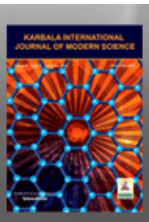

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Chemical composition and antibacterial activity of the essential oil of Myrtus communis leaves

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Chemical composition and antibacterial activity of the essential oil of Myrtus communis leaves

Abstract

The aim of this work is to determine the yield of the essential oil of the *Myrtus communis* leaves, to identify its chemical composition and to evaluate its antibacterial properties. The plant is harvested from Sidi Ahmed Chrif, a region in Ouazzane, Morocco. The extraction of the essential oil was carried out by hydrodistillation in a Clevenger apparatus type. The average yield was 0.7%. The analysis of this oil by Gas Chromatography coupled with Mass Spectrum (GC/MS) allows the identification of 32 compounds. Eucalyptol was the main compound with 42.43%, followed by myrtenyl acetate (21.25%) and α-pinene (19.39%). Myrtle essential oil has a moderate inhibitory activity against *Staphylococcus aureus* (18 mm) with a minimum inhibitory concentration (MIC of 1.32 mg/mL) followed by *Acinetobacter baumannii* (15 mm, MIC of 2.64 mg/mL) and lately *Klebsiella pneumoniae* and *Staphylococcus epidermis* share the same inhibition diameter (11mm and even MIC 6.6 mg/mL). While *Pseudomonas aeroginosae* was very resistant to this essential oil. We have also noticed the existence of a bactericidal activity against the four previously mentioned bacteria.

Keywords

Myrtus communis, Essential oil, Chemical composition, Antibacterial activity

Authors

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1. Introduction

Since their discovery in the early twentieth century, antibiotics have allowed a great advance in therapeutics and have contributed to the rise of modern medicine. The introduction and use of antibiotics in the clinic have considerably reduced mortality due to previously incurable diseases [1]. But unfortunately, the emergence of antibiotic-resistant bacteria has put an end to this wave of optimism. Faced with this resistance, health professionals and researchers throughout the world have taken a major interest in aromatic and medicinal plants [2], whose antimicrobial properties are mainly due to the fraction of the essential oil [3]. Over the last decade, several clinical studies have shown antimicrobial activity of multiple essential oils [4], the later are concentrated substances fragrant whose development has affected several fields ranging from agri-food to chemical and pharmaceutical industries [3,5].

Myrtle (*Myrtus communis*) is a well-known medicinal and aromatic plant, belonging to the family of myrtaceae [6], which comprises about 2 genera and more than 1 specie [7]. This plant is an evergreen shrub, petiolate oval, lanceolate 3 cm long by 1 cm wide. They have a bright green color [8]. Its stems are very numerous and ramified. The flowers are white solitary, pedunculate arranged in the leaf axils [9]. Essential oils of leaves and fruits are widely used by Moroccan people for their medicinal treatment. They are used for their digestive [10], anti-spasmodic [11], Antiseptic and antimicrobial properties [12], antioxidant [13] and Hemostatic activity [14]. Myrtle is also known for its anti-genotoxic properties [15], anti-inflammatory [16], antidiabetic [17], hypo-cholesterolemiant [18], scolicidal effect [19] and in the prevention and treatment of Alzheimer's disease [20].

The objective of this work is to calculate the yield of *M. communis* essential oil leaves, to determine their chemical composition and antimicrobial activity.

2. Material and methods

2.1. Plant material

The leaves of *M. communis* were harvested in June 2015 in Sidi Ahmad Chrif region (Ouazzane, Morocco), and dried in the shade in a dry and ventilated place for two weeks before extraction of the essential oil.

2.2. Extraction of the essential oil

The essential oil was obtained by hydro-distillation in a Clevenger apparatus type [21], For this, a plant mass of 100 g is immersed in distilled water in a 2-L flask. The whole is then boiled (100 °C) for 3h. The oil obtained was recovered, dehydrated with sodium sulfate anhydrous (Na_2SO_4) and stored in the refrigerator (+4 °C) in dark bottles to preserve them from light and heat [22].

2.3. Microbiological tests

The microorganisms used to undertake microbiological tests are: *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii, Staphylococcus epidermidis, Klebsiella pneumoniae* and *Pseudomonas aeroginosae*. They were isolated from several pathological samples (urinary, intestinal, respiratory, skin, etc …). After the isolation and identification of these germs at the El Idrissi Hospital in Kenitra, they were maintained by transplanting on nutrient favorable agar for their growth for 24 h in the dark at 37 °C (Table 1).

2.4. Performance calculation

The yield is defined according to the AFNOR (1986) [23] standard, as being the ratio between the mass of essential oil obtained after the extraction (M') and the mass of the plant material used (M).

The yield is calculated by the following formula (1):

$$R_{HE}(\%) = (M'/M) \times 100 \quad (1)$$

R_{HE} (%): Yield of the essential oil.
M': mass of the essential oil.
M: mass of dry plant material.

2.5. Chemical composition of the essential oil

Analysis of the chemical composition of *M. communis* essential oil was carried out by gas chromatography (Clarus 580) coupled with standard mass spectrometry of Clarus SQ 8S (GC/MS). The apparatus is equipped with a Rxi®-5 ms capillary column (Crossbond®5% diphenyl/95% dimethylpolysiloxane), 30 m long, 0.25 mm diameter and 0.25 µm film

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Table 1
Chemical composition of *Myrtus communis* essential oil.

Retention Time	Constituent	Area %
5.141	2-hexenal, (E)	0.11
6.367	Propanoic acid,2-methyl-,2methylpropyl ester	0.36
6.684	Bicyclo (3,1,0) hex-2-ene,2-methyl-5-(1-methylethyl)	0.05
6.867	α-pinene	**19.39**
7.151	Camphene	0.08
7.255	Bicyclo (3,1,0) hex-2-ene,4-methylene-1-(1-methylethyl)	0.10
7.730	Bicyclo (3,1,1) heptane,6,6-dimethyl-2-methylene, (1S)	0.27
7.972	β-myrcene	0.13
8.014	3-octadecyne	0.06
8.201	Butanoic acid,2-methyl,2-methylpropylester	0.26
8.702	Benzene,1-methyl-3 (1-methylethyl)	0.43
8.893	Eucalyptol	**42.43**
10.169	1,6-octadien-3-ol,3,7-dimetyl	1.37
10.244	Butanoic acid,2-methyl,2-methylbutyl ester	0.31
11.020	Bicyclo (3,1,1) heptane-3-ol,6,6-dimethyl-2-methylene	0.55
11.741	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl), (R)	0.34
11.887	Naphthalene	0.44
11.995	α-terpinol	4.47
12.112	Estragol	2.13
13.075	2,6-octadien-1-ol,2,7-dimethyl	0.27
13.967	Pinocarvyl acetat	0.54
14.459	Myrtenyl acetat	**21.25**
15.301	Geranyl acetat	2.08
15.430	Bicyclo (3,1,1) heptane-2-methanol,6,6-dimethyl acetat	0.19
15.676	Methyl eugenol	1.88
17.361	Phenol,2,6-dimethoxy	0.30
17.615	Durohydroquinone	0.74
19.228	1,2,4-Cyclopentanetrione, 3-(2-pentenyl)	0.21
19.466	2,3,7-trimethyl-3-vinyl-oct-6-enoic acid	0.07
19.724	2R-acetoxymethyl-1,3,3-trimethyl-4T	0.09
20.800	Quinoline, decahydro-2,5-dipropyl	0.04
20.900	Cyclohexanecarboxylic acid,4-propyl-2,3-dicyano-4-	0.03

Bold values represent the majority compounds of the essential oil.

thickness. The injection is done by split mode, the temperature programming consists of an elevation of 50 °C–290 °C at 5 °C/min. The carrier gas used was the Helium with a flow rate of 1 mL/min. The spectra were recorded in electronic impact mode with ionization energy of 70 eV. The volume of the sample injected is 1 μL of the essential oil diluted in hexane.

2.6. Antimicrobial activity

2.6.1. Evaluation of antimicrobial activity by agar plate diffusion method

In this test, Mueller Hinton Agar was seeded with 24 h of bacterial suspension containing 10^8 CFU/mL of bacteria [24]. A sterile Wathman disk of paper 6 mm in diameter, previously impregnated with 5 μL of essential oil was deposited on the agar. These Petri dishes, after remaining at 4 °C for 2 h, were incubated at 37 °C for 24 h [25]. After, a zone or a clear halo is present around the disk if the essential oil inhibits the bacterial development. The larger the area surrounding the disk, the more sensitive the germ is [26]. All tests were repeated three times.

2.6.2. Determination of minimum inhibitory concentration (MIC)

The method of Remmal and Satrani et *al*. [27,28] is used to determine the MIC of our extract. Due to the immiscibility of the essential oils to the water and to the medium culture, therefore the emulsification is carried out in a 0.2% agar solution.

Dilutions are prepared at 1/10, 1/25, 1/50, 1/100, 1/200, 1/300 and 1/500 in the above solution. In test tubes, each containing 13.5 mL of Mueller Hinton medium, sterilized in an autoclave (20 min at 121 °C.) and cooled to 45 °C, 1.5 mL of each of the dilutions were added so as to obtain a final concentration of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v/v), which corresponds to the following concentrations 6.6, 2.64, 1.32, 0.66, 0.33, 0.22 and 0.13 mg/mL.

The tubes are then stirred well before being poured into petri dishes.

Controls, containing the medium culture and the 0.2% agar solution alone, were also prepared. The minimum inhibitory concentration (MIC) is the lowest concentration of the essential oil wich inhibits any visible growth to the naked eye after 16–20 h of incubation at 37 °C [28].

2.6.3. Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration MBC corresponds to the lowest concentration of essential oil capable of destroy more than 99.9% of the initial bacterial inoculum. It is reported to be the lowest concentration where no growth is observed after transplanting in fresh nutrient medium [29].

The method consists of remaking new streaks on those of dishes that have no bacterial growth obtained from the MIC method, wich is cultivated on petri dishes containing Mueller-Hinton Agar only, then the dishes are incubated at 37 °C for 18–24 h. And this according to the method advocated by Andrews and Cosentino et *al*. [30,31]. The tests were performed in triplicate. The MBC of the essential oil is deduced from the first dish devoid of bacteria.

3. Results and discussion

3.1. Organoleptic properties and income

The essential oil is a yellowed-color and smells very aromatic. The extraction yield of the essential oil from the dry material is 0.7%. Which is considered important compared to that obtained from the Chefchaouen region of Morocco with a yield of 0.3% [21], we also note a superiority compared to the yield obtained in Algeria whit a percentage of 0.32% [22] and the one obtained from the region of Taounate in Morocco with a percentage of 0.68% [32]. However, El Beyrouthy and Arnold-Apostolides, in Lebanon reported higher yield, with a percentage of 1% [33]. The essential oil yield dependents on several factors, such as the crop season of the plant material, the origin of the plant, the method extraction, and environmental factors such as temperature and soil quality [34,35].

3.2. Chemical composition of the essential oil of M. communis

The chromatographic analysis of the essential oil of *M. communis* leaves, collected from the region of Sidi Amed Chrif (Ouazzan, Morocco), showed 32 compounds (Table 1), wich representing about 99.87% of the total identified compounds. From Table 1 and Fig. 1, we noted that the major components are eucalyptol, myrtenyl acetate and α-pinene (Fig. 2), with values of 42.43%, 21.25%, 19.39% respectively. Our oil also contains α-Terpinol (4.47%), estragol (2.13%), geranyl acetat (2.08%), methyl eugenol (1.88%) and 1,6-octadien (1.37%). A literature survey revealed that *M. communis* essential oil has been the focus of several earlier studies from different parts of the world, and it appears that the essential oil composition is greatly influenced by climatic, seasonal, geographical or genetic differences [36,37]. Thus, myrtenyl acetate, which was the major constituent of *M. communis* essential oil from Tunisia [38], Morocco [39], Spain [40], and Zakynthos [41] was not detected in our study,

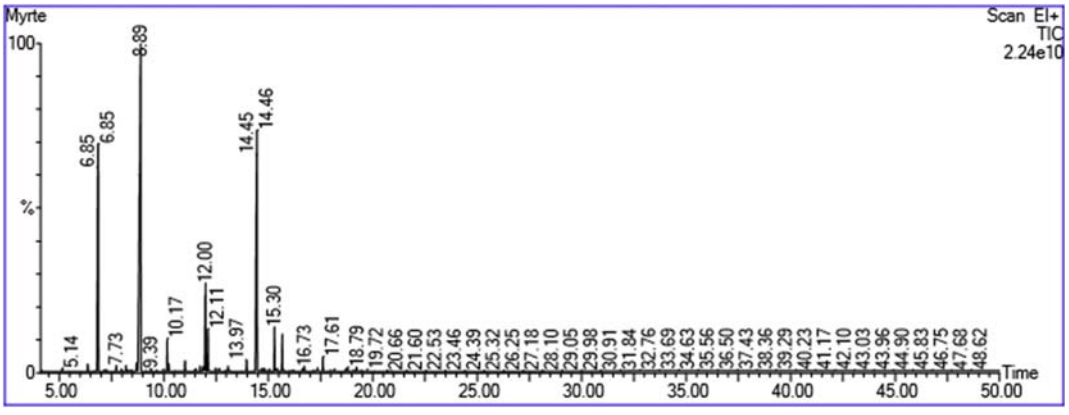

Fig. 1. Chromatogram of *Myrtus communis* essential oil obtained by GC/MS.

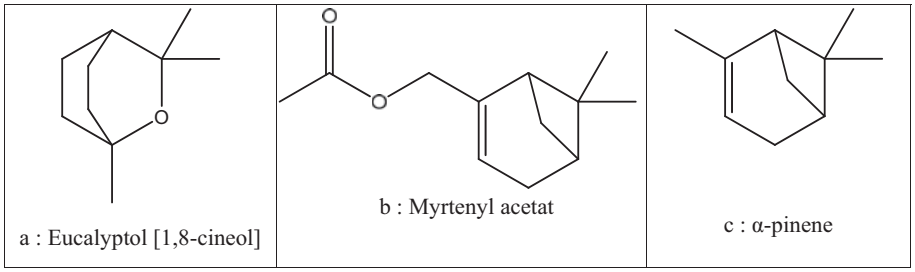

Fig. 2. The structure of three major compounds of *Myrtus communis* essential oil.

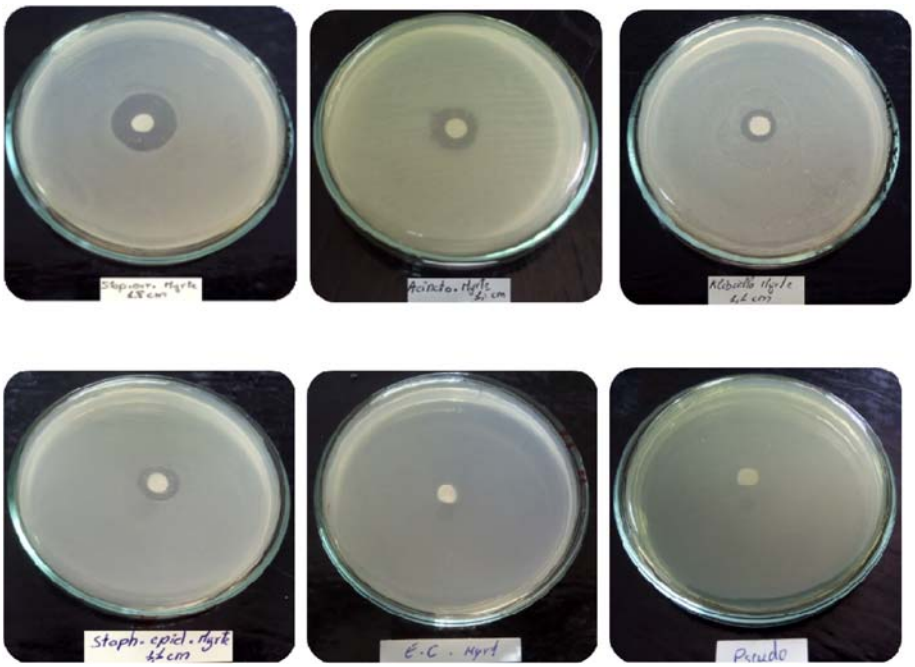

Fig. 3. Effect of *Myrtus communis* essential oil on the growth of the strains tested.

the major constituent is eucalyptol with a percentage of 42.43%. Moreover, most previous studies indicated that α-pinene and 1,8-cineole were the major component of *M. communis* essential oil [22,42–55], while in our study eucalyptol, myrtenyl, acetate and α-pinene were the major constituents. This difference is significantly marked in the study conducted in the Kabylie region of Algeria. On the other hand, Taleb-Toudert et al. [56] found that the three major constituents of their oil are the following: eucalyptol (26.2%), α-pinene (18.96%) and eugenol methyl (1.57%). In a recent study done by Fadil et *al*. [57] who sampled twenty adults *M. communis* plants in the forest of "Ifran" located on the banks of the dam "Sahla" of the city of Taounate in Morocco, they found that the major constituents of the essential oil in this variety of palntes are as follows: α-pinene (0.4–50.3%), 1,8-cineole (8.3–64.9%), myrtenyl acetate (0–61.1%), the α-terpinolene (0–20.8%) and methyl eugenol (0–33.6%).

Table 2
Antibacterial activity of *Myrtus communis* essential oil.

Bacteria	Diameter of inhibition Zones (mm)
Klebsiella pneumoniae	11
Acinetobacter baumannii	15
Staphylococcus aureus	18
Pseudomonas aeroginosae	–
Escherichia coli	–
Staphylococcus epidermidis	11

-: No inhibition zone.

Table 3
The minimum inhibitory concentration (MIC) of *Myrtus communis* essential oil.

	Concentration mg/mL							
	1/100	1/250	1/500	1/1000	1/2000	1/3000	1/5000	C
Bacteria	6.6	2.64	1.32	0.66	0.33	0.22	0.13	
Staphylococcus aureus	-	-	-	+	+	+	+	+
Staphylococcus epidermidis	-	-	+	+	+	+	+	+
Acinetobacter baumannii	-	-	+	+	+	+	+	+
Klebsiella pneumoniae	-	+	+	+	+	+	+	+

-: Inhibition; +: Growth; C: Control.

3.3. Antibacterial activity

The results obtained from the analysis of the antibacterial activity of our extract are expressed in terms of the diameters of inhibition zones measured around the discs (Fig. 3 and Table 2). From the obtained results (Table 2), we can say that the essential oil of *M. communis* possesses an antibacterial activity with regard to the four tested strains; *A. baumannii, S. aureus, S. epidermidis, K. pneumoniae* inactive on the two remaining strains (*Escherichia coli* and *P. aeroginosae*). The largest zone of inhibition is observed in the presence of *S. aureus* (18 mm) followed by *A. baumannii* with a 15 mm inhibition zone. *K. pneumoniae* and *S. epidermidis* shared the same row with an inhibition diameter of 11 mm. *Pseudomonas aeruginosa* and *Escherichia coli* were found to be very resistant to myrtle essential oil. Fadil et *al*. [57] showed that the inhibition of *S. aureus* and *E. coli* have great effects compared to our results with inhibition diameters of 24 ± 1.5 mm and 18 ± 0.6 mm respectively. In contrast, Belmimoun et *al*. [58] found a lower inhibition zone for *S. aureus* with a diameter of 0.7 ± 0.01 mm, and the reverse for *E. coli* with a diameter of 0.7 ± 0.2 mm.

From Table 3, we found that the MIC of 1.32 mg/mL was sufficient to stop the growth of *S. aureus* which was most vulnerable to this Essential oil, followed by *S. epidermidis* and *A. baumannii* that were inhibited from the minimum concentration of 2.64 mg/mL. On the other hand, *K. pneumoniae* was inhibited at the essential oil MIC of 6.6 mg/mL. Fadil et *al*. [57] showed that the minimum inhibitory concentration for *S. aureus* (with MIC of 0,125% (v/v) is lower compared to *Echerichia coli* (with MIC of 0.5% (v/v)). Also the work carried out by Chebaibi et *al*., on the antibacterial activity of essential oil of myrtle from the Region of Taounate (Ghafsaye), showed a significant efficiency on all the following strains (*Escherichia coli, K. pneumoniae, Enterobacter cloacae, Proteus rettgeri, P. aeruginosa* and *S. aureus*). The MIC value is 0.500% for all the strains tested [59].

Table 4
The minimum bactericidal concentration (MBC) of the essential oil *of Myrtus communis*.

Strains tested	1/100 6.6 mg/mL	1/250 2.64 mg/mL	1/500 1.32 mg/mL
Staphylococcus aureus	−	−	+
Acinetobacter baumannii	−	−	+
Staphylococcus epidermidis	−	−	+
Klebsiella pneumoniae	−	+	+

-: Inhibition; +: Growth.

The determination of the minimum bactericidal concentration is deduced from the first biolite devoid of bacteria. On the basis of this definition, it emerges that the minimum bactericidal concentration reported in Table 4 is 2.64 mg/mL for *S. aureus, A. baumannii and S. epidermidis*. For *K. pneumoniae*, we found that 6.6 mg/mL is the minimum bactericidal concentration.

The difference observed in the sensitivity of different microorganisms is essentially due to their chemical composition, especially the presence of monoterpenes [60] such as eucalyptol (1,8-cineole) and α-pinene. These compounds belong to the ether and hydrocarbon groups [61], and they are well known for their antimicrobial properties [62]. Indeed Eucalyptol is a natural saturated monoterpene, which has been used for the symptomatic treatment of sinusitis and bronchitis due to its mucolytic, antimicrobial and spasmolytic properties [62]. And it is not forgotten that probably the synergistic effect between all components of the essential oil of *M. communis* may be attributed to the antimicrobial activity of the essential oil. Moreover, it has been reported that myrtle essential oils of different chemotypes have shown significant inhibitory activity against Gram-positive bacteria more than Gram-negative [55].

4. Conclusion

This work was carried out to study the chemical composition of the essential oil of *M. communis* leaves and to evaluate its antibacterial activity in vitro. Based on the results of composition analysis of oil by CG/SM, the essential oil contained 32 chemical elements and the yield of essential oils was 0.7%. The chemical profile of the investigated oil is highlighted by Eucalyptol (42.43%), myrtenyl acetate (21.25%) and α-pinene (19.39%) as major compounds. This oil reveals in vitro antibacterial activity on the studied bacterial, confirmed by the inhibition zone diameter ranging from 11 to 18 mm and a MIC value between 1.32 mg/mL and 6.6 mg/mL depending on the microorganism being tested. Overall, this study further supports the view that the essential oil of myrtle is promising as naturel source with antibacterial activity and thus confirms its potentiel uses as antimicrobial agents for industrial applications such as pharmaceutical products.

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