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Chemical composition of the essential oils from leaves of *Melissa officinalis* extracted by hydrodistillation, steam distillation, organic solvent and microwave hydrodistillation

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Abstract

Traditional hydrodistillation (HD), steam distillation extraction (SD), organic solvent extraction (SE), and microwave assisted hydrodistillation (MWHD) techniques were compared and evaluated for their effectiveness in the isolation of essential oil of *Melissa officinalis* from Algeria. The microwave assisted hydrodistillation technique was optimized in terms of both delivered power and time duration. The oils were analyzed by GC and GC-MS. The four oils contained the same dominant two components neral (24.04%, 24.06%, 38.18% and 18.86%) and geranial (33.79%, 37.91%, 35.57% and 27.79%) respectively in oils obtained by HD, MWHD, SD and SE. Citronellal (15.29%) is present in the oil of HD in comparison with MWHD (1.44%), SD (0.44%) and SE (1.39%).

Keywords: *Melissa officinalis*, Essential oil analysis, Hydrodistillation, Solvent extraction, Steam distillation extraction, Microwave assisted hydrodistillation.

1. Introduction

Melissa officinalis L. (Lamiaceae*)* is a perennial edible herb native to the Mediterranean region. The plant is cultivated in various parts of the world and grows especially in western Asia, south-western Serbia and North Africa. In Algeria it grows especially in the moist ravines of the mountains, Babors, Djurdjura and Mouzaia. This species is often grown as a honey plant. This plant is known locally by the names tizizoult, touroudjan [1, 2]. It is used in the folk medicine for the treatment of headaches, indigestion, colic, nervousness, cardiac failure and depression. Infusion of the leaves has been used to treat rheumatism [3]. For external use the powdered *M. officinalis* is used as sternutatory, against headaches. Applications of the plant crushed or as infusion on wounds lotions; relieve pain and inflammation [3].

There are three subspecies of *M. officinalis*. subsp. *officinalis*, *M. officinalis*. subsp. *inodora* and *M. officinalis*. subsp. *altisssima*; however only subsp. *officinalis* (with characteristic lemony odor *)* has commercial value [4, 5]. *M. officinalis*. subsp. *officinalis* oil is frequently used in the pharmaceutical, food and perfumery industry [6]. The chemical composition of the *M. officinalis*. subsp. *officinalis* essential oil has been previously studied and concerned plants from various origins [7-15]. All the investigated leaf oils were characterized by the occurrence of oxygenated monoterpenes as major components. However, several compositions were observed with respect to the contents of the four principal components limonene, citronellal, neral and geranial. The oxygenated compounds of the *M. officinalis.* subsp. *officinalis* oil are reputed to possess sedative, spasmolytic, antimicrobial, antiviral, anti inflammatory and antioxidative properties [16-24].

To the best of our knowledge, no report has been found concerning Algerian *M. officinalis* essential oils constituents extracted by hydrodistillation, microwave assisted hydrodistillation steam distillation and organic solvent extraction techniques.

In the present work, organic solvent extraction, hydrodistillation, steam distillation and microwave-assisted hydrodistillation, were applied to the isolation of *M. officinalis* essential oil. The volatile fraction profiles were determined by GC-FID and GC-MS; the qualitative and quantitative profiles of the distillates, obtained by all

techniques, were compared. Consequently, in continuation with our work on the characterization of aromatic and medicinal plants from Algeria [25, 26], the authors now report the chemical composition of the leaves oil of *M. officinalis* collected from Bejaia region located eastern Algeria and a rigorous comparison is provided between microwave-assisted hydrodistillation, classical hydrodistillation, organic solvent extraction and steam distillation techniques to obtain essential oils.

2. Materials and methods

2.1. Plant materials

The sample of *M. officinalis* was collected in June 2011 at Bejaia region located in north-eastern Algeria. The plant was identified in the botanical department of National Institute Agronomic of Algiers (NIA), Algeria.

2.2. Hydrodistillation Apparatus and Procedure (HD)

100g fresh plant material leaves and 1000mL distilled water were placed in a 2000mL round-bottom flask and connected to a Clevenger-type apparatus according to the European Pharmacopoeia [27]. Hydrodistillation was performed for 3h after boiling. Oils yields (w/w) obtained from the experiments were calculated on moisture free basis. The essential oil was collected, dried under anhydrous sulphate and stored at +4°c until used.

2.3. Steam distillation extraction Apparatus and Procedure (SD)

For a rigorous comparison, the same operating conditions have been used for conventional steam distillation at the bottom of the steam producing section there is an electrical resistance heater whose power is controlled with a rheostat and used to heat and boil water. The essential oil is collected, dried with anhydrous sodium sulphate and stored at +4°C until used.

2.4. Organic solvent extraction (SE)

Air dried plant material (100g) was extracted for 6 h at 25°C with hexane (500ml). After filtration hexane was removed under vacuum using a rotary evaporator and the viscous residue was submitted to steam distillation [28].

2.5. Microwave –assisted hydrodistillation apparatus and procedure (MWHD)

Microwave–assisted hydrodistillation (MWHD) was performed at atmospheric pressure using a microwave laboratory oven operating at 2450MHz (Model: Midea AG823ABI). 100g of fresh leaves *M. officinalis* were heated using a fixed power of 800W for 20min with the addition of 50mL distilled water [29-31]. The oil extraction was continuously conducted at 100°C until no more essential oil was obtained. The essential oil was collected, dried under anhydrous sodium sulphate and stored at +4°C until used.

2.6. GC analysis

A Hewlett Packard HP5890 series II GC-FID system was used chromatographic analysis, fitted with a fused silica capillary column with an apolar stationary phase HP5MS (30 m x 0.25 mm, 0.25 µm film thicknesses). The temperature program was 60°C for 5 min increased at 3°C/min to 250°C for 5min. Injection was performed at 250° C in the split mode; 1/50. 0.1µL of the oil was injected. A flow rate of 1 mL/min carrier gas (N_2) was used. The percentage composition of the individual components were obtained from electronic integration measurements using flame ionisation detection (FID; 260°C) n-alkenes (C_5-C_{28}) were used as reference points in the calculation of retention indices (RI).

2.7. GC/MS analysis

The GC/MS analysis was performed with a Hewlett Packard GC (HP5890 series II) / quadripole MS system (model HP MSD5971), equipped with an electronic impact source at 200°C, fitted with a fused silica-capillary column with an apolar stationary phase HP5MS (30 m x 0.25 mm, 0.25 µm film thickness). The chromatographic conditions were the same with GC analysis, the electron impact spectra were recorded at an ion voltage of 70 eV over a scan range of 30-600 uma.

2.8. Identification of the compounds

The compounds were identified by comparison of their retention indices (RI), on apolar column with those reported in the literature [32, 33] and by comparison of their mass spectra with the internal Wiley library of GC/MS system or with authentic samples.

3. Results and discussion

The importance of *M. officinalis* essential oil is shown by the large amount of published work, describing chemical composition, biological properties, and applications [4, 5, 6, 11, 13]. The most commonly reported main constituents of *M. officinalis* essential oil are citral (geranial, neral), citronellal, β-caryophyllene and caryophyllene oxide.

Microwave assisted hydrodistillation, which can be considered quite an innovative isolation technique, is based on the interaction between water contained in the vegetal material and microwaves generated from a source.

Table1 listed the grouped components of the essential oil: oxygenated and non-oxygenated fractions and composition of chemical families of *M. officinalis* essential, oil obtained by different isolation methods. Comparisons of yields, the percentage content of the individual components, retention indices, isolation times and chemical class distribution are summarized in Table1.

The hydrodistillation (180min) of the fresh leaves of *M .officinalis* gave clear oil with a yield of 0.24 % (w/w) on dry weight basis; when extracted by microwave-assisted hydrodistillation (20min) 0.30 % (w/w) on dry weight basis were obtained; SD (120min) and SE gave respectively 0.42% (w/w) and 0.56% (w/w) on dry weight basis. The oils were analysed by GC and GC/MS. Sixty four compounds were identified, which represented about (97.34%, 95.29%, 97.23% and 89.51%) of total oils obtained by (HD, MWHD, SD and SE) respectively.

A total of 64 compounds were identified in *M. officinalis* essential oils using the four techniques. Using HD 51 compounds were detected and then 47compunds were detected in SE, while 43 and 40 compounds were detected in MWHD and SD extract respectively. SD and MWHD enabled the detection of the most volatile active compounds in essential oil of *M. officinalis* such as neral and geranial, but their proportions depend strongly on the extraction technique. Substantially higher amounts of oxygenated monoterpenes compounds and lower amounts of monoterpene hydrocarbons were present in the essential oils of the aromatic plants extracted by HD and SD in comparison with MWHD and SE.

GC/MS analysis of essential oils from leaves *M. officinalis* (Table1) obtained by four techniques (HD, MWHD, SD and SE) showed that all are mainly composed of oxygenated monoterpenes family with (80.69%, 67.70%, 77.80 % and 50.30%) from (HD, MWHD, SD and SE) respectively.

Oxygenated sesquiterpenes family are present in this oils with (2.13%, 6.71%, 3.63% and 9.29%) and quantity of hydrocarbon sesquiterpenes family present with (8.06, 3.30, 9.02 % and 18.23%) obtained respectively by (HD, MWHD, SD and SE). The hydrocarbon monoterpenes family were present in smaller quantity with (1.79%, 0.64%, 1.81% and 2.48%) by (HD, MWHD, SD and SE) respectively. Quantity of hydrocarbon sesquiterpenes family present with (8.06%, 3.30%, 9.02 % and 18.23%) obtained respectively by (HD, MWHD, SD and SE). The hydrocarbon monoterpenes family present of smaller quantity with (1.79%, 0.64%, 1.81% and 2.48%) by (HD, MWHD, SD and SE) respectively.

Also we report the classification of oil components. As shown in Table1, the major compounds in the oils types aldehydes: such as neral (24.04%, 24.06%, 38.18 % and 18.86%) and geranial (33.79%, 37.91%, 35.57% and 27.79%) respectively in oils obtained by (HD, MWHD, SD and SE). Present other aldehyd with less percentage of citronellal (1.44%, 0.44% and 1.39%) in oils obtained by (MWHD, SD and SE) respectively, but higher quantity with 15.29% of oil was obtained by hydrodistillation.

Interestingly SD gives a higher content of citral (neral and geranial) with respectively (38.18% and 35.57%). The same MWHD method gives a higher content of (neral and geranial) with (24.06%, 37.91%) than HD with (24.04%, 33.79%) and less percentage obtained of neral and geranial by SE with (18.86%, 27.79%) respectively.

Sesquiterpene hydrocarbons was present of β- caryophyllene higher content obtained by SE (11.81%) than (SD and HD) with (4.55% and 4.10%) and lower content of MWHD with (0.25%). Oxygenated sesquiterpenes was present in height content of compound caryophyllene oxide with (7.71%, 5.84%, 1.62% and 1.28%)

respectively by (SE, MWHD, HD and SD). Two alcohol diterpenes are present by compound phytol (0.18% and 0.13%) obtained by (SD and SE) respectively and absent this compound in HD and MWHD techniques. The second diterpenes alcohol, 13-epi-manool was present with (1.57%, 0.49%, 0.43% and 0.2%) respectively by (SE, MWHD, SD and HD) isolation techniques.

Table1: Yields, extraction times, grouped compounds and chemical compositions of essential oils obtained by HD, MWHD, SD and SE from leaves *Melissa officinalis*

N°	RI	RI_A	Compounds*	HD%	MWHD%	SD%	SE%
1	975	973	*trans*-Pinane	0.84	0.23	0.11	-
2	979	978	1-Octene-3ol	0.22	-	-	0.17
3	982	985	6-Methyl-5-hepten-2-one	0.30	0.20	-	0.24
4	1018	1018	α-Terpinene	0.22	0.10	0.47	0.10
5	1022	1022	*o*-Cymene	-	0.14	-	0.21
6	1030	1032	Limonene	0.54	-	1.23	0.20
7	1046	1050	*trans*-β-Ocimene	0.19	0.17	-	-
8	1077	1074	*cis* –Linalool oxide	-	0.1	-	-
9	1099	1097	Linalool	0.52	0.29	1.37	0.70
10	1105	1103	n-Nonanal	0.28	0.10	0.10	0.90
11	1111	1111	*cis*- Rose oxide	0.29	-	0.12	0.14
12	1128	1127	*trans*-Rose oxide	0.39	-	0.64	0.13
13	1145	1145	*neo*-Isopulegol	0.56	0.20	0.98	-
14	1156	1153	Citronellal	15.29	1.44	0.44	1.39
15	1163	1162	Isogeranial	0.25	0.33	-	-
16	1173	1173	Menthol	0.10	0.16	-	-
17	1180	1182	Isomenthol	0.45	0.34	-	-
18	1191	1189	α- Terpineol	-	0.45	-	-
19	1217	1217	*trans*- Carveol	0.10	0.39	-	0.16
20	1228	1228	Nerol	1.40	0.53	0.35	0.13
21	1246	1240	Neral	24.04	24.06	38.18	18.86
22	1258	1255	Geraniol	3.9	1.21	-	0.88
23	1278	1270	**Geranial**	**33.79**	**37.91**	**35.57**	**27.79**
24	1282	1280	Nonanoic acid	0.41	0.42	-	0.22
25	1295	1290	Thymol	-	0.29	-	-
26	1312	1306	Undecanal	0.21	0.32	-	1.01
27	1318	1320	Dihydro citronellol acetate	0.29	0.64	-	0.13
28	1323	1323	Methyl geranate	0.11	-	0.50	0.17
29	1331	1331	Eugenol	0.13	3.78	0.15	0.12
30	1344	1351	α- Cubebene	tr	-	-	0.49
31	1353	1354	Citronellyl acetate	0.37	1.13	0.31	0.24
32	1364	1365	Geranyl acetate	0.22	8.24	1.84	0.26
33	1372	1372	α-Ylangene	2.12	1.61	-	0.16
34	1376	1376	α-Copaene	0.15	-	-	1.30
35	1386	1383	β-Bourbonene	-	tr	-	0.66
36	1391	1391	β-Elemene	-	0.37	-	-
37	1405	1404	β-Caryophyllene	4.10	0.25	4.55	11.81
38	1412	1409	α-Gurjunene-	0.06	0.58	0.6	0.25
39	1443	1443	(Z)-β- Farnesene	0.34	-	0.35	0.12
40	1475	1476	γ -Himachalene	0.51	0.14	0.14	-
41	1480	1480	Germacrene D	0.10	0.22	1.27	1.22

42	1489	1491	Valencene	0.324	-	-	-
43	1500	1500	Pentadecane	-	0.23	0.13	0.95
44	1504	1508	(E,E)-α-Farnesene	0.18	-	2.11	2.51
45	1520	1521	*cis*-Calamenene	0.24	-	-	0.13
46	1529	1529	Citronellyl n-butyrate	-	0.39	-	-
47	1538	1538	α-Cadinene	-	0.13	-	0.24
48	1549	1549	Elemol	-	0.14	0.17	0.82
49	1572	1574	Germacrene D-4-ol	0.10	-	1.60	0.76
50	1580	1581	Caryophyllene oxide	1.62	5.84	1.28	7.71
51	1604	1606	Humulene epoxide II	0.10	0.22	0.17	-
52	1649	1649	β-Eudesmol	0.31	0.26	0.17	-
53	1665	1671	β-Bisabolol	-	0.25	0.24	-
54	1762	1762	Cedryl acetate	-	0.11	-	0.24
55	1841	1843	(E,E)-Farnesyl acetate	0.15	-	0.27	0.65
56	1873	1872	(Z-β)-Santalol acetate	-	0.11	0.14	0.19
57	1947	1949	Phytol	-	-	0.18	0.13
58	1959	1961	13- *epi*-Manool	0.43	0.49	0.2	1.57
59	1995	1994	1-Eicosane	0.19	-	0.5	0.54
60	2005	2009	Hexadecyl acetate	0.05	-	0.13	-
61	2028	2026	Geranyl linalool	0.11	-	0.11	2.18
62	2082	2082	Octadecanol	0.2	-	0.16	0.33
63	2103	2100	n-Heneicosane	0.38	-	0.26	0.13
64	2130	2128	Methyl octadecanoate	0.17	0.78	0.14	0.27
			Total percentage (%)	**97.34**	**95.29**	**97.23**	**89.51**
			Monoterpene hydrocarbons	**1.79**	**0.64**	**1.81**	**2.48**
			Oxygenated monoterpenes	**80.69**	**67.70**	**77.8**	**50.3**
			Sesquiterpene hydrocarbons	**8.06**	**3.30**	**9.02**	**18.23**
			Oxygenated sesquiterpenes	**2.13**	**6.71**	**3.63**	**9.29**
			Others	**4.67**	**16.94**	**4.97**	**9.21**
			Yield (%)(W/W)	**0.24**	**0.30**	**0.42**	**0.56**
			Extraction time	**180min**	**20min**	**120min**	**6 h**

*Compounds listed according with crescent RI order

RI_A: retention indices relative to C_5-C_{28} n-alkenes on HP5MS capillary column

tr: trace(<0.05%)

The composition of the oil from *M. officinalis* collected in Bejaia region located in north-eastern Algeria was dominated by neral, geranial and citronellal. This composition was qualitatively the same that the oils from Serbia [16], Slovak [6], Egypt [11], France [9] and Iran [15]; as seen in Table 2. However, limonene was the major component in the samples from Scotland [24] (57.50 %), neral was found with only (4.30 %) and geranial was completely absent. Basta et al. (2005) reported that caryophyllene oxide (12.6 %) and β-pinene (18.20 %) were also the most abundant constituents in the oil of *M. officinalis* from Greece [34] but neral and geranial were not detected in the oil. Oils from Cuba [10] and Brazil [14] were dominated by neral (29.90 % and 39.30 %) and geranial (41.00 % and 47.30 %) respectively. A low content (0.20 %) of citronellal was found in leaves of Cuba [10] and it is not detected in oil from Brazil [14]. A typical composition from Turkey [20] is characterized by the occurrence of β-carophyllene (14.20 %), which is drastically different from Algerian oil. Minor compounds were punctually reported. Geraniol in Scotland (5.73 %) [24], in Egypt [11] (4.20 %), in Serbia [16] (3.40 %) and the percentage of the oil from Algeria (3.90%). Geranyl acetate was present at (5.90 %) in Slovak [6] and (7.10 %) in Iran [15]. Otherwise, several sesquiterpenes have been reported at appreciable content like β-carophyllene (4.90 %) in Egypt [11], (4.60 %) in Serbia [16], (4.20 %) in Slovak [6], (4.90 %) in Iran [15], (2.40 %) in France [9], the same content in our oil (4.10 %). An oxygenated sesquiterpene, caryophyllene oxide was identified at (10.0 %) in Egypt [11], (8.35 %) in Slovak [6], (5.30 %) in

Cuba [10], (2.70 %) in Iran [15], (1.70 %) in Serbia [16] and (1.62 %) in Algerian oil. Similar results were obtained with essential oil of *M. officinalis* [4, 5, 6, 11, 13]. The most dominant constituent obtained was citral (geranial and neral).

Table 2: Main constituents of chemical composition of *Melissa officinalis* of various origins

Constituents	Various origins										
	This Work	Serbia 2004	Slovak 1997	Cuba 1999	Egypt 1995	France 1998	Brazil 2005	Scotland 1995	Iran 2003	Turkey 2004	Greece 2005
β-pinene	-	-	-	-	-	-	-	-	-	-	18.20
Limonene	-	2.20	0.10	-	0.70	-	-	57.50	-	-	tr
Linalool	0.52	0.50	0.08	0.60	0.20	0.60	0.80	0.60	0.90	1.30	tr
Citronellal	15.29	13.70	11.30	0.20	13.30	39.50	-	24.90	12.90	2.90	-
Neral	24.04	16.40	22.20	29.90	19.70	20.40	39.30	4.30	24.50	5.80	-
Geraniol	3.90	3.40	-	-	4.20	0.20	-	5.70	0.70	0.40	-
Geranial	33.79	23.40	33.60	41.00	26.80	27.80	47.30	-	35.50	6.60	-
Geranyl acetate	0.22	0.80	5.90	4.40	1.80	0.60	1.50	-	7.10	-	-
β- Caryophyllene	4.10	4.60	4.20	-	4.90	2.40	0.90	-	4.90	14.20	15.30
Caryophyllene oxide	1.62	1.70	8.30	5.30	10.00	-	1.20	-	2.70	-	12.60

Conclusion

M. officinalis oils obtained by hydrodistillation, microwave hydrodistillation, steam distillation and solvent extraction were investigated by capillary GC and GC/MS and compared in terms of isolation times, yields and chemical composition. Results support the possibility to use solvent extraction as alternative method to produce essential oils. MWHD offers many important advantages, including higher extraction yield than HD, shorter extraction time and the highest percentage of the active component geranial. The present study gives a better insight on the volatiles contained in leaves of *M. officinalis* which grows in Algeria.

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