

# Essential oil composition and antioxidant activity of *Marrubium vulgare* L. growing wild in Eastern Algeria

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## ABSTRACT

In previous work [1], the essential oil of the aerial parts of *Marrubium vulgare* L. obtained by hydrodistillation was analysed by gas chromatography coupled to mass spectrometry (GC-MS) in order to determine their chemical composition. Fifty (50) components in the oil of *M. vulgare* were identified. The results demonstrated that the major components of the essential oil were: 4,8,12,16-Tetramethyl heptadecan-4-olid (16.97 %), Germacrene D-4-ol (9.61 %),  $\alpha$ - pinène (9.37 %), Phytol (4.87 %), Dehydro-sabina ketone (4.12 %), Piperitone (3.27 %),  $\delta$ -Cadinene (3.13 %), 1-Octen-3-ol (2.35 %) and Benzaldehyde (2.31 %). In this study, the antioxidant properties of essential oil were examined. The results showed that this oil can be considered an effective source of antioxidants of natural origin. This is the first report on chemical composition of *M. vulgare* essential oil cultivated in Algeria and the original study on the antioxidant activity of *M. vulgare* essential oil. The antioxidant activity was investigated with one method: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method.

**Keywords:** *Marrubium vulgare*, Lamiaceae, Essential oil composition, GC, GC/MS, antioxidant.

## 1. INTRODUCTION

In recent years, essential oils of plants and their other products from secondary metabolism have been in high demand from the manufacturers of foods flavoring, fragrance, cosmetics, and pharmaceutical industries due to the growing interest of consumers in ingredients from natural sources. Many plants have been used for different purposes, such as food, drugs and perfumery. They have been screened for their potential uses as alternative remedies for the treatment of many infections and preservation of foods from the toxic effects of oxidants [2].

Lamiaceae is composed of more than 240 genera, most of them are highly aromatic due to the presence of external glandular structures, namely peltate and capitate trichomes that

produce essential oils. According to Lawrence [3], it is possible to distinguish between the Lamiaceae oil-rich and oil-poor species. The latter being characterized by hydrocarbon-rich oils, such as germacrene D,  $\beta$ -caryophyllene, (E)- $\beta$ -farnesene,  $\delta$ -cadinene and  $\alpha$ -humulene, among others. The *Marrubium* genus is represented by about 30 species [4]. Considered oil-poor species [3], little is known about their essential oils since more importance has been given to their maceration extract, which is consisted of the known and dominant active component marrubiin [5]. *Marrubium vulgare*, commonly known as horehound or boarhound, is native in Europe, Western Asia and North Africa, and is cultivated worldwide as a source for food flavoring and for medicinal purposes [6,7]. The name "marrubium" refers to the bitter taste of the herb and "hoar" to the white pubescence covering the plant [8].

Under Polish climatic conditions, *Marrubium vulgare* L. is a perennial plant. Medicinal properties of horehound have been long known and the origin of its use goes back to ancient Egypt. The medicinal raw material is the herb of horehound (*Marrubi herba*) [9]. The herb consists of whole or crushed flowering aerial parts of *Marrubium vulgare* L [10], and it shows multiple effects on human organism [11-13]. The essential oil of *Marrubium vulgare* L. has a relaxant and expectorant effect as well as a vasodilator [14].

In Algeria, *Marrubium vulgare* is used in folk medicine to cure several diseases of the digestive tract, such as diarrhoea, as well as diabetes, rheumatism, cold and respiratory pains [15,16]. Pursuing our studies on the Algerian flora, this work reports the morphology and distribution of the glandular trichomes of *M. vulgare* growing spontaneously in Algeria, and the composition of its oil during the flowering and vegetative phases.

Synthetic antioxidants are widely used to retard undesirable changes as a result of oxidation in many foods. Excessively, oxidized fats and oils are not suitable for nutritive purposes. Because the oxidation products of oils have toxic effects, many synthetic substances such as propylgallate and citric acid are commonly used in lipids to prevent oxidation. Recently, these synthetic substances have been shown to cause effects, such as enlarging the liver size and increasing the microsomal enzyme activity.

The use of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been restricted in food because of its carcinogenic effect. Therefore, the search for new natural antioxidant sources has been greatly intensified. In this field, plant originated antioxidants have been widely used in oils or lipid containing foods in order to prevent oxidative deterioration. The main purpose of this study was to investigate the chemical composition of *M. vulgare* essential oil and to determine its antioxidant activity by Scavenging of DPPH (1,1-diphenyl-2-picrylhydrazyl) test.

## 2. EXPERIMENTAL

### 2. 1. Chemicals, reagents and plant material

Plant materials (aerial parts) of *M. vulgare* L. were grown in the zone of Nigrine district of El-Ater in the wilaya of Tebessa, north east of Algeria. The whole plants were collected during the period of May to June 2009.

### 2. 2. Distillation of essential oil

The samples were dried in the shade in natural air far from moisture and all pollutants for a fortnight in the room temperature.

100 g of ground rosemary were submitted to water distillation for 4 h using a Clevenger apparatus. The distilled essential oils were dried over anhydrous sodium sulfate, filtered and stored at 4 °C.

### 2. 3. Gas chromatography

The gas chromatographic analyzes were performed using a Hewlett Packard 6890 chromatograph equipped with a nonpolar column HP5MS (30 x 0.25 mm d.i. , Film thickness 0.25 microns) and a flame ionization detector. The procedures conditions were as follow: carrier gas: nitrogen, flow rate 0.8ml/min, injector temperature: 250 °C, detectors temperature: 300 °C, temperature program: from 60 to 250 at 2 °C / min, with two levels: 8 minutes at 60 °C and 15 min at 280 °C, injection of 0.4 µl of pure essential oil and 1µl of absolute mode: mode split 1: 20.

In order to determine retentions indices (RI) a series of n-alkanes (C5–C28) mixture was analysed under the same operative conditions on HP-5 columns and the sample indices were calculated following Van den Dool and Kratz [17].

### 2. 4. Gas chromatography and mass spectrometry (GC/MS) analysis conditions

The essential oils were analyzed on an apparatus of gas chromatography coupled to mass spectrometry brand Hewlet Packard 5973 A, equipped with an a polar capillary column (HP5MS, 30 m x 0.25 mm, phase thickness: 0.25 µm). the detection mode: electronic impact, ionization current: 70 eV, carrier gas: helium, flow rate: 0.7 ml/mn, the source pressure: 10-7 mbar, interface temperature: 280 °C, injection: 250 °C, the programming of the oven: 2 °C / min from 60 °C to 280 °C, with isothermal: 8min at 60 °C and 15 minutes at 280 °C 0.1 to 0.2 µl of pure essential oil and 1µl absolutely were injected in split mode 1: 20.

The identification of the essential oil constituents was based on a comparison of their retention times to *n*-alkanes, compared to published data and spectra of authentic compounds using their mass spectra compared to the Wiley version 7.0 library [18-20]. as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature (Adams, 2007). The chromatographic conditions were identical to those used for GC analysis.

### 2. 5. Antioxidant activity

The ability of *M. vulgare* oil to scavenge free radicals were assayed with the use of a synthetic free radical compound, 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method employed by Bersuder [20], a volume of 500 µl of each sample was mixed with 50 lml of ethanol and (0.02 %, w/v) of DPPH in 99.5 % ethanol. The mixture was shaken vigorously and incubated in the dark. After 30 min, the absorbance was measured at 517 nm using a spectrophotometer. The DPPH radical-scavenging activity was calculated as follows:

$$\text{Radical-scavenging activity} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

where,  $A_{\text{blank}}$  and  $A_{\text{sample}}$  are the absorbance of the control (blank) and the sample, respectively.

The IC<sub>50</sub> value is defined as the amount of antioxidant necessary to inhibit DPPH radical formation by 50 %.

The synthetic antioxidant reagent BHT was used as a positive control. The values are presented as the means of triplicate analysis.

### 3. RESULTS AND DISCUSSION

#### 3. 1. Chemical composition

The study showed that the essential oil content in the dry herb of *Marrubium vulgare* L. was on average 0.05 % [21].

Figure 1 shows the peaks of GC-MS spectrum. The search analysis in the digital library. The percentages and the retention indices of the identified components are listed in Table 1 in the order of their elution on the HP-5MS column. GC-MS analysis of *M. vulgare* essential oil led to the identification of fifty (50) compounds, accounting for 82.42 % of the total oil. The yield of essential oil obtained by hydrodistillation from aerial part of plant was 0.04 %.

Table 1 illustrates also the nine components with a supremacy of three major components: 4,8,12,16-Tetramethyl heptadecan-4-olid (16.97 %), Germacrene D-4-ol (9.61 %),  $\alpha$ - pinene (9.37 %). They represent about 36 % of 56 % and shows the different chemical groups with a dominance of other compounds with 41.64 % of the total rate of volatile oil, followed by Oxygenated sesquiterpene with a lower rate (13.17 %) and, Monoterpene hydrocarbon (12.61 %) Oxygenated monoterpene (9.46 %), Sesquiterpene hydrocarbon (5.58 % ) respectively.

#### 3. 2. Antioxidant activity

Relatively stable organic radical DPPH has been widely used in the determination of the antioxidant activity of the essential oil. DPPH radical decreased in the presence of a hydrogen donor, that is, a free radical-scavenging antioxidant.

In the DPPH-test, the ability of the essential oil to act as the donor of hydrogen atoms or electrons in the transformation of DPPH into its reduced form DPPH-H was measured spectrophotometrically. Assessed samples were able to reduce the stable violet DPPH radical to the yellow DPPH-H, reaching 50 % of reduction with IC<sub>50</sub> values. Lower IC<sub>50</sub> value indicates higher antioxidant activity.

The results represented in Figure 2 of the DPPH radical scavenging activities (% inhibition) of various concentrations of *M. vulgare* oil showed a concentration dependent activity profile. As shown, it is clear that as the concentration increased, the scavenging effect also increased with inhibitory activity observed as was in the case of *M. vulgare* oil, reaching as high as 254 at 1000  $\mu\text{g/ml}$ .

This value is too close to the activity potentials of synthetic antioxidants BHT (35  $\mu\text{g/ml}$ ) at the same concentration. The amount of the essential oil needed for 50 % inhibition of free radical activity is expressed by IC<sub>50</sub> (the concentration reducing 50 % of DPPH).

The lower the IC<sub>50</sub> value is, the greater the free radical-scavenging activity. The results depicted in Figure 3 indicate that *M. vulgare* essential oil exhibited an IC<sub>50</sub> value of 153.84  $\mu\text{g/ml}$ , which is about 2 times higher than the synthetic antioxidant (BHT).

The efficiency of an antioxidant component to reduce DPPH essentially depends on its hydrogen donating ability, which is directly related to the less content of phenolic hydroxyl moieties.

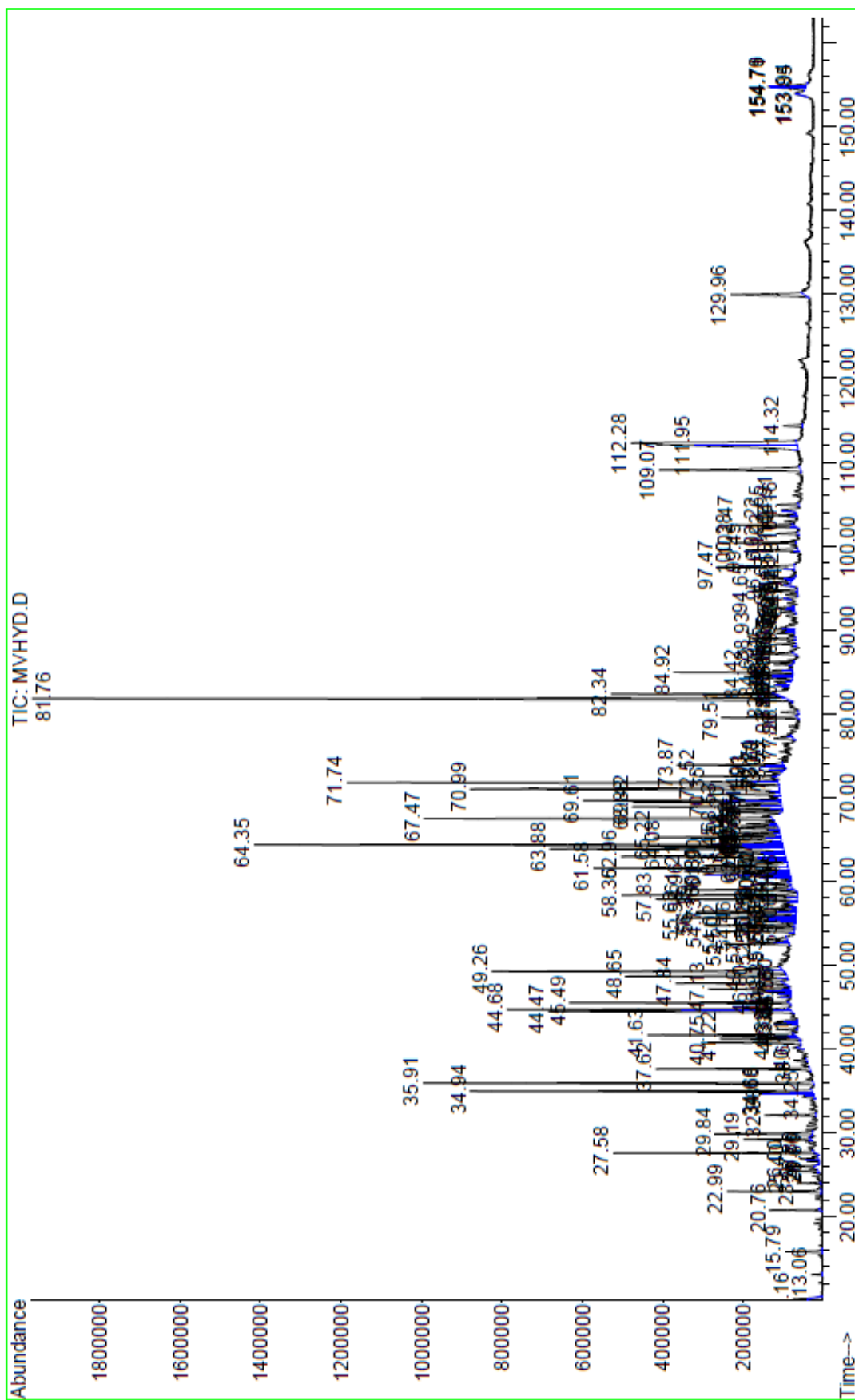


Figure 1. GC-MS of Essential oil.

**Table 1.** Chemical composition, retention indices (IR) and percentage composition of the *M. vulgare* essential oil.

N°	IR	Compound	%	Identification
1	823	Trans -2-Hexanal	0.75	GC,GC/MS
2	903	Heptanal	0.1	GC
3	906	Santolina triene*	0.71	GC,GC/MS
4	937	<b><math>\alpha</math>- pinene</b>	<b>9.37</b>	GC,GC/MS
5	946	Camphene	0.51	GC,GC/MS
6	953	<b>Benzaldehyde</b>	<b>2.31</b>	GC
7	962	Sabinene	0.37	GC
8	983	<b>1-Octen-3-ol</b>	<b>2.35</b>	GC
9	990	Myrcene	0.47	GC,GC/MS
10	995	Octanol-3	0.64	GC
11	1000	Dichlorobenzene<1,>*	0.72	GC,GC/MS
12	1022	p-Cymene	0.63	GC,GC/MS
13	1029	1-8-cineole	0.1	GC
14	1038	cis-Ocimene	0.2	GC
15	1055	$\gamma$ -Terpinene	0.85	GC,GC/MS
16	1113	$\beta$ -Thujone	0.81	GC,GC/MS
17	1117	<b>Dehydro-sabina ketone*</b>	<b>4.12</b>	GC,GC/MS
18	1122	Camphor	0.83	GC,GC/MS
19	1219	Carvone	0.89	GC
20	1224	<b>Piperitone</b>	<b>3.27</b>	GC
21	1239	Neral	0.27	GC,GC/MS
22	1250	Geraniol	0.92	GC
23	1261	Anethole<E>	0.47	GC,GC/MS
24	1275	Geranial	0.78	GC
25	1282	Thymol	0.17	GC,GC/MS
26	1284	2-Undecanone	0.98	GC,GC/MS
27	1291	Cymen-7-ol<p>*	0.95	GC
28	1467	$\alpha$ -Humulene	0.12	GC
29	1481	Germacrene D	0.88	GC
30	1491	$\beta$ - Guaiene	0.23	GC,GC/MS
31	1501	$\alpha$ - Farnesene	0.23	GC,GC/MS
32	1505	$\gamma$ - Cadinene	0.44	GC,GC/MS
33	1510	Trans -calamenene	0.21	GC
34	1513	<b><math>\delta</math>- Cadinene</b>	<b>3.13</b>	GC,GC/MS
35	1521	Trans-Cadina-1-4-diene*	0.21	GC,GC/MS
36	1529	$\alpha$ - calacorene	0.73	GC
37	1538	<b>Germacrene D-4-ol</b>	<b>9.61</b>	GC,GC/MS
38	1566	Spathulenol	0.87	GC
39	1574	Salvial-4(14)-en-1-one*	0.96	GC,GC/MS
40	1579	$\beta$ - oploponone	0.63	GC,GC/MS
41	1845	trans-trans-Farnesyl acetate*	0.8	GC
42	1862	cis-cis-Farnesyl acetone*	0.98	GC,GC/MS
43	1876	Trans-cis-Farnesyl acetone*	0.77	GC,GC/MS
44	1895	Nonadecane	0.53	GC,GC/MS
45	1921	<b>Phytol</b>	<b>4.87</b>	GC,GC/MS
46	2102	n-Heneicosane*	0.8	GC,GC/MS
47	2135	Linoleic acid*	1.0	GC,GC/MS
48	2210	Sclareol*	0.81	GC,GC/MS
49	2303	Tricosane*	0.96	GC,GC/MS
50	2327	<b>4,8,12,16Tetramethyl heptadecan-4-olid*</b>	<b>16.97</b>	GC,GC/MS
	<b>Total</b>		<b>82.46</b>	
		Grouped Compounds		
		Monoterpene hydrocarbon	12.61	
		Oxygenated monoterpene	9.46	
		Sesquiterpene hydrocarbon	5.58	
		Oxygenated sesquiterpene	13.17	
		Others Compounds	41.64	

\*New Compounds

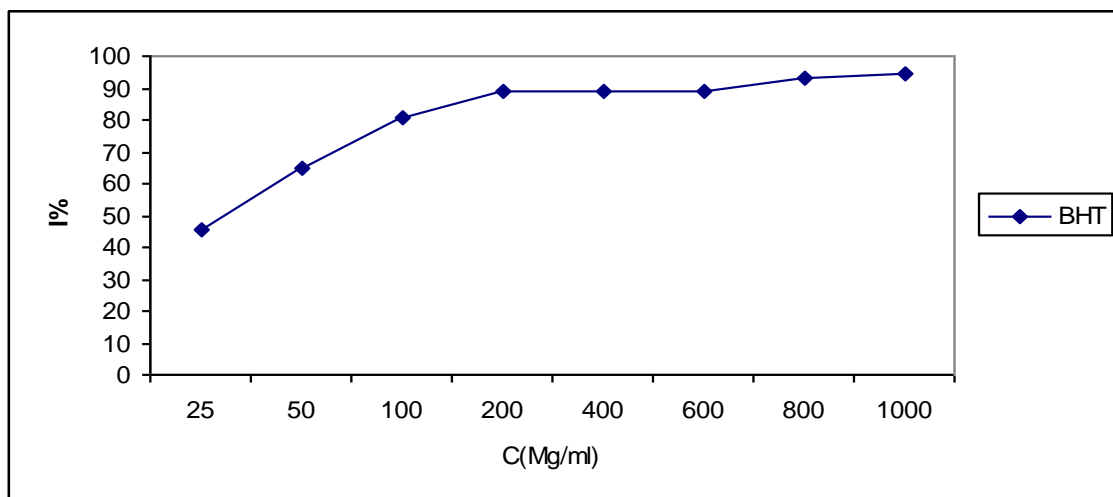


Figure 2. The synthetic antioxidant BHT.

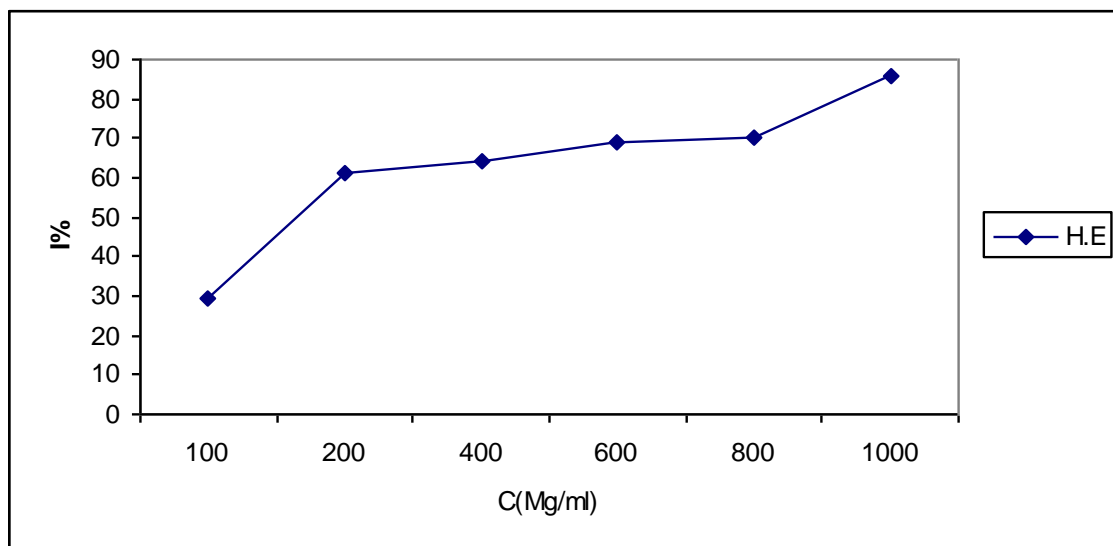


Figure 3. Antioxidant activity of *M. vulgare* essential oil.

#### 4. CONCLUSION

Essential oil of *M. vulgare* from Algeria had significant differences in the chemical composition as compared to the same essential oil from other country, which can be attributed to several factors.

The results demonstrated that the major components of the essential oil were : 4,8,12,16-Tetramethyl heptadecan-4-olid (16.97 %), Germacrene D-4-ol (9.61 %),  $\alpha$ - pinène (9.37 %), Phytol (4.87 %), Dehydro-sabina ketone (4.12 %), Piperitone (3.27 %),  $\delta$  – Cadinene (3.13 %), 1-Octen-3-ol (2.35 %) and Benzaldehyde (2.31 %). The results showed that this oil can be considered an effective source of antioxidants and enhance the human health as natural antioxidant.

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## REFERENCES

- [1] A. Abadi, A. Hassani, *Inter. Lett. Chem. Phys. Astro.* 8(3) (2013) 210-214.
- [2] Barlow S. M., Hudson B. J. F., (Ed.), Elsevier, Amsterdam 1990, pp. 23.
- [3] B. M. Lawrence, Science. Edits., R. M. Harley, T. Reynolds, *Royal Botanic Gardens, Kew* 1992, pp. 399-436.
- [4] M. S. Abu-Asaband, R. D. Cantlno, In: *Advances In Lablate Science*. Edits. R. M. Harley, T. Reynolds, Royal Botanic Gardens, Kew (1992, pp. 97-112.
- [5] V. Schiemper, A. Ribas, M. Nicolau, V. Cechinel-Fliho, *Phytomedecine* 3 (1996) 211-216.
- [6] W. Letchamo, S. Mukhopadhyay, *J. Hortic. Sci.* 72(1997) 741-748.
- [7] S. Sahpaz, et al., *Journal of Ethnopharmacology* 79 (2002) 389-392.
- [8] J. E. Slmon, A. F. Chadwickand, L. E. Craker, *Herbs-anIndexedblblllograptry* 1971-1980, Elsevier, Amsterdam 1984, p. 48.
- [9] Wolski T., Matosiuk D., Baj T., Ziewiec A., , *Postępy Fitoterapii* 1 (2007) 39-45.
- [10] *Polish Pharmacopoeia* VIII. PTFarm., Warszawa 2008, pp. 2249-2250.
- [11] Bradley P. R., *British Herbal Medicine Association* 1 (1992) 218-220.
- [12] El Bardai S., Wibo M., Hamaide M-Ch., Lyoussi B., Quetn-Leclerq J., Morel N., *British Journal of Pharmacology* 140 (2003) 1211-1216.
- [13] Kohlmünzer S., *Farmakognozja*, Wydawnictwo Lekarskie PZWL Warszawa, 2007, p. 308.
- [14] Wyk B. E., Wink M., *Rośliny lecznicze świata*, MedPharm Polska, 2008, p. 198.
- [15] Rachid Belhattab, Larbi Larous, *J. Essent. Oil Res.* 18 (2006) 369-373.
- [16] Y. Mahmoudi, *La thérapeutique par les plantes communes en Algérie*. Ed. Palais du livre, Blida(1990).
- [17] H. Van den Dool, P. D. Kratz, *J. Chromatogr.* 11 (1963) 463.
- [18] F. Macchioni, P. L. Cioni, G. Flamini, I. Morelli, S. Maccioni, M. Ansaldi, *Flavour Frag. J.* 18 (2003) 139.
- [19] V. Roussis, P. Katerina, V. Constantinos, P. V. Catherine, O. Antonio, *J. Essent. Oil Res.* 13 (2001) 118.
- [20] R. P. Adams, *Identification of Essential Oils by Ion Trap Mass Spetroscopy*, Allured Publishing Corporation, Carol Stream, IL, USA, 1995.
- [21] Zawisłak G., *Herba Pol.* 55(3) (2009) 63-68.

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