	1527	4.56	2.21	3.15	2.81
	34	δ -Cadinene	1530	-	1.56	-	-
	35	Epiglobulol	1588	-	-	1.50	1.02
	36	Caryophyllene oxide	1606		3.93	3.88	3.63
				11.84			
	37	Selina-6-en-4-ol	1624	-	-	1.06	-
	38	α -Acorenol	1629	-	-	1.65	-
	39	α -Cadinol	1653	-	-	-	1.36

40	γ -Muurolene	1741	-	-	-
			1.19		
	Total Percentage (%)		91.99	92.17	85.00
			91.05		
	Number of Compounds		18	20	26
				26	20

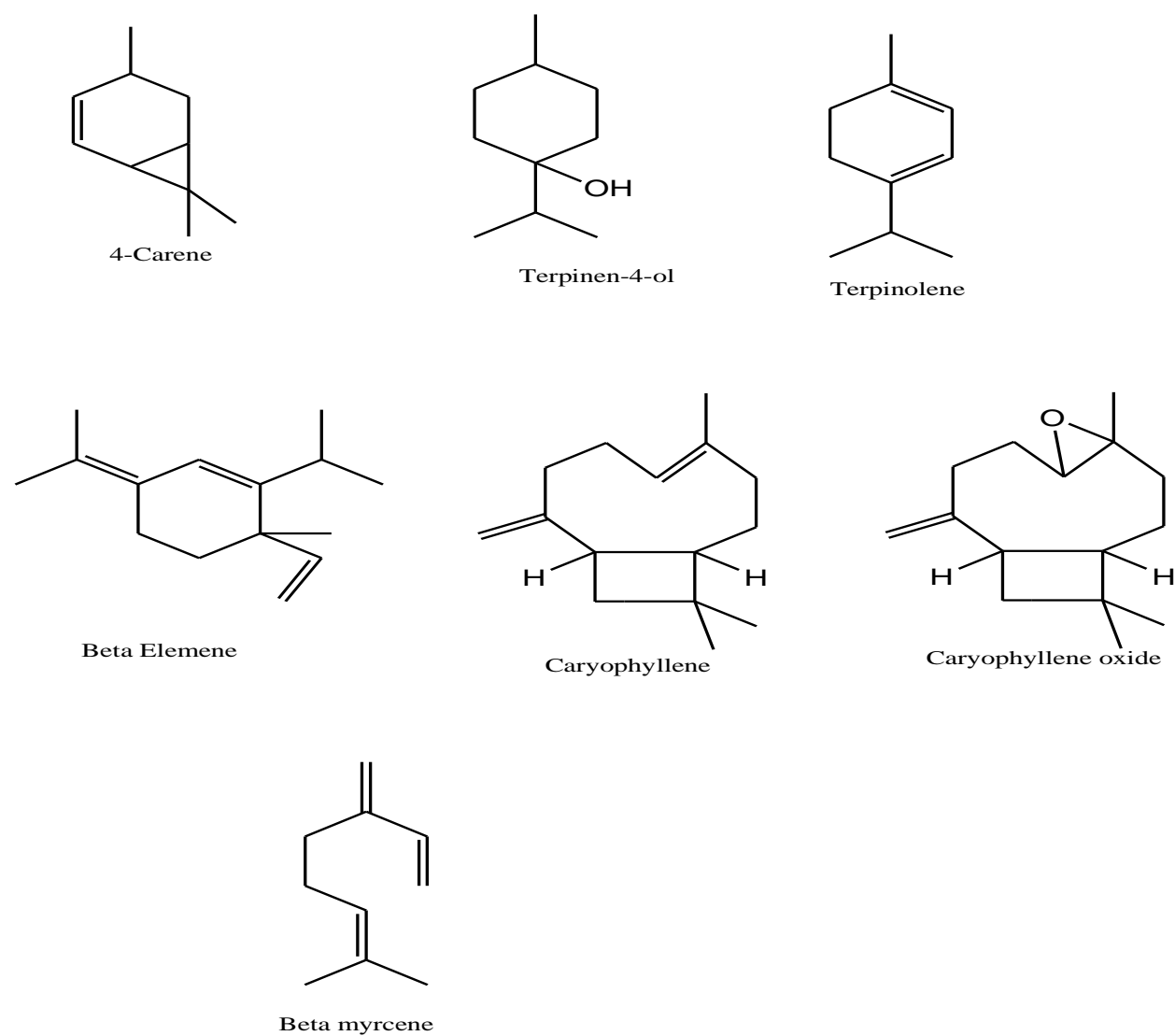


Figure 1: The structures of major compounds in the dried leaf oils

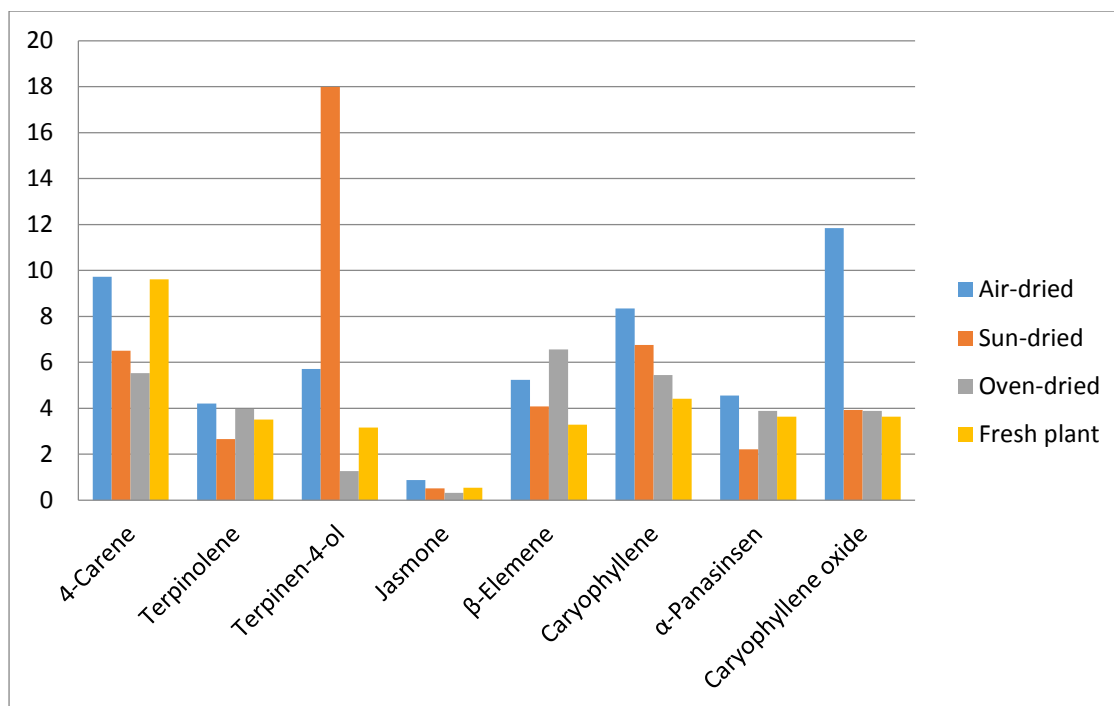


Figure 2: Essential oil composition by different drying methods

Conclusion

The essential oil of *O. gratissimum* was composed mainly of monoterpenoids and sesquiterpenoids. The yield and chemical composition of the essential oil was affected by the various methods of drying. Essential oil of the dried leaves of this plant has the highest. Therefore, on considerations of yield and

chemical composition of the essential oil of *O. gratissimum*, air-drying is suggested as the best method for the plant in order to retain the components responsible for its biological activity.

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