

## Essential oil and fatty acid composition of *Melissa officinalis* L.

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**Summary.** The leaf material of lemon balm (*Melissa officinalis* L.) from different origins was evaluated for their chemical composition under the semi arid conditions of Tunisia. Qualitative and quantitative variations of the chemical composition of essential oils according to the origin were shown. The main compounds of Tunisian samples were germacrene-D (29.17-24.6%) and caryophyllene (14.91-13.44%) in Tabarka and Nefza, the samples were characterized by the absence of citral and citronellal. Fatty acids from cultivated *M. officinalis* leaves were analyzed by GC-MS. The major fatty acids of all studied samples were polyunsaturated fatty acids, linoleic acid (73.93-66.74%), versus (16.25-13.32%) saturated ones, oleic acid and (6.29-4.26%) of monounsaturated fatty acid palmitic acid.

**Key words:** *Melissa officinalis* L., essential oil, fatty acids, tunisia

### Introduction

*Melissa officinalis* L. (Lamiaceae) is a medicinal plant native to Southern Europe and Northern Africa (1). Due to several applications in pharmaceutical, food and hygiene industries, *M. officinalis* has been one of the most important commercial plants in recent decades (2). The plant germinate naturally in sandy and scrubby areas (3) but has also been reported to grow on damp wastelands, at heights ranging from sea level to the mountains (4).

There are three subspecies of *M. officinalis*: subsp. *officinalis* L, subsp. *inodora* (Bornm) and subsp. *altissima* (Sibth. & Sm.) Arcangeli or *M. romana* Miller (5). However, only subsp. *officinalis* has commercial value and the characteristic lemony odour of lemon balm (6).

*M. officinalis* has been credited with many medicinal attributes such as tonic, antispasmodic carminative, diaphoretic, antidepressant, antispasmodic, antibacterial, hypoallergenic (7, 8).

Essential oil yield ranged from 0.03 to 0.47% (9). This is quite low compared to other members of the Lamiaceae family and is considered to be the reason for the high production cost and price of essential oil in the market. Moradkhani, Sargsyan (10); Usai, Atzei (5) reported that the main constituents of the essential oil are citral (geranial and neral), citronellal,  $\beta$ -pinene,  $\alpha$ -pinene,  $\beta$ -caryophyllene, totalizing 96% of the oil ingredients.

The essential oil of *M. officinalis* is a prominent antimicrobial agent against food-borne pathogens and food spoilage bacteria (11). It is currently used in medicine and pharmacology (anti-tumor, anti-bacterial, antimicrobial, antihistaminic, antispasmodic and antioxidant, by means of its antiviral effect curing of herpes (12, 13). It was also reported that *M. officinalis* contains substances that inhibit protein biosynthesis in cancer cells (14, 15) antiulcerogenic, moderate Alzheimer's disease, modulation of mood and cognitive performance, and stimulation of the immune system (against anti-HIV-1) (16). Unsaturated fatty acids (UFA) function

as major nutrients, constituents of cell membranes and precursors of various signal molecules, and they are important in both the medical and, as they are involved in the human inflammatory response, blood-pressure regulation, cholesterol metabolism and brain development (17, 18). Epidemiologic prospective cohort studies have suggested that replacing saturated fatty acids with carbohydrates is modestly associated with a higher risk of ischemic heart disease, whereas replacing SFAs with polyunsaturated fatty acids is associated with a lower risk of ischemic heart disease (19).

Due to the continental and geographical conditions, Tunisia is a suitable location for the growth of many medicinal plants, which are genetically valuable resources in fundamental and applied research in plant breeding. However *M. officinalis* has been cultivated in some European and Balkan countries, but not in Tunisia. Its oil is sometimes adulterated with *Cymbopogon* spp.

The Tunisian *M. officinalis* has received little attention, and previous studies have been focused mainly on other species from Lamiaceae family, *M. officinalis* vanished from many parts of Tunisia (20), but some still preserved in two locations in the North West of the country: Tabarka and Nefza (21). Therefore, an investigation of this genetic resource was needed for the assessment of its biochemical composition. The objective of the present study is to determine the chemical composition of the essential oil and fatty acid of *M. officinalis* cultivated in Tunisia.

## Material and methods

### *Plant Material*

The plant material was botanically characterized by Dr Ben Brahim N. (Laboratory of science and agricultural techniques, National Agricultural Research Institute of Tunisia (INRAT) according to the morphological descriptions in the Tunisian Flora (20). Tunisian seeds were harvested from sites found in northern Tunisia (Tabarka and Nefza). Seeds of French *M. officinalis* were provided by National Institute for Agricultural Research, and the German seeds were provided by the Institute for Food and Resource Economics (ILR) University of Bonn.

### *Isolation of essential oil*

The leaves were air-dried (for 30 days) at room temperature in a shadowy place, protected from direct light. Each sample was powdered and mixed to ensure sample uniformity. The essential oils were obtained from 100 g (dry weight) of plant material using a Clevenger-type Apparatus for 3 h. The hydrodistillation was performed in three replicates, and the oils were stored at 4°C until analysis by GC/MS. The average oil yields were estimated on the basis of the dry weight of the plant material.

### *GC/MS analysis*

The oils were analyzed with a Hewlett-Packard 6890N/5975B inert GC-MSD system (Agilent, USA) equipped with two cap. Columns, a HP-INNOWAX (30 m×0.25 mm i.d., film thickness 0.25 µm) and an HP-5MS (30 m×0.25 mm i.d., film thickness 0.25 µm) column (J&W Scientific, USA). The oven temperature was programmed isothermal at 50°C for 1 min, then rising from 50 to 250 °C at 28/min, and finally held isothermal at 250°C for 15 min; injector temperature, 250 °C; ion source temperature, 230 °C; carrier gas, He (high purity 99.99%; 1.2 ml min<sup>-1</sup>); injection volume, 1 µl; split ratio, 100:1. The electron impact ionization mode was used with an ionization voltage of 70 eV. Total ion chromatograms were obtained over the scan range of 30–800 a.m.u in the full-scan acquisition mode, and the compounds were identified using the NIST05 and Wiley 7 databases with a resemblance percentage above 85%. Semi-quantitative data were calculated from the GC peak areas without using correction factors and were expressed as a relative percentage (peak area %) of the total volatile constituents identified. Retention indices (RI) were determined for all the detected compounds based on the retention times (tr) of a homologous series of n- alkanes (C8–C30) (22).

### *Fatty acid methyl ester preparation*

Triplicate samples of 1 g of *M. officinalis* leaves were subjected to lipid extraction using a modified version of the Bligh and Dyer (23) method. Thus, leaf samples were kept in boiling water for 5 minutes then ground manually using a mortar and pestle; chloroform/methanol mixture (2:1 v/v) was used for lipid extraction. After washing by fixation water, the organic layer containing lipids was recovered and dried under a nitrogen stream. Total fatty acids (TFAs) of total lipids were

transmethylated using sodium methoxide solution (3% in methanol) (24). Methyl heptadecanoate (C17:0) was used as an internal standard. The fatty acids methyl esters (FAMES) obtained were subjected to GC analyses.

## Results

### Essential oil

The *M. officinalis* samples cultivated under the climatic conditions of the INRAT yielded a small amount of essential oil. The oils were analyzed by GC/MS. Forty-two compounds were identified, representing about (86.11%, 83.1%, 96.72% and 71.83%) of the total oils obtained from Nefza, Tabarka, Germany and France respectively. In addition to the differences in the essential oil yield, the GC/MS analyses revealed qualitative and quantitative differences in the composition between the oils of the four origins (Table 1).

GC/MS analysis showed that the oils of the German population were characterized by the presence of a significant aldehyde fraction (39.31; 27.71%) with geranial and neral being the dominant components, together with the sesquiterpene (12.23%)  $\beta$ -caryophyllene. The sesquiterpene caryophyllene oxide (27.06%) was found to have the highest value in the French population, which exhibited lower levels (7.12–4.29% respectively) geranial and neral. Germacrene-D (32.08–27.06%) was the highest in the Tunisian samples Tabarka and Nefza, together with the sesquiterpene caryophyllene (16.4– 14.7% respectively).

### Fatty acids

The total fatty acids (TFAs) extracted from the French, German, Tabarka and Nefza populations account for 95.38, 98.91, 94.65, 88.02 mg/g DW, respectively (Table 2).

Leaves of the German, Tabarka and Nefza populations have the same FA composition. Linoleic acid is the major compound reaching over (74.08, 70.75, and 66.74% respectively) of TFA followed by palmitic acid (15.77, 15.82, and 13.32% resp), C18:1n-9 (oleic acid) (6.29, 5.89 and 4.26 % resp) and C20:0 and (arachidic acid) (1.06, 1.19 and 1.31% resp). The main FA of the French samples was C18:2n-6 linoleic acid (73.93%), C16:0 (palmitic acid) (16.25%), C18:1n-9 (oleic acid)

(4.62%) C20:0 (arachidic acid) (1.60%); and the C16:1 (palmitoleic acid) was not detected. *Melissa officinalis* leaves were characterized by a high proportion of polyunsaturated fatty acid (PUFA) linoleic acid (73.93–66.74%) versus (16.25–13.32%) of saturated ones (SFA) oleic acid and (6.29–4.26%) of monounsaturated (MUFA) palmitic acid (Table 2). To the best of our knowledge, the foliar fatty acid composition of *M. officinalis* is reported herein for the first time. The proportion of the fatty acids did not show any differences according to the origin of samples.

## Discussion

### Essential oil

All the sampled populations of *Melissa officinalis* yielded a small content of essential oil. These results were similar to those found with Iranian *M. officinalis* which produced 0.06–0.16% (2). However, this yield was lower for *M. officinalis* grown in other countries such as Turkey, for example, the total essential oil content ranged between 0.27–0.36%, (25). In Spain, the yield was 0.5% as reported (26).

In previously investigated oils from cultivated *M. officinalis*, the major components are aldehydes such as citronellal, neral and geranial, and the sesquiterpenes such as (*E*)-caryophyllene and caryophyllene oxide were also important compounds (27). The Iranian oils presented the citral (geranial and neral), citronellal and geraniol as the main components of *M. officinalis* (10). Algerian populations showed that the most dominant constituents obtained were citral, citronellal and caryophyllene oxide (28). The main components of oil from Turkey, were citronellal (39%), citral (33%), citronellol, linalool and geraniol (29). Those results are similar to our findings for the German and the French samples. Essential oil content shows a strong dependence genetic constitution of the different origins (30).

As it is known in the literature, the essential oil of *M. officinalis* subsp. *officinalis* contains significant amounts of citral and/or citronellal, whereas for the *M. officinalis* subsp. *altissima* a strong belong to chemotype germacrene D.(30)

It is noteworthy that the main components of the leaf oils of cultivated *M. officinalis* subsp. *altissima* from Greek as  $\alpha$ -caryophyllene (7.27–12.66%), ger-

**Table 1.** Comparison of the essential oils isolated from different *M. officinalis*.

N°	Components	Content (%)				
		RI	Nefza	Tabarka	Germany	France
1	Camphene	954	-	-	-	1.29
2	<i>£</i> -3-carene	1011	0.32	-	-	-
3	( <i>Z</i> )- $\alpha$ -Ocimene	1026	-	0.5	-	-
4	Citronellol	1229	-	-	1.88	-
5	Neral	1240	-	-	27.71	4.29
6	Geraniol	1267	-	-	39.31	7.12
7	Thymol	1290	-	-	0.4	-
8	$\alpha$ -ylangene	1372	0.42	-	-	-
9	$\alpha$ -Copaene	1376	0.72	0.54	-	-
10	Geranyl acetate	1381	-	-	1.42	-
11	Dehydro-ar-ionene	1389	0.84	-	-	-
12	( <i>E</i> )- $\alpha$ -Bergamotene	1412	0.55	-	-	1.24
13	( <i>E</i> )- Caryophyllene	1419	1.36	1.25	-	1.06
14	$\beta$ -Caryophyllene	1420	14.7	16.4	12.23	8.92
15	$\alpha$ -cedrene	1432	0.52	0.27	-	-
16	Alloaromadendrene	1439	-	0.59	-	-
18	Aromadendrene	1441	0.3	-	-	-
19	$\alpha$ -Cubebene	1475	1.45	1.34	1.23	0.42
20	Germacrene D	1468	27.06	32.08	1.67	2.0
21	Bicyclogermacrene	1495	0.18	-	-	-
22	<i>Cis</i> -Calamenene	1521	0.75	-	-	-
23	$\beta$ -sesquiphellandrene	1522	2.75	0.4	-	0.97
24	delta-Cadinene	1523	0.73	-	-	-
25	$\alpha$ -Cadinene	1524	0.34	-	-	-
26	gamma-Cadinene	1538	4.96	-	0.76	1.77
27	$\alpha$ -Calacorene	1542	0.71	1.23	-	0.76
28	Nerolidol	1559	0.9	-	-	-
29	Globulol	1568	0.42	-	-	-
30	Caryophyllenol	1572	0.91	1.47	0.5	2.23
31	Germacrene D-4-ol	1574	0.72	-	-	-
32	Caryophyllene oxide	1576	9.54	16.61	8.76	27.06
33	Spathulenol	1578	0.49	-	-	-
34	Humulene oxide II	1606	0.56	1.01	0.26	1.29
35	$\alpha$ -Cadinol	1654	4.61	3.24	-	5.64
36	t-Muurolol	1627	-	-	0.59	-
37	<i>iso</i> Aromadendren epoxide	1641	0.77	-	-	0.46
38	Farnesol	1743	-	0.49	-	-
39	( $\beta$ - <i>Z</i> )Curcumen-12-ol	1756	0.53	-	-	-
40	Phytol	1949	6.96	5.68	-	3.64
41	<i>epi</i> manoyl oxide	1993	0.22	-	-	-
42	( <i>E-E</i> )-Geranyl linalool	2027	0.82	-	-	1.59
Total compound			86.11	83.1	96.72	71.88
Monoterpene hydrocarbons			0.32	0.5	-	1.29
Oxygenated monoterpenes			-	-	70.72	11.41
Sesquiterpene hydrocarbons			57.79	54.1	15.89	15.98
Oxygenated sesquiterpenes			20.82	22.82	10.11	39.52
Oxygenated diterpenes			7.18	5.68	-	3.64
Yield (%(w/w))			0.038	0.032	0.164	0.140

**Table 2.** Fatty acid percentages content of *M. officinalis* leaves

Fatty acid	France	Germany	Tabarka	Nefza
C16:0 (palmitic acid)	16.25	15.77	15.82	13.32
C16 :1 (palmitoleic acid)	-	1.71	1.00	1.47
C18 :1n-9 (oleic acid)	4.62	6.29	5 ,89	4.26
C18 :2n-6 (linoleic acid)	73.93	74.08	70.75	66.74
C20:0 (arachidic acid)	1.60	1.06	1.19	1.31
Total (%)	95.38	98.91	94.65	88.02

macrene-D (34.79-51.50%), sabinene (0.91-14.68%) and  $\alpha$ -pinene (0.53-8.03%) (31). These compounds have also been detected as the main ones in the studied *Melissa* oils of Greek origin from natural populations, whereas no citral or citronellal was detected. The subspecies *M. officinalis* subsp. *altissima* exhibits a different chemical profile and the slight odor of lemon perceptible during a short period of flowering is not attributable to the presence of citral (32).

The presented results suggest the existence of two different chemotypes in the species *M. officinalis*. We declare the three chemotypes ct. caryophyllene oxide, ct. citral and ct. germacrene D.

The present work provides for the first time data about the chemical composition, qualitative and quantitative patterns of essential oils extracted from the Tunisian *Melissa officinalis*. The analysis implies that the samples from Tunisia, Tabarka and Nefza can belong to the *M. officinalis* subsp. *altissima*, according to the previous studies (31-30).

### Fatty acids

To the best of our knowledge, the foliar fatty acid composition of *M. officinalis* is reported herein for the first time. The proportion of the fatty acids did not show differences according to the origin of samples. The comparison between different *Melissa* evidenced a similarity, at least with reference to the presence of the main fatty acid constituents. It is noteworthy that previous findings showed that the genus *Satureja*, *Origanum* and *Thymus* of the Lamiaceae family had some minor variations in fatty acid composition, which are dominated by the chemotypes of linoleic acid, palmitic acid and linolenic acid.(33)

In conclusion, the Tunisian lemon balm has not been studied before, it is founded in small fragmented habitats. Quantitative and qualitative differences between the es-

sential oils of Tunisian and introduced *M. officinalis* have been revealed and three chemotypes have been detected. Interestingly, in terms of the dominant compounds, not even trace amounts of neral, geranial, or of citronellal, were detected in Tunisian samples Tabarka and Nefza, which were dominated by germacrene-D. No significant difference was identified with the composition of FA from the four populations which are dominated by the unsaturated fatty acids linoleic acid.

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### Notes on contributors

Mouna SOUHI: Collected *Melissa officinalis* specimens from Tabarka and Nefza (north of Tunisia), cultivated the materials and followed their growth and life cycle, interpreted the results and wrote the draft manuscript.

Ismail AMRI: Interpretation of the chemical composition.

Amir SOUSSI: Performed the statistical analyzes.

Karim HOSNI: Performed the chemical analyses of essential oil and fatty acid.

Nadia BEN BRAHIM: Article revision.

Mohamed ANNABI: Data analysis and article revision

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