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Research Article

COMPARISON OF CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *MENTHA PULEGIUM* ESSENTIAL OIL FROM TWO ECOTYPES (EL BAYADH AND DJELFA)

Z. Boukhobza[1], A. Cheriti[1*], M. Kacimi El Hassani[2], M. B. Lahreche[2]

[1]Phytochemistry & Organic Synthesis Laboratory, Faculty of Medicine, UTMB, Algeria

[2]Organic Chemistry & Natural Substances Laboratory, Faculty of Sciences, Z. Achour University, Djelfa, Algeria

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ABSTRACT

Mentha pulegium, collected from two different sites in Algerian high plains (El Bayadh and Djelfa), were analyzed to determine essential oil constituency. Essential oils were extracted by hydrodistillation and subsequently analyzed by GC/MS. Quantitative differences were recorded between the percentages of some constituents between plants from the two geographical origin. The most important major components were: 1,8-cineole, menthone, menthol, cis-Pulegone and Pulegone. The antimicrobial activity of the essential oils was tested using the disc-diffusion assay. The results showed that the essential oil of *Mentha pulegium* had great potential of antibacterial activity against selected bacteria

Keywords: Essential oil; *Mentha pulegium*; GC/MS; antibacterial activity; ecotypes; high plains.

Author Correspondence, e-mail: karimcheriti@yahoo.com

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1. INTRODUCTION

Plants have formed the basis of Traditional Medicine (TM) systems that have been in existence for thousands of years and continue to provide mankind with new remedies, such as, the oldest known medicinal systems of the world: Ayurveda, Arabian medicine, Chinese and Kempo medicine [1-3]. Since ancient time, volatile essential oils and plants extracts are used for preserving human health in most ancient civilizations. Thus, the World Health Organization (WHO) has recognized the potential utility of traditional remedies and strives to preserve primary health care involving medicinal plants. Nearly 50,000 species of higher plants have been used for medicinal purposes. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world and in which higher plants contribute to no less than 25% [1]. Actually, there has been a rise interesting in the usage of aromatic medicinal plants and their essential oils in technical research and industrial applications including nutritious therapeutic and cosmetic uses [4,5].

The genus *Mentha* consisting of aromatic perennial herbs belonging to the Lamiaceae (Labiatae) family, in the Order Lamiales, which includes many other families, such as Verbenaceae, Scrophulariaceae and Acanthaceae which have achieved high economic value. The *Mentha* genus includes approximately 25-30 species and hybrids that spread mostly in temperate and subtemperate regions of the world, which 6 are grows in different Algerian ecotypes [6]. Most *Mentha* species are used in different ethpharmacopeae for treatment of gastrointestinal disorders, as astringents, tonics, laxatives, mouth fresheners, for flatulent colic, nervous disorders, gout, stomach ailments, insect repellent and as culinary herbs [7-9].

Algeria with its large area and diversified climate has a varied flora about 4450 taxa of which 3950 indigenous with 6.5% endemic [10]. This richness flora is a source of rich and abundant medical matter. In Algeria, *Mentha pulegium*. (syn.: *M. gibraltarica Willd., M. numidica Poiret, M. albarracinensis* Pau; *M. aromatica* Salisb.; *M. aucheri* Perard; *M. hirtiflora* Opiz; *M. montana* Lowe; *M. pulegioides)* commonly known as '*Fliou*' is widely used in folk medicine as a spice and for the treatment of various diseases such as gastrointestinal tracts, gallbladder disorders, cephalic pains, bronchitis, carminative, sedative, anti-inflammatory and dysmenorrheal… [11-13].

Some interesting biological activities of *Mentha pulegium* essential oil have been described in the literature [7,14], thus It is exhibited several activities such as: Antimicrobial, antioxidant, spasmolytic, hepatotoxicity, anti-genotoxic, antimyometrium, relaxant, acaricidal and anti-steel corrosion effects.

Tracing the current literature, nothing was found concerning the chemical composition and biological activity of *Mentha pulegium* essential oils growing wild in Algerian high plains (El Baydh area). Thus, as a part of our investigation into Algerian medicinal plants [15-23], in this study we investigate for the first time the variability of the chemical composition and antibacterial activity of the essential oils from *Mentha pulegium* specie collected from two regions (El Bayadh and Djelfa).

2. RESULTS AND DISCUSSION

2.1. Chemical composition of the essential oil

A yellowish oils with a characteristic minty pleasant-smelling odor were obtained with a yield of 2.7% and 2.1% respectively from *M. pulegium* collected from El Bayadh (EO32) and Djelfa (EO17)

The chemical composition of the *M. pulegium* oil is presented in Table 1. The components are listed in order of their elution on the CBP-5 capillary column. Twenty six compounds were identified representing 87.17% of the oil from El Bayadh (EO32) and twenty five compounds were identified representing 83.24% of the oil from Djelfa (EO17).

The essential oil of *M. pulegium* contained mainly oxygenated monoterpenes, with pulegone as the main constituent in both EO32 (39.24%) and EO17 (63.19%). In the El Bayadh Oil (EO32), in additional to pulegone we found other main constituents such as: 1,8-Cineole (12.43%), Menthone (5.82%), Menthol (4.09%), Cis pulegone (6.28%). Whereas these compounds were found with small contents in *M. pulegium* oils from Djelfa (EO17), which is characterized only by Pulegone (63.19%) and a remarkable amount of Carvone (2.72%).

Table 1. Chemical composition of the *Mentha pulegium* essential oil samples

N°	Components	KI*	EO32	EO17
1	α-Pinene	929	2.62	1.09
2	Camphene	941	0.12	0.24
3	β-Pinene	970	2.14	1.62
4	α-Phellandrene	997	-	0.17
5	Limonene	1020	0.98	0.63
6	1,8 Cineol	1032	12,43	0.78
7	y-Terpinene	1049	1.09	1.17
8	Linalool	1091	0.28	0.33
9	Ocimène	1121	-	0.10
10	Trans pinocarveol	1137	0.26	-
11	Isopulegol	1140	0.08	0.12
12	Trans-p-menth-2-ene-1-ol	1143	0.39	-
13	Camphor	1145	2.71	0.52
14	Menthone	1153	5.82	1.06
15	Iso menthone	1156	0.78	-
16	Chrysanthenol	1164	1.03	0.38
17	Menthol	1168	4.09	0.29
18	Cis pulegone	1170	6.28	1.15
19	α-Terpineol	1188	0.76	1.73
20	Pulegone	1227	39.24	63.19
21	Carvone	1236	1.52	2.72
22	Piperitone	1249	1.62	0.56
23	Linalyl acetate	1244	-	1.59
24	iso-pulegyl acetate	1270	-	1.49
25	Carvacrol	1282	0.26	-
26	Neryl acetate	1355	-	0.16
27	Z-Caryophyllene	1411	1.04	1.36
28	Germacrene D	1470	0.53	0.41
29	Spathulenol	1567	0.84	-
30	4-epi-cubedol	1537	0.09	-
	Total Identified (%)		**87.17**	**83.24**

Origin of Essential Oils: EO32 (El Bayadh); EO17 (Djelfa)

* Retention indices on CBP-5.

Previous studies on *M. pulegium* EOs harvested in Uruguay, Cuba, Iran, India, Turkey, Bulgaria, Greece, Portugal, Morocco, Tunisia and Algeria (Jijel, Djelfa) reported Menthone and Pulegone as the major components of the essential oil, but in different proportions [24-27], with the predominance of pulegone as major compound: 73.40% and 85.40% respectively in Uruguay [28] and Morocco [29]. Other studies confirm the predominance of piperitone and piperitenone [30,31].

There is a great variability in the chemical composition of *M. pulegium* essential oil among the studies performed so far, thus three chemotypes have been established, pulegone type, piperitenone / piperitone type and isomenthone / neoisomenthol type [32]. Benyache team [26] in their analysis of several samples of *M. pulegium* from Jijel (eastern Algeria), conclude that these oils can be classified into two chemotypes: one of pulegone and a new chemotype poor in pulegone and rich of terpenic hydrocarbons fractions and relatively high level of 1,8-cineol. Thus, according to the composition of the essential oils (EO32, EO17), we noted that the *M. pulegium* collected from El Bayadh is related to this new chemotype and specie collected from Djelfa is related to pulegone type.

It's well known that chemical variability may be related with different vegetative phases of the plant, environmental and growing conditions (e.g. seasonal and geographical variations, soil composition [33, 34].

2.2. Antibacterial activity

Medicinal plants have been successfully applied in clinics and as an important source of pharmacological substances around the world. One of the most efficient ways of finding new bioactive compounds is collecting data on the use of medicinal plants in traditional pharmacopeia [1]. Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global healthcare problem in the 21st century [35]. We summarized in table 2, the results of the *in vitro* antibacterial activity of the essential oils (EO32, EO17) against pathogens causing urinary tract, lung and gastrointestinal infection: *Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aereginosa* and *Staphylococcus aureus*. The inhibition zone, measured in millimeters, including the diameter of the paper disk, was used as the criterion for measuring the antibacterial activity.

Table 2. Antibacterial activity of *Mentha pulegium* essential oil samples

	EO32	**EO17**	**AM** (15µg/ml)	**CH** (25µg/ml)
	Volume/disk (20µl)			
	Inhibition Zone Diameters (mm)			
Enterococcus faecalis	09.3	08.1	19.4	28.1
Escherichia coli	13.4	10.2	25.7	32.3
Klebsiella pneumoniae	09.1	12.4	08.6	13.7
Pseudomonas aereginosa	06.4	08.3	05.8	24.1
Staphylococcus aureus	11.6	10.3	26.2	14.9

Origin of Essential Oils: EO32 (El Bayadh); EO17 (Djelfa)

AM: Amoxilline, CH: Chloramphenicol

The results of the current research showed a sensitivity of the microorganisms towards the two EOs and revealed that *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus* are highly susceptible to the antimicrobial activity of the essential oil of *M. pulegium* (EO32, EO17) with displaying inhibition values ranging between 09,1 and 13,4 mm. Whereas, a moderate antibacterial effect against *Enterococcus faecalis and Pseudomonas aereginosa* was observed. Thus, it was assumed that the antibacterial activity of *M. pulegium* essential oils (EO32, EO17) could be attributed to the presence of high amount of pulegone in addition to the synergistic action between all components. It has been demonstrated the strong antimicrobial activity of pulegone against bacteria and cytotoxicity of *M. pulegium* essential oil appears to include bacterial membrane damage [36-38].

3. EXPERIMENTAL

3.1. Plant material

Aerial parts of *Mentha pulegium* were collected in May 2017 from tow sites in the high plains: El Bayadh (Latitude: 33° 40′ 49″ N; Longitude: 1° 01′ 13″ E; Altitude: 1313m) and Djelfa (Latitude: 34°40′ 00″ N; Longitude: 3° 15′ 00″ E; Altitude: 1140m). The plant was identified by Pr A. Marouf (Department of Biology, University Center Naama – Algeria) and a voucher specimen is kept in the Herbarium of POSL Laboratory, (UTMB, Algeria) under

N° CA 99/46

3.2. Isolation of the Essential Oil

The *Mentha pulegium* essential oil (EOs) was obtained from dry plant material (100 g) by hydrodistillation using Clevenger apparatus for 3 h, in accordance with the 3rd Edition of the European Pharmacopoeia cited by [39]. The obtained oil was dried over anhydrous sodium sulphate and stored in colored glass at 4 °C until analysis.

3.3. GC-MS Analysis

GC/MS analysis was performed on Shimadzu GC-17A gas-chromatograph, interfaced with Shimadzu QP5000 mass spectrometer, operating at electron impact of 70 eV with an ion source temperature at 250°C, scan mass range of 40-400 m/z at a sampling rate of 0.5 scan/s. A Supelco CBP-5 capillary column (30 m x 0.25 mm, film thickness 0.25 Lm) was used. The oven temperature programmed as follows: 50°C for 2 min and then up to 240°C at 3°C/min, then to 300°C at 10°C/min, ending with a 10 min at 300°C. The carrier gas was He (1.0 mL/min), injector and detector temperature were 240°C. Samples were injected by splitting and the split ratio 1:5.

The EOs component identification was confirmed by comparison of mass spectral fragmentation patterns with the computer library (NIST MS Library), and verified by comparison of their retention indices (determined relatively to the retention times of a n-alkanes homologous series) of the identified compounds with literature [24, 40-42]. The relative amounts of the individual components found in the oil are based on the peak areas obtained, without FID response factor corrections.

3.4. Antibacterial activity

The antibacterial activity of *M. pulegium* essential oil was determined using the paper disc diffusion method [43], against five pathogens bacteria, two gram positive bacteria [*Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923)] and three gram negative bacteria [*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (Isolated), *Pseudomonas aereginosa* (ATCC 27853)], which were obtained from Pasteur institute (Algiers, Algeria). The bacteria were maintained by frequent sub-culturing on Mueller Hinton agar plates (pH 7.4) and stored at 4°C. Bacterial strains grown on nutrient agar at

37°C for 18 h were suspended in saline solution (0.9% NaCl) and the suspension was used to inoculate. A sterile filter paper disc was impregnated with 20 µL of essential oil and was placed on the inoculated agar. The plates were incubated at 37°C for 24 h. Antibacterial activities were evaluated by measuring the inhibition zone diameters. Amoxilline (15µg/ml) and Chloramphenicol (25µg/ml) were included in the test as reference (positive control). The experiments were conducted in triplicate and the zones inhibition was measured in mm [35,44,45].

4. CONCLUSION

In this work, we studied for the first time the chemical composition and antibacterial activity of the essential oil of *Mentha pulegium* from two different sites in Algerian high plains (El Bayadh and Djelfa). Chemical analysis of essential oil by GC/MS identified twenty six (87.17%) and twenty five (83.24%) compounds respectively from specie collected in El Bayadh (EO32) and in Djelfa (EO17). The results allowed concluding that the yields and chemical composition of essential oils vary according to the plant origin. Thus, essential oil originating from Djelfa (EO17) is dominated by pulegone (63.19%). Whereas, oil from El Bayadh (EO32) is characterized by diverse chemical profiles dominated by pulegone (39.24%) and other constituents: 1,8-Cineole (12.43%), Cis pulegone (6.28%), Menthone (5.82%) and Menthol (4.09%), The results obtained in this study show that the essential oils (EO32, EO17) have significant activity against the five tested bacteria, this probably explains the use of this aromatic plant in traditional medicine against a number of human diseases for generations. Further experiments, are planned to establish the influence of the components of these oils on other biological activities.

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