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RESEARCH ARTICLE

Antibacterial study of the ethanolicextract of local common myrtle leaves *Myrtus communis L.*(Myrtaceae)

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ABSTRACT:
This study aims to investigate the antibacterial activity of the ethanolicextract of common myrtle "*Myrtus communisLinn*." leaves (Myrtaceae), specifically against *Escherichia coli* and *Staphylococcus aureus* isolated from patients at Tishreen University Hospital, Syria. Leaves were subjected to ultrasound-assistedextraction using thedistilled-ethanol as a solvent. The dried extract, with a yield of 25.3%, was dissolved in two types of solvent Dimethylsolfoxide(DMSO), and a mixture of Tween 80 solution10% and DMSO 8:2 and then tested for antibacterial activity using the standard disk diffusion technique. In this work, DMSO showed better properties in the improvement of the solubility and the diffusion of the prepared extract, thus enhancing its antibacterial action.The inhibition zones showed that the tested ethanolic extract has interesting activity against both tested strains with higher activity towards *S. aureus*.The overall results encourage the application of this extract to combat bacterial resistance, and as an additive in the food industry to control the foodborne disease caused by *S. aureus.*

KEYWORDS: *Myrtus communis L.*, Antibacterial activity, Antibiotic resistance, Ethanolic extract, Dimethylsolfoxide (DMSO), Tween 80.

INTRODUCTION:
The "Myrtaceae" family has a rich history in the field of medicinal plants. Which has medical value as well as traditional value such as *Syzygiumcaryophyllatum*this plant has been given a prominent place in many traditional Ayurvedic medicine systems**Error! Reference source not found.**and *Syzygium Cuminii* (L) which also has been used in traditional medicine as a treatment for various diseases, the leaves and seeds are used in the treatment of bronchitis, asthma, dysentery, ulcers and other diseases**Error! Reference source not found.**.*Callistemon lanceolatus* DC had a role as traditional bush medicine. The genus is known in folk medicine for its anti-cough, bronchitis and insecticides effects and its volatile oils have been used as antimicrobials and antifungals**Error! Reference source not found.**.

Myrtus communis Linn. (Family Myrtaceae) is a small, perennial, evergreen shrub, 1.8-2.4m high. It has small leaves and deep cracked bark. It is native to southern Europe, northern Africa, and western Asia. It is widespread in the Mediterranean region, in South America, the northwest of Himalayas and Australia**Error! Reference source not found.Error! Reference source not found.,Error! Reference source not found.Error! Reference source not found.,Error! Reference source not found.**. It is also grown in gardens for its fragrant flowers. *Myrtus* is the Greek name for Myrtle and *communis* means a common plant that grows in groups. In Britain, the common myrtle was introduced in 1597 and Linnaeus described it in 1753. This plant occupies a prominent and important position in the writings of many scholars and researchers such as Hippocrates, Pliny, Dioscorides, Galen and Arab writers.

Various parts of this plant such as berries, branches and leaves have been widely used as a folk medicine for a long time. Thanks to the astringent, tonic and antiseptic

properties of its leaves, it is used in wound healing and digestive and urinary disorders. The oil is antiseptic and anti-catarrhal.Therefore, it can be used to treat chest diseases**Error! Reference source not found.**. According to data on *in vitro* and *in vivo* studies, myrtle has the potential to be used in pharmaceutical development as a drug. Many of the traditional uses of this herb have been validated from a scientific point of view. In this regard, further studies are required to reveal the potential roles of myrtle as an alternative in the treatment of microbial, cardiovascular, gastrointestinal, dermatological, neurological, and other diseases**Error! Reference source not found.Error! Reference source not found.**.

Antibiotic Resistance:

The discovery of penicillin by Alexander Fleming marked a milestone in modern medicine. Which saved millions of lives during World War II. This paved the way for new antibiotics against deadly infections. However, this efficacy y quickly decreased due to antibiotic resistance. The development of antibiotic resistance is primarily attributed to increased drug use**Error! Reference source not found.**.The excessive and misuse of antibiotics clearly drive the evolution of resistance**Error! Reference source not found.**but there are other reasons such as (patientnoncompliance, inadequate diagnosis, agricultural uses, and vast availability of few new antibiotics).Wilma Mary Thomson *et al***Error! Reference source not found.**suggested tracking antibiotic consumption patterns over time and across countries or aiding investments in alternatives to antibiotics as ways to limit antibiotic resistance**Error! Reference source not found.**.

Chemical Composition of The Ethanolic Leaf Extract of The Plant:

The compounds of the *M. communis* leaf extract were identified by Gas chromatography–mass spectrometry analysis. Fifty compounds were detected, which constitute 71% of the whole extract. The most dominant identified compounds were 1,1,8a- trimethyloctahydro-2,6-naphthalenedione (27.6%), pyrogallol (9.1%), 1,8-cineole (3.9%).The most representative identified compounds were α-terpineol (1.6%), linalool (2.8%), squalene (1.15%), α-terpinyl acetate (1.02%), D-limonene (0.65%) and linalyl acetate (0.97%). The other constituents present were β-caryophyllene (0.56%), acetol (0.64%), linalyl formate (1.93%) and α-tocopherol-β-D-mannoside (1.78%). The presence of newly identified compounds especially 1,1,8a-trimethyloctahydro-2,6-naphthalenedione (27.6%) and pyrogallol (9.1%) or their combination with other secondary metabolites contribute to the antibacterial activityagainst Gram-positive bacteria**Error! Reference source not found.**.

MATERIALS AND METHODS:

Sample preparation:

The leaves samples were collected in the month of March from Al-Dahriyeh district of Al-Haffa, Latakia, Syria, then dried under shade conditions for 7 days Humidity test was conducted to determine the percentage of humidity in the dried leaves using 10g of the crude drug placed in an oven (Memmert D24080) at 100°C for 3 hours.

The crude drug was grinded using a laboratory grinder (Sokany-China, SM-3017) and stored in a dry place until extraction.

Extraction:

An 80 g of grinded dried leaves were placed in an Erlenmeyerflask, and 700 ml of distilled ethanol were added, then the flask was placed in an ultrasonic bath (Elma-Germany, Elmasonic S 60 H) and the extractionwas performedat room temperature for two hours.

After that, the extract was filtered in two steps. The extract was collected and evaporated using the rotary evaporator (Scilogex, RE100-Pro). After the evaporation process was completed, the dry extract was stored in a desiccator at room temperatureuntil the antibacterial test is performed.

Microscopical examination:

The powdered drug was examined under a light microscope (Micros Austria MCX51LED) at a magnification of 10x and 40x. Different intracellular components were identified and photographed using a digital camera.

Biological study and examination of antibacterial efficacy:

The disc diffusion technique was used to verify the antibacterial activity (*Antibiogram*).

- **Preparation of the bacterial suspension:**

Three bacterial colonies were suspended using a sterile culture loop in a tube containing 10 ml of sterile distilled water.The medium was homogenized using a tube-vibrator to prepare the bacterial suspension from the studied bacterial strains. The bacterial suspension turbidity was adjusted to 0.5 McFarland equivalence ($1.5x10^8$ CFU/ml)**Error! Reference source not found.**by the measurement of the optical density (OD) at 625nm using a UV-visible spectrophotometer (Dlab, SP-UV1100) OD ∈ [0,08-0,13]**Error! Reference source not found.Error! Reference source not found.**.

- **Preparation of the extract solution for biological examination:**

Two different solvents were tested; Dimethylsolfoxide (Carloerba reagents) (DMSO) the extract was dissolved in the DMSO (D.ex), and a mixture of Tween 80solution (ScienceLab chemicals and laboratory equipment) (10%) in sterile distilled water and DMSO 8:2 (T.ex). Both samples were applied to the disc with a final amount of 0.6mg per disc.

- **Preparation of Petridishes:**

The dishes were prepared by pouring 20 ml of autoclave (Systec, CE2561) pre-sterilized Mueller-Hinton nutrient solution (MERCK) into each plate, and then kept in a sterilized place until solidification.

Brushing was done using a cotton swab impregnated with the prepared bacterial suspension. The two prepared extract-solutions D.ex and T.ex were added to the sterile discs with a final concentration of 0.6mg/disc. The impregnated discs were placed inthe infected plates, with a negative control of each solventand a positive controlcontaining the specific antibiotic (Abtek antimicrobial susceptibility test disc). Then, the plates were incubated at 37°C in an incubator (Memmert 30-1060) and the diameters of the inhibition zones were read and measured after 18 hours of incubation. The experiments were performed in triplicate and all data are expressed as the mean of triplicate.

RESULTS AND DISCUSSION:

The micro-morphological survey using distilled water showed the occurrence of helical vessels (figure1-a), secretory structures, oil cavities (figure1-b), with a pale-yellow cotenant due to the accumulation of essential oils**Error! Reference source not found.**. Druses crystals ofcalcium oxalate (figure1-c) and unicellular non glandular trichomes (figure1-d) were observed,suggesting the correct botanical identification of the plant.

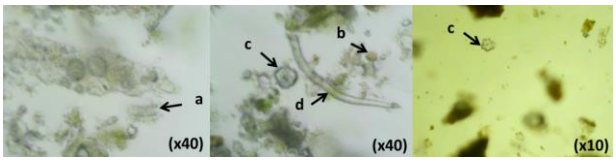

Figure.1: intracellular components of *Myrtus communis L* leaves; a: helical vesselsb:oil cavities c: Druses crystals ofcalcium oxalate d:non-glandular trichomes.

E. coli is the most predominant uropathogen**Error! Reference source not found.** and *S. aureus* is a major cause of hospital acquired infections, causing high morbidity and mortality across the world**Error! Reference source not found.** That's why we chooseto test the antibacterial activity of the plant against them.

The dried extract was obtainedwith an extraction yield of 25.31% of the dried leaves., the antibiotic sensitivity test was imposed using Antibiotic discs (Company: Abtek Biologicals Ltd) to verify the best antibiotic for positive control since the tested bacteria were isolated from patients and not of referenced strains.This means that the tested bacteriaisresistant to antibiotics and The World Health Organization "WHO" considers antibiotic resistance to be one of the most important global healththreats**Error! Reference source not found.** The inhibition zonesare summarized in Table 1.

Table 1: inhibition zone of antibiotic sensitivity test using the standard disc diffusion method.

Inhibition zone (mm)			
Antibiotic	***Staphylococcus aureus***	***Antibiotic***	***Escherichia coli***
Imipenem (10µg)	*55*	*Imipenem (10µg)*	*30.3*
Cotrimoxazole (25µg)	*28*	*Cotrimoxazole (25µg)*	*25*
Vancomycin (30µg)	*20*	*Ceftriaxone (30µg)*	*0*

Our study compares the effect of thesolvent on the antibacterial activity of the extract using Dimethylsolfoxide DMSO (D. ex) and the aqueous solution of tween 80 (10%). The tween 80 solution showed a solubility problem. Therefore, DMSO was added to improve the solubility of the extract in a ratio of tween 80 (10%): DMSO 8:2 (T.ex).Both solutionswere tested in triplicate using the standard disc diffusion (Ø: 6 mm) with a unified concentration of 0.6 mg/disc against *S. aureus*and *E. coli*.

The inhibition zones of the ethanolic extract of common myrtle leaf against selected bacteria were measured and the meanof triplicates are displayed in (Table 2).

None of the solvents (negative control) showed any antibacterial activity. In all of the triplicate repetitions, the extract dissolved in DMSO D.ex showed a higher antibacterial inhibition zone than the extract dissolved in tween 80 (10%) + DMSO, in agreement with a previous study which suggested that Tween 80 lowers the antibacterial efficacy of some hydrophobic antimicrobial such as essential oil constituents**Error! Reference source not found.Error! Reference source not found.**, which is the case of our extract**Error! Reference source not found.Error! Reference source not found.**.

Table 2: Results of the antibiogram study on the tested bacteria

Inhibition zone (mm)		
Solution	**D.ex DMSO**	**T.ex: Tween 80 (10%): DMSO) 8:2)**

Con. per disc		0.6mg/disc	0.6mg/disc
Bacteria	*Staphylococcus aureus*	24	13.3
	Escherichia coli	17	11.3

Thus, according to Table 1 and Table 2,the leaves extract dissolved in DMSO showed a very interesting inhibition zone value of 24 mm against*Staphylococcus aureus* compared to that of vancomycin (20 mm).However, the inhibition zone is lower than that of cotrimoxazole (28 mm), suggesting the beneficial effect of this extract in foodborne infection caused by *S. aureus*.

As for the antibacterial activity of *Escherichia coli*, the inhibition zone of 17 mm was lower than that reported against *S. aureus* but still interesting and promising to investigate the antibacterial spectrum against other Gram positive and Gram-negative bacteria.

CONCLUSION:

Antibiotic resistance represents a major challenge in the field of infectious diseases. Aromatic plants containing essential oils are the best candidates to participate in the search for alternatives. The leaves oflocal common myrtle *Myrtus communisL.* can be considered as a potential candidate as an additive in the food industry or for applications in the treatment of foodborne infections caused by *S. aureus*.The presented data are important for any application of this plant as antibacterial since they allow proposing possible associations with other agents or antibiotics.

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CONFLICT OF INTERESTS:

The authors declare that they have no conflict of interest.

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