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RESEARCH ARTICLE

EVALUATION OF THE CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM LEAVES OF SUDANESE *OCIMUM GRATISSIMUM* AND *OCIMUM BASILICUM*.

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Abstract

The genus *Ocimum* (Lamiaceae) involves economically the most important medicinal and aromatic herbs and comprises more than 150 species distributed in tropical and subtropical regions. The objective of this research is to investigate the chemical constituents in the essential oils from leaves of *O. gratissimum* and *O. basilicum* grown in Sudan. The essential oils from the leaves were obtained by hydro-distillation method and analyzed by GC-MS. The yield of the hydro-distilled essential oils obtained from leaves of *O. gratissimum* and *O. basilicum* L. were 0.5% and 0.8% (v/w) respectively. Twenty four compounds were detected in *O. gratissimum* oil; eugenol (41.74 %), caryophellene (21.99 %), isocaryophellene (12.36%), piperitone (3.43%) and methyl chavicol (2.74%) were the major constituent identified. Thirty nine compounds were detected in *O. basilicum* oil; methyl chavicol (39.26%), linalool (17.41 %), 1,8-cineole (5.75%) and m-eugenol (3.51%) were the major constituent identified. It could be concluded that *O. gratissimum* and *O. basilicum* cultivated in Sudan could be classified as eugenol/caryophellene chemotype and methyl chavicol (estragole) chemotype, respectively.

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Introduction:-

The genus *Ocimum* (Lamiaceae) involves economically the most important medicinal and aromatic herbs and comprises more than 150 species distributed in tropical and subtropical regions of Asia, Africa and Central and South America (Paton 1992).

It represents an important source of essential oils and aroma compounds used in foods, perfumery and cosmetics industry (Simon et al., 1984; Simon et al., 1990).

The sub-species and varieties in the genus *Ocimum* vary significantly in their essential oil composition, especially on the main constituents.

Essential oils of *O. gratissimum* (clove basil) and *O. basilicum* (sweet basil) from various origins were investigated by many researchers. The essential oil of *O. gratissimum* from Brazil contained eugenol (34.6 - 73.1%) and 1,8-cineole (5.6 - 22.6%) as the main constituents (Silva et al., 2004); that from Colombia contained eugenol (43.2%), 1,8-cineole (12.8%) and β -selinene (9.0%); β -caryophellene and germacrene-D were also detected in

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considerable amounts (Benitez et al., 2009). Indian *O. gratissimum* essential oil characterized by presence of eugenol (47.45%) and citronellal (3.56%) and lesser amounts of 1,8-cineole, geraniol and vanillin (Saha et al., 2013); that from Kenya characterized by presence of eugenol (68.8%), methyl eugenol (13.21%), cis-ocimene (7.47%), germacrene D (4.25%), trans-caryophyllene (1.69%) and traces of other hydrocarbon terpenes (Matasyoh et al., 2007).

Thymol (32.7%) was found to be the main constituent in *O. gratissimum* essential oil from Nigeria, followed by p-cymene (25.4%) and γ -terpinene (10.8%) as concluded by Asawalam et al., (2008). *O. gratissimum* essential oil from Togo contained (Thymol (31.79 %), p-cymene (15.57 %) and γ -terpinene (12.34 %) among twenty six identified components (Koba et al., 2009). Essential oil of *O. gratissimum* from Benin was dominated by p-cymene (28-54 thymol (9-25%), followed by β -myrcene and α -thujene (Quetin-Leclercq et al., 2012).

On the other hand, essential oil of *O. basilicum* from Thailand characterized by presence of methyl chavicol (estragole) as the dominant constituent (92.48%) (Bunrathep et al., 2007) whereas that from Iran contained methyl chavicol (52.4%) and linalool (20.1%) for the cv. Purple; methyl chavicol (40.5%), geraniol (27.6%) and neral (18.5%) for the cv. green (Sajjids, 2006).

Indonesian *O. basilicum* essential oil was dominated by methyl chavicol (85%) and linalool (4%), followed by linalool and linalool oxide (Mindaryani and Sri Rahayu, 2007); that from Prague composed of methyl chavicol (up to 45%), Linalool (15-26%), eugenol (10-22%), bergamotene and methyl cinnamate were also detected among the other constituents (Holadova et al. 2008). *O. basilicum* essential oil from Malaysia contained methyl chavicol (30.87 - 80.95%) as the major constituent (Vani et al., 2009).

O. basilicum essential oil from Turkey was dominated by methyl eugenol (78.02%) followed by α -cubebene (6.17%) and traces of other compounds (Ozcan and Chalchat, 2002); that from Pakistan contained linalool (56.7-60.6%), epi- α -cadinol (8.6-11.4%), α -bergamotene (7.4-9.2%) as the major constituents (Anwar et al., 2008).

Linalool (69.2%) was reported to be the main constituent in essential oil of *O. basilicum* from Serbia (Beric-Bjedovet et al., 2007). The oil from China contained linalool (29.68%) followed by (Z)- cinnamic acid methyl ester (21.49%) among the detected compounds (Wu et al., 2009).

Geraniol (34.89%) and citral (23.51%) were the dominant constituents documented in the essential oil of *O. basilicum* from India (Saha et al., 2013). Linalool (69.87%) was found to be the major constituent in the essential oil isolated from the aerial parts of *O. basilicum* grown in Oman (Khan et al., 2015).

Generally, *O. basilicum* oils have been classified according to their geographical source into three large groups which are the European type, the Reunion type and the tropical type. They are also classified as chemotypes based on their major constituents in to, for example, methyl chavicol, methyl cinnamate, methyl eugenol and linalool chemotypes (Lawrence, 1988; Koutsos et al., 2009; Koba et al., 2009; Singh et al., 2014). On the other hand, nine chemotypes were reported for *O. gratissimum*: linalool/methyl chavicol, eugenol/1,8-cineole/sesquiterpenes, methyl cinnamate, methyl-eugenol/eugenol, ethyl cinnamate, citral, geraniol, eugenol and thymol-rich-chemotypes (Brada et al., 2011).

The objective of this research is to investigate the chemical constituents in the essential oils from leaves of *O. basilicum* and *O. gratissimum* grown in Sudan.

Materials and methods:-

Plant material:-

O. basilicum and *O. gratissimum* leaves were collected from the Nursery of the Horticulture Sector Administration, Ministry of Agriculture, Khartoum, Sudan.

Essential oils preparation:-

The essential oils were obtained from the leaves of *O. basilicum* and *O. gratissimum* by hydro-distillation, using Clevenger apparatus, for four hrs. The yield was expressed in percent of the dry material (v/w). The oil was dried over anhydrous sodium sulfate.

GC-MS analysis of the oil:-

The essential oil was analyzed by gas chromatography coupled with mass spectrometry (GCMS-QP 2010). The sample was dissolved in dichloromethane (1%) and injected at 250 °C (Injector temperature) into the capillary column type HP-1, 30m x 0.25 mm i.d., stationary phase (95 % diethyl-5% diphenylsiloxane, 0.25 µm film thickness), using helium as a carrier gas at a flow rate of 1.2 ml/min. The injected volume was 1 µl and the injection mode used was split (split ration 50:1). The initial oven temperature was 35 °C held for 3min, then raised at a rate of 5 °C/min up to 240 °C, then at a rate of 3 °C/min up to 280 °C, held for 4 min. Detector temperature was 200 °C. The MS was operated in the EI mode at 70 eV. The mass and scan range was set at m/z 35-450.

Compounds identification:-

Identification of compounds from the essential oils was achieved by matching their mass spectra with reference spectra from the computer library of NIST or Wiley Registry of Mass Spectral Data.

Results and Discussion:-

The yield of the hydro-distilled essential oils obtained from leaves of *O. gratissimum* and *O. basilicum* L. were 0.5% and 0.8% (v/w) respectively.

Gas chromatogram of *O. gratissimum* essential oil revealed detection of twenty four compounds. About 99% of the constituents were identified. The constituents of the essential oil of *O. gratissimum*, as well as their percentage abundance, are shown in Table (1) according to their retention times.

Table 1:- The chemical composition of the essential oils of *O. gratissimum*.

Peak	RT	Compound	percentage
1	12.933	(+)-4-carene	0.24
2	13.082	n-octanal	0.43
3	13.903	Limonene	0.31
4	13.999	1,8-cineole	0.22
5	15.275	1-octanol	0.16
6	16.192	linalool	1.60
7	19.233	Methyl chavicol	2.74
8	20.882	Piperitone	3.43
9	23.908	Eugenol	41.74
10	24.440	Eudesmo-4(14),11-diene	1.54
11	24.713	Isocaryophellene	12.36
12	25.555	Caryophellene	21.99
13	26.258	α -humulene	2.38
14	26.744	γ -selinene	0.66
15	27.071	β - selinene	1.87
16	27.285	α -selinene	2.04
17	27.553	β - elemene	1.84
18	27.692	γ -cadinene	0.44
19	28.510	Elemol	1.1
20	29.403	Caryophellene oxide	0.86
21	30.66	Δ -cadinene	0.48
22	31.141	Selina-6-en-4-ol	0.6
23	46.899	Unidentified	0.41
24	49.067	Unidentified	0.55

O. gratissimum essential oil consisted mainly of oxygenated monoterpenes (50.63%) and hydrocarbon sesquiterpenes (45.12%) followed by oxygenated sesquiterpenes (4%) and traces of hydrocarbon monoterpenes (Fig. 1).

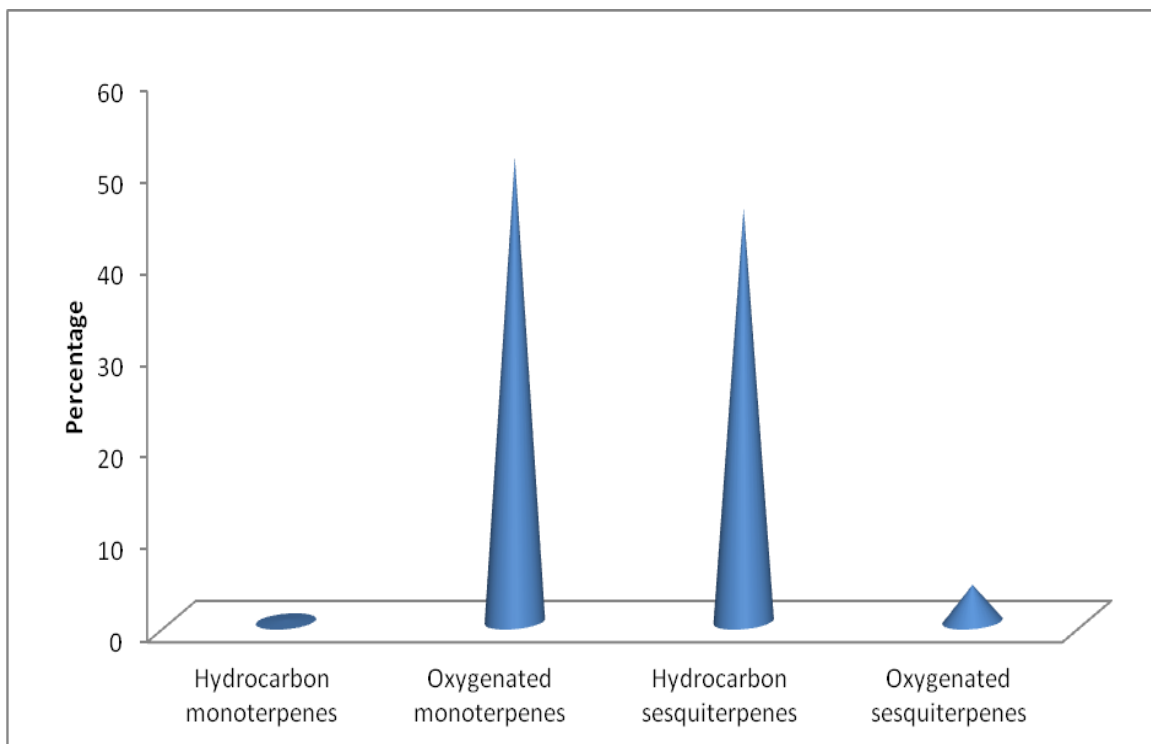


Figure 1:- Terpenoidal composition of the essential oil of *O. gratissimum*

Within the chemical constituents of the essential oil of *O. gratissimum*, eugenol (41.74 %) was the main constituent followed by caryophellene (21.99 %), isocaryophellene (12.36%), piperitone (3.43%) and methyl chavicol (2.74%).

The present investigated essential oil was characterized by absence of thymol which comprised a major constituent in the essential oil of *O. gratissimum* from many African countries (Asawalam et al., 2008; Benedicta et al., 2012).

Based on the chemotype classification system proposed by Grayer et al. (1996), which is based on the combination of the essential oil major components rather than the sole dominant compound, *O. gratissimum* under investigation could be classified as eugenol-caryophellene chemotype.

The majority of the of the reported *O. gratissimum* chemotypes, as proposed by Grayer et al. (1996), were eugenol/1,8-cineole ones (Vasconcelos et al., 1999; Silva et al., 2004; Cristiana et al., 2006; Benitez et al., 2009).

The GC/MS analysis of the hydro-distilled *O. basilicum* oil revealed detection and identification of thirty nine compounds (Table 2).

Table 2:- The chemical composition of the essential oil of *O. basilicum*

Peak	Retention time	Compound	percentage
1	10.74	α -pinene	0.57
2	11.23	Camphene	0.10
3	12.09	β -phellandrene	0.52
4	12.18	β - pinene	1.06
5	12.69	β -myrcene	1.26
6	13.78	p-cymene	0.25
7	13.93	Terpinolene	0.79
8	14.03	1,8-cineole	5.75
9	14.55	β -transocimene	0.24
10	15.17	Trans-sabinene hydrate	0.12
11	15.34	Epoxy linalool	0.31
12	15.85	Fenchone	0.38
13	16.36	Linalool	17.41
14	17.63	camphor	0.26
15	18.29	Myrcenol	0.29
16	19.07	α -terpineol	1.21
17	19.40	Methyl chavicol (estragole)	39.26
18	19.53	octylacetate	0.73
19	20.05	Nerol	0.30
20	20.43	Neral	0.86
21	20.85	geraniol	0.70
22	21.27	Geranial	0.94
23	21.73	Bornyl acetate	1.36
24	23.72	m-eugenol	3.51
25	24.18	α -copaene	0.45
26	24.60	β -elemene	1.87
27	24.87	β -cubebene	1.18
28	25.36	Caryophellene	1.31
29	25.71	Bergamotene	3.59
30	25.80	α -guaiene	0.86
31	26.23	cadina-3,5-diene	0.99
32	26.44	Epibicyclosquiphellandrene	0.59
33	26.91	Germacrene D	1.64
34	27.50	Δ -guaiene	1.65
35	27.71	γ -cadinene	2.45
36	28.28	α -bisabolene	1.17
37	29.25	Spathulenol	0.54
38	30.10	Cubenol	0.65
39	30.69	Δ -Cadinol	2.58

O. basilicum essential oil consisted mainly of oxygenated monoterpenes (73.63%) followed by hydrocarbon sesquiterpenes, hydrocarbon monoterpenes and oxygenated sesquiterpenes (Fig. 2).

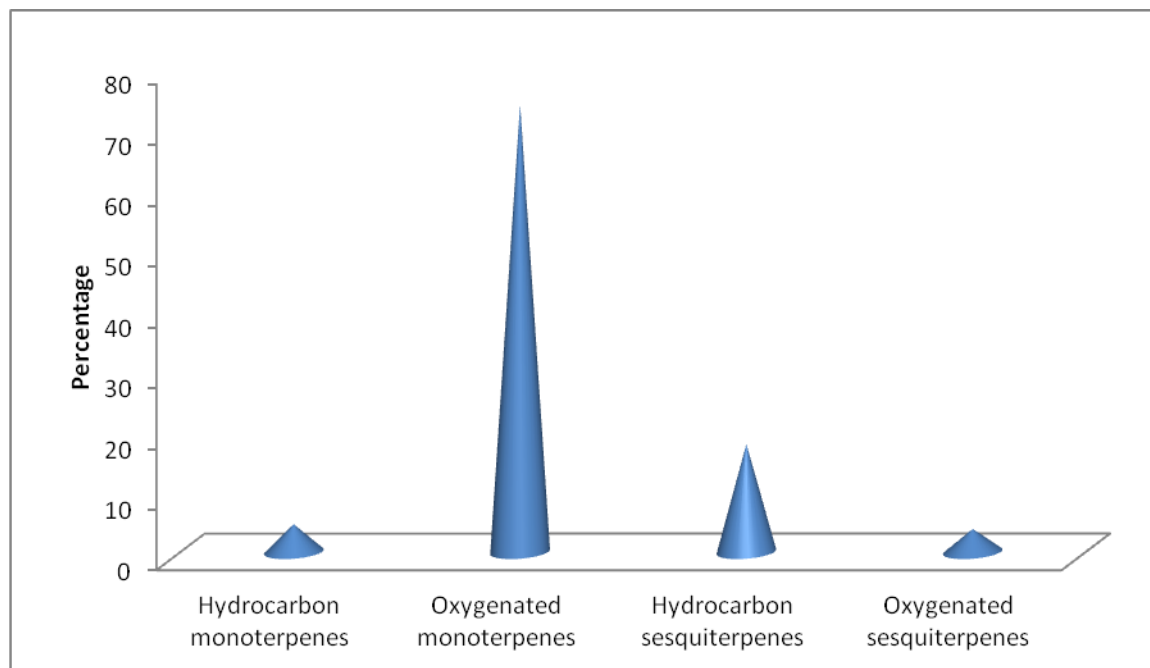


Figure 2:- Terpenoidal composition of the essential oil of *O. basilicum*.

Among the detected monoterpenes, the main constituent identified was methyl chavicol, representing about (39.26%) of the oil, followed by linalool (17.41 %), 1,8-cineole (5.75%) and m-eugenol (3.51%).

The chemistry of the essential oil from leaves of *O. basilicum* in this study is similar to some previously investigated *O. basilicum* essential oil, from different origins, in presence of methyl chavicol as the main constituent (Sajjids, 2006; Bunrathep et al., 2007; Mindaryani and Sri Rahayu, 2007; Vani et al., 2009).

Conclusion:-

It could be concluded that *O. gratissimum* and *O. basilicum* cultivated in Sudan could be classified as eugenol/caryophellene chemotype and methyl chavicol (estragole) chemotype, respectively.

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