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Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils

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ABSTRACT

The analysis and identification of essential oils hydrodistilated from two mint species (Mentha spicata and Mentha pulagium) by means of gaz chromatography and mass spectroscopy was realized in this investigation. In addition, their antagonistic activity against some pathogenic bacteria was screened. Acceptable yield (1.0 and 0.87 % for M. Pulagium and M. Spicata respectively) was obtained. From the leaf essential oil Mentha spicata 57 compounds were separated, representing 97,022% of the total essential oil mass from which 44 compounds were elucidated. The major compound was carvone (59.40 %), other components present in appreciable contents were: limonène (6.12%), 1,8-cinéol, germacrène-D (04.66%), β-caryophyllène (2.969 %), β-bourbonène (2.796 %), α-terpinéol (1.986 %) , Terpinéne-4-ol (1.120 %). The essential oil of Mentha pulegium, 43 compounds were separated, representing 99.52 % of the total essential oil mass from which 29 compounds were elucidated. The major component was pulegone (38.815 %), other components present in appreciable contents were: menthone (19.240 %), pipériténone (16.528 %), pipéritone (6.348 %) and isomenthone (6.096 %), Limonène (4.293 %), Octaan-3-ol (1.854 %). Furthermore, screening of the two essential oils for their antagonistic activity against pathogenic bacteria reveals that they have not an appreciable activity except that observed against Streptococcus pyogenes (20 and 16 mm for M. Spicata and M. Pulagium respectivelly). This growth inhibition was obtained by the undiluted essential oils. However, more research on the factors influencing the biosynthesis and bioactivity of essential oils is needed as essential oils are gained important applications in food and pharmaceuticals industry.

Keywords: *Mentha spicata*, *Mentha pulagium*, essential oil, chemical composition, antimicrobial activity.

INTRODUCTION

Essential oils (EOs) are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition. Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure, mitigate or prevent diseases, infections and indispositions by means of inhalation [1]**.** The antibacterial properties of essential

oils and their components are exploited in such diverse commercial products as dental root canal sealers, antiseptics and feed supplements for lactating sows and weaned piglets. A few food preservatives containing EOs are already commercially available. Numerous publications have presented data on the composition of the various EOs. Detailed compositional analysis is achieved by gas chromatography and mass spectrometry of the EO or its headspace. EOs can comprise more than sixty individual components. Major components can constitute up to 85 % of the EO whereas other components are present only as a trace. The phenolic components are chiefly responsible for the antibacterial properties of EOS [2]**.**

The genus *Mentha* includes 25–30 species that grow in the temperate regions of Eurasia, Australia and South Africa. The mint species have a great importance, both medicinal and commercial. Indeed, leaves, flowers and stems of *Mentha spp.* are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavour. In addition, *Mentha spp.* has been used as a folk remedy for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints due to its antinflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharrhal activities [3]**.**

Mentha pulegium L. is one of the *Mentha* species commonly known as pennyroyal. It is native species of Europe, North Africa and in Asia Minor and near East. The flowering aerial parts of *Mentha pulegium* L. has been traditionally used for its antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis, and also as antiflatulent, carminative, expectorant, diuretic, antitussive, menstruate. Some pharmacological effect of *Mentha pulegium* L. essential oil such as abortifacient effect in rat myometrium, cytotoxic activity against different human cell lines and its antioxidant effect were confirmed. The ingredients of *Mentha pulegium* L. oil have been subjected to a number of studies which have shown a difference in its constituents depending on the region of cultivation and there have been some variations in the constituents from different countries. It has been found *Mentha pulegium* L. oil from Bulgaria contains pulegone (42.9–45.4%); from Uruguay; pulegone (73.4%), isomenthone (12.9%); from Egypt; pulegone (43.5%), piperitone (12.2%); from Tunisia, pulegone (41.8%), isomenthone (11.3%). These studies showed three chemotypes of *Mentha pulegium* L. with the following major oil components (1) pulegone, (2) piperitenone and/or piperitone and (3) isomenthone/ neoisomenthol. Though the flowering aerial part of this plant commonly is used because of its antiseptic properties, heretofore there is no report that investigated the antimicrobial activity of this plant [4]**.** This study aimed to investigates the chemical composition of essential oils of two cultivated mint species largely used in algeria (*M. spicata and M. pulegium*), as well as their antimicrobial activities to validate its traditionally uses.

MATERIALS AND METHODS

Plant Material

Aereal parts of *Mentha spicata* and *Mentha pulegium* were harvested during June 2008 from Amoucha, wilaya of Sétif (north east Algeria). Then, plant parts were washed with tap water, to eliminate soil and other surface contaminants. After the dryness, at laboratory temperature and obscurity, the plant material was cut to small pieces with universal knife.

Extraction of the essential oil

100 g of the air-dried aerial parts of the two species were subjected to hydrodistillation for 3 h with 500 ml distilled water using a Clevenger-type apparatus. The oil obtained was collected and

dried over anhydrous sodium sulfate and stored in screw capped glass vials in a refrigerator at 4–5°C prior to analysis. Yield based on dried weight of the samples was calculated.

Gas chromatography (GC-FID) analysis

The gas chromatography analysis of the two volatile oils was performed using a HP 6890 equipped with: Flame ionization detectors (FID), HP Innowax (DB5; polyethylenglycol) 30 m 9 x 0.25 mm ID, 0.25 µm film thickness fused capillary column. The carrier gas was Hydrogen: (1ml min $^{-1}$). The oven temperature program was 5min isothermal at 50°C, then 50–300°C (BP-20) at rate of 5°C/min and held isothermally (300°C) for 5 min. The injection port temperature was 280°C, detector 300°C. Volume injected: 1 µl of 1: 60 (v : v in hexane) solution. Percentages of the constituents were calculated by electronic integration of FID peak areas.

Gas chromatography-mass spectrometry (GC/MS) analysis

The analyses of the volatile constituents were run on a Hewlett-Packard GC-MS system (GC: 7890; MSD-HP 5975-C.). The fused-silica DB5 capillary column (30 m 9 x 0.25 mm ID, film thickness of 0.25 µm) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1.0 ml min-1. Oven temperature was programmed (50°C for 5 min, then 50-300°C at 5°C/min) and subsequently, held isothermally (300°) for 5 min. Injector port: 250°C, detector: 280°C, split ratio 1:100. Volume injected: 1 µl of 1% solution (diluted in hexane): HP 5975 recording at 70 eV; scan time 1.5 s; mass range 40–300 amu.

Compounds identification

The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library [5] [6], and those described by [7], as well as on comparison of their retention indices either with those of authentic compounds or with literature values [8] [7]**.**

Antibacterial Activity

Four Gram positive bacteria (*Staphylococcus aureus ATCC25923, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogene*) and five Gram negative bacteria (*Pseudomonas aeruginosa ATCC27853, Escherichia coli ATCC 25922, klebsiella pneumoniae, Schegella somei, and Salmonella typhi*) were used in this study. The non reference strains used were isolated from human specimens at the laboratory of bacteriology of the university hospital center of Sétif. The bacterial inoculums was prepared from overnight broth culture in physiological saline (0.8 % of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar) and MH agar supplemented with 5 % sheep blood for fastidious bacteria (*Streptococcus pneumoniae, Streptococcus pyogenes*) were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria, filled with 10 µl of mother solution and diluted essential oil (1:1,1:2, 1:5, and 1:10 v:v of DMSO). DMSO was used as negative control. The following antibiotics, ceftazidine (CAZ), Ceftriaxome (CRO), Pristinamycine (PT), Chloramphénicol (C), and Ciprofloxacine (CIP) were used as positive controls. Then, Pétri dishes were incubated at 37°C during 18 to 24h aerobically, except that containing *S. pneumoniae* which incubated at an atmosphere enriched with 05% CO_2. After incubation, inhibition zone diameters were measured and documented.

RESULTS AND DISCUSSION

Extraction and composition analysis

The retention time and chemical composition of essential oils of *Mentha spicata* are presented in table 01, and 02. The gas chromatogram of the oil on a HP-5 MS capillary column is shown in

Fig. 01 and 02. The constituents of *M. spicata* and *M. pulegium* leaf oils were listed in order of their elution on the non polar column (Figure 1 and 2).

The greater essential oil yield (g 100g-1 of dry plant materials) was acquired from leaves of *Mentha pulegium* (1.00 g 100g-1) and the minimum was from leaves of *M. spicata* (0.87 g 100g-1). Our results are in agreement with those of [9] who investigated the yield of essential oil from wild *M. spicata,* grown in Greece ranging from 0.1-1.8 %. Asekun *et al*., [10] and Mimica-Dukic *et al*., [8] examined the oil contents of other *Mentha* species (*M.longifolia* and *M. arvensis*) to be 0.79% and 0.36-1.36%, respectively. It has been reported that *Mentha* species demonstrated the highest essential oil yield in summer (1.08 - 1.20, 1.70 g 100g-1), when the plants were at full bloom, than in winter (0.62 - 0 1.05 g 100g-1) when the plants reached at the end of their growing cycle. Also, differences in the yield of *Mentha* essential oils with respect to geographical regions were reported [11]**.** Literature revealed that essential oil contents depend not only on temperature, relative humidity, but also duration of sunshine, air movement and rainfall [12][13]**.** The chemical compositional data of the essential oils from four *Mentha* species (*Mentha spicata*, and *M. pulegium*) are reported in Table 1 and 2. The essential oil yield of the study was 0.87 and 1.0 %. It is relatively comparable to other plants industrially exploited as a source of essential oils: lavender (0.8-1.8%), menthe (0.5-1%), neroli (0.5-1%), Laurel (0.1-0.35%), *Lippia rotundifolia* (0.01%) [14]**.** In contrast, *Mentha rotundifolia* and *Mentha pulegium* essential oils yield 4.33% and 2.33% respectively, as obtained in other countries of the North Africa**.** In the leaf essential oil *Mentha spicata* (Table 1), 57 compounds were separated by GC-MS, representing 97,022% of the total essential oil mass from which 44 compounds were elucidated. The major component was carvone (59.40 %), other components present in appreciable contents were: limonène (6.12%), 1,8-cinéol, germacrène-D (04.66%), β-caryophyllène (2.969 %), β-bourbonène (2.796 %), α-terpinéol (1.986 %) , Terpinéne-4-ol (1.120 %). In other studies (Soković, 2009), carvone (49.5 %) is also the major constituent, followed by menthone (21.9 %) and limonene (5.8 %) of the extractable essential oils from this plant. In contrast, in studied *M. spicata* essential oil, menthone is absent.

The essential oil of *Mentha pulegium* (Table 2), 43 compounds was separated by GC-MS, representing 99.52 % of the total essential oil mass from which 29 compounds were elucidated. The major component was pulegone (38.815 %), other components present in appreciable contents were: menthone (19.240 %), pipériténone (16.528 %), pipéritone (6.348 %) and isomenthone (6.096 %), Limonène (4.293 %), Octaan-3-ol (1.854 %). Mahboubi and Haghi [4] were obtained that the flowring plant (*M. pulegium*) originated from Iran yields 0.27 %. In contrast, Benayad et *al***.** [15] reported that the same plant yields 2.33 %. However, in other study, *M. spicata* and *M. pulegium* yield 0.59 and 0.90 % respectively. There are some reports in literature on the chemical composition of *Mentha* essential oils. In *M. spicata* essential oils from Greece, carvone was recognized as the main component [16]**.** Sokovic and Griensven [17] also reported that carvone (49.52%), menthone (21.92%), limonene (5.77), and 1,8-cineole (3.06%) were the major compounds identified in the essential oil of *M. spicata* from Montenegro. In hydrodistilled *M. arvensis* essential oils from India, menthol, *p*-menthone, *iso*-menthone, and *neo*-menthol, were found to be as the main components [17]**.** Singh *et al*. [18] examined a high level of menthol in the *M. arvensis* essential oil from India. Viljoen *et al*. [19] reported piperitenone oxide (15-66%) as the main compounds identified in the essential oils of piperitenone oxide chemotype of *M. longifolia*. Gulluce *et al*. [20] reported *cis*piperitone epoxide, pulegone, piperitenone oxide, as the main components of essential oil from *M. longifolia*, growing in Turkey. The minor variations in the chemical compositions of different species of *Mentha* essential oil across countries might be attributed to the varied agroclimatic (climatical, seasonal, geographical) conditions of the regions, isolation regimes and adaptive

metabolism of plants. Studies reported by [4] show clearelly that *M. pulegium* essential oil contains the following compounds: piperitenone 33.0%, α-terpineol 04.7%, pulégone 02.3% from which piperitone was the majore constituent (38.00 %). In contrast, pulegone (88 %) was the main component in the same plant analysed by [21]. In addition, results published by Vian et ***al.*** [22] present that pulegone (83.70 %) was reported as the main component of *M. pulegium* essential oil. whereas, Hilan et *al*., [23] reported that menthol is the main constituent (50 %). The essential oils of *Mentha spicata,* and *M. pulegium* mainly consisted of oxygenated monoterpenes. Carvone, menthol and menthone were the principal oxygenated monoterpenes in the essential oils of *M. spicata, M pulegium*.

Antibacterial Activity

Antibacterial activity of the essential oil was tested by the disc diffusion assay. Table 3, show diameter of inhibition of the different concentrations of the essential oil in DMSO (v/v) against either Gram positive and negative pathogenic bacteria. The concentration 10 % has no effects on tested bacteria, and 20 % exhibited a weak activity only against *Streptococcus pneumoniae*. The other concentrations (50 and 100 %) present weak activity except that observed against tested *Streptococcus* species. Moreover, *Pseudomonas aeruginosa* ATCC 27853 was resistant to the essential oil. It seems clearly that the essential oil of *M. spicata* has a dose dependent activity against susceptible species. Similarly, the essential oil of *M. pulegium* exhibits weak inhibitory effects toward most tested bacteria. In addition, *Pseudomonas aeruginosa* ATCC 27853 was resistant to the essential oil. In general, tested Gram negative bacteria appear more resistant than Gram positive ones. Both *Streptococcus* species and *Staphylococcus aureus* ATCC 25923 were sensitive and they were more susceptible to *M. pulegium* than *M. spicata* essential oils (Table 03). Gram positive bacteria are known to be more susceptible to essential oils than Gram negative bacteria. However, the weak antibacterial activity of Gram negative bacteria observed in this investigation is in accord with other studies. This phenomenon was ascribed to possession of these bacteria to a hydrophilic polysaccharide chains as a barrier to hydrophobic essential oils [24]. Essential oils rich in phenolic compounds are reported to possess high level of antimicrobial activity [25]**.** It is believed that the phenolic components of essential oils show strongest antimicrobial activity, followed by aldehyde, ketones and alcohols [26]**.** Our results are in good agreement with the findings of Cantore *et al*., [27] who reported that Gram-positive bacteria are more sensitive to plant essential oils than Gram negative bacteria. There is evidence in the literature that the essential oils of some *Lamiaceae* plants possess a moderate to good antibacterial activities [28] [8] [29-32] [17] investigated that *M. spicata* oil possessed better antibacterial activity than the standard drugs streptomycin + penicillin. Sivropoulou *et al***.,** [33] investigated that antimicrobial activity of *M. spicata* oil might be attributed to high contents of carvone and *cis*-carveol. Our results are in good agreement with the findings of Bader *et al*., [34] who studied that carvone exhibited better antimicrobial activity than the entire oil. In an other report [4] piperitone and piperitenone were found the main components of *M. pulegium* essential oil (71.1%) and exhibits a remarkable antibacterial activity against Gram positive bacteria than Gram negative ones. Antimicrobial activity of the essential oils from M. spicata and M. pulegium was proved. The high concentrations of carvone and pulegone can be used as explanation for traditional uses of the two *Mentha* species for treating microbe related illnesses. The increasing antibiotic resistance of pathogens that associated with infectious diseases as well as undesirable side effects of antibiotics suggested the use of *Mentha* essential oils as antibiotics or alternatives. However, further research is required to better understand the scientific and biotechnological basis values of applied phytotherapy.

Table 1. Chemical composition, Kovats indices and percentage composition of the essential oil extracted from *Mentha spicata*

Pic	RT	Compound	IR	%
01	9.86	α-thujène	926	tr
02	10.128	α-pinène	934	0.322
03	10.72	Camphène	950	tr
04	11.527	Sabinène	973	0.327
05	11.701	β-pinène	978	0.607
06	11.797	Oct-1èn-3-ol	981	0.125
07	12.098	Myrcène	989	0.379
08	12.391	Octan-3-ol	998	0.305
09	13.048	α-terpinéne	1017	0.161
10	13.534	Limonène	1032	**6.129**
11	13.637	1,8 cinéole	1035	**3.800**
12	13.688	(Z)- β-ocimène	1037	0.331
13	14.029	(E)- β-ocimène	1047	0.118
14	14.431	γ- terpinéne	1059	0.360
15	14.870	Cis hydrate de sabinène	1073	0.975
16	14.305	terpinolène	1086	0.098
17	15.575	linalol	1100	0.212
18	15.838	nd	1102	0.118
19	17.998	Delta terpinéol	1173	0.202
20	18.066	Endo bornéol	1176	0.484
21	18.309	Terpinéne-4-ol	1184	1.120
22	18.821	α-terpinéol	1201	1.986
23	19.006	trans dihydrocarvone	1207	1.555
24	19.675	Néoiso-dihydro carvéol	1031	0.221
25	19.842	Cis carvéol	1237	1.176
26	19.967	pulégone	1242	0.224
27	20.485	carvone	1260	**59.40**
28	21.538	nd	1297	0.218
29	22.286	Acétate dedihydroiso carvéol	1326	0.374
30	22.706	pipériténone	1342	0.147
31	23.021	nd	1354	0.183
32	23.190	Acétate de cis carvyle	1360	0.613
33	23.938	β-bourbonène	1389	**2.796**
34	24.021	β-éléméne	1392	0.838
35	24.102	(Z)-jasmone	1395	0.632
36	24.493	nd	1410	0.155
37	24.860	β-caryophyllène	1425	**2.969**
38	25.071	β-copaène	1434	0.347
39	25.424	nd	1484	0.490
40	25.551	(E- β-farnésène	1453	0.542
41	25.715	α-humulène	1460	0.187
42	25.866	nd	1466	0.431
43	26.031	γ-muurolène	1473	0.258
44	26.389	Germacrène-D	1487	**4.665**
45	26.690	bicyclogermacrène	1499	0.722
46	26.978	γ-cadinène	1511	0.109
47	27.184	Delta cadinène	1520	0.271
48	27.256	Cis calaménène	1523	0.152
49	28.616	spatulénol	1581	0.664
50	28.747	Oxyde de caryophyllène	1587	0.649
51	29.470	nd	1619	0.268
52	29.851	nd	1636	0.153
53	30.053	nd	1645	0.024
54	30.366	α-cadinol	1660	0.470
55	31.010	nd	1689	0.362
56	31.242	nd	1699	0.239
57	36.561	nd	1961	0.231

Tableau 2. Composition chimique de l'huile essentielle de *M. pulegium*

Pic	RT	Composés	IR	%
1.	10.128	α-pinène	934	0.509
2.	11.527	Sabinène	973	0.642
3.	11.699	β-pinène	978	0.896
4.	11.969	Octan-3-one	986	0.660
5.	12.094	Myrcène	989	0.206
6.	12.410	Octaan-3-ol	998	1.854
7.	13.313	Para cyméne	1025	0.072
8.	13.493	Limonène	1031	4.293
9.	13.583	1,8 cinéole	1034	0.059
10.	14.02	(E)- β-ocimène	1027	tr
11.	14.423	γ- terpinène	1059	0.051
12.	14. 84	Cis sabinène hydrate	1072	tr
13.	15.30	terpinolène	1085	tr
14.	15.84	transhydrate de sabinène	1102	tr
15.	16.352	Acétate d'octan-3-yle	1119	0.204
16.	17.658	menthone	1161	19.240
17.	17.889	isomenthone	1170	6.096
18.	17.985	menthol	1173	0.302
19.	18.08	bornéol	1176	tr
20.	18.138	isopulégone	1178	0.548
21.	18.290	Terpinéne-4-ol	1183	0.063
22.	18.4	Décan-3-one	1187	tr
23.	18.56	néoisomenthol	1192	tr
24.	18.77	α- terpinéol	1199	tr
25.	18.89	Cis pipéritol	1203	tr
26.	19.500	schisofurane	1225	0.353
27.	19.55	Cis carvéol	1227	tr
28.	20.139	pulégone	1248	38.815
29.	20.495	pipéritone	1260	6.348
30.	20.826	Isopipériténone	1272	0.224
31.	22.842	Pipériténone	1347	16.528
32.	23.894	β- bourbonène	1387	0.056
33.	24.191	nd	1398	0.049
34.	24.318	β- caryophyllène	1423	0.160
35.	25.710	α-humulène	1460	0.597
36.	26.320	Germacrène-D	1484	0.064
37.	28.729	Oxyde de caryophyllène	1586	0.180
38.	29.109	nd	1603	0.084
39.	29.221	nd	1608	0.063
40.	29.370	humulène époxyde ll	1614	0.449
41.	29.693	nd	1629	0.055
42.	29.861	Eléma-1,3,11(13)-trién-12-al	1637	0.143
43.	30.006	Caryophyll-5-èn-12-al	1643	0.136

Table 3. Antibacterial activity of *M. pulegium* and *M. spicata* essential oils measured as diameter of inhibition (mm)

	M. pulegium essential oil				*M. spicata* essential oil				control
Dilutions (v/v in DMSO)	1/1	1/2	1/5	1/10	1/1	1/2	1/5	1/10	
P. aeruginosa ATCC 27853	-	-	-	-	-	-	-	-	+
Escherichia coli ATCC 25922	10	07	-	-	09	07	-	-	+
Staphylococcus aureus ATCC 25923	12.5	07	-	-	11	07	-	-	+
Staphylococcus epidermidis	12	07	-	-	10	07	-	-	+
Streptococcus pneumoniae	14	11	07	-	13	10.5	07	-	+
Streptococcus pyogenes	20	14	-	-	16	13	-	-	+
Klebsiella pneumoniae	10	07	-	-	08	07	-	-	+
Salmonella typhi	10	07	-	-	08	07	-	-	+
Schigella sonnei	09	07	-	-	09	07	-	-	+

DMSO : Demethysulfoxide ; + : positive ; - : negative.

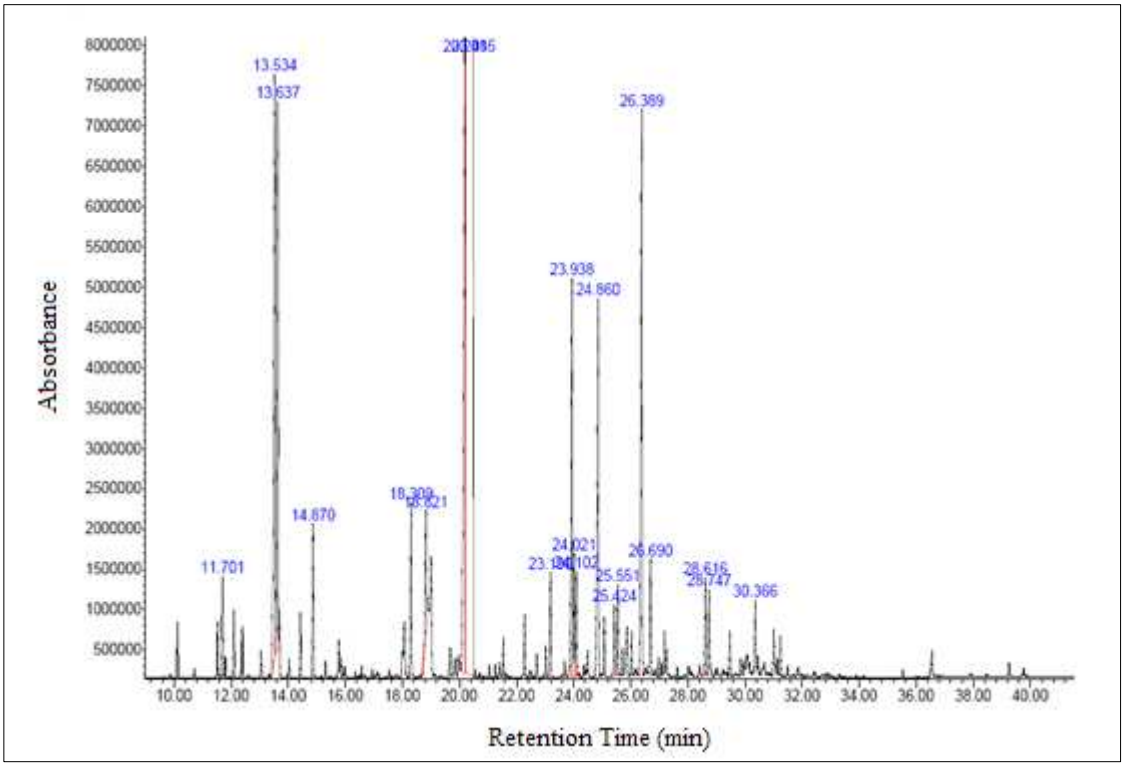

Figure 1. GC/FID profiles of *M. spicata* L. essential oil (for numbering see Table 1).

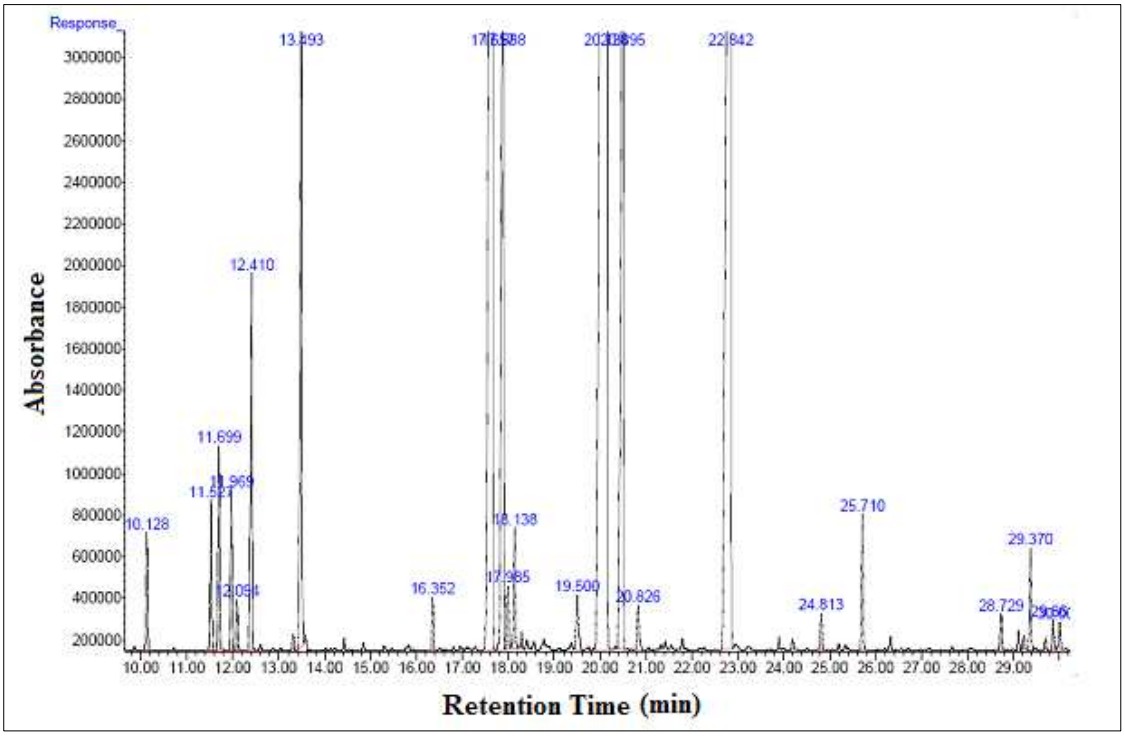

Figure 2. GC/FID profiles of M. pulegium L. essential oil (for numbering see Table 2)

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