January 2014, Vol. 8, No. 1, pp. 35-41
Journal of Life Sciences, ISSN 1934-7391, USA

Chemical Composition and Antibacterial Activity of *Artemisia herba-alba* and *Mentha pulegium* Essential Oils

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Received: October 07, 2013 / Accepted: December 05, 2013 / Published: January 30, 2014.

Abstract: The chemical composition of essential oils obtained from *Artemisia herba-alba* and *Mentha pulegium* were determined. The essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC/MS). Their antibacterial activity was studied *in vitro* against three standard strains: *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and five clinical strains: *Enterobacter cloacae*, *Staphylococcus aureus*, *Pseudomonas pyocyanique*, *Enterococcus faecium*, and *E. coli*. Nineteen constituents were identified in *A. herba-alba* essential oil representing 99.57% of the total composition. The major component was α-thujone (59.07%). The bacterial strains were inhibited at concentrations ranging from 1.25 µL/mL to 5 µL/mL and killed at concentrations ranging from 1.25 µL/mL to 10 µL/mL. *M. pulegium* resulted in the identification of eighteen constituents representing 99.48% of the total composition. The main component was pulegone (78.07%). The minimal inhibitory (MIC) and bactericidal (MBC) concentrations were ranging from 1.25 µL/mL to 2.5 µL/mL.

Key words: *Artemisia herba-alba*, *Mentha pulegium*, GC/MS (gas chromatography-mass spectrometry), antibacterial activity.

1. Introduction

Therapy of bacterial infection is mainly based on the use of antibiotics. The widely and sometimes the inappropriate use of these agents, led to the development of multidrug resistant strains, hence the importance of directing research towards new ways especially toward herbal medicine that have always been a source of inspiration for new drugs.

Essential oils are volatile, natural, complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites [1]. They are widely used in medicine as constituents of different medical products, in the food industry as flavouring additives and also in cosmetics as fragrances [2].

Indeed, several studies have confirmed the antigenotoxic [3, 4], antibacterial [5, 6] and antifungal effects [7] of some essential oils and their components.

Artemisia herba-alba is a medicinal and aromatic dwarf shrub, that commonly grown in Mediterranean basin [8]. In Morocco, this plant is found in the Oriental regions, the Eastern Rif, the Middle Atlas, the High Atlas and the Saharan Anti-Atlas [9]. It is used extensively in traditional medicine to treat helminthiasis, diabetes mellitus and other

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conditions such as jaundice [10]. Also, the antihyperglycaemic [11], antimicrobial [12], antioxidant, antispasmodic, anti-venom, nematicidal, anthelmintic, anti-leishmanial, neurological, pesticidal and inhibitor activities of this plant have previously been reported [13].

Mentha pulegium is one of the *Mentha* species known as pennyroyal, a native herb of Asia and near East [14]. In Morocco, this plant grows in wet areas [9]. It has been traditionally used as antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis [15].

The aim of this study was to determine the chemical composition of essential oils of *A. herba-alba* and *M. pulegium* grown in Morocco and to investigate antibacterial activities against some clinical strains that exhibit multidrug resistance to commonly used antibiotics.

2. Material and Methods

2.1 Essential Oils

Essential oils used in this study were provided by Santis Company. They are extracted by steam distillation from flowers, leaves and stems (Table 1).

2.2 Microorganisms

The tested strains included the following bacteria: three standard strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 (Microbiology Laboratory, Faculty of Pharmacy, University of Barcelona, Spain) and five clinical isolates *Enterobacter cloacae*, *Staphylococcus aureus*, *Pseudomonas pyocyanique*, *Enterococcus faecium* and *Escherichia coli* (Microbiology Laboratory, CHU Ibn Rochd, Casablanca, Morocco).

2.3 Gas Chromatography-Mass Spectrometry Analysis

The chromatographic analysis of essential oils was performed with a gas chromatograph (Trace GC Ultra) coupled to a mass spectrometer (Polaris Q ion trap MS), with a VB-5 capillary column (methylpolysiloxane with 5% phenyl; 30 m × 0.25 mm; film thickness 0.25 µm). Fragmentation was performed by electron impact at 70 eV. Helium (1.4 mL/min) was used as carrier gas. Split-type injector was heated to a temperature of 200 °C. The volume injected was 1 µL. The column was initially maintained at a temperature of 40 °C for 2 min, increased to 180 °C at a rate of 4 °C/min, and finally raised to 300 °C for 2 min at 20 °C/min.

2.4 Disc Diffusion Method

The tested as described previously [16]. Sterile filter paper disc (6 mm diameter) were impregnated with 10 µL of essential oil and transferred into the Luria Bertoni Agar present in Petri dishes, which had previously seeded by spreading 1 mL of bacterial suspension adjusted to 10^6 CFU/mL. Standard antibiotics amoxicillin (25 µg/disk) were used as positive control. After incubation at 37 °C during 24 h, the diameters of inhibition zones were measured in millimeters. Tests were carried out in triplicate.

2.5 Determination of MIC and MBC

MIC was determined in this work by the method of macro-broth dilution [17]. A serial of dilution of essential oil ranging from 20 µL/mL to 0.15 µL/mL were prepared in test tubes containing Broth Luria Bertoni medium with 0.15% Agar [18]. Each tube was inoculated with the same volume of bacterial suspension adjusted to 10^6 CFU/mL. The tubes were then incubated at 37 °C for 18 h. MIC values were defined as the lowest concentration of the EO at which the absence of growth was recorded. Controls of medium with either microorganisms or the essential oil alone were included. From tubes where it was no trouble, aliquots of 10 µL were inoculated on Muller Hinton Agar medium. The MBC was the lowest concentration that gives no subculture. Each assay was repeated thrice.

3. Results and Discussion

3.1 Chemical Composition of Essential Oils

Analysis of the chemical composition of *A. herba-alba* essential oil by gas chromatography coupled with mass spectrometry (GC/MS) revealed the presence of nineteen compounds representing 99.57% of the total composition (Table 2).

Table 1 Region and period of collection of each plant studied.

Plant species	Region of collection	Period of collection
Artemesia herba-alba	Taroudant: Southeast Morocco	April-June 2009
Mentha pulegium	Taounate: Northeast Morocco	April-July 2009

Table 2 Chemical composition (%) of two essential oils from *Aremisia herba-alba* and *Mentha pulegium*.

Compunds	Percentage of compounds (%)	
	Artemisia herba-alba	*Mentha pulegium*
α-Pinene	0.68	0.41
Artemisia triene	3.75	
Sabinene	3.05	
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	0.93	
ç-Terpinene	0.27	
α-Thujone	59.07	
2,4-Hexadiene, 2,3-dimethyl-	11.73	
α-CAMPHOLENE ALDEHYDE	12.71	
Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl- (CAS)	0.72	
2-α-PINENE	0.25	
Patchoulane	0.30	
trans-2-Caren-4-ol	0.13	
α-Muurolene	0.16	
GERMACRENE-D	0.44	0.19
1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1aà,4aá,7à,7aá,7bà)]-	0.14	
1,1,3,3,5,5,7,7,9,9,11,11-DODECAMETHYL-HEXASILOXANE	5.24	13.37
3-Carene		0.29
(+)-Camphene		0.80
Cyclohexene,4-ethyl-3-ethylidene-4,8-		0.50
Bis(2-propylamino)-2,6-dichloro-1,5- naphoquinone		0.14
3-Cyclopentene-1-ethanol, 2,2,4-trimethyl-		0.35
l-MENTHONE		0.74
Isomenthone		0.36
Cyclohexanone, 5-methyl-2-(1-methylethenyl)-, trans		1.45
Pulegone		78.07
α-Cedrol		0.49
Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-		0.80
α-Caryophyllene		1.19
10aH-2,12a-Methano-1H,4H-cyclopropa[5,6][1,3]dioxolo[2',3']cyclopenta[1',2':9,10]cyclodeca[1,2-d][1,3]dioxin-15-ol, 1a,2,7a,13,14,14a-hexahydro-1,1,6,6,9,9,11,13-octamethyl-,acetate,[1aR-aà,2à,7aà,7bR*,10aà,12aà,13à,14aà,15S*)]-		0.13
10aH-2,12a-Methano-1H,4H-cyclopropa[5,6][1,3]dioxolo[2',3']cyclopenta[1',2':9,10]cyclodeca[1,2-d][1,3]dioxin-15-ol, 1a,2,7a,13,14,14a-hexahydro-1,1,6,6,9,9,11,13-octamethyl-, [1aR-(1aà,2à,7aà,7bS*,10aà,12aà,13à,14aà,15R*)]-		0.20
Total	99.57%	99.48%

Among these compounds, five of them can be considered as the main constituents: α-thujone (59.07%); campholene aldehyde (12.71%); 2,4-Hexadiene, 2,3-dimethyl- (11.73%); Artemisia triene (3.75%) and Sabinene (3.05%). The α-thujone was also identified as the majority constituents of the essential oil of Matmata in Tunisia (44%) [19] and Jordan (16%) [20].

The chemical composition of *Artemisia herba-alba* essential oil of Taroudant is vastly different from that of M'sila (Algeria), wich is dominated by camphor (19.4%) trans-pinocarveol (16.9%) chrysanthenone (15.8) and β-thujone (15%) [21]. Previous studies have shown that camphor was the main component of the *Artemisia herba-alba* of Algeria, Spain and Israel with a percentage between 15% and 68% [22-24].

The constituents of *Mentha pulegium* essential oil from Taounat are listed in Table 2. Chromatographic analyzes have identified eighteen compounds representing 99.48% of the total composition. The essential oil of *M. pulegium* is characterized by the presence of the pulegone as the main component with a percentage of 78.07%.

These results are similar to most of the work already done in Morocco [22-24]. Also, the work undertaken by Snoussi, et al. [25] and Hajlaoui, et al. [26] in Tunisia showed that pulegone was the major compound of *M. pulegium* with concentrations 44.27% and 61.11%, respectively. While work of Mahboubi, et al. [27] in Iran as well as those of Derwich, et al. [28] in Morocco highlighted another chemotype whose major compounds are piperitone and piperitenone with low levels of pulegone. In addition to pulegone (43.3% to 87.3%), Beghidji, et al. [29] found in different sources of Algeria, a chemotype of *M. pulegium* characterized by its richness in monoterpene (α and β-pinene, camphene, sabinene, α-terpinene and myrcene).

The chemical composition of *Artemisia herba-alba* and *Mentha pulegium* essential oils shows a large interspecies variability, due to climatic and soil variations, to the vegetative cycle, and to seasonal variation.

3.2 Antibacterial Activity

The *in vitro* antimicrobial activity of *A. herba-alba* and *M. pulegium* essential oils against microorganisms was qualitatively and quantitatively assessed by the diameter of inhibition zone, MIC and MBC values.

To determine the MIC and MBC, the authors adopted the method of broth dilution using 0.15% agar to ensure the homogeneity of the oil-water mixture [33]. The diameter of inhibition zone, MIC and MBC results are shown in Tables 3 and 4.

The data indicated that the oils exhibited varying levels of antimicrobial activity against the investigated bacteria. With the exception of *Pseudomonas* strains that shows resistance to the bactericidal and bacteriostatic action of *A. herba-alba* and *M. pulegium* essential oils, all other bacteria are inhibited at concentrations ranging from 1.25 μL/mL to 5 μL/mL for essential oil of *A. herba-alba*, and from 1.25

Table 3 Diameter of inhibition zone in (mm).

	Microorganism species	Inhibition zone diameter in (mm)		Positive control (AMX)
		A. herba-alba	*M. pulegium*	
Gram−	*E. coli* ATCC 25922	16 ± 0	15 ± 0	25 ± 0
	E. coli clinique	17 ± 0	12.5 ± 0.7	0 ± 0
	Ps. aeruginosa ATCC 27853	0 ± 0	0 ± 0	0 ± 0
	Ps. pyocyanique	8 ± 0	0 ± 0	0 ± 0
	Enterobacter cloacae	13 ± 0	11 ± 0	12 ± 0
Gram+	*St. aureus clinique*	11 ± 0	12 ± 0	12 ± 0
	St. aureus ATCC 29213	13 ± 0	15 ± 0	16 ± 0
	Enterococcus faecium	12 ± 0	12 ± 0	18 ± 0

Table 4 MIC and MBC of essential oils.

	Microorganism species	MIC (μL/mL)		MBC (μL/mL)		MBC/MIC	
		A. herba-alba	*M. pulegium*	*A. herba-alba*	*M. pulegium*	*A. herba-alba*	*M. pulegium*
Gram−	*E. coli* ATCC 25922	1.25	1.25	1.25	1.25	1	1
	E. coli clinique	2.5	1.25	2.5	1.25	1	1
	Ps. aeruginosa ATCC 27853	>20	>20	>20	>20		
	Ps. pyocyanique	>20	>20	>20	>20		
	Enterobacter cloacae	1.25	2.5	1.25	2.5	1	1
Gram+	*St. aureus clinique*	2.5	1.25	5	1.25	2	1
	St. aureus ATCC 29213	2.5	1.25	5	1.25	2	1
	Enterococcus faecium	5	2.5	10	2.5	2	1

μL/mL to 2.5 μL/mL for the essential oil of *M. pulegium*. And killed at concentrations ranging from 1.25 μL/mL to 10 μL/mL for essential oil of *A. herba-alba* and from 1.25 μL/mL to 2.5 μL/mL for the essential oil of *M. pulegium*.

Ps. aeruginosa which proved resistant to the antibacterial effect of the essential oils tested is known by a high level of intrinsic resistance to virtually all known antimicrobial compounds including essential oils [34, 35]. This resistance seems to be related with the nature of the outer membrane which is composed of lipopolysaccharides that form an impermeable barrier to hydrophobic compounds [36, 37], but this was not true about *A. herba-alba* essential oil which showed a strong antibacterial effect on Gram− bacteria.

Among the gram-positive bacteria, *Enterococcus faecium* was less sensitive to the action of the essential oils tested with MIC = 5 μL/mL for *A. herba-alba* essential oil and MIC = 2.5 μL/mL for *M. pulegium* essential oil, while *E. coli* ATCC 25922 was more sensitive with MIC = 1.25 μL/mL for both essential oils tested.

The MBC/MIC ratio is used to classify antibiotics according to their characters as bactericides (close to 1) or bacteriostatic (greater than 4) [38]. Our results showed that *A. herba-alba* and *M. pulegium* essential oils has a bactericidal activity against all bacteria tested excepted *Pseudomonas*. The presence of pulegone in *M. pulegium* essential oil and α-thujone in *A. herba-alba* essential oil may be responsible for their antibacterial activity. Indeed, it has reported that pulegone play an important role in antibacterial activity [39, 40]. However, Other Artemisia oils rich in camphor and 1,8-cineole were previously demonstrated to have potent antimicrobial activities in vitro [41, 42]. Thus, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the antimicrobial activity exhibited. Possible synergistic and antagonistic effect of compounds in the oil should also be taken into consideration.

4. Conclusions

In this work, we studied the chemical composition and antibacterial activity of the essential oil of *Artemisia herba-alba* from Taroudant and *Mentha pulegium* from Taounat. Chemical analysis by GC/MS identified respectively nineteen and eighteen constituents. The α-thujone (59.07%) was the major component of *A. herba-alba* while *M. pulegium* was dominated by pulegone (78.07%). The results obtained in this study show that the essential oils tested in vitro have significant activity against most bacteria tested.

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