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Chemical Composition and Antibacterial activity of Essential Oil of *Marrubium vulgare* from Eastern Algeria

Abderazak Abadi[1], Aicha Hassani[2,*]

[1]Laboratoire de Molécules Bio-active et Valorisation de la Biomasse, École Normale Supérieure, BP 92, Kouba-Algiers, Algeria

[2]Laboratoire de Chromatographie, Faculté de Chimie, USTHB, Algiers, Algeria

*E-mail address: ryadabadi@gmail.com

ABSTRACT

The essential oil from flowering *Marrubium* (*Marrubium vulgare* L.) an aromatic member of the Lamiaceae family, from Algeria, obtained by hydrodistillation, was analysed by GC/FID and GC/MS. The constituents were identified by their mass spectra and Kovats' indices. Fivety (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 %), α- pinéne (9.37 %), Phytol (4.87 %), Dehydro-sabina ketone (4.12 %), Piperitone (3.27 %), δ-Cadinene (3.13 %), 1-Octen-3-ol (2.35 %) and Benzaldehyde (2.31 %). Essential oil of *M vulgare* was evaluated for its antibacterial activities against Gram-positive and Gram negative pathogenic bacteria: *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli* and *Klebsiella pneumoniae*.

***Keywords*:** *Marrubium vulgare*; Essential oil composition; GC/MS; antibacterial activities

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 [1].

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 [2].

The *Marrubium* genus is represented by about 30 species [3]. Considered oil-poor species [2], 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 [4] .

Marrubium vulgare, commonly known as horehound or hoarhond, is native in Europe, Western Asia and North Africa, and is cultivated worldwide as a source for food flavoring

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and for medicinal purposes [5,6]. The name ‘’marrubium’’ refers to the bitter taste of the herb and ‘’hoar’’ to the white pubescence covering the plant [7].

The chemical compositions of M. vulgare essential oil from various origins have been the subject of many studies. The literature reveals the occurence of several chemotypes. From Lithuania, (Z)-b-farnesene, b-caryophyllene, (E)-2-hexenal, a-humulene and germacrene D were the main components of M. vulgare essential oil [8]. From Czech Republic, the main constituents of M. vulgare essential oil were b-caryophyllene and germacrene D [9]. From different region of Iran, the main constituants of M. vulgare essential tricyclene, b-pinene, bisabolol, b-elemone and isomenthon-8-thiol [10], b-bisabolene, 8-cadinene and isocaryophyllene [11], and bisabolene, b-caryophyllene, germacrene D and E-bfarnesene [12], caryophyllene oxide, b-caryophyllene and germacrene D [13].

Under Polish climatic conditions, *Marrubium vulgare* L. is aperennial 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*) [14]. The herb consists of whole or crushed flowering aerial parts of *Marrubium vulgare* L. [15], and it shows multiple effects on human organism [16-18]. The essential oil of *Marrubium vulgare* L. has a relaxant and expectorant effect as well as avasodilator [19]. The plant is reported to possess hypoglycemic [20], vasorelaxant [21], antihypertensive [22], analgesic [23,24], anti-inflammatory [25], antioxidant activity [8,26], antiedematogenicactivity [27], and many other biological activities.

In Algeria folk medicine, it was used as hypotensive, hypoglycemic and cardiotonic. Recently, a large number of essential (volatile) oils and their constituents have been investigated for their biological activity, notably antibacterial and antioxidant properties [28,29]. Essential oils and their components are gaining increasing interest as a natural alternative to synthetic drugs [30], particularly against microbial agents because of their relatively safe status wide acceptance by consumers and their exploitation for potential multipurpose functional use.

The interest in plants with antibacterial properties has been revived because of current problems associated with the use of antibiotics [31]. Therefore, essential oils and other naturally occurring antibacterial are attractive to the food industry as well as imparting flavor [32]. More recently, the essential oil of this plant was advocated for their use as an antioxidant agent [26], but to the best of our knowledge, there are no reports on the antimicrobial properties and the cytotoxicity has been published. Therefore, this paper was conducted to investigate for the first time the antibacterial properties against clinical and pathogenic microorganisms and the cytotoxicity of M. vulgare essential oil grown in Algeria.

2. MATERIALS AND METHODS

2. 1. 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 0.8 ml/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 [33].

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 apolar 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 [34-36]. 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. Antibacterial assay dilution method

The minimal inhibitory concentration (MIC) of essential oil was determined using the Mueller Hinton broth (MHB) dilution method [37]. All tests were performed in MHB supplemented with ethanol 5% [38]. Bacterial strains were cultured overnight in MHB at 37 °C. Tubes of MHB containing various concentrations of oils were inoculated with 10 µl bacterial inoculums adjusted to 106 CFU/ml. They were incubated under shaking conditions (100-120 rpm) at 37 °C for 24 h [39,40]. Control tubes without tested samples were essayed simultaneously. The essays were performed in triplicate. The MIC was defined as the lowest concentration preventing visible growth [41,42].

3. RESULTS AND DISCUSSION

3. 1. Chemical composition of the 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	**α- pinene**	**9.37**	GC,GC/MS
5	946	Camphene	0.51	GC,GC/MS
6	953	**Benzaldehyde**	**2.31**	GC
7	962	Sabinene	0.37	GC
8	983	**1-Octen-3-ol**	**2.35**	GC
9	990	Myrecene	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	γ –Terpinene	0.85	GC,GC/MS
16	1113	ß-Thujone	0.81	GC,GC/MS
17	1117	**Dehydro-sabina ketone***	**4.12**	GC,GC/MS
18	1122	Camphor	0.83	GC,GC/MS
19	1219	Carvone	0.89	GC
20	1224	**Piperitone**	**3.27**	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	α-Humulene	0.12	GC
29	1481	Germacrene D	0.88	GC
30	1491	ß- Guaiene	0.23	GC,GC/MS
31	1501	α- Farnesene	0.23	GC,GC/MS
32	1505	γ – Cadinene	0.44	GC,GC/MS
33	1510	Trans –calamenene	0.21	GC
34	1513	**δ – Cadinene**	**3.13**	GC,GC/MS
35	1521	Trans-Cadina-1-4-diene*	0.21	GC,GC/MS
36	1529	α- calacorene	0.73	GC
37	1538	**Germacrene D-4-ol**	**9.61**	GC,GC/MS
38	1566	Spathulenol	0.87	GC
39	1574	Salvial-4(14)-en-1-one*	0.96	GC,GC/MS
40	1579	ß- oplopenone	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	**Phytol**	**4.87**	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	**4,8,12,16Tetramethyl heptadecan-4-olid***	**16.97**	GC,GC/MS
	Total		82.*46*	
		Grouped Compounds		
		Monoterpene hydrocarbon	12.61	
		Oxygenated monoterpene	9.46	
		Sesquiterpene hydrocarbon	5.58	
		Oxygenated sesquiterpene	13.17	
		Others Compounds	41.64	

*New Compounds

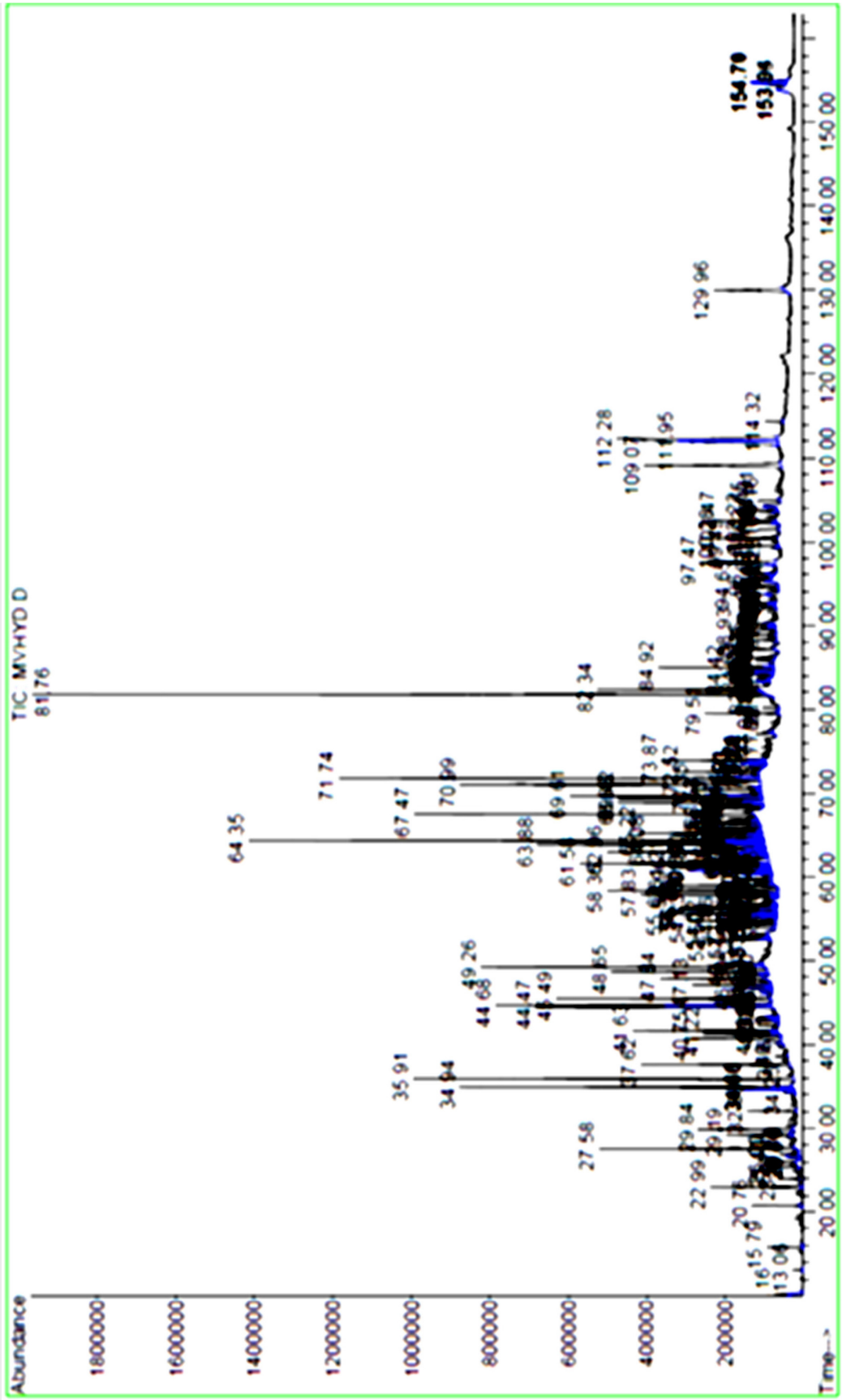

Fig. 1. GC-MS of Essential oil.

The study showed that the essential oil content in the dry herb of *Marrubium vulgare* L. was on average 0.05 % [43]. 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 compononents: 4,8,12,16Tetramethyl heptadecan-4-olid (16.97 %), Germacrene D-4-ol (9.61 %), α- pinene (9.37 %). They represnt about 36 % of 56 % and shows the different chemical groups with a dominance of other compounds with 41.64 % of the total rate of volatil 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. Antibacterial activity

The **Antibacterial** activities of M. vulgare essential oil against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter (DD), the medium inhibitory concentration (IC50) and the minimal inhibitory concentration (MIC) values. This essential oil displayed varied antibacterial activity. As can be seen from Table 2, essential oil inhibited the growth of bacterial for Gram (+)and Gram (-) bacterias, along with IC50 and MIC values ranging from 0.1-15 µl/ml, respectively. the activities of essential oil tested against all strains (*Bacillus subtilis* (ATCC6663), *Micrococcus luteus* (ATCC9314), Escherichia coli (CIP54.8) and *Klebsiella pneumoniae*(CIP82.91)) has been revealed.

Table 2. Minimal inhibitory concentration (MIC) of essential oil from *M. vulgare.*

The concentrations (µl/ml)	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**1**	**2**	**3**	**4**	**5**	**10**	**15**
Bacterias of Gram +												
***Bacillus subtilis* (ATCC6663)**	+	+	+	+	+	+	+	+	+	+	+	-
***Micrococcus luteus* (ATCC9314)**	+	+	+	+	+	+	+	+	+	+	-	-
Bacterias of Gram -												
***Escherichia Coli* (CIP54.8)**	+	+	+	+	+	+	+	+	+	+	-	-
***Klebsiella pneumoniae* (CIP82.91)**	+	+	+	+	+	+	+	+	+	+	+	-

4. CONCLUSION

The essential oil of M. vulgare showed significant antimicrobial activity. Analysis by GC-MS and GC/FID of M. vulgare essential oil of eastern Algeria and tested represent an inexpensive source of natural antibacterial substances for use in pathogenic systems to prevent the growth of bacteria and extend the shelf life of the processed food.

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References

[1] Barlow S. M., Hudson B. J. F., (Ed.)., Elsevier, Amsterdam 1990, pp. 23.

[2] B. M. Lawrence, Science. Edits., R. M. Harley and T. Reynolds, *Royal Botanic Gardens*, Kew, 1992, pp. 399-436.

[3] M. S. Abu-Asaband, R. D. Cantlno, In: *Advances In Lablate Science*. Edits. R, M. Harley and T. Reynolds, Royal Botanic Gardens, Kew, 1992, pp. 97-112.

[4] V. Schiemper, A. Ribas, M. Nlcolau, V. Cechinel-Fliho, *Phytomedecine* 3 (1996) 211-216.

[5] W. Letchamo, S. Mukhopadhyay, *J. Hortic. Sci.* 72 (1997) 741-748.

[6] S. Sahpaz et al., *Journal of Ethnopharmacology* 79 (2002) 389-392.

[7] J. E.Slmon, A. F. Chadwickand, L. E.Craker, *Herbs-anIndexedblbllograptry 1971-1980,* Elsevler, Amsterdam 1984, p. 48,

[8] A. Abadi, A. Hassani, *International Letters of Chemistry, Physics and Astronomy* 9(1) (2013) 17-24.

[9] Nagy M., Svajdlenka E.,*J. Essent Oil Res.* 10 (1998) 585-587.

[10] Saleh M. M., Glombitza K. W., *Planta Med* 55 (1989) 105-108.

[11] Morteza-Semnani K., Saeedi M., *J. Essent Oil Bearing Plants* 7 (2004) 239-242.

[12] Asadipour A, Mehrabani M, Nazeri V, Tabarraii M., *Ulum-i-Daroei* 2 (2005) 77-82.

[13] Khanavi M., Ghasemian L., Hosseiny Motlagh E., Hadjiakhoondi A., Shafiee A., *Flav. Fragr. J.* 20 (2005) 324-326.

[14] Wolski T., Matosiuk D., Baj T., Ziewiec A., *Postępy Fitoterapii* 1 (2007) 39-45.

[15] *Polish Pharmacopoeia* VIII. PTFarm., Warszawa, 2008, 2249-2250.

[16] Bradley P. R., *British Herbal Medicine Association* 1 (1992) 218-220.

[17] El Bardai S., Wibo M., Hamaide M-Ch., Lyoussi B., Quetn-Leclerq J., Morel N., *British Journal of Pharmacology* 140 (2003) 1211-1216.

[18] Kohlmünzer S., *Farmakognozja*, Wydawnictwo Lekarskie PZWL Warszawa, 2007, 308.

[19] Wyk B. E., Wink M., *Rośliny lecznicze świata*, MedPharm Polska, 2008, 198.

[20] Roman R. R., Aharcon A.F., Lara L.A., Flores S. J.,L., *Arch. Med. Res.* 23(1) (1992) 59-64.

[21] El-Bardai S., Morel N., Wibo M., Fabre N., Llabres G., Lyoussi B., Quetin L., *Plant Med* 69(1) (2003) 75-77.

[22] El-Bardai S., Lyoussi B., Wibo M., Morel N., *Clin Exp Hyprtens* 26(6) (2004) 465-474.

[23] DeSouza M. M., DeJesus R. A. P., Cechinel-Filho V., Schlemper V., *Phytomed* 5(2) (1998) 103-107.

[24] Sahpaz S., Garbacki N., Tits M., Bailleul F., *J. Ethnopharmacol* 79(3) (2002) 389-392.

[25] Schlemper V., Ribas A., Nicolau M., Cechinel-Filho V., *Phytomedicine* 3 (1996) 211-216.

[26] Kadri A., Zarai Z., Bekir A., Gharsallah N., Damak M., Gdoura R., *African Journal of Biotechnolgy* 10(198) (2011) 3908-3914.

[27] Stulzer H. K., Tagliari M. P., Zampirolo J. A., Cechinel-Filho V., Schlemper V. *J. Ethnopharmacol* 108(3) (2006) 379-392.

[28] Baratta M. T, Dorman H. J. D., Deans S. G., Figueiredo A. C., Barroso J. G., Ruberto G., *Flav. Fragr. J.* 13 (1998) 235-44.

[29] Bounatirou S., Smiti S., Miguel M. G., Faleiro L., Rejeb M. N., Neffati M., Costa M. M., Figueiredo A. C., Barroso J. G., Pedro L. G., *Food Chem* 105 (2007) 146-155.

[30] Fabio A., Cermelli C., Fabio G., Nicoletti P., Quaglio P., *Phytother Res.* 21 (2007) 374-377.

[31] Voravuthikunchai S. P., Sririrak T., Limsuwan S., Supawita T., Iida T., Honda T., *J. Health Sci.* 51(5) (2005) 590-596.

[32] Burt S., *International Journal of Food Microbiology* 94 (2004) 223-253.

[33] H. Van den Dool, P. D. Kratz, *J. Chromatogr.* 11 (1963) 463.

[34] F. Macchioni, P. L. Cioni, G. Flamini, I. Morelli, S. Maccioni, M. Ansaldi, *Flavour Frag. J.* 18 (2003) 139.

[35] V. Roussis, P. Katerina, V. Constantinos, P. V. Catherine, O. Antonio*, J. Essent. Oil Res.* 13 (2001) 118.

[36] R. P. Adams, *Identification of Essential Oils by Ion Trap Mass Spetroscopy,* Allured Publishing Corporation, Carol Stream, IL, USA, 1995.

[37] May J., Chan C. H., King A., Williams L., French G. L., *J. Antimicrobial Chemoter* 45 (2000) 639-643.

[38] Ferreira A., Proenca A. C., Serralheiro C. M. L. M., Araujo M. E. M., *J. Ethnopharmacology* 108 (2006) 31-37.

[39] Saidana D., Mahjoub M. A., Boussaada O., Chriaa J., Cheraif I., Daami-Remadi M., Mighri Z., Helal A. N., *Microbiology Res.* 163 (2008) 445-455.

[40] Boussaada O., Ammar S., Saidana D., Chriaa J., Chraif I., Daami-Remadi M., Helal A. N., Mighri Z., *Microbiology Res.* 163 (2008) 87-95.

[41] Hammer K. A., Carson C. F., Riley T. V., *J. App. Microbiology* 86 (1999) 985-990.

[42] Delaquis P. J., Stanich K., Girard B., Mazza G., *Int. J. Food Microbiology* 74 (2002) 101-109.

[43] Zawiślak G., *Herba Pol*. 55(3) (2009) 63-68.

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