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Essential Oil Content and Composition of Some *Ocimum* Species and Subspecies Grown in Sudan I[1]

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Abstract: This research was conducted to study the variation among *Ocimum* species and subspecies grown in Sudan on basis of the essential oil content of their leaves and flowers and oil profiles. Four different plant materials were used in this experiment. The layout of the experiment was Randomized Complete Block Design with three replicates; treatments were the four *Ocimum* species and sub-species. The highest oil content of leaves (3 %) was obtained from *O. americanum* L. (Wild), while the lowest oil content of leaves and flowers 1 % and 1.3 % respectively were obtained from *O. tenuiflorum* L. (Clove). The highest oil content of flowers (2.8 %) was obtained from *O. basilicum* L. (Baladi). Linalool was the most predominant component in leaves' and flowers' oil of *Ocimum* spp. followed by Eugenol and Eucalyptol (Cineole). Other important components were Methyleugenol, Caryophyllene, Citral, Neral (β-Citral), α-Citral, Acetic acid octyl ester, 1-Octanol, α-Bergamotene, β-Cadinene(-), α-Caryophyllene (Humulene), β-elemene and Germacrene D. Oxygenated monoterpenes were the most predominant components in the oils followed by sesquiterpene hydrocarbons and phenylpropanoids.

Key words: *Ocimum* spp*;* essential oil; oil profile

[1] Part of M.Sc. thesis by the first author

INTRODUCTION

The genus *Ocimum* which belongs to the family Lamiaceae, collectively called, basil comprises annual and perennial herbs and shrubs. Because of the popularity of basil, the plant is often referred to as the "king of the herbs". The geographical distribution of the genus *Ocimum* shows three main centers of biodiversity *i.e.* (a) Tropical and subtropical regions of Africa (b) Tropical Asia and (c) Tropical parts of America (Brazil) (Paton *et al.*1999).

The leaves of basil are often hairy and possess epidermal glands which secrete volatile oils giving characteristic scents to many of the species. The essential oils found in leaves, flowers and seeds of *Ocimum* species are used as medicine. Basil has been used as a medicinal and aromatic plant for centuries. It is important for the pharmaceutical industry and is still being used in traditional medicines in many parts of the world. Basil grows in Sudan as a wild plant and is also cultivated for ornamental purposes. It has also limited folk medicinal utility *e.g*., use as anti-malarial in Southern Sudan, or insecticidal use in Western Sudan (Abduelrahman *et al*. 2009).

Essential oil of basil showed promising insecticidal activity against crop pest and insects. Basil has been traditionally employed as a medicinal herb in the treatment of headaches, coughs, diarrhoea, constipation, warts, and kidney malfunction (Saha *et al*. 2013). The curative properties of basil result from the presence of essential oil, phenolic compounds, flavonoids and other substances revealing antibacterial (Nour *et al.* 2009), anti-mycotic (Oxenham *et al.* 2005) and antioxidant activities (Taie *et al.* 2010). Essential oils derived from several *Ocimum* species have been reported to be active against several Gram-positive and Gram-negative bacteria as well as against yeasts and fungi due to their terpenic constituents (Lang and Buchbauer 2012).

The cosmetic industry uses basil oil in lotions, shampoos, perfumes, soaps, dental creams and mouth washes (Orafidiya *et al.* 2006). Moreover, oil from African basil can be employed industrially in a small proportion to enhance normal vegetable oil used in soap making due to its high saponification value and the aroma (Aladekoyi and Orungbemi 2016). The presence of essential oils and their composition determine the specific aroma of plants and the

flavour of the condiment. Not only the type of cultivar but also the agronomic practices and environmental conditions affect the composition of sensory important compounds (Vina and Murillo 2003). Regardless of these factors linalool, 1,8-cineole, methylcinnamate, methylchavicol and methyl eugenol are generally the main compounds responsible for the typical basil aroma (Lee *et al.* 2005).

The genus *Ocimum*, has long been recognized as a diverse and rich source of essential oil, the genus is characterized by great variability among its constituent species, including morphology, growth habit, the color of flowers, leaves, stems and chemical composition (Svecova and Neugebauerov 2010). The most popular species are *O. basilicum* L., *O. americanum* L. (*syn. O. canum*), *O. gratissimum* L., *O. kilimandscharicum* L., *O. tenuiflorum* L. (syn. *O. sanctum*), (Paton *et al.* 1999; Rewers and Jędrzejczyk 2016).

Further advancement in the classification of *Ocimum* was based on the volatile oil composition. It uses the most prevalent aromatic compounds for classifying the different basil chemotypes. The components which represent more than 20% are taken into consideration. Essential oil varies with the cultivar type. Many *Ocimum* species contain primarily monoterpene derivatives such as limonene, camphor, 1,8-cineole, linalool and geraniol. Others contain primarily phenayl derivatives, such as eugenol, methyleugenol, chavicol, estragole, methyl-cinnamate, often combined with various amounts of linalool (Grayer *et al.* 1996).

Based on the main constituents of the essential oils, Nour *et al.* (2009) classified the Sudanese basil accessions into 7 chemotypes, namely, high methyl-chavicol, high linalool, high geraniol, linalool-methyl cinnamate, linalool-geraniol, methyl cinnamate-linalool and eugenol-linalool.

The chemical composition varies not only among species but also cultivars, which can be further affected by cross-hybridization, morphogenesis, polyploidization, stages of harvesting, drying and storage, process of oil extraction, *etc*. (Chowdhury *et al.* 2017). When basil is grown for its dried leaves, it is harvested just prior to the appearance of flowers. For essential oil,

it is harvested during full bloom (Keita *et al.* 2001). Other factors affecting the composition of volatile oils include the method for extraction and analysis (Ayala and De-Castro 2001), the organ of the plant analyzed and plant growth stage (Bonnardeaux 1992), environmental factors (Hiltunen and Holm 1999), use of fertilizers (Alder *et al.* 1989) and water supply (Hiltunen and Holm, 1999). For these reasons, chemotype classification should not be based only on one major compound because frequently there are two or more major compounds that may be present in almost equal amounts. It would be better if classification were based on all of the major compounds (Grayer *et al.* 1996).

The still existing uncertainty in the classification within the genus *Ocimum* depends on the fact that species identification relied on morphological characters which expression is known to be affected by developmental and environmental factors. That is, the taxonomy of basil from different geographical locations is complicated by the presence of many varieties and cultivars without significant differences in morphology (Golparvar *et al.* 2015). Hence the objectives of this research were to study the variations in essential oil contents and profiles of four different species and subspecies of basil grown in Sudan, under different local names, as a means of chemotaxonomy.

MATERIALS AND METHODS

Experimental Site

A field experiment was conducted at the Research Farm of the Department of Horticulture, Faculty of Agriculture, University of Khartoum at Shambat from June 18th 2017 to April 26th 2018.

Plant Materials

Four different plant materials were used in the study, namely *Ocimum basilicum* L. (Baladi), *Ocimum basilicum* var. *thyrsiflorum* L. (White), *Ocimum americanum* L. (Wild) and *Ocimum tenuiflorum* L. (synonym: *Ocimum sanctum*) (Clove). These species were used to investigate the variations among them based on oil content of leaves & flowers and oil composition, under Shambat environment.

Experimental Design
The layout of the experiment was Randomized Complete Block Design with three replicates. Treatments were the four *Ocimum* species and sub-spcies.

Cultural methods
Before the initiation of the experiment, the land was ploughed, harrowed, leveled and divided into three blocks each one contained 4 experimental units (plots) each plot contained 3 ridges 3m long & 0.7m wide.

Seeds were planted in the nursery on May, 2017 till plants reached transplanting stage. The plants were transplanted in the field on June18th, 2017. Pre-transplanting irrigation was applied, followed by the first irrigation immediately after transplanting. The plants were transplanted on the shoulders of the ridges. There were seven plants per ridge at 40 cm spacing, the direction of the ridges were North to South. Irrigation was carried out every 5 days during summer and every 7 days during winter, weeding was done when needed.

Herb preparation for oil extraction
Leaves during flowering stage were picked in the morning from each species as composite samples from the three replications, washed gently to remove the dust particles, spread in thin layers on clean flat benches under complete shade and well ventilation to ensure good dried material without any molds or decomposition of the volatile oil quality and quantity.

The flowers were picked at full flowering stage in the morning from each species at all replication and dried in the same way as leaves.

After the completion of drying process for leaves and flowers they were packed in air tight bags, and kept at room temperature until extraction process.

Oil extraction
Oil was extracted by hydro distillation using a Clevenger apparatus, according to the technique of British Pharmacopoeia (GMC 1968).

Oil Preparation

After separation of the oil, anhydrous sodium sulphate was added to remove any excess water. The mixture was shaken vigorously from time to time during a period of 2 hours. The pure sample of oil was stored in sterilized, dry, air tight and opaque glass containers at 4±1°C.

Determination of Oil Composition (Profile)

Oil composition was analyzed using Gas Chromatography- Mass Spectrometry (GC-MS). Sample preparation was performed by taking 10 µL of the volatile oil and dissolving it in 1 ml ethanol, then 10 mg anhydrous sodium sulphate was added to absorb the traces of moisture. The diluted sample was passed through syringe filter, after that 1µL of the sample was injected in the GC-MS.

The analysis of the sample was carried out using GC-MS model (GC/MS-QP2010-Ultra) from Japans 'Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm × 0.25 µm).

The sample was injected using split mode, instrument operation in EI mode at 70 eV. Helium as the carrier gas with purity 99.9993 % passed with flow rate 1.69 ml/min, pressure: 100 kPa, linear velocity 47.2 cm/sec. The temperature program was started from 50°C with rate 7°C/min to 180°C then the rate was changed to 10°C/min reaching 280°C as a final temperature degree. The injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C.

The sample was analyzed using scan mode in the range of 40-500 m/z charges to ratio and the total run time was 30 minutes. Identification of components of the sample was achieved by comparing their retention times and mass fragmentation patterns with those available in the library of the National Institute of Standards and Technology (NIST).

Data analysis

Data recorded were statistically analyzed using the procedure described by Gomez and Gomez (1984). Means separation was performed using the Least Significance Difference (LSD) test.

RESULTS AND DISCUSSION

Oil content of leaves:

There was significant difference in oil content of leaves among *Ocimum* spp. (Table 1).

Table 1. Oil content of leaves of four *Ocimum* spp. at flowering stage

Treatments	Oil content (%)
O. tenuiflorum L. (Clove)	1
O. basilicum var. *thyrsiflorum* L. (White)	1.2
O. basilicum L. (Baladi)	2.2
O. americanum L. (Wild)	3
Mean	1.85
SE±	0.92

The highest oil content of leaves was obtained from *O. americanum* L. (Wild) (3 %). It was not significantly different from the *O. basilicum* L. (Baladi) (2.2 %), but they were significantly higher than the other species.

The lowest oil content of leaves (1 %) was obtained from *O. tenuiflorum* L. (Clove). It was not significantly different from the *O. basilicum* var. *thyrsiflorum* L. (White) (1.2 %), but they were significantly lower than the other species.

Oil content of flowers:
There were highly significant differences in oil content of flowers among *Ocimum* spp. (Table 2)

Table 2. Oil content of flowers of four *Ocimum* spp.

Treatments	Oil content (%)
O. tenuiflorum L. (Clove)	1.3
O. basilicum var. *thyrsiflorum* L. (White)	1.5
O. basilicum L. (Baladi)	2.8
O. americanum L. (Wild)	1.6
LSD ($P \leq 0.05$)	0.58

The highest oil content of flowers was obtained from *O. basilicum* L. (Baladi) (2.8 %) it was significantly higher than the other species.

The lowest oil content of flowers was obtained from *O. tenuiflorum* L. (Clove) (1.3 %). There were no significant differences among *O. tenuiflorum* L. (Clove), *O. basilicum* var. *thyrsiflorum* L. (White) and *O. americanum* L. (Wild).

Results indicated that there were differences in oil content among leaves and flowers of *Ocimum* spp. The oil content of flowers was higher than leaves in all species except *O. americanum* L. (wild).

Said-Al Ahl *et al.* (2015) reported that oil percentage values recorded for basil varieties during two seasons, respectively for leaves and flowers were as follows, *O. basilicum* var. *purpurascens* (0.2041 % and 0.1966 %), *O. basilicum* var. *odoratus* (0.1999 % and 0.1812 %), *O. basilicum* var. *thyrsiflorum* (0.1728 % and 0.1770 %) and *O. basilicum* var. *alba* (0.1583 % and 0.1666 %).

The essential oils content varied with a range of 0.29 % to 0.33 % for flowers and 0.32 % to 0.48 % for leaves in sweet basil accessions of *O. basilicum* L. the Silate-Egyptian accession had the lowest essential oil content obtained from leaves and flowers. However, South Darfur accession had the highest oil content but did not significantly differ from Kennana accession (Aburigal *et al.* 2016). Basil plants cultivated in middle Africa contained from 0.02 % to 2.1% of essential oil (Tchoumbougnang *et al*. 2006). An experiment was conducted on 38 genotypes of sweet basil (*O. basilicum* L). Oil content of the tested accessions varied from 0.07 % to 1.92 % in dry herbage (Zheljazkov *et al*. 2008) and 0.65 % to 1.90 % (Beatovic *et al.* 2015). Rawat *et al.* (2016) studied six varieties of sweet basil (*O. basilicum* L.) and they reported that essential oil contents varied from 0.05 % to 5 % in the dry herbage. Therefore, in the light of previously reported results on the oil content, the investigated *O. basilicum* L. varieties in our study can be classified as of rich oil content varieties.

There was marked variability in essential oil content among *Ocimum* spp. as reflected in the results and the literature findings above. Such variability could probably be attributed to the variety, genetic factor, environmental factor, nutritional & physiological status of the plant, cultivation region, plant part, harvesting time of the day (morning or noon), stage of harvest, drying method, storage period and oil extraction method.

Essential oil composition:

The essential oil composition of *Ocimum* spp. was determined by Gas Chromatography-Mass Spectrometry (GC-MS); a total of 100 % of *Ocimum* spp. oil components were identified. Considerable variation in the chemical composition was recorded and a broad spectrum of components which were different in quality and quantity was shown.

The components were categorized into three groups *i.e.* major components (>10 %), minor components (<10% >1 %) and traces (<1 %) according to Said-Al Ahl *et al*. (2015)

Table 3. Important essential oil components and percentages of leaves & flowers of *O. basilicum* L. (Baladi).

No	Component name	Formula	Percentage %	
			Leaves	Flowers
1	Linalool	$C_{10}H_{18}O$	60.98	58.51
2	Eucalyptol (cineole)	$C_{10}H_{18}O$	8.02	7.66
3	Eugenol	$C_{10}H_{12}O_2$	6.20	0.67
4	α-Bergamotene	$C_{15}H_{24}$	4.97	1.07
5	β-Cadinene(-)	$C_{15}H_{24}$	3.53	4.20
6	L-α-Terpineol	$C_{10}H_{18}O$	2.82	1.14
7	Camphor, (1R,4R)-(+)-	$C_{10}H_{16}O$	2.04	1.76
8	Germacrene D	$C_{15}H_{24}$	1.81	4.94
9	β-elemene	$C_{15}H_{24}$	1.07	3.99
10	γ-Muurolene	$C_{15}H_{24}$	1.37	2.08
11	Caryophyllene	$C_{15}H_{24}$	0.81	1.90
12	δ-Guaiene	$C_{15}H_{24}$	0.41	1.63
13	α- Caryophyllene (Humulene)	$C_{15}H_{24}$	0.45	1.02
	Total components		36	55

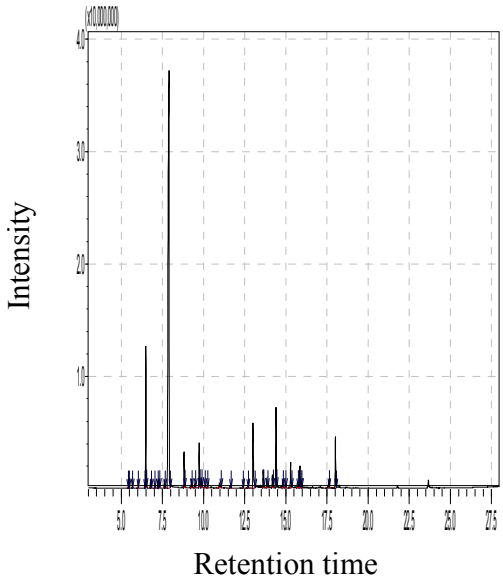

Fig. 1. Gas chromatogram of leaves' essential oil of *O. basilicum* L. (Baladi).

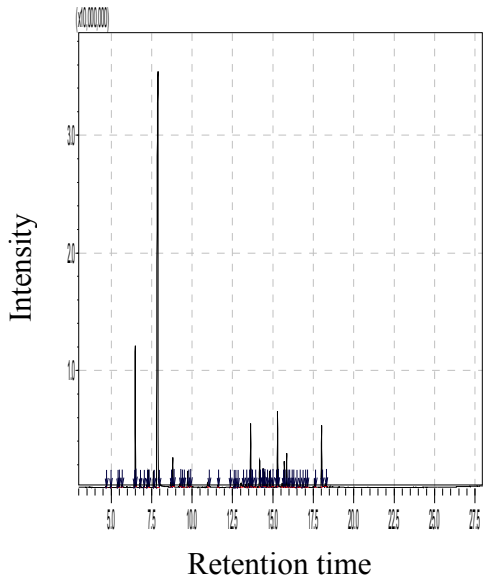

Fig. 2. Gas chromatogram of flowers' essential oil of *O. basilicum* L. (Baladi).

As shown in Table 3, and Fig. 1, Linalool was the major and highest (60.98 %) component detected in leaves' oil of *O. basilicum* L. (Baladi) followed by Eucalyptol (8.02 %) and Eugenol (6.20 %) which were detected in minor concentrations. A total of 36 components were identified which represent 100 % of leaves' oil of *O. basilicum* L. (Baladi). The oil was rich in oxygenated monoterpenes which represented 76.62 % of total oil components *e.g.* Linalool, Eucalyptol, L-α-Terpineol and Camphor,(1R,4R)-(+)-. Monoterpenes hydrocarbons were detected in trace concentrations which represented 0.67% of total oil components. Phenylpropanoids represented 6.87 % of total oil components. Eugenol is an important phenylpropene detected in minor percentage (6.20 %). Sesquiterpene hydrocarbons

represented 15.19 % of total oil components *e.g.* α-Bergamotene, β-Cadinene(-), Germacrene D, γ-Muurolene and β-elemene.

A total of 55 components were identified which represent 100 % of flowers' oil of *O. basilicum* L. (Baladi) (Table 3. and Fig. 2). Linalool was the major and highest (58.51 %) component detected in flowers' oil of *O. basilicum* L. (Baladi) followed by Eucalyptol (7.66 %) and Germacrene D (4.94 %) which were detected in minor concentrations. The flowers' oil of *O. basilicum* L. (Baladi) was rich in oxygenated monoterpenes that represented 71.21 % of total oil components *e.g.* Linalool, Eucalyptol, Camphor, (1R,4R)-(+)- and L-α-Terpineol. Monoterpene hydrocarbons represented 1.52 % of total oil components each component of them detected in trace concentration.
On the other hand sesquiterpene hydrocarbons represented 24.4 % of total oil components *e.g.* Germacrene D, β-Cadinene(-), β-elemene, γ-Muurolene, Caryophyllene, δ-Guaiene, α-Bergamotene and α-Caryophyllene (Humulene). Oxygenated sesquiterpene and phenylpropanoids were detected in trace concentrations which represent 0.41 % and 0.78 % of total oil components respectively.

Table 4. Important essential oil components and percentages of leaves & flowers of *O. tenuiflorum* L. (Clove).

No	Component name	Formula	Percentage %	
			Leaves	Flowers
1	Eugenol	$C_{10}H_{12}O_2$	42.52	28.63
2	Methyleugenol	$C_{11}H_{14}O_2$	6.65	1.31
3	Caryophyllene	$C_{15}H_{24}$	19.74	33.76
4	α- Caryophyllene (Humulene)	$C_{15}H_{24}$	2.15	4.61
5	α-Guaiene	$C_{15}H_{24}$	3.87	-
6	δ-Guaiene	$C_{15}H_{24}$	-	3.85
7	β-elemene	$C_{15}H_{24}$	1.04	16.53
8	β-Selinene	$C_{15}H_{24}$	3.26	3.33
9	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	$C_{15}H_{24}$	13.29	-
10	11,11-Dimethyl-spiro[2,9]dodeca-3,7-dien	$C_{14}H_{22}$	2.71	2.27
	Total components		26	26

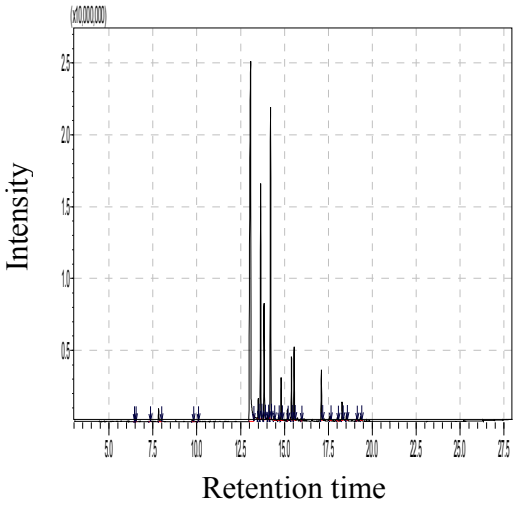

Fig. 3. Gas chromatogram of leaves' essential oil of *O. tenuiflorum* L. (Clove).

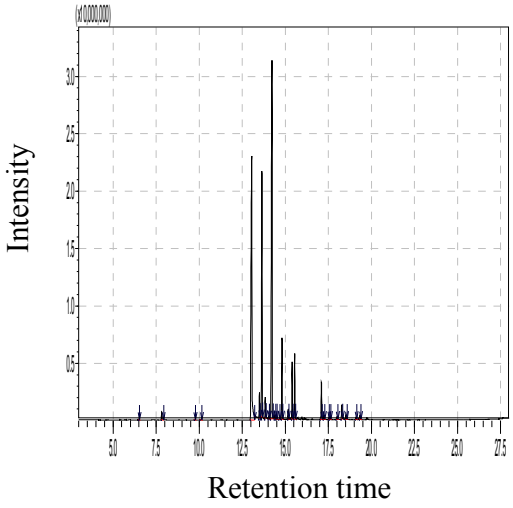

Fig. 4. Gas chromatogram of flowers' essential oil of *O. tenuiflorum* L. (Clove).

Table 4 and Fig.3 show that a total of 26 components were identified which represent 100% of leaves' oil of *O. tenuiflorum* L. (Clove). There were three major components *i.e.* Eugenol was the highest (42.52%), followed by Caryophyllene (19.74) and 8-Isopropenyl-1,5-dimethyl- (13.29%). The oil was rich in phenylpropanoids that represented 49.17 % of total oil components. Eugenol was the most important phenylpropene which characterized leaves' oil of *O. tenuiflorum* L. (Clove) from other *Ocimum* spp. Methyleugenol also belongs to phenylpropanoids and was detected in minor (6.65%) concentration. Sesquiterpene hydrocarbons represented 44.52% of total oil components *e.g.* Caryophyllene, 8-Isopropenyl-1,5-dimethyl-, α-Guaiene, β-Selinene, α-Caryophyllene (Humulene) and β-elemene. Oxygenated monoterpenes, monoterpene hydrocarbons and

oxygenated sesquiterpenes were detected in trace concentrations which represented 0.95%, 0.25% and 0.81% of total oil components respectively.

A total of 26 components were identified which represented 100 % of flowers' oil of *O. tenuiflorum* L. (Clove) (Table 4 and Fig.4). There were three major components in flowers' oil of *O. tenuiflorum* L. (Clove) *i.e.* Caryophyllene (33.76 %) was the highest followed by Eugenol (28.63 %) and β-elemene (16.53 %). The oil was rich in sesquiterpene hydrocarbons which represented 65.01 % of total oil components *e.g.* Caryophyllene, β-elemene, α-Caryophyllene (Humulene), δ-Guaiene and β-Selinene. Phenylpropanoids represented 29.94 % of total oil components namely Eugenol and Methyleugenol. Oxygenated monoterpenes and oxygenated sesquiterpenes represented 0.64 % and 1.4 % of total oil components, respectively.

Table 5. Important essential oil components and percentages of leaves & flowers of *O.basilicum* var. *thyrsiflorum* L. (white).

No	Component name	Formula	Percentage %	
			Leaves	Flowers
1	Methyleugenol	$C_{11}H_{14}O_2$	77.88	31.86
2	α-Bergamotene	$C_{15}H_{24}$	6.48	12.89
3	β-Cadinene(-)	$C_{15}H_{24}$	3.79	7.15
4	Eucalyptol (Cineole)	$C_{10}H_{18}O$	2.74	7.26
5	Linalool	$C_{10}H_{18}O$	2.56	5.73
6	γ-Muurolene	$C_{15}H_{24}$	1.52	3.35
7	Camphor, (1R,4R)-(+)-	$C_{10}H_{16}O$	0.77	3.46
8	β-elemene	$C_{15}H_{24}$	0.67	2.06
9	Isocaryophyllene	$C_{15}H_{24}$	-	1.77
10	α- Caryophyllene (Humulene)	$C_{15}H_{24}$	-	1.63
11	β-curcumene	$C_{15}H_{24}$	-	1.46
12	L-α-Terpineol	$C_{10}H_{18}O$	0.25	1.36
13	δ-Guaiene	$C_{15}H_{24}$	-	1.15
14	Bornyl acetate	$C_{12}H_{20}O_2$	0.52	1.31
	Total components		17	75

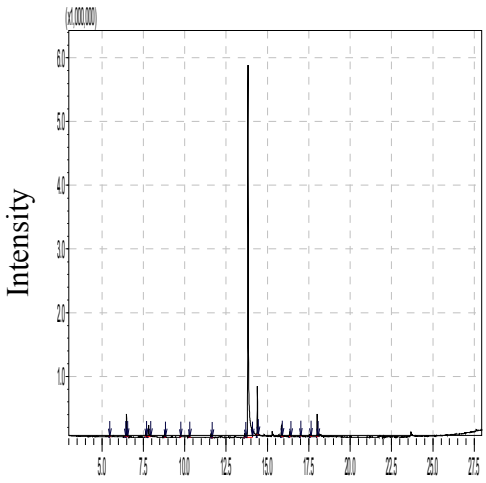

Fig. 5. Gas chromatogram of leaves' essential oil of *O. basilicum* var. *thyrsiflorum* L. (white)

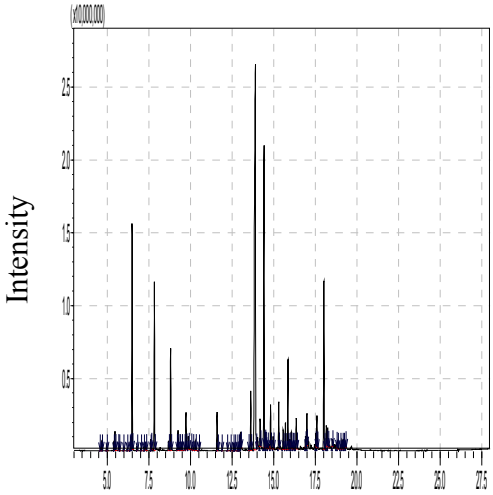

Fig. 6. Gas chromatogram of flowers' essential oil of *O. basilicum* var. *thyrsiflorum* L. (white)

Table 5. and Fig. 5. show that a total of 17 components were identified which represented 100 % of leaves' oil of *O. basilicum* var. *thyrsiflorum* L. (white). Methyleugenol was the only major (77.88 %) component detected. Phenylpropanoids represented (77.88 %) of total oil components comprising only Methyleugenol. Sesquiterpene hydrocarbons represented 12.84 % *e.g.* α-Bergamotene, β-Cadinene(-) and γ-Muurolene. Oxygenated sesquiterpenes were detected in trace concentrations that represented 0.91 % of total oil components. Oxygenated monoterpenes represented 7.81 % *e.g.* Eucalyptol (Cineole) and Linalool. Monoterpene hydrocarbons were detected in trace concentrations which represented 0.16 % of total oil components.

A total of 75 components were identified which represent 100 % of flowers' oil of *O. basilicum var. thyrsiflorum* L. (white) (Table 5 and Fig.6). There were two major components in flowers oil of *O.basilicum var. thyrsiflorum* L. (white) *i.e.* Methyleugenol was the highest (31.86 %) followed by α-Bergamotene (12.89 %). Phenylpropanoids represented 32.94 % of total oil components in flowers' oil *e.g.* Methyleugenol was the important phenylpropene that was detected. Sesquiterpene hydrocarbons represented 36.49 % *e.g.* α-Bergamotene, β-Cadinene(-), γ-Muurolene, β-elemene, Isocaryophyllene, α-Caryophyllene (Humulene), β-curcumene, and δ-Guaiene. Oxygenated Sesquiterpene represented 3.8 %. of total oil components. Oxygenated monoterpenes represented 20.74 % of total oil components; Linalool, Eucalyptol (Cineole), Camphor,(1R,4R)-(+)-, L-α-Terpineol and Bornyl acetate were the important Oxygenated monoterpenes that were detected. Monoterpene hydrocarbons represented 2.09 % of total oil component; each component was detected in trace concentrations.

Table 6. Important essential oil components and percentages of leaves & flowers of *O. americanum* L. (Wild).

No	Component name	Formula	Percentage %	
			Leaves	Flowers
1	Citral	$C_{10}H_{16}O$	-	5.18
2	α-Citral	$C_{10}H_{16}O$	44.92	-
3	Neral (β-Citral)	$C_{10}H_{16}O$	37.71	3.75
4	Acetic acid, octyl ester	$C_{10}H_{20}O_2$	1.77	47.52
5	Acetic acid, hexyl ester	$C_8H_{16}O_2$	-	1.21
6	1-Octanol	$C_8H_{18}O$	0.64	33.52
7	1-Cyclohexene-1-acetaldehyde,α ,2-dimethyl	$C_{10}H_{16}O$	1.32	0.17
8	β-Bisabolene	$C_{15}H_{24}$	2.69	2.23
9	cis-Verbenol	$C_{10}H_{16}O$	1.79	-
10	Carane, 4,5-epoxy-,trans	$C_{10}H_{16}O$	3.07	-
11	α- Caryophyllene (Humulene)	$C_{15}H_{24}$	-	1.21
12	Linalool	$C_{10}H_{18}O$	0.30	1.05
	Total components		28	27

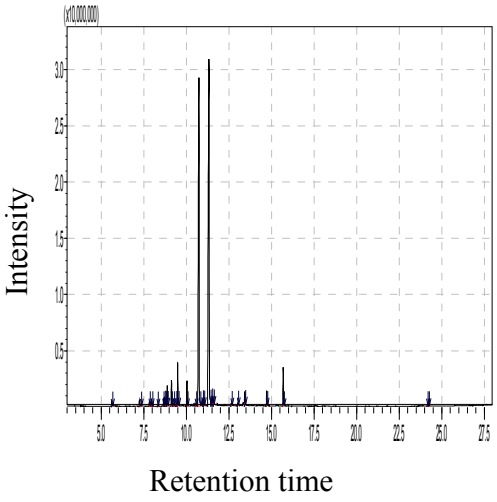

Fig. 7. Gas chromatogram of leaves' essential oil of *O. americanum* L. (Wild).

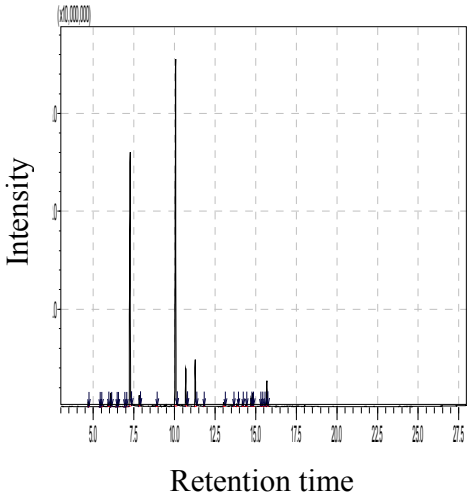

Fig. 8. Gas chromatogram of flowers' essential oil of *O. americanum* L. (Wild).

Table 6. and Fig.7 show that a total of 28 components were identified which represented 100 % of leaves' oil of *O. americanum* L. (wild); there were two major components in leaves' oil *i.e.* α-Citral was the highest (44.92 %) followed by Neral (β-Citral) (37.71 %). The oil was rich in oxygenated monoterpenes that represented 91.17 % *e.g.* α-Citral, Neral (β-Citral), Carane,4,5-epoxy-,trans, cis-Verbenol and 1-Cyclohexene-1-acetaldehyde. Monoterpene hydrocarbons were detected in trace concentrations which represented 0.54 % of total oil components. Sesquiterpene hydrocarbons represented 3.63 % of total oil components. β-Bisabolene was the important sesquiterpene hydrocarbon detected. Acetic acid, octyl ester is an organic compound known as fatty alcohol ester (ester derivatives of 1-Octanol which is fatty alcohol) represented 1.77 % of total oil components.

A total of 27 components were identified which represented 100 % of flowers' oil of *O. americanum* L. (Wild) (Table 6. and Fig.8). There were two major components in flowers' oil of *O. americanum* L. (Wild) *i.e.* Acetic acid, octyl ester was the highest (47.52 %) followed by 1-Octanol (33.52 %). Oxygenated monoterpenes represented 10.18 % of total oil components in flowers' oil *e.g.* Citral, Neral (β-Citral) and Linalool.
Monoterpene hydrocarbons represented 1 % of total oil components; each component was detected in trace concentrations. Sesquiterpene hydrocarbons represented 5.01 % of total oil components *e.g.* β-Bisabolene and α-Caryophyllene (Humulene). Acetic acid, hexyl ester, which belongs to the class of organic compounds known as carboxylic acid esters, represented 1.21 %. Phenylpropanoids represented 1.19 % each component was detected in trace concentrations.

Table 6 shows that there were marked quantitative and qualitative differences in the components of leaves' and flowers' oil of *O. americanum* L. (Wild). That is, α-Citral was the highest (44.92 %) in leaves but it was absent in flowers. Acetic acid, octyl ester was the highest (47.52 %) in flowers but it was very low (1.77 %) in leaves. Neral (β-Citral) was detected in major concentration (37.71 %) in leaves but it was minor (3.75 %) in flowers. 1-Cyclohexene-1-acetaldehyde and β-Bisabolene were also higher in leaves than in flowers. On the other hand 1-Octanol was detected in major concentration (33.52 %) in flowers but it was trace (0.64 %) in leaves. Linalool also was higher in flowers than in leaves. While α-Citral, cis-Verbenol and Carane,4,5-epoxy-,trans were detected only in leaves, Citral, Acetic acid hexyl ester and α-Caryophyllene (Humulene) were detected only in flowers.

The above results are in good agreement with those of most published studies on the chemical composition of *O. basilicum* essential oil in which linalool was found to be the predominant constituent *i.e.* 69 % (Kéita *et al*. 2001) and 41.2 % (Gurbuz *et al*. 2006). Omer *et al.* (2008) analyzed nine *O. basilicum* accessions and found that, the dominant constituent in all samples was linalool, ranging between 19 and 38 % of total oils.
A total of 75 components representing 99.8% of Omani basil oil were identified; linalool represented 69.9 % as the major component, followed by geraniol 10.9 %, 1,8-cineole 6.4 %, α-bergamotene 1.6 % and geranyl acetate 1.4 % (Hanif *et al.* 2011).

Linalool as a chemotype of the essential oils of basil varieties was also reported in different regions of the world (Vieira *et al.* 2014 and Aburigal *et al.* 2016). Methyleugenol was detected as the main component in *O. basilicum* leaves oil in Thailand (Suppakul *et al*. 2003). This result is in agreement with our result of *O. basilicum* var. *thyrsiflorum* L. (White).

Part of the results of this research are in good agreement with that result of Said-Al Ahl *et al.* (2015) who evaluated the volatile oil and chemical constituents of some basil varieties in Egypt and found that, linalool and eugenol found as majors, 1,8-cineol, methyl eugenol and farnesol were represented as minors, and α-pinene, β-pinene, myrcene, ocimene, linalyl acetate and geraniol were considered as traces.

Analysis of essential oil of basil accessions (Aburigal *et al*.2016) revealed that terpenes were the most predominant constituents. The major terpenes present were linalool and germacrene-D. Methyl eugenol was the major constituent of the essential oil of Kennana accession. While the major constituent of essential oil of South-Darfur and Silate-Egyptian accessions were germacerene-D and linalool, respectively. Nour *et al.* (2009) confirmed the classification of the essential oil of *O. basilicum* from Sudan as linalool and eugenol chemotype.

Results of this research are in line with Mondal *et al.* (2012) who reported that, the major constituents of fresh leaves' essential oil of *O. sanctum* L.

(*O. tenuiflorum*) from Delhi which constituted 93.23±6.24 percent of the total identified components were eugenol (57.94 ±2.56 %), β-caryophyllene (15.32 ±0.87 %), germacrene-A (9.10 ±1.01 %), β-elemene (7.57 ±1.20 %) and caryophyllene oxide (3.30 ±0.60 %). Of the 26 essential oil components identified in the *Ocimum tenuiflorum* L accessions, eugenol, β-caryophyllene, E-methyl cinnamate and (trans)-β-guaiene were the most abundant constituents. The level of these essential oil constituents varied significantly in all accessions at all harvest stages (Sims *et al.* 2014).

Results are in conformity with that of Smitha *et al.* (2014) who reported that major essential oil constituents of *O. americanum* were Citral and for *O. sanctum* L. as eugenol.

Omer *et al.* (2008) reported that, the most abundant components observed in all basil studies were: linalool (4.15 – 55.26 %); 1,8- cineol (3.69 -21.21 %); t-cadinol (1.58-16.91 %); γ-elemene(0.15-6.13 %); geramacrene(0.12-5.0 5 %); cadinene (0.68-4.64 %); guaiene (0.48-2.43 %); α humulene (0.19 – 1.51 %); sabinene (0.10– 1.48 %) and epi-bicyclo sesqui phellandrene (0.09 - 1.10%).

Researchers found that the Sweet basil (*O. basilicum*) essential oil extracted from the dried leaves, gave stronger sweet and woody attributes than the essential oil extracted from fresh leaves (Calín-Sanchez *et al.* 2012). Kumar and Tripathi (2011) reported that, oxygenated derivatives of monoterpenes and sesquiterpenes are more important than terpene hydrocarbons as aroma chemicals, because the characteristic odour of essential oils is representative of the combined odours of the oxygenated compounds, such as citral, citronellol, geraniol, linalool and nerol. They are also important starting materials for the synthesis of other aroma compounds. In our results oxygenated monoterpenes were the most predominant components in leaves' and flowers' oil of *Ocimum* plants.

Németh (2005) reported that, changes in essential oil composition were affected by enzyme activity or structural changes in tracts that accumulate the essential oil during ontogenesis in the Lamiaceae species. These changes are not influenced by genetic reasons but by other parameters like abiotic factors,

phase of development, harvesting time, and storage. So, the conventional taxonomic evaluation and chemotaxonomy have to be taken together with DNA molecular tools for the best classification of *Ocimum* spp. (Labra *et al*. 2004; Carović-Stanko *et al*. 2010).

Finally, the wide range of quantitative as well as qualitative variability that were observed in the oil composition of the genus *Ocimum,* not only among species but also within varieties, might be correlated with different environmental conditions, cross-hybridization, genetic background, Ontogenic factors, use of fertilizers, soil conditions, water supply, harvesting time of the day (morning or noon), stage of harvest, plant part harvested, drying method, storage period and oil extraction method.

CONCLUSIONS

- *Ocimum* species and sub-species vary significantly in their oil content of leaves and flowers.
- The essential oil composition differs quantitatively as well as qualitatively among *Ocimum* species and sub-species.
- *O. americanum* L. (Wild) has the highest oil content of leaves.
- *O. basilicum* L. (Baladi) has the highest oil content of flowers.
- *O. tenuiflorum* L. (Clove) produces the lowest oil content of leaves and flowers.
- Linalool is the most predominant component in leaves' and flowers' oil of *Ocimum* spp. followed by Eugenol and Eucalyptol (Cineole).
- Oxygenated monoterpenes are the most predominant components in leaves' and flowers' oil of *Ocimum* spp. followed by sesquiterpene hydrocarbons and phenylpropanoids.

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محتوى الزيوت الطيارة ومكوناتها في بعض انواع وأصناف الريحان التي تزرع في السودان[2]

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المستخلص: أجري هذا البحث لدراسة محتوى الزيوت الطيارة ومكوناتها في بعض أنواع و أصناف الريحان التي تزرع في السودان. استخدم أربعة انواع واصناف للريحان في التجربة. كان تصميم التجربة القطاعات العشوائية الكاملة بثلاثة مكررات والمعاملات كانت انواع وأصناف الريحان. أعلي محتوي زيت في الأوراق (3 %) رصد في (البري) *O. americanum* L. و أقل محتوي زيت فى الاوراق (1 %) وفى الأزهار(1.3 %) رصدا في (القرنفلي) *O. tenuiflorum* L. . أعلي محتوي زيت في الأزهار (2.8 %) رصد في (البلدي) *O. basilicum* L. Linalool هو أكثر مركب سائد في زيت الأوراق و الأزهار يليه Eugenol و (Cineole) Eucalyptol. إحتوت زيوت الأوراق و الأزهار علي مركبات أخري مهمة و هي: Methyleugenol، Caryophyllene ، Citral (β-Citral) ، Neral ، α-Citral ، Acetic acid octyl ester ، 1-Octanol ، α-Bergamotene ، β-Cadinene(-)، Germacrene D و β-elemene ، α-Caryophyllene (Humulene) ، Oxygenated monoterpenes هي أكثر مجموعات المركبات شيوعا في هذه الزيوت تليها مجموعتا ال sequiterpene hydrocarbons و ال phenylpropanoids.

[2] المقالة مستلة من أطروحة الماجستير للمؤلف الاول