

Comparative Chemical Composition and Antibacterial Activities of *Myrtus communis* L. Essential Oils Isolated from Tunisian and Algerian Population

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Abstract

The chemical composition of Essential oils isolated by hydrodistillation from leaves of Tunisian and Algerian *Myrtus communis* L. population was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Twenty three compounds were identified, representing 93.73% of total oil, which was found to be rich in monoterpenes hydrocarbons (53.38%) particularly α -Pinène (35.30%) and α -limonène (14.76%). The physico-chemical properties were determined. The percentage of all components varied within and among population. The highest percentages of α -Pinène (45.4%) and 1,8-Cineole (35.7%) were observed in the Algerian population. The percentage of α -limonène was significantly higher in the Tunisian population (18.16%). The study of antibacterial activity revealed that 10 μ l of *Myrtus communis* L. essential oil significantly inhibited the growth of five tested bacteria especially *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella* sp., and *Listeria* sp.

Keywords: *Myrtus communis* L; Myrtaceae; Essential oils; Hydrodistillation; α -Pinène; 1,8-Cineole; Antibacterial activity

Introduction

Essential oils are volatile organic compounds found in various plant tissues such as fruits, leaves, flowers, bark, stem, seeds, wood and roots. The quality of essential oils depends on the several factors including the part of the plant used, the plant variety and its country of origin, the method of extraction and the refining process [1]. The volatile oil isolated from aromatic plants provides a number of ecological advantages to the plant. For example, they provide protection against predators and other enemies, and mediate plant-plant interactions including allelopathy [2,3]. Until recently, essential oils have been studied mostly from their flavor and fragrance viewpoints only for flavoring foods, drinks and other goods [4]. Actually, however, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multi-purpose functional use [5]. Specially, Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the family of Myrtaceae that grows spontaneously throughout the Mediterranean area. In Italy it grows along the coast and in the inner hills, and it is spread especially in the islands, where it is one of the most characteristic species. *Myrtus communis* L. is an annual plant that has been used since ancient times for medicinal, food and spices purposes. The leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils [6,7]. The fruits of this plant are mostly composed of volatile oils, tannins, sugars, flavonoids and organic acids such as citric and malic acids [6,8]. In past times, ripe fruits were used as food integrators because of their high vitamin contents. The fruit decoction was used to bathe new-borns with reddened skin, while the decoction of leaves and fruits was useful for sore washing. The decoction of the leaves is still used for vaginal lavage, enemas and against respiratory diseases [9,10]. The essential oil obtained from the leaves by steam distillation is also important in perfumery [6]. Besides, the *M. communis* L. essential oil obtained from the leaves was used in the past for the treatment of lung disorders [11]. The essential oil obtained from this species has been widely investigated. Its composition is quite variable [12-14]. One of the main constituents of myrtle essential oil is 1,8-cineole [15]. The isolation of essential oils from *Myrtus communis* leaves is usually obtained by

hydrodistillation method with a Clevenger-type apparatus, according to the Italian Official Pharmacopoeia. The chemical composition of the essential oils, analysed by Gas/Cromatography (G/C), generally exhibits α -pinene, 11%; 1,8-cineole, 16%; linalool, 12%; α -terpineol, 7%; and limonene, 5% [16]. The oil composition is highly influenced by the geographic origin of the plant [17,18]. According to the numerous published papers on the topic, myrtle essential oil possesses strong antimicrobial activity that makes it a valuable raw material for the cosmetic, pharmaceutical and foodstuff industries [19,20]. Earlier studies showed the antimicrobial properties of Myrtle essential oil against several clinical strains and in particular against *Helicobacter pylori* [21]. Moreover, Romani et al. [22] showed the biological activities of tannins, including anticancer and antioxidant. Other, studies have indicated that myrtle plant could be used as a source of antioxidant and antibacterial activities [23]. Generally, these studies were mainly focused towards the phenolic compounds in myrtle extracts [17].

Thus, essential oils are gaining remarkable interest for their potential multipurpose use as antioxidant, antibacterial, and antiseptic agent [24,25].

The present work therefore, attempts to determine the chemical composition and evaluate antibacterial activity of essential oil isolated from leaves of Tunisian and Algerian *Myrtus communis* L. population. So, we assessed the antibacterial activity of the essential oils against five tested bacteria.

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Materials and Methods

Plant material

Samples of *Myrtus communis* L., were collected at random from Tunisian and Algerian population. Ten individuals for each population were used for the analysis of their essential oil. Because the species multiplies through both sexual and vegetative reproduction, samples were collected at a distance of >20 m apart to avoid sampling from the same parent. Vouchers specimens are deposited in the herbarium of the National Institute of Applied Science and Technology.

Isolation of the essential oils

The essential oils were extracted by hydrodistillation of fresh plant material using a Clevenger-type apparatus. For each sample, the oil was isolated from 20 g of fresh leaves, which had been ground in liquid nitrogen. The powder obtained was macerated in 200 ml n-hexane for 12 h. After filtration, the essential oil was dried using a rotary evaporator (60°C). The yield represents 0.5% of the fresh sample weight. The oils were stored at 4°C in the dark until analysis.

Analysis of the essential oils

Oils were analyzed using an Agilent 6980 series gas chromatograph, equipped with a flame ionization detector (FID, 280°C) and a split-splitless injector (220°C) attached to an HP INNOWAX column (30 m, 0.25 mm; 0.25 µm film thickness). Helium (He) was used as the carrier gas at a flow rate of 2 ml/min. Temperature was programmed to range from 50 to 220°C at 8°C/min and the final temperature was held for 10 min. A 2 µl aliquot of sample, diluted in 10 ml n-hexane, was injected. GC-MS analysis was performed on an HP 5890 series II instrument under the following conditions: injection of 2 µl of sample; HP-5MS capillary column (30 m×0.25 mm; coating thickness, 0.25 µm); oven temperature programmed to range from 50 to 240°C at a rate of 5°C/min; carrier gas, He; flow rate, 1.2 ml/min; split ratio, 1:60; ionization energy, 70 eV; scan time, 1 s; mass range from 40 to 300 m/z. The mass spectrometer was a HP 5972 and the total electronic impact mode at 70 eV was used.

Physicochemical properties of essential oil

The essential oil components for each individual were identified through retention indices, determined by standards injected under the same chromatographic conditions. In addition, the compounds were also identified by comparison of their retention indices with those reported in the literature and by comparison of their mass spectra with the HP chemstation database HP NBS 75 K. L. Percentages of compounds were determined from their GC peak areas. Injections were repeated twice for each sample.

Antibacterial activity

Microbial strains: The antimicrobial activity was tested using oils from each population against *Escherichia coli* ATCC10536, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Salmonella* sp., and *Listeria* sp. Bacterial strains were cultured overnight at 37°C in nutrient broth (Scharlau Microbiology, Spain). For the antimicrobial tests, LB medium was used.

Antibacterial activity assays

The antimicrobial activity of oils was determined through the agar disc diffusion and the broth dilution methods. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. All tests were performed in duplicate.

Disc diffusion method: The disc diffusion method was made according to Sacchetti et al. [26]. Tryptic soy agar (TSA) was distributed into sterilized Petri dishes with a diameter of 9 cm (15 ml). One hundred microliters of suspension of the tested microorganisms, containing 5×10^5 CFU/ml of bacterial strains was poured in TSA. The filter paper discs (6 mm in diameter) were individually impregnated with 10 µl of each oil and then placed onto the agar plates. Before incubation, all Petri dishes were kept in the refrigerator (4°C) for 2 h and incubated after at 37°C for 24 h for bacteria growth. After incubation, the diameters (mm) of the inhibition zones were measured including the diameter of discs. The antimicrobial potentials were estimated according to indices reported by Rodriguez Vaquero et al. [27]. Gentamycin (30 µg/disc) and DMSO served as a positive and negative control.

Determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations: Serial dilutions of 1/10, 1/20 and 1/30 were made with dimethylsulphoxide (DMSO) and 10 µl of each dilution were put down on sterile paper discs (6 mm diameter) placed on the surface of inoculated Petri dishes. The MIC was defined as the lowest concentration of the total essential oil at which the microorganism does not demonstrate visible growth [28]. Referring to results of the MIC assay, the minimum bactericidal concentration (MBC) was determined. Fifty microliters from each dilution of essential oil, showing growth inhibition zone in disc diffusion method, were added to 5 ml of TSA broth tubes then incubated at 37°C for 24 h in an incubator shaker. From tubes without microbial growth, 0.1 ml of cells was spread on TSA agar plates. MBCs were determined as the highest dilution at which no growth occurred on the plates.

Statistical analysis

For each sample from each locality, we calculated the mean percentage of compounds established on the three samples. The variations of oils among populations and of their biological activities were tested by a variance analysis at $p < 0.001$ and $p < 0.05$ [29]. The significance of differences between means was determined by Duncan's multiple range test (DMRT) at $p < 0.05$.

Results

Chemical composition of *Myrtus communis* L. essential oil

Essential oil obtained by hydrodistillation of fresh leaves of *Myrtus communis* L. had a light yellow color and a pungent odor. The chromatographic analysis showed a complex mixture of components with a consistent fraction of monoterpenes and sesquiterpenes. The list of the compounds, in order of elution, and the quantitative data, are reported in Table 1. Twenty three oil compounds were identified accounting for 93.73% of total oil, while 6.27% of the oil remained unidentified. The monoterpenes hydrocarbons displayed the highest contribution (53.38%) among which α -Pinène (35.30%) and α -limonène (14.76%) were the most abundant. Whereas, the oxygenated monoterpenes represented only 34.31% of the total oil. In comparison with monoterpenes, the esters were relatively weak with 4.31%. The sesquiterpene were the poorest fraction (1.73%). The percentage of all components varied within and among population (Table 1). The highest percentages of α -Pinène (45.4%) and 1.8-cineole (35.7%) were observed in the Algerian population, respectively. The percentage of α -limonène was significantly higher in the Tunisian population (White Ballouta "WB": 18.16%).

Antibacterial activity assays

The *in vitro* antimicrobial activity of the essential oils estimated by

Compound	RI	Populations			P % (Over all population)
		Algerian population	Tunisian population (BB)	Tunisian population (WB)	
α-Thujene	931	0.32e	0.27f	0.21f	0.26
α-Pinene	938	45.4a	30.50a	30.00a	35.30
β-Pinene	978	0.22f	0.32f	0.48f	0.34
α-Phellandrene	1006	0.11f	0.22f	0.23f	0.18
3-Carene	1008	0.18f	0.29f	0.34f	0.27
2-Carene	1012	0.85e	1.50e	0.83f	1.06
ρ-Cymene	1026	0.48e	0.95e	0.55f	0.66
α-Limonene	1031	8.36c	17.78b	18.16b	14.76
1-8-cinéole	1040	35.7b	20.90b	22.30b	26.30
β-Ocimene	1050	0.38e	3.40d	3.18d	2.32
α-Terpinolene	1088	0.45e	0.41	0.41f	0.42
β-linalool	1105	1.93d	10.1c	9.24c	7.09
Trans-Pinocarveol	1161	0.15f	0.29e	0.23f	0.22
Terpinen-4-ol	1177	0.35e	0.29e	0.27f	0.30
Myrtenyl acétate	1335	0.13f	0.84e	0.89f	0.62
β-caryophyllène	1418	0.34e	0.39e	0.37f	0.36
α-Humulene	1454	0.30e	1.30e	1.17e	0.92
β-caryophyllene epoxide	1534	0.71e	0.34f	0.42f	0.49
Caryophyllene oxide	1581	0.22f	0.50f	0.63f	0.45
α-Caryophyllene	1600	0.11f	tr	tr	0.03
β-fenchyl alcohol	1633	0.46e	tr	tr	0.15
α-Amorphene	2018	0.12f	tr	tr	0.04
Hydroquinone	2032	0.44e	1.58e	1.41	1.14
Total identified (%)		97.71	92.17	91.32	93.73
Oil yield		0.54	0.48	0.50	0.50

Table 1: Chemical composition of *Myrtus communis* L. essential oils isolated from Algerian and Tunisian population: (Black Ballouta: BB and White Ballouta: WB).

Bacteria	Source no.	Inhibition zone (mm)											
		Populations											
		Algerian population				Tunisian population (BB)				Tunisian population (WB)			
		C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
Gram-negative													
<i>Escherichia Coli</i>	ATCC10536	15b	8c	8c	8c	20a	8c	7c	NA	15b	9c	10c	8c
<i>Salmonella</i>	-	14b	7c	7c	6c	12b	8c	6c	6c	11c	6c	9c	12b
Gram-positive													
<i>Staphylococcus aureus</i>	ATCC 6538	10c	7c	7c	8c	23a	22a	8c	1d	21a	19a	8c	8c
<i>Bacillus subtilis</i>	ATCC 6633	9c	8c	8c	8cc	12b	9c	7c	6c	10c	8c	8c	7c
<i>Listéria</i>	-	10c	7c	7c	7c	15b	8c	7c	7c	7c	9c	NA	8c

Where C1: 10µl of essential oil and C2: 1/10, C3: 1/20, C4: 1/30.

Values followed by the same letter under the same line are not significantly different (Duncan's multiple range test at P>0.05).

Table 2: Antibacterial activity estimated by diameter of inhibition of *Myrtus communis* L. essential oils isolated from Algerian and Tunisian population: (Black Ballouta: BB and White Ballouta: WB).

the diameter of inhibition varied according to populations and bacteria strains (Table 2). The highest activity was observed against *E. coli* ATCC10536 with strongest inhibition zones (15 mm) recorded for the essential oils isolated from Algerian population of *Myrtus communis* L.

However, for the essential oils isolated from Tunisian population (Black and White Ballouta: "BB" and "WB") of *Myrtus communis* L., the highest activity was observed against *Staphylococcus aureus* ATCC 6538 with strongest inhibition zones (23 and 21 mm respectively) the variety: WB). Also our results showed a significant inhibition of antimicrobial activity with the dilution of the essential oil for the tunisian and algerian population (Table 2).

Discussion

The essential oil of *Myrtus communis* L. was previously investigated. In agreement with our result, most studies indicated that α-Pinene

and 1,8-Cineole (35.30% and 26.30% respectively) were the major components of the *Myrtus communis* L. oil [30-35]. Nevertheless, in our study, the percentage of all components varied within and among population (Table 1). The highest percentages of α-Pinène (45.4%) and 1,8-cineole (35.7%) were observed in the Algerian population. The percentage of α-limonène was significantly higher in the Tunisian population (18.16%). This significant difference in the chemical composition of the extracted oil according to populations may be due to one of the following reasons:

- Nutrients of different soils and their accumulation in the leaves may result in different metabolism and production of different bio-products and volatile oils,
- The change in genes through generations and hybridizations, naturally and induced, may result in production of a variety of volatile

oils compared to nectars or those of different habitat, the out-crossing species breeding system would have to play a major role in this genetic diversity [30,36].

- Acclimation of species to the environment in which it was grown in the past,
- Differences may be due to different ecotypes of the species.

These differences could be related to the environmental factors (climate, season and soils), the genetic diversity of the species, the geographic conditions, the harvest period and the isolation technique [37]. These factors influenced the available resources, the plant's biosynthetic pathways, the metabolism and consequently the relative proportion of the main characteristic compounds, their nature and their production. This leads to the existence of different origin, as well as seasonal variation throughout the plant's vegetative cycle [38]. Also, it is likely that the observed heterogeneities between populations could correspond to particular adaptive selection pressure traits (climate, soil, etc.).

Subsequently, we study antibacterial activity of *Myrtus communis* L. essential oils isolated from Tunisian and Algerian population.

For the antibacterial activity, we revealed that this activity varied according to population and bacteria strains (Table 2). The highest activity was observed against *E. coli* ATCC10536 with strongest inhibition zones (15 mm) recorded for the essential oils isolated from Algerian population of *Myrtus communis* L. However, for the essential oils isolated from Tunisian population (Black and White Ballouta: "BB" and "WB") of *Myrtus communis* L., the highest activity was observed against *Staphylococcus aureus* ATCC 6538 with strongest inhibition zones (23 and 21 mm respectively) the variety: WB). The results presented in this study (Table 2) are in line with the small number of published papers on the effects on bacterial growth of *Myrtus communis* L. essential oils. Salvagnini et al. [39] reported the antimicrobial activity of essential oil from leaves of *Myrtus communis* L. against *Staphylococcus aureus*, *S. epidermidis*, *E. coli*, *B. subtilis* and *Serratia marcescens*. Yadegarinia et al. [20] have demonstrated the activity of *Myrtus communis* L. essential oil against *E. coli*, *S. aureus* and *Candida albicans*.

Besides, the antibacterial effect of *Myrtus communis* L. essential oils have been recently reported by Akin et al. and Janetti et al. [4,11]. The antibacterial activity of Myrtle infusions was also previously investigated [40] and their effects are generally attributed to their major components.

Nevertheless, in this study, we will find reports for the first time of the Comparative chemical composition and antibacterial activity of *Myrtus communis* L. essential oils isolated from Tunisian and Algerian population.

Our study showed that the composition of *Myrtus communis* L. essential oil is rich in oxygenated monoterpenes, especially 1,8-Cineole and α -Pinene which are known to possess a significant antibacterial activity [11]. However, it is difficult to compare the data with the literature because several variables influence the results, such as the different chemical composition due to the environmental factors (such as geography, temperature, day length, nutrients, etc) of the plant [4]. Our study on Tunisian and Algerian essential oil of *Myrtus communis* L. obtained by hydrodistillation demonstrates a high variation in the composition of oils and their biological activity potentials according to populations. This points out the importance of the genetically and morphologically characterization of varieties within the species and

of populations within varieties wherever, antimicrobial activity of oils were determined. Algerian populations were chemically distinct and showed oils rich in α -Pinène (45.4%) and 1,8-cineole ((35.7%).

Based on our preliminary results, the essential oils of *Myrtus communis* L. could be for various commodities of medicinal and pharmacological attributes. Besides, the ease of cultivation and rapid growth of *Myrtus communis* L. makes it, potentially, a very valuable natural resource for the commercial production of pharmaceuticals, over and above the present production of Myrtle oil for medicinal purposes. It is likely, that in the years ahead *Myrtus communis* L. metabolites other than volatile constituents will form part of the armory drugs available to the physician for the treatment or prevention of human diseases.

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