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Physiochemical characterization of essential oil from *Mentha piperita* L. cultivated in Sudan

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Abstract
Mentha piperita leaf essential oil was obtained by hydrodistillation method. It yielded about 2.4% (v/w). The Physicochemical properties of *M. piperita* leaf oil were determined. The chemical composition of the essential oil of *M. piperita* was investigated using GC-MS technique. The gas chromatogram revealed detection of seventeen compounds. Monoterpenes, represented about 83% of the oil, of which 79.87% were oxygenated. Among the oxygenated monoterpenes, the most abundant components found was menthone (57.7%) followed by menthofuran (7.2%), 1,8- cineole (5.5%), isomenthone (3.8%), menthyl acetate (2.3%), pulegone (1.7%) and isomenthol (1.5%), whereas the most abundant hydrocarbon monoterpene is limonene (1.6%). This study confirmed that *M. piperita* grown under Sudan climatic conditions is menthone chemotype (*M. piperita* ct. menthone).

Keywords: *mentha piperita*, hydrodistillation, GC-MS, chemotype, menthone

1. Introduction
Mentha Piperita L. **(**Peppermint**),** which belongs to Family Lamiaceae (Labiatae)**,** is a perennial herb. It's a native of the Mediterranean, but also cultivated in Europe e.g. Italy, Great Biritian, and naturalized in the Northern United States of America and Canada. It has been cultivated since ancient times in Japan, China and Egypt. Also it is cultivated in North Africa in Morocco and has been cultivated recently in Sudan. Generally, it is extensively cultivated in soils with high water holding capacity, it requires a daytime length of 15-16 Hrs [1, 2]. *M. Piperita* **(**Peppermint) oil is a colorless, Pale green or pale yellow liquid that has strong, penetrating odor of peppermint and a pungent taste that is followed by a sensation of cold when air drawn into the mouth [2]. It is used as an antiseptic, stimulant, carminative agent or it is further used as a flavoring agent in cosmetic and pharmaceutical industries throughout the world.

The essential oil contents and composition of *M. piperita* leaves shows variation in plants of different geographical origin. In a sample analyzed in Turkey, the major components detected were menthone (44.1%), menthol (29.5%), menthyl acetate (3.8%) and menthofuran (0.9%) [3]. Chemical composition of Bulgarian peppermint essential oils characterized by presence of menthol (35.2 – 46.2%) and menthone (8.7 – 25.9%) [4]. The major constituent in essential oil from leaves of *M. piperita* from Morocco was menthone (29.01%) followed by menthol (5.58%), menthyl acetate (3.35%), menthofuran (3.01%), 1,8-cineole (2.4%), isomenthone (2.12%), limonene (2.1%), α-pinene (1.56%), germacrene-D (1.5%),β-pinene (1.25%), sabinene (1.13%), and pulegone (1.12%) [5].

In another sample from Turkey, (+)-Menthol (38.06%), menthol (35.64%), neomenthol (6.73%) and cineole (3.62%) were the main components detected in the oil [6].

Essential oil of leaves of *M. piperita* studied in Korea showed the presence of linalyl acetate (28.2%), menthol (33.4%), 1,8-cineole (32.1%) as major components [7].

Previously studied essential oil of *M. piperita* from Sudan revealed detection of menthone (47.38%), menthofuran (9.79%), menthol (8.58%), pulegone (7.92%), 1,8-cineole (5.29%), isomenthone (4.64%) and limonene (2.73%) as the major components [8].

The main constituents in essential oil samples, obtained by hydrodistillation method, from Brazil, were menthol (12 - 92.7%), mentone (2.2 - 56.9%), and neomenthol (2.9 - 12.1%) [9].

Based on GC/MS analysis of an essential oil of peppermint from Taiwan, the major components determined were menthol (30.35%), menthone (21.12%), and trans-carane (10.99%) [10].

Twenty three compounds were identified in the essential oil of *M. piperita* from Iran; the major components detected were menthol (38.33%), menthone (21.45%) and menthyl acetate (12.49%) [11].

In another sample of peppermint essential oil analyzed in Iran, menthol (36.9%), menthone (28.8%) and methyl acetate (4.5%) were the main components, followed by carveone (3.8%), neomenthol (3.8%), 1,8-cineole (3.8%) and limonene (3.29%) [12].

The main objective of this research is characterization of the essential oil of *M. pipperita* grown in Sudan (Khartoum North).

2. Materials and Methods
2.1 Plant material
M. piperita plant was brought from Gasim nursery, Khartoum North (Shambat area) and authenticated in Medicinal and Aromatic Plants Institute, Khartoum (MAPRI).

2.2 Essential oil content determination
100 g of shade dried, crushed *M. piperita* leaves were

hydrodistilled, using Clavenger apparatus, for four hours. The percentage of the oil (v/w) was calculated based on the dry material.

2.3 Physiochemical properties determination
All the physical parameters of the oil under investigation were determined as described in British Standards [13].

2.4 (GC-MS) analysis
GC-MS analysis was carried out on Gas Chromatography-Mass Spectrometer-Quadrupole (QP) -2010- SHIMADZU, with (30 m × 0.25 mm i.d, film thickness 0.25 µm) capillary column at flow rate 1.74ml/min. Injection temperature was 280°C and injection mode was split. The detector temperature was 280°C. Helium was used as the carrier gas with velocity 47.6 cm/sec. Oven temperature programmed at 45°C for 4min, then was raised to 240°C at rate 6°C/min, hold time 2min, then raised to 280°C at rate 39°C/min. The interface temperature was 260°C. The quadrupole mass spectrometer scanning range was 40-350 m/z, with an ionizing temperature 200.00°C.

3. Results and discussion
The *M. piperita* leaf oil which was obtained by hydrodistillation method was found to be 2.4% (v/w). This yield is relatively high compared to values found in the literature (0.28 - 2.6%) [14, 15].
The Physicochemical properties of present investigated *M. piperita* leaf oil, are shown in Table (1), compared to standard parameters [16].

Table 1: Physicochemical properties of *M. piperita* oil

No.	Parameters	Experimental values	Standards values [16]
1	Colour	Pale green	Pale green –Pale yellow
2	Refractive index(at 30ºC)	1.4641	1.457 –1.467
3	Specific gravity	0.898	0.900 – 0916
4	Solubility in ethanol	Sol. in 4 vols. 70% & in 1 vol. 80%	Sol in 1 vol. 95%
5	Acid value	1.47	Not more than 1.5
6	Ester value	29	12 – 30

The chemical composition of the essential oils of *M. piperita* was investigated using GC-MS technique. The gas chromatogram revealed detection of seventeen compounds; their identification based on their mass spectra. Identification of the mass spectra of the constituents of the oil was achieved by searching in NIST library [17]. The percentage composition of components in each oil was determined by computerized peak area measurements. The constituents of *M. piperita* essential oil are listed in order to their retention time in Table (2).

Table 2: Chemical constituents of essential oil of *M. piperita*

Peak	R. time	Compound	Area%
1	2.141	Propane2,2diethoxy	0.73
2	3.627	α-pinene	0.50
3	4.392	sabinene	0.26
4	4.451	β-pinene	0.76
5	5.215	limonene	1.57
6	5.564	1,8-cinneole (eucalyptol)	5.47
7	6.203	Terpeneol,cis,beta	0.34
8	7.694	isomenthol	1.45
9	7.932	menthone	57.68
10	8.050	menthofuran	7.18
11	8.228	isomenthone	3.80
12	9.483	menthylacetate	2.26
13	9.767	pulegone	1.69
14	10.893	Unidentified	0.24
15	16.821	Hexadecanoic acid,methyl ester	4.05
16	19.971	10-octadecanioc acid,methyl ester	8.66
17	20.233	9,12-octadecanoic acid,methyl ester	3.35

The majority of essential oil constituents are monoterpenes, representing about 83%, of which 79.87% are oxygenated monoterpenes. Ketones represent the major constituents of the oil (Fig. 1).

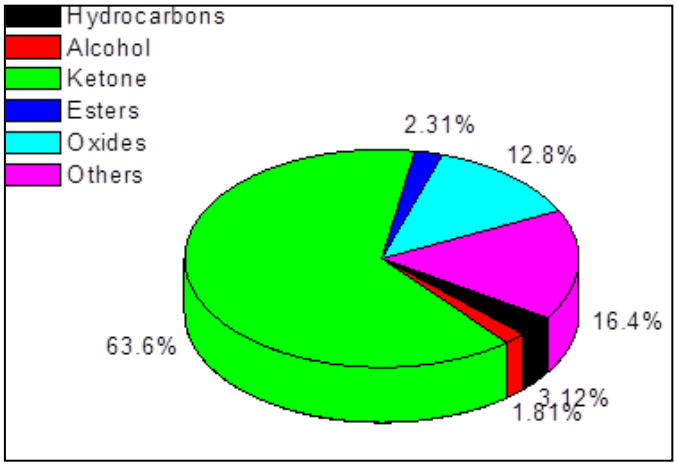

Fig 1: M. Pioerita essential oil constituents groups

Among the oxygenated monoterpenes, the most abundant components found in the leaf oil were menthone (57.7%) followed by menthofuran (7.2%), 1,8- cineole (5.5%), isomenthone (3.8%), menthyl acetate (2.3%), Pulegone (1.7%) and Isomenthol (1.5%), whereas the most abundant hydrocarbon monoterpene is limonene (1.6%). It is surprising that, menthol, which represents one of the major constituents in previously studied *M. piperita* oil, did not detected in the oil under investigation, but its isomer isomenthol was detected in low concentration (1.45%). On the other hand, the present result confirmed that menthone is the main constituent in the essential oil from leaves of *M. piperita* grown in Sudan, irrespect to the variation in the minor constitutes detected in the present and previously investigated essential oil [8]; which may be attributed to the seasonal variation.
The main chemical constituents detected in the investigated oil are shown in Fig. (2).

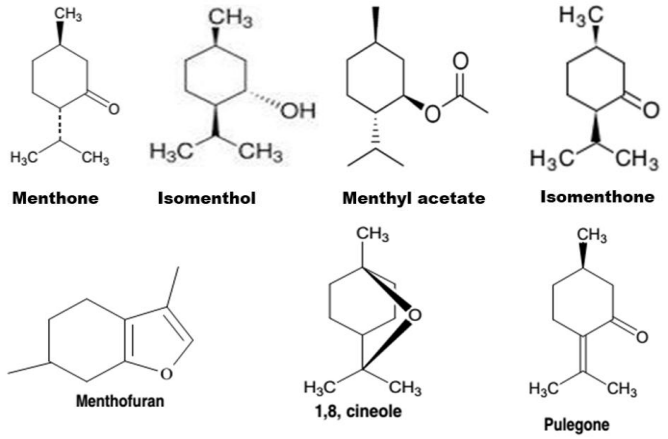

Fig 2: Main chemical constituents detected in oil of M. piperita cultivated in Sudan

Comparing the present study findings with some of the previous studies from other countries (Fig. 3), it could be noticed that there is a clear difference, qualitatively and quantitatively, among the oil constituents from different geographical locations.

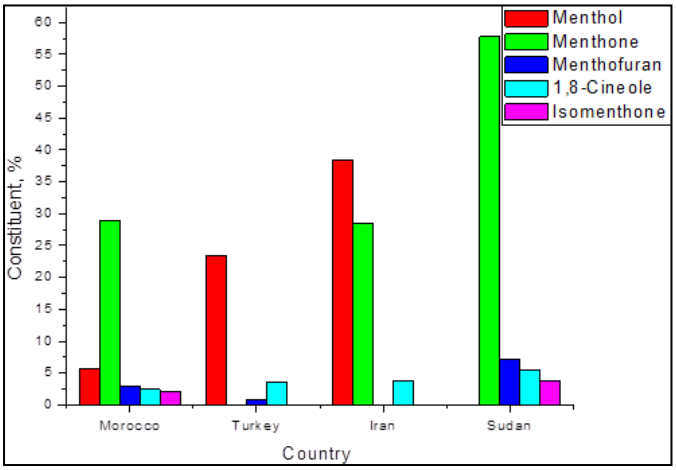

Fig 3: Major constituents of M. piperita oil from different locations

This variations in constituents and percentage may be due to the impact of environmental factors such as temperature, relative humidity irradiance, photoperiod and cultivation practices which influence the composition of essential oils, as well as the growth stage [15, 18, 19]. Also variation may be due to the state of material (fresh or dry state) or the influence of the extraction method of the oil [20].

4. Conclusion

The essential oil contents of leaves from *M. piperita* under investigation showed variation in constituents and composition from those obtained from other different geographical origin (locations). It was found that menthone is the main constituent in the essential oil under investigation, whereas menthol isomer, isomenthol was detected as a minor constituent.

It could be concluded that, *M. piperita* grown under Sudan climatic conditions comprises a menthone chemotype one (*M. piperita* ct. menthone).

5. References

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