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Antibacterial activity of *Citrus Aurantium* and *Myrtus Communis* extracts on some pathogenic bacteria

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ABSTRACT

Background: *Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa* are regarded as harmful organisms. These germs exhibit resistance to multiple antibiotics; therefore, new therapies must be employed to combat the infection. **Objective:** The antimicrobial efficacy of extracts from *Citrus aurantium* and *Myrtus communis* leaves was examined against bacteria. **Methods:** Between September 2023 - July 2024, a combined total of 50 clinical isolates were collected from cotton swabs taken from patients experiencing diabetic foot, respiratory infections, and urinary tract infections. Visiting specialized laboratories within the city of Baqubah, the diffusion method was employed to examine the impact of plant extracts on the development of the isolated bacteria under investigation. **Result**: Ethanol extracts of Myrtus communis showed varying efficacy against *S. aureus and S. epidermidis* at concentrations of 100, 75, 50, 25, and 12.5 mg/ml, but extracts of Citrus aurantium exhibited ineffectiveness against both S. aureus and S. epidermidis at concentrations of 25 and 12.5 mg/ml. However, *Citrus aurantium* extract demonstrated efficacy at concentrations of 100, 75, and 50 mg/ml against both *S. aureus and S. epidermidis*, suppressing their growth with inhibition zones. Ethanol leaves extract of Myrtus communis and leaves extract of *Citrus aurantium* showed varying efficacy versus *E. coli* at concentrations of 25, 50, 75, and 100 mg/ml, whereas Myrtus communis was effective at a dose of 12.5 mg/ml. Conversely, *Citrus aurantium* at 12.5 mg/ml was ineffective against *E. coli*. All concentrations for Ethanol extracts of Myrtus communis and Ethanol leaves extract of *Citrus aurantium* were effective against the *K. pneumoniae*. Ethanol leaves extract of *Myrtus* communis and extracts of *Citrus aurantium* demonstrated varying efficacy against P. aeruginosa at concentrations of 50, 75, and 100 mg/ml, while concentrations of 25 and 12.5 mg/ml were ineffective. The ethanol extract of *Myrtus* communis showed varied efficacy against *P. mirabilis* in various concentrations, while the leaves extract of *Citrus aurantium* was not effective at all concentrations. **Conclusion:** Our findings indicate that ethanol extracts from plant leaves *(Citrus aurantium* and *Myrtus communis*) possess the capability to function as antibiotics against prevalent bacterial isolates responsible for infections in our community

Introduction

Citrus aurantium, this evergreen tree, commonly referred to as sour orange, bitter orange, Seville orange, or bigarade, can reach a height of up to five meters. Esteemed for its fragrant white blossoms, it is thought to have originated in Eastern Africa and Syria, subsequently cultivated in the USA, Spain, and Italy**[1]** . Leaves of *Citrus* constitute a substantial supply of bioactive

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compounds, including flavonoids, ascorbic acid, and phenolic molecules, acknowledged for their inherent antioxidant qualities. The leaves of *C. aurantium* can be utilized in the drug industry as components in medicinal preparations.[2]. *Myrtus communis, also* known as myrtle (also available under the Arabic name myrtle or hades), is a renowned therapeutic herb belonging to the Myrtaceae family [3]. The Mediterranean region, in conjunction with countries like Iraq and Jordan, is home to this evergreen shrub that is full of fragrant compounds and has medicinal properties, antibacterial, anti-inflammatory, disinfecting, hypoglycemic, and antioxidant properties [4]. The various components of the herb, such as its fruits, berries, and leaves, were employed in traditional medicine for many years [5] . It demonstrates a broad spectrum of pharmacologic and medical properties, encompassing anti-diabetics, antioxidants, anti-viral, anti-microbial, anti-fungal, anti-cancer, liver protection, and neurological activities[6]. The ethanolic leaves extract from *M.communis* demonstrates various biological functions, including antinociceptive, anti-diabetic, anti-inflammation, and antioxidant properties [7, 8].

Aromatic and therapeutic plants, validated by nature, extracted through scientific methods, and corroborated by researchers, have served as traditional remedies for many human ailments for millennia throughout diverse regions globally[9]. *Staphylococcus* constitutes a component of the human indigenous microflora and is asymptomatically harbored in several anatomical locations , but transmission of it from these locations , become opportunistic and cause several diseases [10].

Staphylococcus aureus, a member of the Staphylococcic family, manifests as Gram-positive cocci in clusters. *S. aureus* infection is a significant contributor to skin, soft tissue, respiratory, bone, joint, and endovascular conditions. Numerous strains of *S. aureus* are acquiring resistance to existing antibacterial drugs, posing a significant challenge in medical microbiology [11]. Methicillin-resistant *S. aureus* (MRSA) infections can elicit a wide array of symptoms, contingent upon the affected body regions. Medicinal plants have served as treatments for infectious disorders in numerous tropical nations, justifying the exploration of natural items for addressing MRSA infections[12]. Additionally, *S. epidermidis* is classified as a coagulase-negative staphylococcus. It is a commensal microbe that represents a significant component of the typical skin and mucosal microbiota. The pathogenicity of *S.epidermidis* depends on its capacity to attach to and build biofilms on the surfaces of previously mentioned medical devices [13]. *P. aeruginosa* is a sophisticated gram-negative facultative anaerobe equipped with a variety of strategies to manipulate and undermine host defensive mechanisms. The bacteria are a prevalent cause of nosocomial infections and an antibiotic-resistant priority pathogen. *P. aeruginosa* compromises upper and lower airway homeostasis in the lungs by inflicting physical damage on the epithelium and avoiding both innate and adaptive immune responses [14]. *E. coli*, a member of the Enterobacteriaceae family and a primary contributor to foodborne infections, is a prevalent inhabitant of the gastrointestinal system in both animals and humans. The presence of *E. coli* in meat products presents a considerable public health threat, as these bacteria may possess various antibiotic-resistant genes[15]. Research indicates that *E. coli* bacteria derived from contaminated meat and meat products demonstrate resistance to prevalent antibiotics, such as ampicillin, tetracycline, and trimethoprim-sulfamethoxazole[16]. *K. pneumoniae*, a member of the Enterobacteriaceae family, has emerged as a significant pathogen associated with nosocomial infections, particularly affecting the urinary tract (UTIs), respiratory tract, and bloodstream. While it frequently colonizes the skin, gastrointestinal tract, and nasopharynx, it poses a risk to individuals who are hospitalized for extended durations and have impaired immune systems. It accounts for around 70% of infections in humans. When structured in biofilms, the virulence factor renders it an exceptionally resilient microbe, providing significant resistance to the penetration of antimicrobial agents[17].

The primary aim from the present research was to assess the inhibitory effects of two distinct plant leaves *(C. aurantium* and *M. communis*) extracts from chosen medicinal plants on specific human pathogens.

Material and Methods

Design of Study and Participants

This cross-sectional comparative study was conducted between September 2023 and July 2024 at Baqubah Technical Institute/Middle Technical University, Iraq, in collaboration with Baqubah

Teaching Hospital. Fifty clinical samples were collected using cotton swabs from patients with diabetic foot, respiratory tract infections, and urinary tract infections who were visiting the specialized laboratories in Baqubah city. After conducting the necessary diagnostic tests to identify the samples using the Vitic device, the samples were used to test the ability of the extracts to inhibit bacterial growth using the diffusion method by using Muller-Hinton media in Petri dishes.

Collection of plants leaves

The leaves of *Citrus aurantium* and *Myrtus communis* utilized in this study were gathered in September 2023 from the Al-Khalis District in Diyala Governorate, Iraq.

Preparation the alcoholic extract of *Citrus aurantium* and *Myrtus communis*

The leaves of the plant were rinsed with tap and distilled water to eliminate dirt and dust, thereafter dried in the shade at ambient temperature, and then pulverized into powder utilizing an electric grinder. Approximately 100 cc of 70% ethanol was introduced into a flask holding 10 grams of *C. aurantium* and *M. communis*. The mixture was then incubated in a shaker at 35 °C for 24 hrs. The extract was further filtered using Whatman No. 1 filter paper. The resulting extract has been concentrated with a rotary evaporator, followed by drying the filter in an electric oven at 40 °C until the alcohol fully evaporated, resulting in a dry extract powder from the alcoholic extract, which was stored in a sterile, sealed glass vial at 4 °C for subsequent analyses[18].

Determination the Antimicrobial activity of *C. aurantium* and *M. communis* by agar well diffusion method

The diffusion method was employed to examine the impact of plant extracts on the development of the isolated bacteria under investigation. The bacterial isolates were first grown in nutrient broth for 12-18 hours before use and standardized according to McFarland 0.5 standards. The solid Mueller-Hinton agar medium was inoculated with sterile cotton from the bacterial suspension containing 1.5×108 cells/ml. Holes were made on the surface of the culture medium using a cork borer, and the prepared concentrations of each extract were placed at a rate of 0.1 ml for each hole. The dishes were maintained at the ambient temperature for 20 minutes, followed by incubation at 37°C for 24 hrs. The extract's effectiveness was evaluated by measuring the diameter of the inhibitory regions around every hole in millimeters [19].

Statistical analysis

The findings of the present research have been analyzed about the objectives and presented according to the general description of the sample. Microsoft Excel 2010 and SPSS (version 25) software were used for statistical analysis. Microsoft package (Excel and Word). The data are expressed as mean ± SD One-way ANOVA and T-test were utilized for the substantial comparison of means. The Chi-square test was utilized to compare percentages significantly at the 0.05 and 0.01 probability thresholds.

RESULTS

This investigation demonstrated that the leaves of *Myrtus communis* and *Citrus aurantium* have substantial bactericidal activity against the assessed clinical and environmental isolates. The examined isolates comprised *S. aureus, S.epidermis, E. coli, K. pneumoniae , P. mirabilis, and P. aeruginosa. The* antibacterial activity results for the two plant extracts demonstrated that *S. aureus, S. epidermis, E. coli, K. pneumoniae, P. mirabilis*, and *P. aeruginosa* constituted the most susceptible strains., as illustrated in Tables 1-6 and Figure 1.

In Table 1 the inhibiting effects of *M. communis* extract commenced at (100,75,50,25,12.5) mg/ml with inhibition zones of 27 mm, 24mm, 25mm, 24mm and 22mm against *S. aureus* respectively, whilst the *Citrus aurantium* extract inhibition zones of 17 mm, 16mm, 12mm, 0, 0 against these bacteria, *Staphylococcus aureus* shows significant susceptibility to both *M. communis* and *C. aurantium* extracts, indicating strong antimicrobial properties against it.

While result in table 2 show the inhibiting effects of *M.communis* extract commenced at (100,75,50,25,12.5) mg/ml with inhibition regions of [31 , 30 , 25 , 25 and 22] mm against *S. epidermidis* respectively , whilst the *C. aurantium* extract inhibition regions of [23 , 20 , 14] mm, 0 , 0 against this bacteria , *S.epidermidis* Similar to *S. aureus*, this strain also exhibits high susceptibility to the extracts, suggesting their potential effectiveness in treating infections caused by this organism.

Rustle in Table 3 show the inhibiting effects of *Myrtus communis* extract commenced at (12.5, 25, 50, 75, and 100) mg/ml with inhibition

regions of [20, 18, 15, 14 and 12] mm against *E. coli* respectively, whilst the *C. aurantium* extract inhibition regions of [16, 15, 14, 13] mm, 0 against these bacteria, The extracts demonstrate varying levels of effectiveness against *E. coli*, with some concentrations showing notable inhibition.

While rustle in table 5 show the inhibiting effects of Myrtus communis extract commenced at (12.5, 25, 50, 75, and 100) mg/ml with inhibition regions of [15, 14, 12] mm, 0 and 0 against *P. aeruginosa.* respectively, whilst the *Citrus Aurantium* extract inhibition regions of [17, 16, 12] mm, 0 and 0 against this bacterium. *Pseudomonas aeruginosa* is known for its robust resistance to various antimicrobial agents, and the data suggest that it shows significant resistance to both *Myrtus communis* and *Citrus aurantium* extracts. The effectiveness of these extracts against *P. aeruginosa* is notably lower compared to other strains.

Rustle in table 6 show the inhibiting effects of *Myrtus communis* extract commenced at (12.5, 25, 50, 75, and 100) mg/ml with inhibition regions of [28, 26, 20, 18 and 15] mm against *P. mirabilis.* respectively, whilst the *Citrus Aurantium* extract non effect against these bacteria, The bacterium *Proteus mirabilis* also demonstrated susceptibility to both extracts.

Tables 7 and 8 present statistical analyses conducted Employing ANOVA to evaluate the significant of variations among the means of varied concentrations of *Myrtus communis* and *Citrus aurantium* extracts. The significant level was established at $P < 0.05$, implying that any p-value beneath this threshold implies there was a statistically significance impact of the extracts on the examined bacterial strains.

Table 1. Effect of *Myrtus communis* and *Citrus aurantium* ethanol extract against *S. aureus.*

S. aureus	*Concertation mg\ml*	100	75	50	25	12.5
inhibition zones (mm)	*Myrtus communis*	27 mm	24 mm	25 mm	24 mm	22 mm
	Citrus aurantium	17 mm	16 mm	12 mm	0 mm	0 mm

Table 2. Effect of *Myrtus communis* and *Citrus aurantium* ethanol extract against *S. epidermidis.*

S. epidermidis	*Concertation mg\ml*	100	75	50	25	12.5
inhibition zones (mm)	*Myrtus communis*	31 mm	30 mm	25 mm	25 mm	22 mm
	Citrus aurantium	23 mm	20 mm	14 mm	0 mm	0 mm

Table 3. Effect of *Myrtus communis* and *Citrus aurantium* ethanol extract against *E. coli.*

E. coli	***Concertation mg\ml***	**100**	**75**	**50**	**25**	**12.5**
inhibition zones (mm)	***Myrtus communis***	20 mm	18 mm	15 mm	14 mm	12 mm
	Citrus aurantium	16 mm	15 mm	14 mm	13 mm	0 mm

Table 4. Effect of *Myrtus communis* and *Citrus aurantium* ethanol extract against *K. pneumonia.*

K. pneumonia	***Concertation mg\ml***	**100**	**75**	**50**	**25**	**12.5**
inhibition zones (mm)	***Myrtus Communis***	20 mm	18 mm	15 mm	11 mm	10 mm
	citrus aurantium	20 mm	19 mm	17 mm	13 mm	11 mm

Table 5. Effect of *Myrtus communis* and *Citrus aurantium* ethanol extract against *P. aeruginosa.*

P. aeruginosa	***Concertation mg\ml***	**100**	**75**	**50**	**25**	**12.5**
inhibition zones (mm)	***Myrtus communis***	15 mm	14 mm	12 mm	0 mm	0 mm
	Citrus aurantium	17 mm	16 mm	12 mm	0 mm	0 mm

Table 6. Effect of *Myrtus communis* and *Citrus aurantium* ethanol extract against *P. mirabilis.*

P. mirabilis	***Concertation mg\ml***	**100**	**75**	**50**	**25**	**12.5**
inhibition zones (mm)	***Myrtus communis***	28 mm	26 mm	20 mm	18 mm	15 mm
	Citrus aurantium	0 mm	0 mm	0 mm	0 mm	0 mm

Table 7. Statistical analysis using (ANOVA) between concentration of *Myrtus Communis* and significance of variations between the means of inhibition zones (mm) was assessed at $P < 0.05$.

Concentration of *Myrtus Communis* mg\ml	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Escherichia coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***	***Proteus mirabilis***	***Mean ± S.E***
100	27	31	20	20	15	28	23.50 ± 2.487
75	24	30	18	18	14	26	21.67±2.445
50	25	25	15	15	12	20	18.67±2.261
25	24	25	14	11	0	18	15.33±3.792
12.5	22	22	12	10	0	15	13.50±3.840
Mean ± S.E	24.40±0.814	26.60±1.691	15.80±1.428	14.80±1.934	8.20±3.3.382	21.40±2.441	$P < 0.05$

Table 8. Statistical analysis using (ANOVA) between Concentration of *Citrus aurantium* and Significance of variations between the means of inhibition zones (mm) was assessed at $P < 0.05$.

Concentration of *Citrus aurantium* mg\ml	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Escherichia coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***	***Proteus mirabilis***	***Mean ± S.E***
100	17	23	16	20	17	0	15.50+3.274
75	16	20	15	19	16	0	14.33+2.974
50	12	14	14	17	12	0	11.50+2.419
25	0	0	13	13	0	0	4.30+2.741
12.5	0	0	0	11	0	0	1.83+1.833
Mean ± S.E	9.0±3.768	11.40±4.874	11.60±2.943	16.00±1.732	9.0±3.768	0.0	$P < 0.05$

Figure 1. Effect of *Myrtus Communis* and *Citrus Aurantium* ethanol extract against some bacteria using (12.5, 25, 50, 75, and 100) mg/ml constriction and control in center.

Myrtus Communis ethanol extract

Citrus Aurantium ethanol extract

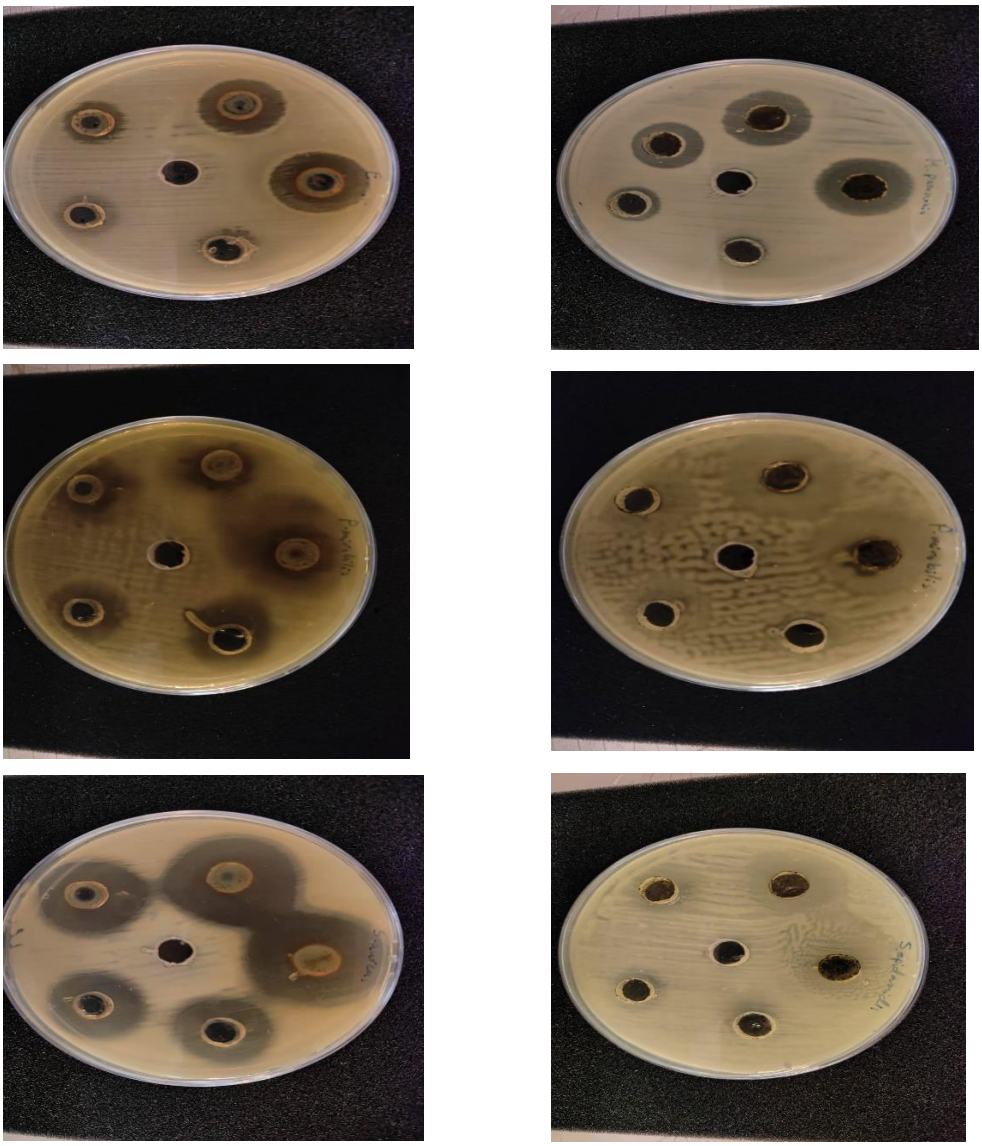
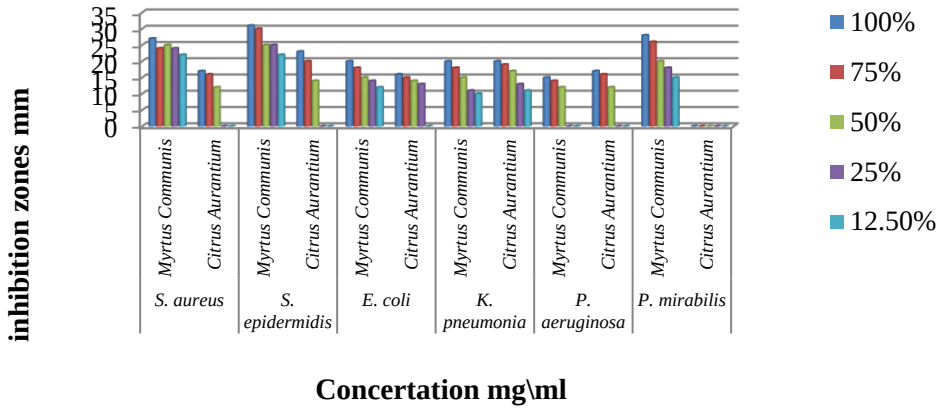


Figure 2. Inhibition zones of *Myrtus communis* and *Citrus aurantium* ethanol extract against some bacteria using (12.5, 25, 50, 75, and 100) mg/ml constriction and control in center.



DISCUSSION

Citrus aurantium, commonly known as bitter orange, has been reported to have many therapeutic potentials in folk medicine. The reason for this is that the plant extracts comprise numerous bioactive components, including saponins, tannins, flavonoids, terpenoids, as well as glycosides. This explains the ability of plant extracts to inhibit the bacteria used in this study. The finding of this research are confirmed by the study of Rao et al.[20]. which identified the presence of active compounds like tannins, flavonoid and terpenoids, in the aqueous and ethanol extracts of *C. auratium*. The research aligns with the findings of Khudair *et al*. [21]

Exhibited the effectiveness of ethanol extracts from *Myrtus communis* as an antibiotic against *S. aureus* and *P. aeruginosa*, in comparison to *Citrus aurantium* which exhibited intermediate effectiveness [22]. Plant extracts have a significant role in disease therapy and human health care, as they contain numerous bioactive compounds that can be employed in the production of pharmaceuticals derived from natural sources[23]. This work presents the examination of *M. communis* and *C. aurantium* leaf extracts against several pathogenic bacteria, as indicated by inhibitory zones in the agar well diffusion assay. Most of the examined extracts had antibacterial action, with varying selectivity for each bacterium. The beneficial effects of myrtle extract on hospital pathogens have been documented. A study evaluated the effects of various plants, including myrtle, against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* isolated from hospital patients. The results indicated that myrtle extract produced growth inhibition zones of 30, 50, and 22 mm in the diffusion penetration test; thus, the use of myrtle extract for treating sinusitis and bronchitis was recommended [24].

CONCLUSION

Our findings indicate that ethanol extracts from plant leaves (*M. communis* and *C. aurantium*) have the potential to serve as antibiotics against prevalent bacterial isolates responsible for infections in our community. Antibiotics derived from these extracts would be beneficial in mitigating infections by utilizing natural antibiotics devoid of the adverse effects associated with conventional antibiotics**.**

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Competing interests

The authors declare that they have no competing interests.

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