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In vitro Antibacterial Effect of Essential Oil and Two Extracts of *Myrtus communis* Leaves

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ABSTRACT

Our study aims at evaluating the efficacy of extracts and essential oil of Myrtus communis leaves from the Syrian coast against several bacterial strains. The leaves were collected and dried, then extracted for essential oil and ethanolic and hexan extracts were prepared. The yield of essential oil was measured and the chemical composition of the essential oil was determined using Gas Chromatography (GC). The yield of essential oil was 1.3 ml/100 g and the richest component was alpha-pinene. Our results showed that the ethanolic and hexan extracts of Myrtus communis had higher antibacterial activity against gram positive bacteria (Staphylococcus aureus and Streptococcus pyogenes) than gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The ethanolic extract showed the greatest inhibitory effect against Staphylococcus aureus. These results demonstrate that Myrtus communis leaves extract has high antibacterial activity, making it a source of compounds to be used for the treatment of gram-positive bacterial infections.

Keywords: *Myrtus comminus*; Antibacterial; Essential oil; Extract

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INTRODUCTION

Nowadays, many bacterial strains are resistant to chemical antibiotics. This encourages researchers to find natural source of antibacterial agents [1]. One of the most studied plants is *Myrtus communis* which is known of its biological effects. Myrtle (*Myrtus communis*) belongs to the Myrtaceae family is evergreen and aromatic plant with numerous stems and branches [2]. Recently, several studies have been conducted on the antimicrobial properties of the leaf and stem extracts of myrtle against pathogenic bacteria and good results have been obtained about its effects on *staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa* [3-6].

Different parts of the plant have been used in the food industry, for example for flavoring meat and sauces, and in the cosmetic industry [7]. Foods flavored with the smoke of myrtle are common in rural areas of Italy or Sardinia [8]. Plant extracts and essential oils have long constituted a natural source of antimicrobial compounds [9,10]. Essential oils and

purified components are used as natural antimicrobials in food, as well as to prevent the growth of food borne bacteria and molds, resulting in a longer shelf life for processed foods [11]. This study aims at evaluating the efficacy of extracts and essential oil of *Myrtus communis* leaves from the Syrian coast against several bacterial strains in order to provide valuable information in this field.

MATERIALS AND METHODS

Plant Collection

The leaves of *Myrtus communis* were collected during September 2018, from Banias district in Tartous, Syria. The study was carried out at the department of pharmacognosy and department of microbiology, faculty of pharmacy, Al Andalus University, Tartous, Syria. The plant was air dried in the shade for one week at room temperature 25-27°C [12].

Ethanolic and Hexan Extract Preparation

To prepare an ethanolic extract, the *Myrtus communis* dried leaves were ground into a fine powder, and thereafter 2 g of the *Myrtus* was mixed with 10 ml of 95% ethanol to obtain 20% (w/v) extract. Extraction was carried out at room temperature in the dark for 7 days, with periodical hand shaking [5]. After extraction, the mixture was centrifuged and supernatants were designated as an ethanolic extract. Then, the ethanol was evaporated in 50°C and the residue was dissolved in Dimethyl Sulfoxide (DMSO). The same was performed for the N-hexane extract.

Essential Oil Extraction

Samples of 30 g of the *Myrtus* leaves were soaked with 300 ml of N-hexane, another 30 g was soaked in 300 ml of ethanol, for 24 hours and 110 g of powder was hydro distilled using 1100 ml of distilled water for each, the process was obtained by Clevenger type apparatus for 4 hours, the yield was recorded and the pure oil was saved in sealed glass vials at 4-5°C until analysis.

Gas Chromatography Analysis

The chemical composition of the essential oil of the myrtle plant was determined in the central laboratory of the higher institute for marine research at Tishreen university in Latakia city using a Gas Chromatography (GC) device equipped with MS mass spectrometry (Agilent-7890A gas chromatograph)**.** The analysis was performed using a capillary column of the type: DB-5 dimensions: (30 m × 320 micro m i.d) and a layer thickness of 0.25 micro m and connected to the mass spectrometry detector**.**

The thermal program of the oven starts from 70°C to 270°C at a rate of one degree every four minutes, and the carrier gas is helium, with a flow rate of >1,2 ml / min**.** Then, the components of the essential oil extracted from myrtle leaves were identified by comparing the mass spectra obtained for each vertex of the GC/MS chromatograms with the mass spectra found in the libraries available on the NIST and Wiley computer.

Culture Preparation

The essential oil, ethanolic extract and hexan extract of *Myrtus* leaves were tested against tow gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and tow gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). These strains were obtained from laboratory section of Tishreen university hospital in Lattakia city and maintained on nutrient agar at 4°C.

Sensitivity Test of the Extracts

The agar disc diffusion method was employed to determine the sensitivity of the extracts, discs (6 mm) were infused with 10 µl extracts dried for 3 days and then placed on the agar surface after culturing of previously mentioned bacterial strains. All plates were incubated for 48 hours in 37°C.

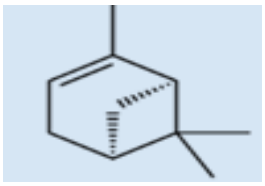
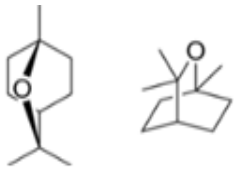
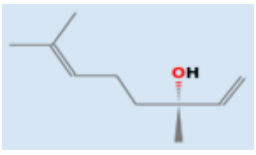
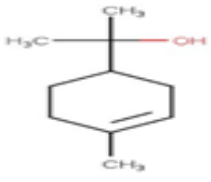
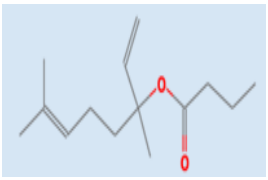
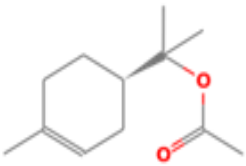
RESULTS

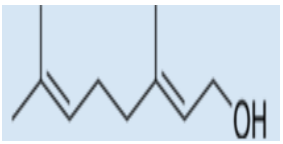
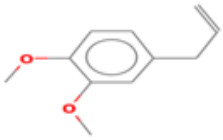
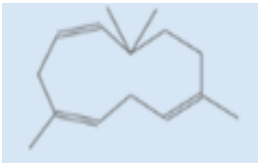
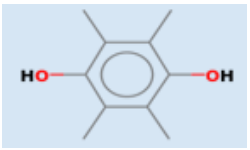
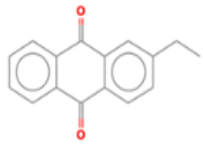
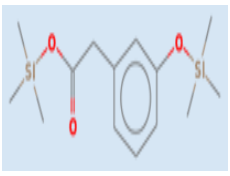
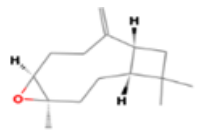
The Yield and Chemical Composition of the Essential Oil

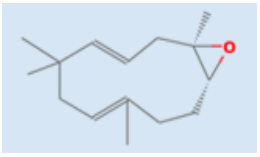
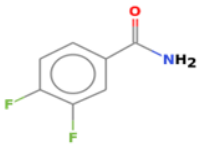
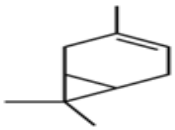
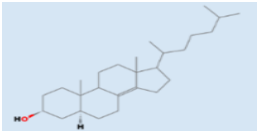
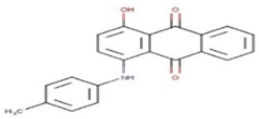
The yield of the essential oil was 1.43 ml for the 110 gram of *Myrtus* leaves, which is equal to 1.3 ml/100 g. The chemical composition of the essential oil of the myrtle plant was determined in the central laboratory of the higher institute for marine research at Tishreen University in Latakia city using a Gas Chromatography (GC) device equipped

with MS mass spectrometry (Agilent-7890A gas chromatograph as mentioned in the methods**.** After analyzing the essential oil with the GC-MS device, the most abounded compound was Alpha-pinene with a percentage of 9.67% of all detected compounds? The overall detected compounds are present in Table 1 and Figure 1.

Table 1: The chemical composition of the essential oil of *Myrtus communis*

Formula	percentage	Element Name
	9.67	Alpha-pinene
	8.23	Eucalyptol
	7.37	3,7-dimethyl,1,6-Octadien-3-ol
	5.07	Alpha-terpinol
	5.04	Linalyl butyrate
	2.06	Alpha terpenyl acetate
	4.64	geraniol

		
	2.23	D-methyl eugenol
	1.28	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl, Z,Z,Z
	0.5	Alpha Farnesene
	6.59	Durohydroquinone
	0.46	9,10-Anthracenedione, 2-ethyl
	1.06	Benzene acetic acid,3-hydroxy,TMS
	0.47	Cyclododecene, (Z)

	1.08	Alpha-humulene epoxide II
	3.08	3,4-fluoro- Benzamide
	0.92	Balmetic acid
	0.40	Phytol
	0.53	Oleic acid
	0.22	Carene
	0.21	Doristerol
	7.47	C.T.sovent blue 90
	7.28	Decamethylcobaltocene

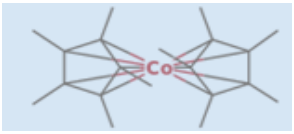
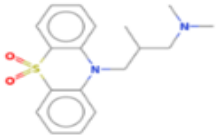
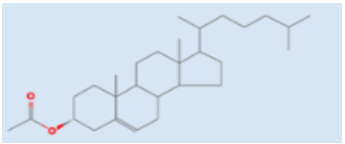
		
	1.97	Oxomemazine
	0.49	Pentacosane

Figure 1: Efficacy against *Staphylococcus aureus*

Sensitivity of the Essential Oil

The sensitivity of *Myrtus communis* L. was examined against isolated bacteria by disc diffusion method. *Myrtus communis* L. essential oil exhibited significant susceptibility with 29 mm inhibition diameter while the ethanolic extract 26 mm and 31 mm for the hexan extract, in comparison with 27 mm for Trimethoprim/Sulfamethoxazole against *Staphylococcus aureus* which is known by its high resistant rates to antibiotics.

The effect on *Pseudomonas aeruginosa* was 15 mm for the essential oil, 12 mm for the ethanolic extract and 14 mm for the hexan extract in comparison with 12 mm for Trimethoprim/Sulfamethoxazole. The effect on *Streptococcus pyogenes* was 11 mm for the essential oil, 15 mm for the ethanolic extract, 17 mm for the hexan extract in comparison with 26 mm for Trimethoprim/Sulfamthoxazole.

Finally, the effect of the essential oil on *E. coli* was 15 mm, 12 mm for the ethanolic extract and no inhibition zone for the hexane tract in comparison with 31 mm for Trimethoprim/Sulfamethoxazole, and all previous results are shown in Table 2 and Figure 2.

Table 2: Diameter of inhibition zone of essential oil and extracts of myrtle leaf in millimeters

Bacterial strain	N-hexane Extract	Ethanol Extract	Essential oil	Trimethoprim/ Sulfamethoxazole
Staphylococcus aureus	31	24	15	27
Streptococcus pyogenes	22	20	11	R
E. coli	R	12	15	31
Pseudomonas aeruginosa	R	12	15	R

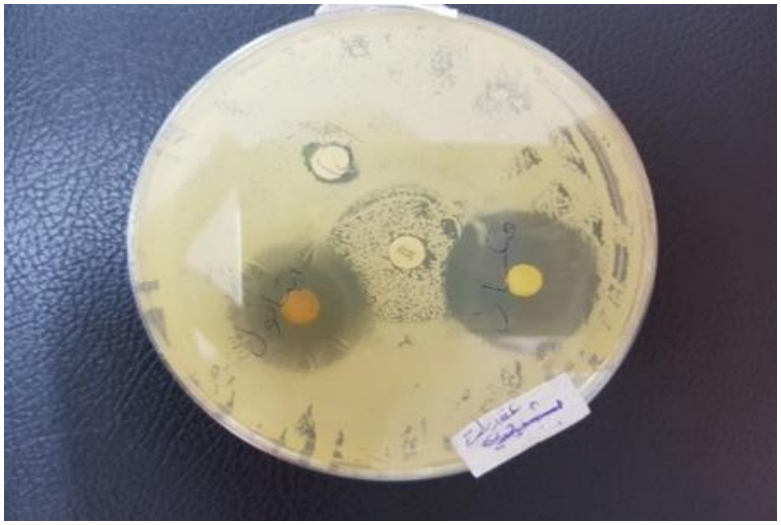


Figure 2: Efficacy against *Streptococcus pyogenes*

DISCUSSION

This study is the first study to show the composition and the antimicrobial effect of the essential oil of *Myrtus communis* leaves from the Syrian coast. In our study, the yield of essential oil from *Myrtus communis* leaves was 1.3 ml/100 g, which is similar to an Iranian study where the yield was 1.6%. The main constituents obtained from gas chromatography/mass spectrometry analysis were α-pinene with a percentage of 9.67% and this result is compatible with the same Iranian paper [13]. Our results showed that the ethanolic and hexan extracts of *Myrtus communis* had higher antibacterial activity against gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) than gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). This result is similar to Mir, et al. findings where the ethanolic extract showed strong inhibitory effect against gram positive and acid fast bacteria. The inhibition zone diameter was 24 mm for the ethanol extract against *Staphylococcus aureus* in our study which is similar to Mir, et al. results [14].

CONCLUSION

These results demonstrate that *Myrtus communis* leaves extract exhibits high antibacterial activity against Gram positive bacteria due to the rich component of Alpha-pinene, which makes this plant a source of compounds to be used for the treatment of Gram-positive bacterial infections.

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