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Chemical composition of *Ocimum basilicum* L. essential oil from different regions in the Kingdom of Saudi Arabia by using Gas chromatography mass spectrometer

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Abstract
Ocimum basilicum L. is one of the most popular plants found in Kingdom of Saudi Arabia. This study was conducted to identify the different essential oils of *Ocimum basilicum* L. in different regions. essential oils was extracted from the mature leaves by Hydro distillation method, proportion of oil was 0.1-0.4%.The components of the oil were measured by GC-MS. 59 compounds have been identified in the volatile oils extracted representing 100% of the total chemical composition ratio β- Linalool (9.12-72.59%) 1,8 Cineole (0.4-10.72%) as the main compounds found in all samples. Methyl Chavicol was the highest percentage of the oil (44.90%) while the disappearance of his existence for some samples as well as for each of the trans Methyl Cinmate, Methyl Eugenol, trans- Geraniol, Methyl Cinmate and Eugenol where recorded respectively 46.69, 18.39, 13.10, 11.21, 10.96%. Comparing the composition of the analysed essential oils of *Ocimum basilicum* L. there were revealed both the quantitative and qualitative differences concerning the major compounds.

Keywords: *Ocimum basilicum* L, Gas chromatography - mass spectrometer, essential oils analysis

Introduction

"Ocimum basilicum L. (sweet basil) belongs to family Lamiaceae. The family Lamiaceae comprises the most employed medicinal plants as a worldwide source of spices and also as a consolidated source of extracts. The chemical composition of sweet basil essential oil has been investigated and by now more than 200 chemical components have been reported from many regions of the world as described by Danile *et al.* [1].

Ocimum basilicum (commonly known as scent leaf) is native to Africa, Asia and Pacific Island and is one of the Lamiaceae family. The plant is mostly annual or perennial herbs and the genus contains between 50 and 150 species. They are found in the tropical regions of Asia, Africa, and Central and South America as described by Danile *et al.* [1].

"The plant derived its name from Greek Ozo which means to smell and this is in reference to the strong odors of the species within the genus as described by Gabi *et al*. [2]. In French, it is frequently given the name "Herbe Royale" revealing the positive light in which it is viewed. It is sometimes referred to "King of herb" and this name may have been derived from Greek Basileus, or king as described by Uraku [3].

The chemical constituents showed the presence of monoterpene hydrocarbons, oxygenated monoterpene, sesquiterpene hydrocarbons, oxygenated sesquiterpene, triterpene, flavanoids, aromatic compounds, etc. The compounds have antiproliferative, anticancer, antidyspepsia, antigiardial, antiinflammatory, antioxidant, antiulcer, antiviral, insecticidal and wound-healing activities, also showed antiwormal response, cardiac stimulant, effects on central nervous system, hypoglycaemic and hypolipidemic effects and inhibitory effect on platelet aggregation as described byMarwat *et al*. [4]. Various parts of the plant of *Ocimum basilicum* have been widely used in traditional medicine. Leaves and flowering parts of *Ocimum basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic and tonic agent. They have also been used as a folk remedy to treat various ailments such as feverish illness, poor digestion, nausea, abdominal cramps, gastro-enteritis, migraine, insomnia, depression, gonorrhea, dysentery and chronic diarrhea exhaustion. Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites and skin infections as described by Marwat *et al.* [4].

"Oils have been classified into four chemotypes according to their chemical composition and geographical source. The European type, cultivated in Europe, USA, and Africa, is characterized by linalool and methylchavicol as the major oil constituents. The Reunion type, located in the Comoros and Seychelles Islands, Africa, and Reunion Island, is characterized by a high concentration of methyl chavicol. Tropical type originated from India, Pakistan, Guatemala, Haiti, and Africa is rich in methyl cinnamate. Another basil chemotype, with eugenol as the main component, is common in North Africa, Russia, Eastern Europe, and some parts of Asia. In addition to these, other basil oils have also been reported which contained various quantities of linalool, camphor, methyl chavicol, methyl cinnamate, and eugenol as described by Simon *et al*. [5]; Marotti *et al*. [6]; Pandey *et al*. [7]. Therefore, the objective of this study was to investigatethe different essential oils of *Ocimum basilicum* L. Plant grown in different areas in the Kingdom of Saudi Arabia.

Materials and Methods

Plant material

The plant materials *Ocimum basilicum* L. were collected from Different cities in the kingdom of Saudi Arabia (KSA) (Abha, Dammam, Jeddah, Medina, Mecca, Riyadh, Taif (AL Shafa and AL Hada), and Yanbu). As shown in the map (Figure1), The climate of KSA is influenced by several natural factors, butthe most important factor is the location. KSA is located between latitudes 16 and 33 north and between longitudes 34 and 56 east. That made the largest part of it within dry tropical desert regions in the western continents. Therefore, we have focused our research on the most famous areas between longitude 50-38 and between latitude 26-18 as mentioned in (Table 1):

Essential oil extraction by classical Hydrodistillation procedure

The samples were preparation and the essential oil was extracted from the collected fresh plant samples, the leaves were released, 300 grams of the fresh mature leaves were and hydro distilled for 3 hours using Clevenger type apparatus (Model of this apparatus), producing less colored essential oils at a yield of 0.13-0.37%. Oils were dried over of anhydrous sodium sulphate and filtered. The obtained oils were collected in a sealed vial.

Essential oil analysis

Sample preparation for GC-MS analysis

A quantity of 10 µl from the essential oil was mixed with 1 ml of GC grade n-hexane. The new mixture was agitated for one min, and 1 µl was injected into the GC–MS by using the auto sampler injector.

Gas Chromatography-Mass Spectrometry System

The aim of the selection and definition of chromatographic condition is to achieve a proper separation of the components of the oil, both for the qualitative analysis, and for the proper quantification. The analysis of the samples was performed using gas chromatograph (GC, Model CP-3800, Varian, Walnut Creek, CA, USA) coupled with a mass spectrometer (MS, Model Saturn 2200, Varian) and auto sampler (Model Combi Pal, Varian) system. The separation was done using a VF-5ms fused silica capillary column (5% phenyl-dimethylpolysiloxane, 30 m × 0.25 mmi. d., film thickness 0.25 µm, Varian).For MS detector, electron impact (EI) ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant low rate of 1 ml min^{-1}. Injector and mass transfer line temperature were set at 250 and 300 °C, respectively. The Optimization condition for oven temperature was programmed for 1 min at 50 °C, 50 to 240 °C at 3 $^{\circ}C min^{-1}$ then hold for 5 minutes at 240 °C, all programme 69.33 min. The injection of the samples was carried out with the auto-sampler for 1 µl with a split ratio 1/20. The conditions of analysis and specification of the instrument were optimized for a better separation and resolution. Identification of components was based on matching with mixed standard and Wiley and NIST electronic library.

Results

In (Table 2) illustrate the composition and the percentage of *Ocimum basilicum* L. essential oils.

Overall, the most noticeable information could bethe β-Linalool, Methyl Chavicol Methyl Eugenol, Methyl Cinmate, trans Methyl Cinmate, Eugenol, Methyl Eugenol, 1,8-Cineole was the highest rates in the most of regions.

Abharegion (twenty-four) compounds were identified from the *O. basilicum* L. The most abundant constituents in the *O. basilicum* L. essential oil were β-Linalool (51.93%), Methyl Chavicol (14.85%), trans - Geraniol (13.10%), trans Methyl Cinmate (9.21%)

In the Eastern region in Dammam samples (thirty-four) compounds were identified from the *O. basilicum* L. The most abundant constituents in the *O. basilicum* L. essential oil were Methyl chavicol (44.9%), Methyl Eugenol (15.72%), trans Methyl Cinmate (11.69%), β-Linalool (9.12%), 1,8-Cineole (3.47%).

In Jeddah samples (thirty-nine) compounds were β-Linalool (38.75%), Methyl chavicol (26.27%), 1,8-Cineole (10.72%), trans-Geraniol (8.36%).

In Madina samples (thirty-six) compounds were identified from the *O. basilicum* L. The most abundant constituents in the *O. basilicum* L. essential oil were β- Linalool (43.33%), Methyl Eugenol (18.39%), Eugenol (10.96%), 1,8-Cineole (5.88%), Methyl Chavicol (1.30%).

In Mecca samples, twenty two compounds were found to be trans Methyl Cinmate (46.69%), βLinalool (31.58%), Methyl Cinmate (11.21%).

In the capital city of Riyadh samples (Forty-tow) compounds compounds were identified from the *O. basilicum* L. The most abundant constituents in the *O. basilicum* L. essential oil were Mythyl Chavicol (38.95%), β- Linalool (29.16%), trans-Geraniol (3.10%), Methyl Eugenol (3.01%), trans Methyl Cinmat (2.53%), 1,8-Cineole (1.95%).

In AL-Hada, Taif samples (Forty) compounds were identified from the *O. basilicum* L .The most abundant constituents in the *O. basilicum* L. essential oil were β-Linalool (60.15%), trans - Geraniol (9.82%), Methyl Eugenol (9.04%), 1,8-Cineole (1.59%).

In Al-Shafa, Taif samples (thirty-three) compounds were identified from the *O. basilicum* L. The most abundant constituents in the *O. basilicum* L. essential oil were β-Linalool (72.59%), trans-Geraniol (5.25%), Methyl Chavicol (4.59%), 1,8 Cineole (4.25%).

In Yanbu samples, (thirty-one) compounds were identified from the *O. basilicum* L. The most abundant constituents in the *O. basilicum* L. essential oil were β-Linalool (59.61%), trans-Geraniol (13.09%), Eugenol (10.11%), 1,8 Cineole (1.79%).

Table 1: The altitude and sites of *Ocimum basilicum* L collected from KSA

City	Longitude and latitude		Altitude	Temperatures in Feb 2016
Abha	N 18.216797°	E 42.503765°	2270m	13-20 °C
Dammam	N 26.399250°	E 49.984360°	593 m	15-25 °C
Jeddah	N 21.5169444°	E 39.2191667°	27m	23-28 °C
Medina	N 24.470901°	E 39.612236°	608m	17-30 °C
Mecca	N 21.422510°	E 39.826168°	333m	24-36 °C
Riyadh	N 24.774265°	E 46.738586°	597.6m	13-24 °C
Taif	N 21.422019º	E 40.495617°	1542m	13-23 °C
Yanbu	N 24.186848°	E 38.026428°	7m	18-31 °C

Table 2: The composition together with the percentage and retention time of *Ocimum basilicum* L. essential oils.

			Percentage of oil								
					Taif						
	Oil constituent	R_t	Mecca	Abha	Hada	Shafa	Yanbu	Dammam	Madina	Riyadh	Jeddah
1	α – Pinene	8.15	-	-	0.17	0.13	-	-	0.14	0.09	0.37
2	Camphene	8.79	-	-	0.07	-	-	-	0.07	-	0.07
3	Sabinene	9.68	-	-	0.07	0.13	-	0.07	0.14	-	0.38
4	β – Pinene	9.89	-	-	0.18	0.29	0.18	0.14	0.3	0.15	0.73
5	1 Octen3 ol	9.99	-	-	0.31	0.41	-	-	0.27	0.55	0.46
6	β – Myrcene	10.31	-	-	0.5	0.63	-	0.14	0.58	0.13	0.78
7	3-Octanol (CAS)	10.7	-	-	-	-	-	-	-	0.10	-
8	Ethyl Amyl Carbinol	10.73	-	-	-	0.07	-	-	-	-	-
9	dl-Limonene	12.03	-	-	0.35	0.15	0.07	0.36	0.49	0.18	0.4
10	1,8-Cineole	12.2	0.92	0.40	1.59	4.25	1.79	3.47	5.88	1.95	10.72
11	β-CisOcimene	12.79	0.53	-	1.01	0.38	0.48	1.57	2.57	-	1.01
12	Carene	12.8	-	-	-	-	-	-	-	1.11	-
13	γ –Terpinene	13.33	-	-	0.18	-	-	0.11	-	0.16	0.11
14	trans-Sabinene hydrate	13.92	-	-	0.12	0.08	-	0.13	0.09	0.29	0.2
15	α –Terpinolene	14.55	-	-	0.14	0.09	-	0.2	0.24	0.14	0.15
16	Fenchone	14.76	1.12	-	0.09	-	0.13	0.48	0.19	-	0.13
17	β- Linalool	15.25	31.58	51.93	60.15	72.59	59.91	9.12	43.33	29.16	38.75
18	D-Fenchyl alcohol	16.16	0.5	-	-	-	0.18	0.63	1.03	0.14	0.2
19	Camphor	17.46	1.01	0.48	0.08	0.14	0.46	1.19	0.16	0.10	0.74
20	Borneol	18.61	0.2	0.16	0.28	0.23	0.33	0.23	0.93	0.39	0.15
21	4- Terpineol	18.96	-	0.76	0.89	-	-	0.79	0.08	0.94	0.67
22	α-Terpineol	19.65	0.37	0.23	0.06	0.42	0.62	0.41	0.3	-	0.84
23	MythylChavicol	19.86	-	14.85	0.74	4.59	-	44.90	1.3	38.95	26.27
24	Nonyl acetate	20.35	0.17	-	-	-	-	-	-	-	-
25	Fenchyl acetate	20.62	0.76	0.09	0.33	-	0.34	0.57	1.12	0.17	0.38
26	β-Citronellol	21.16	0.20	-	-	-	-	-	-	-	-
27	Z-Citral	21.64	-	0.51	0.22	-	0.21	-	-	0.32	0.3
28	trans-Ocimene	22.07	-	-	-	0.19	-	-	-	-	-
29	trans-Geraniol	22.19	0.11	13.10	9.82	5.25	13.09	-	0.20	3.10	8.36
30	E-Citral	22.98	-	0.63	0.28	0.12	0.31	-	-	0.36	0.4
31	Bornyl acetate	23.66	0.49	0.34	0.72	0.4	1.08	0.59	2.26	1.09	0.49
32	Methyl Cinmate	24.57	11.21	1.45	-	-	-	1.23	-	0.14	-
33	α-Terpinanly acetate	26.37	-	-	-	-	-	0.13	-	0.09	-
34	Eugenol	26.65	-	-	0.1	-	10.11	-	10.96	-	0.53
35	α-Copaene	27.56	-	-	-	-	-	-	-	0.09	-
36	Neryl acetate	27.74	-	1.16	-	-	0.19	-	-	-	0.92
37	Geranyl acetate	27.75	-	-	0.95	-	-	-	-	1.30	-
38	Linalyl acetate	27.76	-	-	-	0.48	-	-	-	-	-
39	transMethylCinmate	28.12	46.69	9.21	-	-	-	11.69	-	2.53	0.31
40	β- Elemene	28.18	-	-	0.07	-	-	-	-	-	-
41	Methyl Eugenol	28.78	-	-	9.04	-	0.27	15.72	18.39	3.01	0.55
42	trans-Caryophyllene	29.45	-	-	0.35	-	-	-	-	-	-
43	α-Bergamotene	29.97	1.34	1.46	1.93	0.21	2.6	1.33	2.19	1.30	1.1
44	α-Guaiene	30.08	-	-	-	0.15	-	-	-	0.27	-
45	α- Cadinene	30.51	-	-	0.14	0.12	0.09	-	-	0.19	-
46	α- Cubebene	30.80	-	-	-	-	-	0.2	0.12	0.25	-
47	α- Humulene	30.92	-	-	-	-	0.15	0.11	0.14	0.18	-
48	β- Cubebene	31.19	-	0.12	0.32	0.32	0.26	0.15	0.21	0.44	0.11
49	γ- Muurolone	31.97	0.08	0.29	0.82	0.91	0.34	0.78	0.73	1.14	0.28
50	β-Farnesene	32.09	0.08	0.09	-	-	0.16	-	0.17	-	0.07
51	γ-Gurjunene	32.59	0.35	0.29	0.48	0.63	0.67	0.49	0.47	0.89	0.32
52	δ-Guaiene	32.81	-	0.21	0.47	0.34	0.35	0.2	0.23	0.54	0.18
53	α-Seline	33.04	0.22	0.23	0.33	0.28	0.33	0.25	0.24	0.50	0.23
54	γ-Cadinene	33.28	0.33	0.57	1.52	1.39	1.28	0.5	0.98	1.51	0.5
55	β- Sesquiphellandren	33.69	-	-	-	-	0.09	-	-	-	-
56	Germacrene D	33.98	-	-	-	0.08	-	-	-	-	-
57	Cubenol	37.26	-	-	0.66	0.59	0.4	0.24	0.35	0.86	0.23
58	TAU-Cadinol	38.28	0.58	1.41	4.3	3.95	3.55	1.89	3.16	4.92	1.61
59	α-Eudesmol	38.78	-	-	0.16	-	-	-	-	0.27	-

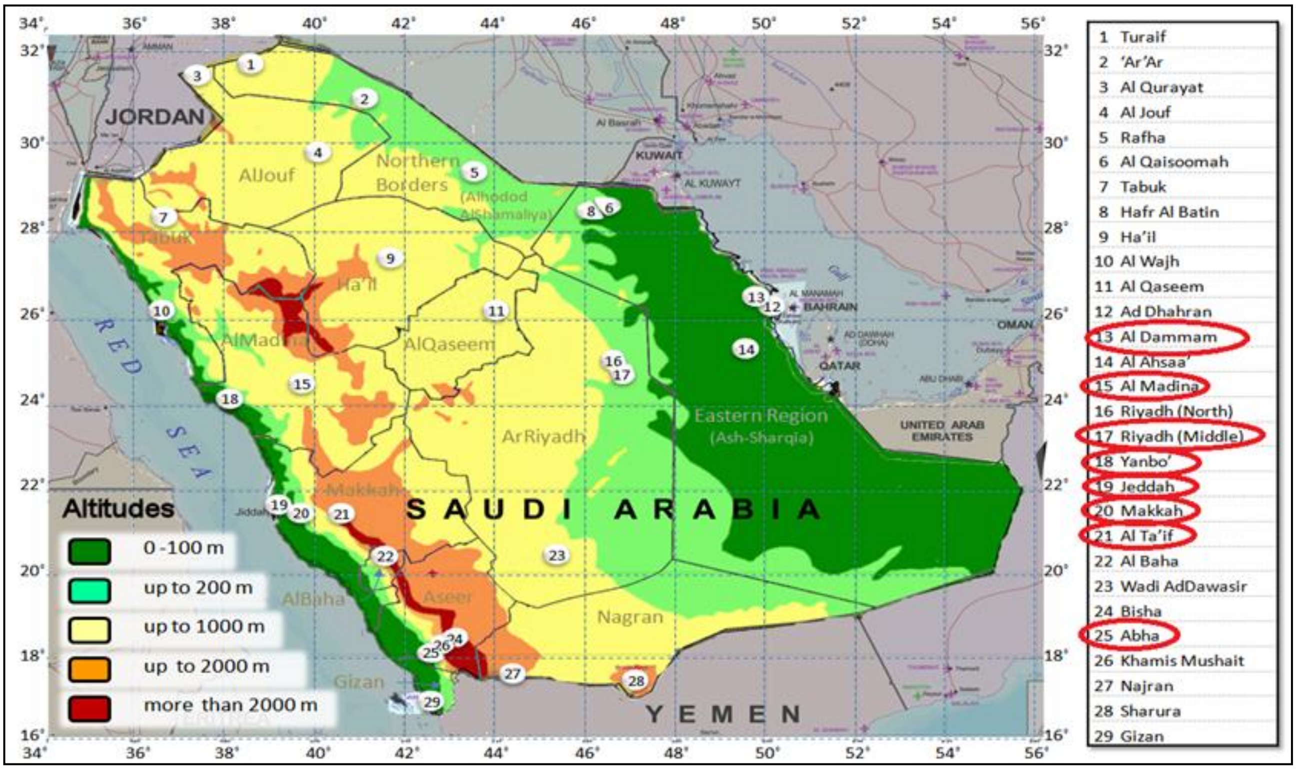

Fig 1: The KSA map explain the sampling areas of *Ocimum basilicum* L.

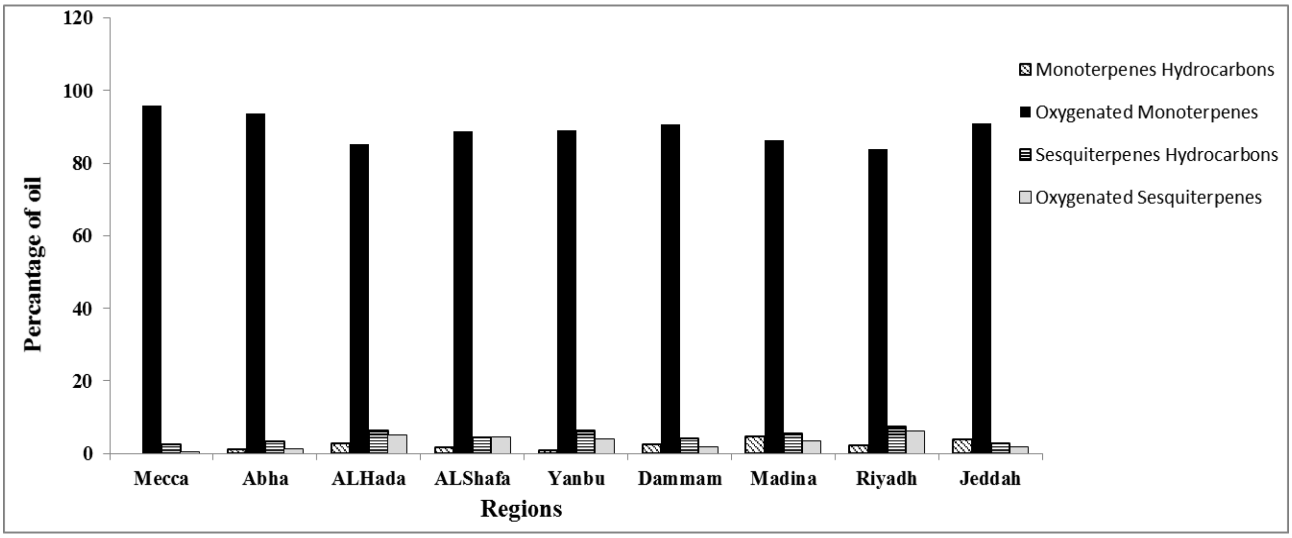

Fig 2: Histograms of chemical classes of *Ocimum basilicum* L. essential oils.

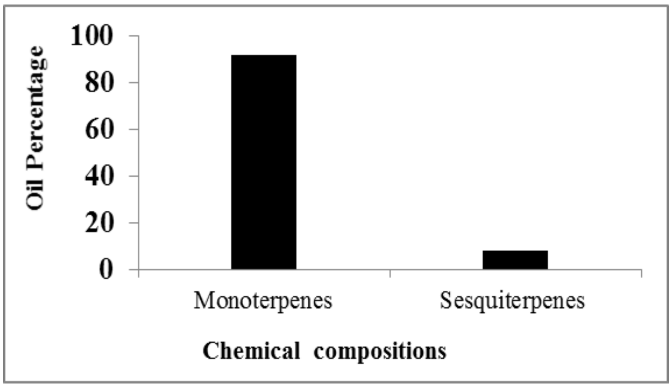

Fig 3: Approximate difference between the two categories for all cities.

Discussion

Plants that grow in different regions and environmental conditions present variation of the chemical constituents. In general, variations in the essential oil content of *Ocimum basilicum* affected by geographic regions might be correlated with variable agro-climatic conditions and/or different agricultural techniques and methods for cultivating. Our results indicated that oxygenated monoterpenes were the main compounds of *Ocimum basilicum* essential oils followed by sesquiterpenes hydrocarbons (Figure 2 and 3). Our results are in agreement to those as described by Hussain *et al.* [8], who reported the oxygenated monoterpenes were the major compounds in *Ocimum basilicum* essential oils (60.768.9%) followed by sesquiterpenes hydrocarbons (16.0-24.3%). The various differences in the constituents of basil essential oils across regions may be due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants.

The composition of *Ocimum basilicum* oil was studied by several researches Al Abbasy *et al.* [9] ;Chhetri*et al.* [10]; El-Soud *et al.* [11]; Imeri *et al.* [12]; Filip *et al.* [13]; Bazaid *et al.* [14];

Nurzynska-Wierdak *et al*. [15]. They indicated that β-Linalool is a prominent part of volatile oil. In our study, the percentagesof β- Linalool at the AL Shafa, AL Hada, Yanbu, Abha, Madina, and Jeddahregions is 72.59%, 60.15%, 59.91%, 51.93%, 43.33%, 38.75%, respectively. On the other hand, some previous studies, reported that main component was not Linalool but Methyl chavicol as described by Shirazi *et al*. [16]; Bunrathep *et al*. [17]; Sajjadi [18]. These studies were similar to the regions of Dammam and Riyadh where the ratios were 44.60%, 38.95% respectively. There are several reports in the literature showing the variation in the yield and chemical composition of the essential oil with respect to geographical regions. For the chemical composition of *Ocimum basilicum*, our results are in excellent agreement to those of El-Soud *et al.* [11]. Who reported that the most important components of essential oil from *Ocimum basilicum* were: linalool (48.4%), 1,8-cineol (12.2%), eugenol (6.6%), methyl cinnamate (6.2%). This study is close to our study of the samplesfrom Jeddah, Medina, Yanbu and Al Hadaas. Also, the same trend was reported by Klimankova *et al*. (19); Hanif *et al*. [20]. Who found that linalool (69.9%), geraniol (10.9%), 1,8-cineole (6.4%), α-bergamotene (1.6%), geranyl acetate (1.4%) were the main components of Oman *Ocimum basilicum* essential oil.

Previous article [14], reported that the most abundant constituents in the essential oil of *Ocimum basilicum* were varied from 0.68 to 0.79% according to the sample location. The results showed that the main components of volatile oil were 1,8-cineole (7.87- 9.13%), Linalool (26.57-27.89%), Estragole (14.14-18.78%), Methyl Cinnamate (5.41- 8.87%), Eugenol (10.26-16.67%), and Limonene (3.15- 4.11%). They indicated thatthe agronomical practices and environmental conditions affect the composition of important compounds of essential oil. The current study was focused on the Taif region in KSA. Results reported for the AL Hada area indicated that Linalool 27.82%, 1,8-cineole 8.95%, Estragole 14.14%, Methyl cinnamate 8.87%, Eugenol 16.67%, and Limonene 4.11% were the major compounds.

Also, Chhetri *et al*. [10] reported that the most abundant constituents in the essential oil of *Ocimum basilicum* from Yemen was dominated by linalool (74.5%) with lower concentrations of 1,8-cineole (7.4%) and stragole (7.2%). This study is similar to Bazaid *et al*. [14]. As well as our study for AL Shafa, AL Hada, Abha, Dammam, Jeddah, Madina and Riyadh.

On the other hand, Joshi [20]. Reported that Methyl eugenol (39.3%) and methyl chavicol (38.3%), accounting about 98.6% for the total oil. This previous result is similar toour result of Dammam region where Methyl chavicol (44.9%) and Methyl Eugenol (15.72%) were the highest compounds. "In Ethiopia, the results were different, the oil contained, as main components, copaene (25.5%), p-menth-2-en-1-ol (7.7%), eugenylacetae (4.8%), bornyl acetate (4.0%), γ-himachalene (3.6%), rosifoliol (3.0%) and α –cubebene (2.5%) as described by Unnithan *et al*. [22].

Comparing the composition of the analysed *Ocimum basilicum* L. essential oils from different places in KSA, there were revealed both the quantitative and qualitative differences concerning the major compounds. There was a study showed the difference (quantitative and qualitative) in Romania by Benedec *et al*. [23]. The observed differences might be the result of different environmental, genetic factors, different chemotypes as well as other factors that can influence the oil composition.

Conclusion

According to our results which proved that there is an obvious difference in the chemical composition between samples of which is regarding the difference in environmental and geographical factors which are known to have a significant influence on the essential oil composition of the plants. From these results, it can be concluded that the chemical composition of the essential oil obtained from the leaves of plants collected from nine different regions of Kingdom of Saudi Arabia has different qualitative and quantitative properties.

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