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Aromatic Index of Three Essential Oils from West Algeria

Attou Amina[1*], Davenne Dominiqu[2], Benmansour Abdelhafid[3] and Lazouni Hamadi Abderrahmene[1]

[1]Department of Biology, Laboratory of Natural Products: Biological Activities and Synthesis, Faculty of Sciences, University Abou Bekr Belkaid, Algeria

[2]Department of Pharmacognosy, University of Rennes, Faculty of Pharmacy, France

[3]Laboratory Rosier Davenne, Company Specialized in Essential Oils, Avignon, France

Research Article

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***For Correspondence**

Attou Amina, Department of Biology, Laboratory of Natural Products: Biological Activities and Synthesis, Faculty of Sciences, University Abou Bekr Belkaid, P.B.119 Tlemcen, 13000, Algeria, Tel: +213552562910.

E-mail: attouamina1986@yahoo.com

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ABSTRACT

Use of medicinal plants is as old as human civilization and continuous efforts are being made to improve medicinal plants or produce their products in high amounts through various technologies, especially for searching new antimicrobial substances to combat microbes that constantly develop resistance to synthetic antibiotics. To evaluate the antibacterial activity of 3 plant essential oils (*Ammoides verticillata*, *Mentha pulegium* and *Ruta chalepensis*) against bacterial strains isolate from inpatients samples, a disc diffusion method was used, and to see the relation between activity and composition, the three essential oils was analyzed by GC/MS. The essential oil of *Ammoides verticillata* shown an excellent activity on both gram-positive and gram-negative bacteria, followed by *Mentha pulegium* that have medium effect, but the two essential oils are inactive against Pseudomonas aeruginosea. Then *Ruta chalepensis* oil is very weak or inactive on all bacteria, this give the *Ammoides verticillata* essential oil the highest aromatic average index of 0.89 on selected bacteria. The chemical composition supports the antibacterial actions of these volatile oils, the *Ammoides verticillata* oil-rich with phenolic compound especially thymol- has the best effect compared to *Mentha pulegium* oil which is characterized by the presence of monoterpenes, and at last ketones in *Ruta chalepensis* oil prevent it to have antibacterial activity

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [1]. World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants which are widely used as medicine and constitute a major source of natural organic compounds.

Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties [2,3]. Some oils have been used in cancer treatment [4]. Some other oils have been used in food preservation, aromatherapy and fragrance industries [5]. Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential [6].

In this research, we choose three aromatic plants still used in west Algeria to flavor food dishes and for their medicinal properties:

Ammoides verticillata

Briq. (Nunkha) belonging to family Apiaceae is a highly valued medicinally important. The roots are diuretic in nature and the seeds possess excellent aphrodisiac properties. The seeds contain 2% to 4.4% brown colored oil known as ajwain oil [7]. The main component of the whole plant or seeds oil is generally thymol, but significant variation of chemical composition from different regions were observed at many researches and may be considered as chemotypes as thymol, p-cymene, carvacrol and

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γ-terpinene [8]. The essential oil is used in the treatment of gastro-intestinal ailments, lack of appetite and bronchial problems. The seeds are used in small quantities for flavoring numerous foods, as preservatives, in medicine and for the manufacture of essential oil in perfumery [9].

Mentha pulegium

L (pulegium means repulse fleas) is an aromatic plant belonging to the family Lamiaceae and widespread in northern Europe, the Mediterranean region and Asia, called by locals Fliou, it is a great emmenagogue, digestive, tonic and sudorific, leaves and flowers are commonly used to repel insects, but also against colds and flu, reduce asthma attacks, combat fever and regulate menstruation, used also in some culinary preparations to flavor sauces, desserts and drinks [10]. It has been found *Mentha pulegium* L. oil from Bulgaria contains pulegone (42.9% to 45.4%); from Uruguay, pulegone (73.4%) and 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: pulegone, piperitenone and/or piperitone and isomenthone/neoisomenthol [11].

Ruta chalepensis

L, herbaceous plant with woody stem at the base, can reach 1 m in height, with aromatic foliage, and dark yellow flowers, with four or five petals; it is an ornamental plant in gardens. The name Ruta comes from the greek « rhyté » which means rescued, prevent, or "REO" means flowing certainly making reference to its emmenagogue properties, and commonly known by the RUE. In cooking, it is used to flavor sauces and prey, cheese, oil and vinegar [12-15]. It was used as emmenagogue and abortifacient, it has a clear stimulating effect on the uterus [16,17].

MATERIALS AND METHODS

Plant Material

The aerial parts of the plants were collected at flowering season (June 2013) from west Algeria, identify in the Laboratory of Botanic, University of Tlemcen, Algeria. The plant was dried at room temperature for two weeks.

The Extraction of Essential Oil

It was done by hydro-distillation of the aerial part of the plant, where 100 g of dry plant is introduced into a flask bi collar, and moistened with water; the mixture is brought to a boil for 2-3 hours. The water laden vapours of essential oil, the refrigerant passing through, condense and drop into a separator funnel; water and oil separate by density difference. The essential oil is stored in dark vials at 4 °C until use.

GC/MS Analysis

The essential oil was analyzed by gas chromatography, type Perkin Elmer CLARUS 500, with Flame Ionization Detector (FID), coupled with mass spectrometry Perkin Elmer Clarus 600, two fused silica capillary columns (30 m x 0.25 mm, 0.25 µm film thickness) are used: The Elite-WAX is a polar column with Polyethylene Glycol as stationary phase and the Elite 1 column of 100% Dimethyl Polysiloxane stationary phase as the nonpolar column. Carrier gas is Hydrogen with a flow of 45 ml/min. the initial temperature is 50 °C and rise with 3 °C/min and then held isothermally at 230 °C (20 min). injection volume: 0.2 µl of pure oil.

Identification of the components was done by the comparison of their relative retention times and mass spectra with National Institute of Standards and Technology (NIST) Atomic Spectra Database, and personal collection of the Rosier Davenne's laboratory.

Antimicrobial Activity by the Disc Diffusion Method

The antimicrobial activity of the essential oil was tested by the disc diffusion method according to the National Committee for Clinical Laboratory Standards (2001) using 100 µl of suspension of the tested microorganisms, containing 2 x 10^8 CFU/ml. Mueller–Hinton agar sterilized was distributed into sterilized Petri dishes of 9 cm diameter, after solidification they have been inoculated with the tested microorganisms. The filter paper discs (6 mm in diameter) impregnated with 10 µl of oil and then placed onto the agar. The Petri dishes were kept at 4 °C for 2 h, and then placed were incubated at 37 °C for 24 h. The diameters of the inhibition zones (mm) were measured including the diameter of discs. All the tests were performed in duplicate.

The antimicrobial activity was evaluated against a 131 hospital isolates (101 Enterobacteriaceae, 14 Staphylococci and 16 Streptococci) were collected from several types of specimens (from the Laboratory of Microbiology at the Regional Military University Hospital of Oran during 2 months from 05/01/2014 to 06/03/2014.

Aromatic Index of the Essential Oils

With the results of aromatogramme, the aromatic index is the ratio of the diameter, in millimeters, of the inhibition halo and that of an ideal essential oil whose maximum germicidal action would be in 100% of cases. First, we define the crosses index

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[18,19]. The diameter of the zone of inhibition measured in mm after 24 hours of incubation at 37 °C is symbolized by a number of cross varying from 1 to 3:

1. Cross when the inhibition halo measure one to two millimeters;

2. Crosses when measuring two to three millimeters;

3. Crosses when measuring three millimeters and above.

The symbol 3 crosses (+++) represents the maximum germicidal activity. Then added the total number of crosses obtained for each essential oil to obtain the index crosses.

To set the cross index of an ideal essential oil whose germicidal action is greatest in 100% of cases; make the product of the number of cases by the number 3. The aromatic index is obtained by the formula:

$$Aromatic\ index = \frac{\text{cross index of the tested essential oil}}{\text{cross index of the ideal essential oil, 100\% effective}}$$

RESULTS

The Content of Essential Oil

The plants collected from west Algeria have interesting yields of essential oil of about 2.58%, 1.36% and 1.90% respectively for *Ammoides vericillata, Mentha pulegium* and *Ruta chalepensis.*

Antibacterial Activity

Fist we can say that enterobacteriaceae are responsible of more than 77% of infections, *Escherichia coli* is the most isolated from several types of samples (especially urinary infections). Results in **Table 1** indicate that *Ammoides verticillata* and *Mentha pulegium* volatil oils have strong antibacterial actions against both Gram positive and Gram-negative strains, except *Pseudomonas aeruginosea*, but the essential oil extracted from *Ruta chalepensis* is very weak or inactive **(Table 2)**.

Table 1. percentage of sensibility and measurement of inhibition zone (IZ).

Strains	Essential oils		
	Ammoides verticillata	Mentha pulegium	Ruta chalepensis
Enterobacteriaceae	92.08 11<IZ<38	84.15 8<IZ<24	1.98 IZ<8
Staphylococci	85.71 24<IZ<44	78.57 9<IZ<15	21.42 IZ<10
Streptococci	93.75 25<IZ<41	68.75 8<IZ<16	25.00 IZ<9

Table 2. Crosses index (CI) and Aromatic index (AI) of essential oils

Strains	Essential oils					
	Ammoides verticillata		Mentha pulegium		Ruta chalepensis	
	CI	AI	CI	AI	CI	AI
Enterobacteriaceae	271	0.89	180	0.59	2	0.006
Staphylococci	36	0.85	23	0.54	3	0.071
Streptococci	45	0.93	24	0.50	4	0.083
The volatile oil extracted from *Ammoides verticillata* is more effective with three crosses index and aromatic index of 0.89 on *enterobacteriaceae*						

GC/MS Analysis

The chemical composition of essential oil of *Ammoides verticillata* was characterized by 12 constituents, representing 96.67% of the total oil. It is dominated by the phenolic compound thymol (45.77%), and monoterpenes: p-cymene (20.19%), limonene (15.70%) and γ-terpinene (8.42%). For *Mentha pulegium*, the analysis characterizes 17 constituents represent 92.11% of the oil, the main components are monoterpenes represented by pulegone (45,17%) and menthone (33,98%). The essential oil of *Ruta chalepensis* is characterized by the presence of more than 87% of 2-undecanone (a ketone).

DISCUSSION

An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable [20,21]. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death [22]. Gram-positive bacteria were

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more resistant to the essential oils than gram-negative bacteria [23]. In the present study, the essential oil of *Ammoides verticillata. Briq* and *Mentha pulegium. L* were active against Gram positive and Gram-negative strains but not against *Pseudomonas aeruginosea*, the essential oil of *Ruta chalepensis* was inactive or has a very weak activity.

CONCLUSION

This is related with the respective composition of the plant volatile oils, the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic interactions between components, phenolic structures such as thymol and monoterpenes are highly active against microbes due to the presence of the hydroxyl group, the inhibitory effect of phenols could be explained by interactions with the cell membrane of micro-organisms and is often correlated with the hydrophobicity of the compounds [24,25].

Despite the antimicrobial characteristics of thymol and carvacrol, Chamberlain and Dagley (1968) found that Pseudomonas strain is able to degrade thymol completely and carvacrol partially, this explain its resistance. Other researchers reported that ketones are weakly antiseptic, but they have antifungal action [26-28].

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REFERENCES

1. Tepe B, et al. In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of Thymus. J Agric Food Chem. 2004;52:1132-1137.
2. Kordali S, et al. Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish Artemisia absinthium, Artemisia dracunculus, Artemisia santonicum and Artemisia spicigera essential oils. J Agric Food Chem. 2005;53:9452-9458.
3. Sylvestre M, et al. Essential oil analysis and anticancer activity of leaf essential oil of Croton flavens L. from Guadeloupe. J Ethnopharmacol. 2006;103:99-102.
4. Faid M, et al. Almond paste: Physicochemical and microbiological characterizations and preservation with sorbic acid and cinnamon. J Food Prod. 1995;58:547-550.
5. Milhau G, et al. In vitro antimicrobial activity of eight essential oils. J Essent Oil Res. 1997;9:329-333.
6. Darokar MP, et al. Detection of antibacterial activity in the floral petals of some higher plants. Curr Sci. 1998;75:187-189.
7. Bairwa R, et al. Trachyspermum ammi. Pharmacognosy Review. 2012;6:56-60.
8. Chauhan B, et al. Composition of volatile oil of Carum copticum Benth & Hook. Fruits. IRJP. 2012;3:131-132.
9. Pruthi JS, et al. Spices and condiments, (4th ed), New Delhi: National Book Trust, India. 1992.
10. Chalchat JC, et al. Essential oil of wild growing Mentha pulegium L from Yugoslavia. J Essent Oil Res. 2000;12:598-600.
11. Mohaddese M and Ghasem H. Antimicrobial activity and chemical composition of Mentha pulegium L. essential oil. J Ethnopharmacol. 2008;119:325–327
12. Baba Aissa F. Useful Plant Encyclopedia: Flora of algeria and the maghreb, Ed: Modern Library Rouiba. 1999.
13. Iserin P. Encyclopaedia of Medicinal Plants, (2nd ed): Larousse. 2001.
14. Bonnier G. La Grande Flore en Couleur. Tome 3rd ed: Berlin. 1999.
15. Takhtajan A. Flowering Plants, (2nd ed): Springer. 2009.
16. Wiart C. Medicinal plants of the Asia Pacific: Drugs for the future? Ed: World Scientific. 2006.
17. NCCLS. Performance Standards for Antimicrobial Susceptibility Testing, 11th Informational Supplement. M100-S11. Wayne. PA. USA. 2001.
18. Belaiche P. Traité de Phytothérapie et d'Aromathérapie : Maladies Infectieuses. Tome 3. Paris. Maloine. 1979.
19. Teuscher E, et al. Plantes Aromatiques: Epices, aromates, condiments et Huiles Essentielles. Paris. Lavoisier. 2005.
20. Knobloch K, et al. Action of terpenoids on energy metabolism. Progress in Essential Oil Research: 16th International Symposium on Essential Oils. Ed. EJ Brunke. De Gruyter, Berlin. 1986;429-445.
21. Sikkema J, et al. Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem. 1994;269:8022-8028.

e-ISSN:2320-0189
p-ISSN:2347-2308

22. Denyer SP, et al. Biocide-induced damage to the bacterial cytoplasmic membrane. Mechanisms of Action of Chemical Biocides. Oxford Blackwell Scientific Publication, Oxford, UK. 1991;171-188.

23. Zaika LL. Spices and herbs: Their antibacterial activity and its determination. J Food Saf. 1988;23:97-118.

24. Dorman HJD and Deans SG. Antimicrobial agents from plants, Antibacterial activity of plant volatile oils. J Applied Microbiology. 2000;88:308-316.

25. Ben Arfa A, et al. Antimicrobial activity of carvacrol related to its chemical structure. Lett Appl Microbiol. 2006;43:149-154.

26. Numpaque MA, et al. Thymol and carvacrol biotransformation and antifungal activity against the plant pathogenic fungi Colletotrichum acutatum and Botryodiplodia theobromae. Tropical Plant Pathol. 2011;36:3-13.

27. Mailhebiau P. La Nouvelle Aromathérapie: Caractérologie des Essences et Tempéraments Humains: [Biochimie Aromatique et Influence Psychosensorielle des Odeurs]. Jakin Ed. Lausanne. 1994.

28. Haddouchi F, et al. Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria. Food Chem. 2013;141:253-258.