

ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Myrtus communis* L BERRIES GROWING WILD IN ALGERIA

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ABSTRACT

The development of microbial resistance to antibiotics is a global concern. The present study was carried out to determine the composition and the antimicrobial potential of the essential oil of *Myrtus communis* L. against 13 pathogenic strains responsible of many infections. The results show that levels of MIC observed range from 0.563 to 36 mg/ml.

Keywords: *Myrtus communis* L., essential oil, antimicrobial activity, MIC.

1. INTRODUCTION

The problems regarding application of conventional antibiotics, including antimicrobial resistance, environmental problems, cancerogenity, side effects and high costs, have reinforced a tendency to replace synthetic antimicrobials with natural alternative agents [1]. Plant based products are among the alternative agents examined in order to replace conventional antibiotics. Accordingly, extensive researches have been carried out in order to evaluate the antimicrobial effect of the essential oils and extracts which showed the ability to inhibit the growth of various pathogenic microorganisms [2].

Myrtle (*Myrtus communis* L. Myrtaceae) is an evergreen shrub which grows mainly in Mediterranean climates, distributed in Europe, Asia, Africa and America [3, 4] and has long been used by locals for its culinary and medicinal properties [5]. In Algeria, the species commonly known as Ryhan is abundant in the north of the country [6].

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It grows wild in different bioclimatic zones extending from the upper semi-arid to the lower humid. Populations of *M communis* grow at altitudes ranging from 250 to 1500 m, under a rainfall ranging from 200 to 800 mm/year.

M communis is an important medicinal and aromatic plant, because of the essential oil (EO) content in its leaf, flower and fruit glands. Leaves and berries are sources of essential oil that have medicinal properties including antimicrobial [7, 8], antioxidant and antimutagenic [9, 10, 11, 12], astringent, antiseptic, anti-hyperglycemic [8, 9, 13, 14], antinociceptive and anti-inflammatory [15], insecticide [16, 17] and nematicidal activity [18, 19]. In folk medicine, a decoction of leaves and fruits is used as stomachic, hypoglycaemic, cough and oral diseases, for constipation, appetizing, antihemorrhagic and externally for wound healing [13, 20].

In addition, myrtle berries and leaves are mostly employed for the industrial formulation of sweet liquors with digestive properties [21]. *M communis* has been used since ancient times for medicinal, food, and spice purposes [22].

The essential oil of *M communis* leaves, growing wild in Iran contains eucalyptol, α -pinene, limonene, linalool, α -terpineol, α -myrcene, cis-isoeugenol, α -terpinyl acetate and linalyl acetate as major components [23].

The antibacterial properties of the essential oils of myrtle leaves and extracts against pathogenic bacteria were reported in many studies. The obtained results are promising and estimated that they have different activities because of its different compounds [24-26]

Myrtle (*M communis*) leaves have been shown to possess antibacterial activity against both Gram positive and Gram negative microorganisms. The Gram positive microorganisms were found to be the most sensitive, particularly *Staphylococcus epidermidis* and *Staphylococcus aureus* [6]. In vitro antiviral activity against influenza type A has been documented [27].

Myrtus communis extraction solution have antiseptic activities when used as a mouth wash. No major side effects or complains following the use of plants extract rinses were reported by the patients [5].

To our knowledge, no documented reports on antibacterial activity of the essential oil of *M communis* berries are available. The aim of this study was to evaluate the antimicrobial activity of the essential oil of *M communis* extracted from berries by using in vitro method.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL AND ESSENTIAL OIL EXTRACTION

The berries of *M communis* were harvested from Ain Romana at Blida-Algeria during February, 2014. The specimens were identified at the National Institute of Agronomy in Algiers (INA).

The berries were dried in shade away from sunlight and at room temperature (25-28 °C) for five days, then grinded to fine powder using electric blender and stored in clean labelled airtight bottles.

250 g of the berries powder was distilled with 1000 ml of water for 3 hours, using a Clevenger-type apparatus according to the method recommended in British pharmacopeia [29]. The separated EO was dried over anhydrous sodium sulphate, and stored in dark glass bottles at (4±1) °C prior to use.

2.2. IDENTIFICATION OF THE ESSENTIAL OIL COMPONENTS

The EO was analysed by an Agilent Technologies 5975 mass system with Agilent Technologies 7890 GC. HP-5 MS column (30 m, 0.25 mm, film thicknesses 0.25 µm). Column temperature was from 60 °C to 280 °C. Programmed temperature increase was 4 °C /min. Split ratio was adjusted at 40:1. The injector temperature was set at 300 °C. The purity of helium gas was 99.999%, 0.1 µl samples were injected manually in the split mode. GC/MS analysis was performed on above mentioned Agilent Technologies 5975 mass system.

Mass spectra were recorded at 70 eV. Retention indices were calculated for all components using a homologous series of n-alkanes (C5-C24) injected in conditions equal to samples ones. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (Wiley/ChemStation data system) [30].

2.3. ANTIMICROBIAL ACTIVITY

2.3.1. MICROBIAL STRAINS

The essential oil was tested against a panel of microorganisms, including *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pneumoniae* (ATCC 49619), *Moraxella catarrhalis* (ATCC 49143), *Bacillus subtilis* (ATCC 11778), *Enterobacter aerogenes* (ATCC 13043), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 4404540), *Shigella flexneri* (ATCC 25936), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10239) and *Candida krusei* (ATCC 6258).

Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar (MHA), with the exception of *S. pneumoniae* (MHA containing 50 ml citrate blood/l). Yeasts were cultured overnight at 30°C in Sabouraud dextrose agar.

2.3.2. DISC DIFFUSION METHOD

The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oil in question [31]. Briefly, a suspension of the tested microorganism (0.1 ml of 10^8 cells/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15 μ l of the oil and placed on the inoculated plates. These plates were incubated at 37°C for 24 h for bacteria and, at 30 °C for 48 h, for yeasts. The diameters of the inhibition zones were measured in millimetres. All tests were performed in triplicate.

2.3.3. DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

A broth micro-dilution assay was used, as recommended by NCCLS [32], for the determination of the MIC. All tests were performed in Mueller Hinton Broth (MHB; BBL) supplemented with Tween 80 detergent (final concentration of 0.5% (v/v), with the exception of the yeasts (Sabouraud dextrose broth-SDB + Tween 80). Bacterial strains were cultured overnight at 37°C in MHB and the yeasts were cultured overnight at 30°C in SDB.

Test strains were suspended in MHB to give a final density of 5×10^5 cfu/ml and these were confirmed by viable counts. Geometric dilutions, ranging from 0.02815 to 36.0 mg/ml of the essential oil were prepared in a 96-well microtitre plate, including one growth control (MHB + Tween 80) and one sterility control (MHB+ Tween 80+ test oil). Plates were incubated under normal atmospheric conditions, at 37°C / 24 h for bacteria, and at 30°C / 48 h for yeasts. The bacterial growth was indicated by the presence of a white “pellet” on the well bottom. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate a visible growth. The microorganism growth was indicated by turbidity.

3. RESULTS AND DISCUSSION

3.1. ESSENTIAL OIL COMPOSITION

About 20 compounds, representing 82.75% of the essential oil (EO), were identified. GC/MS analyses revealed that this EO is characterized by high levels of oxygenated monoterpenes (66.9%) including -Phellandrene: 3.96%, -terpineol: 3.71, Caryophyllene: 3.62% and eucalyptol: 1.37%, followed by monoterpene hydrocarbons (22.3%) including -pinene: 10.01% and limonene: 12.93% (table 1). The sesquiterpenes represent 15% of the whole composition. Limonene and octadienol were identified as major components.

Table 1. Main components of the essential oil extracted.

	RI*	Components	Pourcentage (%)
1	7.533	-Pinene	10,01
2	7.797	-Phellandrene	3,96
3	9.830	Propanoic acid, 2-methyl-, 2-methylpropyl	1,95
4	11.553	3-Carene	2,40
5	13.183	Limonene	12,93
6	13.713	Eucalyptol	1,37
7	14.690	-Terpinene	2,87
8	15.410	-Cymene	3,09
9	15.803	(+)-4-Carene	3,33
10	27.893	Octadienol	12,85
11	24.133	Beta germacrene	1,73
12	26.450	-Terpineol	3,71
13	24.340	Caryophyllene	3,62
14	25.877	Estragole	2,27
15	25.990	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl	4,14
16	26.560	-menthenol	2,92
17	27.243	Muurolene	3,80
18	27.953	Geranyl acetate	0,97
19	29.740	Geraniol	1,68
20	33.150	Methyleugenol	3,15
Total			82.75%

*RI: Retention index determined on HP-5MS column.

The monoterpenes are widespread components of the essential oils and used as fragrances and flavours in the cosmetic, perfume, drug and food industries. The comparison of the different major constituents allows our sample to be assigned to the chemotype Limonene/octadienol, because of the high content of these two compounds. Other studies showed that among the constituents of the essential oil of the berries of *M communis*, the -pinene, myrtenol, myrtenal and myrtenyl acetate are presented [33-36].

However, this chemical composition differs from the EO of *M communis berries* harvested in Miliana, reported in a previous study [34]. However, the chemical composition of this EO differs also from the various compositions of EOs isolated from *Myrtus communis* leaves and berries growing wild all around the Mediterranean basin [19, 7, 21]. This could be due to a number of factors, including the geo-climatic locations and growing conditions (concentration of nutrients, temperature, humidity, soil type, day length, climate, altitude, amount of available water,...ect). The chemical composition also depends on season or vegetative period of plant i.e. before or after flowering [37, 38-42]. According to these factors, plant biosynthetic pathways can change the relative proportions of the essential oil components. These variations in chemical composition led to the notion of chemotypes, which are generally defined as a distinct population within the same species that produces different chemical profiles for a particular class of secondary metabolites [43]. Thus, the same species of plant can produce a similar essential oil, but with different chemical composition and therapeutic activities. Essential oil composition also depends on the plant parts used for oil preparation.

All *M communis* L. essential oil compounds may be classified into three main categories: terpenes (monoterpene hydrocarbons and sesquiterpene hydrocarbons), terpenoids (oxygenated monoterpenes and oxygenated sesquiterpenes) and phenylpropanoids [38, 42, 44-46], but also into hydrocarbons and oxygenated compounds [40, 47-51].

3.2. ANTIMICROBIAL ACTIVITY

The in vitro antimicrobial activity of *M. communis* EO was assessed by the disc diffusion and micro-dilution methods against 13 strains. Antibacterial activity was expressed as diameter of the inhibition zones and MIC values (Tables 2). The EO of *M communis* exhibited varying levels of antimicrobial activity against the investigated pathogens. The diameter of the inhibition zones values of different concentrations were between 11.0 and 24.0 mm.

In general, the EO showed relatively high inhibitory activities against the bacteria tested (Table 2). The MICs were within concentration ranges 0.563-36 mg/ml (Table 2).

Table 2. Antimicrobial activity EO and extract of *M communis* by disc diffusion assay.

Pathogens	Growth inhibition* (mm)	Positive control (Penicillin G)	MIC (mg/ml)
<i>Staphylococcus aureus</i>	19.50±1.13	25	1.125
<i>Staphylococcus epidermidis</i>	18.00±0.50	22	2.25
<i>Streptococcus pneumoniae</i>	24.00±1.31	20	0.563
<i>Moraxella catarrhalis</i>	15.50±1.30	-	4.5
<i>Bacillus subtilis</i>	14.00±0.65	10	9.0
<i>Enterobacter aerogenes</i>	13.00±0.47	12	18.0
<i>Escherichia coli</i>	20.00±0.20	-	1.125
<i>Salmonella typhimurium</i>	14.50±1.20	-	4.5
<i>Shigella flexneri</i>	15.50±0.24	-	4.5
<i>Klebsiella pneumoniae</i>	16.00±0.33	-	4.5
<i>Pseudomonas aeruginosa</i>	13.50±0.65	-	18.0
<i>Candida albicans</i>	12.00±0.24	-	36
<i>Candida krusei</i>	11.00±1.12	-	36

Values are expressed as mean±SD; * Diameter on inhibition zone including well diameter of 6mm; Penicillin G (10UI) is used as a positive reference standard for bacterial strains

Results obtained from disc diffusion method, followed by measurements of MIC, indicate that *S pneumonia* is the most sensitive microorganism with the lowest MIC values (0.563 mg/ml) in the presence of the EO of *M communis* (Table 2). *E coli*, *S aureus* and *S Epidermidis* were other sensitive ones against the EO with MICs values included between 1.125 and 2.25 mg/ml. A moderate activity was observed against six Gram negative microorganisms (*M catarrhalis*, *B subtilis*, *E aerogenes*, *S thyphimurium*, *S flexneri*, *K pneumonia* and *P aeruginosa*) known for their resistance to many antibiotics. As far as this report is concerned, a very weak antifungal activity was observed against *C albicans* and *C krusei*. These results support the use of this species in traditional medicine for the treatment of cough and oral diseases.

The EO that we used for antimicrobial in vitro assay contained a high quantity of monoterpenes that, according to literature, do have antimicrobial activity. The antibacterial activity of *M. communis* essential oil may be attributed to the high level of Limonene, a compound with known antimicrobial properties. As in [52], the myrtle oil compounds oxygenated terpenes, such as eucalyptol, -cymene and -terpineol, exhibit potent antibacterial activity. The antimicrobial activity of most terpenoids is linked to their functional groups and it has been

shown that the hydroxyl group of phenolic terpenoids and the presence of delocalized electrons are important for antimicrobial activity.

In vitro tests indicated that terpenes are inefficient as antimicrobials when applied as single compounds [53-55], while certain terpenoid components of essential oils can act as uncouplers, interfering with proton translocation over a membrane vesicle and subsequently interrupting ADP phosphorylation [56].

There are published papers dealing with antimicrobial activity of essential oil principal components. Regarding the mechanism of action of these components once they crossed the microbial cellular membrane, interactions with membrane enzymes and proteins would cause an opposite flow of protons, affecting cellular activity [57]. The mechanisms by which essential oil can inhibit microorganisms vary a lot. In some cases it may be due to the hydrophobicity of the EO which penetrates into the lipid bilayer of the cell membrane and makes the cells more permeable, leading to leakage of vital cell contents [58, 59].

4. CONCLUSION

Our results demonstrated that EO of *Myrtus communis* berries exhibited a significant antimicrobial activity against a range of pathogenic bacteria and yeasts. Moreover, Additional investigations on curative claims of *M communis* volatile oils are needed to investigate these issues and to complement the considerable number of analytical and in vitro bioactivity researches that are being carried out on these natural fragrances. These results lay the ground work for further studies based on the molecular mechanisms underlying the antimicrobial effect of the EO. This is of particular interest if we consider that the myrtle berries are already used in folk medicine.

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