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Inhibitory Activity of Methanolic and Aquatic Extracts of *Myrtus Communis* on the Biofilm Formation of Methicillin-Resistant *Staphylococcus aureus*

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Abstract

Finding effective medicines against resistant bacteria is extremely urgent given the occurrence of antibiotic resistance. The extract of *myrtle* plant contains compounds that show antibacterial properties. This investigation sought to assess if the methanolic and aqueous extract of *Myrtus communis* can inhibit biofilm formation, which is formed by a serious pathogen, *Staphylococcus aureus*, using standard methods. 82 *Staphylococcus aureus* isolates were isolated and diagnosed from various sources including wounds, nasal swabs, ear swabs, and blood-based on cultural, and microscopical properties, molecular identification, and automatic,(VITEK-2 system). Herein, Sixty-eight *mecA*-positive, *S. aureus* (MRSA) isolates were identified from clinical samples. On these isolates, the antibacterial and anti-biofilm properties of *M. communis's* methanolic and aquatic extract were assessed. All MRSA isolates can produce biofilm. The results displayed that the average biofilm formation of 22 strong isolates was (1.118 ± 0.30). When compared to control groups, The aqueous and methanolic extracts of *M. communis* significantly decreased the biofilm formation ($P < 0.05$). The range of the minimum inhibitory concentration (MIC) for several isolates was 0.0325 to 2 mg/ml. The results of the modified CV assay showed that *Myrtus communis* has a biomass attachment effect that is greater than 70% (percentage inhibition) at 2 MIC. Regarding a comparison of the effects of Myrtle's alcoholic and aqueous extracts, no significant differences exist ($P > 0.05$), both have a significant effect in inhibiting bacterial growth and biofilm formation. The extract prevents growth and biofilm formation, destroys pre-formed biofilm, and kills cells living inside the biofilm.

Keywords: Antibiotics resistance, Biofilms, *Myrtus communis*, *Staphylococcus aureus.*

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Introduction

Antibiotic resistance in bacterial biofilm communities increases the burden of biofilm in the medical field and leads to chronic infections. Hospital-acquired infections continue to be mostly caused by *S. aureus* and Drug-resistant organisms are becoming a serious concern, even though *Staphylococcus aureus* infections were previously curable with conventional antibiotics [1,2]. The most common way for S. aureus to spread to others is through contaminated hands. Normally, the skin and mucous membranes act as a strong barrier to prevent infection. However, S. aureus may be able to enter underlying tissues or the circulation if these barriers are broken (for example, skin injury from trauma or mucosal damage from a viral infection) and cause infection Persons who are immunocompromised or who have invasive medical devices are particularly vulnerable to infection [3-5]. The strong propensity of MRSA to build biofilm on both biotic and abiotic surfaces exacerbates the issue more [6-8]. The virulence of *S. aureus* is attributed to its adhesion and invasion abilities, the capacity to adhere is linked to the production of biofilms and confers protection against antibiotics. A population of microbial cells that cling permanently to the substrate or one another is called a microbial biofilm. This population creates an extracellular polymeric matrix that surrounds it [9,10]. Resistant to methicillin The penicillin-binding protein (PBP2a) is encoded by the *mecA* gene, which *S. aureus* strains known as MRSA have acquired [11]. *mecA* is an important gene that gives MRSA the inherent ability to grow in the presence of penicillin-like antibiotics. *mecA* gene present in all MRSA strains [12]. PBPs are membrane-bound enzymes that are crucial to the growth, division, and structure of microorganisms[13]. Antibiotic treatments commonly used for bacterial infections may reduce biofilm but cannot eliminate it, and often come with significant side effects. The power of biofilm disruption lies in its potential to dismantle intricate microbial communities, offering a key intervention against bacterial infections that involve biofilm formation. Natural antibiofilm substances such as phytochemicals, show promising capabilities in reducing biofilm growth and addressing related health challenges. Researchers from around the world have been inspired by the challenges posed by biofilm to explore new solutions for controlling it. In this case, reliable data demonstrated that the problems associated with biofilms can be addressed by using plant products, a valuable source of bioactive chemicals with antibacterial and chemopreventive qualities [14]. Many recent studies have evaluated the effect of natural antibiofilm products and assessed the potential antibiofilm impacts of ingredients found in natural products. *Myrtus communis L.*, also known by its Arabic name Aas or Hadas, is a small tree or typical evergreen shrub that is fragrant with leaves. It is a member of the Myrtaceae family. It is Indigenous to the Middle East, which includes nations like Jordan, Iraq, and Saudi Arabia, as well as the Mediterranean region [15]. It is traditionally used as a hypoglycemic, disinfectant, and antiseptic[16]. In Turkish villages, myrtle leaves have long been utilized as an antibacterial remedy [17]. Similar to this, this plant's fruit is used in Italian folk medicine to cure a variety of infectious diseases, such as dysentery and diarrhea; the leaves are used as a mouthwash, antiseptic, and inflammatory agent to treat candidiasis[18]. Lung conditions are treated using the essential oil extracted from myrtle leaves[19]. This study examined the *M. communis* extracts as antibacterial and anti-biofilm activity against MRSA *S. aureus* that was isolated from clinical samples.

Methods

Bacterial identification

A total of 82 *S. aureus* isolates were isolated and diagnosed from hospitals in the city of Anbar, western Iraq, during a period of approximately 4 months from September 2023 to January 2024 from patients suffering from various infections suspected of being infected with *S. aureus*. These samples were

collected from "Fallujah Teaching Hospital", "Women and Children's Hospital", "Ramadi Teaching Hospital", and "Ramadi Hospital for Women and Children". where the sources of these samples were different, including (Wound, Nasal swabs, Blood, Ear swabs). *S. aureus* isolates were identified through microscopic diagnosis, biochemical tests, vitek system diagnosis, and genetic diagnosis. For examination of the ability to mannitol fermentation, and hemolysis type, Each sample was grown independently on mannitol salt agar and blood agar and incubated aerobically at 37°C for "24" hours.

DNA extraction and detection of *nuc* and *mecA* genes

Isolates of *S. aureus* were active on mannitol agar for an 18 hr. For 20 h, three to four colonies were grown and incubated at 37°C. DNA was extracted using the Norgen-Canada Microbiome DNA Isolation Kit, stored at -20°C. The manufacturer's instructions were followed to make the 1% "agarose gel", 1 X "(TAE) buffer", and molecular weight markers (100 bp). PCR mixtures (24 µL) contained(14µL of blue master-mix, 1 µL of forward primer 5- GCGATTGATGGTGATACGGTT-3(10 Pm/µL), 1 µL of reverse primer 5-GCCAAGCCTTGACGAACTAAAGC-3(10 Pm/µL) for *nuc*, and the same concentration was also used for the *mecA*, F5-TCCAGATTACAACTTCACCAGG-3, R 5-CCACTTCATATCTTGTAACG-3, DNA template (60 ng) and 6 free nuclease water to complete the volume to 24 µL. Amplification was conducted in a thermocycler (T100; Biorad, Hercules, CA, USA) with 30 cycles of denaturation at 92°C for 45 seconds, annealing at 52°C for 30 seconds, and elongation at 72°C for 1 minute, followed by a final extension step at 72°C for 7 minutes[20]. The gene-specific PCR amplicons were visualized using Image Lab Software (Bio-Rad).

Ethical and approval committee

The study was approved by the Al-Anbar Directorate of Health Ethics and Research Committee (approval number 34, decision No. 2022054, dated October 25, 2023).

Antibiotic sensitivity test

Various classes of antibiotics were used in the antibiotic susceptibility test, which was carried out on (82) bacterial isolates by the disk diffusion method as described by Bauer et al[21]. 15 antibiotic agents (disks) tested against *S. aureus* they are; Penicillin(10µg), ciprofloxacin(5µg), Azethromycin(15µg), Chloramphenicol(30µg), Tetracycline(10µg), vancomycin(30µg), nitrofurantions(100µg), gentamycin(120µg), oxacillin(10µg), amoxicillin(10µg), doxycyillin(10µg), sulfamethoxazole(25µg), cefixime(5µg), amikacin(30µg) and cefoxitin(30µg) manufactured by (Bioanalyse Company Turkey origin).The sensitivity of antibiotics was determined by measuring the inhibition zone diameter to identify whether the bacteria were susceptible (S), intermediate (I), or resistant (R) to the antibiotics according to CLSI 2023 recommendation.

Biofilm formation

Biofilm formation was assessed semi-quantitatively using 96-well flat bottom plates, following established methods[22]. Bacterial inoculations were prepared by diluting bacteria in TSB with 1% glucose at a 1:100 ratio. Each well of a 96-well flat-bottomed polystyrene plate received 200 µL of bacterial suspension, then incubated overnight at 37°C. After incubation, plates were washed with PBS and stained with 0.1% Crystal Violet for 15 minutes at room temperature. Excess stain was removed by washing, and biofilm was quantified by measuring OD570 nm after solubilization in ethanol. Biofilm assays were performed in triplicate, and isolates with OD570 values ≥ the positive control were classified as biofilm-positive and studied *Myrtus communis* extract against biofilm development. To assess cell attachment, the

modified crystal violet (CV) test was employed. Using a microplate reader, absorbance at 595 nanometers was measured. The average absorbance (OD595 nm) and the following equation were used to determine the biomass formation inhibition percentage for each concentration of the test materials:

(Percentage inhibition = 100 - [(OD595 nm experimental well with test material / OD595 nm control well without test material) x 100]).

Plant collection

Myrtus communis was collected in summer from Fallujah city's public nurseries in western Iraq, The plant leaves were washed with tap water, allowed to air dry at room temperature, and then ground into a powder and saved the powder.

Preparation of extracts:

A. Alcoholic extraction.

In a sterile flask, 100 g of dried powder *Myrtus communis* was soaked in 500 ml of 70% methanol, and shaken vigorously for 30 min. at 37 C. The extract was filtered with "Whatman filter paper", and centrifugation at 2500 rpm for 10 min. was done. The supernatant fraction was taken and dried under pressure at 40 C using rotary evaporate. Finally, the extract powder was stored in a refrigerator in a dark sterile bottle until use. Different concentrations (2mg/ml, 1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml, 0.0625mg/ml) were prepared from stock solution.

B. Aqueous extraction.

Parekh methods were used [23], 50 grams of plant powder was added to 500 ml of distilled water, with shaking for 30 min. at 37 C. The extract was filtered with "Whatman filter paper", and centrifugation at 2500 rpm for 10 min. was done. The supernatant fraction was taken and dried under pressure at 40 C using rotary evaporate. Finally, the extract powder was stored in a refrigerator in a dark sterile bottle until use. Different concentrations (2mg/ml, 1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml, 0.0625mg/ml) were prepared from stock solution.

Determination of Minimum Inhibitor Concentration (MIC)

Using the Resazurin Microtitre-plate Assay, the minimum inhibitory concentration (MIC) of the methanolic and aquatic extract solutions was assessed (REMA)[24]. Each well of the microtitre plate received 100 µl of Brain Heart Broth (BHI) under aseptic conditions. The first row of the 96-well plates was then filled with 100 µl of extract solutions (2 mg/ml for the methanolic extract and 2 mg/ml for the aquatic extract), each one independently. Pipetting 100 µl of the substance test in successively decreasing concentrations (1/2,1/4,1/8,1/16,1/32,1/64, and 1/2048) was how serial dilutions were carried out. Each well-received 10µl of bacterial solution containing 1.5x10 CFU/ml. After lightly wrapping them with Parafilm, they were incubated at 37°C for 18 to 24 hours. Following the incubation period, 30 microliters of resazurin solution were added to every well, and the plate was then incubated again for four hours to monitor any color changes.

The variations in resazurin's color were used to visually examine the outcomes. The MIC value was determined by using the lowest concentration at which there was no change in the color of resazurin[25].

Inhibitory effect of extract on biofilm formation

A- Inhibition of Initial Cell Attachment

The extract of *Myrtus communis* was used to evaluate its effect on the attachment of planktonic cells. Aqueous and Alcoholic Extract solutions (equivalent to 2 MIC, 1 MIC, 0.5 MIC, 0.25 MIC, 0.125MIC, 0.0625MIC) were prepared. Biofilm development was quantified using the modified crystal violet test (CV) by using microtiter plates[26].

B- Inhibition of preformed biofilm

The effect of the extract on biofilm development and maturity was calculated. Before adding material of extract, the bacteria were grown for 24 hours and allowed to form biofilms. The 200 µL diluted *S. aureus* cell suspension was transferred to a 96-well plate and incubated at 37 °C. After biofilm formation, the different concentrations of extract (2mg/ml,1mg/ml,0.5mg/ml,0.25mg/ml,0.125mg/ml ,0.0625mg/ml) were added to the pre-formed biofilm, Then after that, the plates were incubated for 8, 12, 16, 20, and 24 hours after test material was used on biofilms that had formed. Following the removal of the supernatant-containing cells scattered from biofilms, the plates were cleaned, dried, and stained for fifteen minutes using 200 µL of crystal violet. A modified CV test was used to quantify the biofilm, and biofilms were assessed for biomass attachment [26].

Statistical analysis

Numerical data were statistically analyzed for significance and presented as Mean ± SD. Significance was determined at the 5% level ($P < 0.05$) using an F test. Data analysis was conducted using SPSS version 22 software.

Result and Discussion

Staphylococcus aureus identification.

Eighty-two isolates from 200 clinical specimens were determined to be *Staphylococcus aureus*. *S. aureus* was distributed in chronic burns wounds swabs from patients who the government hospitalized, and wounds Inflammation (Wound 48 (58.53%), Nasal swabs 18(21.95), Blood 10 (12.19), and Ear swab 6 (7.3%)). bacteremia patients 10 (12.19). *S. aureus* isolates were identified through cultural tests, microscopic diagnosis, biochemical tests, and genetic diagnosis (Table 1)(Fig.1). The finding that *S. aureus* was the most prevalent isolate from wounds is consistent with many earlier publications and other research, particularly those from affluent nations, that show *S. aureus* as the main bacterium [27], [28]. In another study in Iran, *S. aureus* was common Pathogens in burn wound infections after Pseudomonas aeruginosa, at a rate of 20.2% [29]. Results of previous studies,[30-32] indicate that the most prevalent isolates in wound injuries are *S. aureus* and P. aeruginosa. *S. aureus* is frequently found in burn wounds because it colonizes human skin and mucous membranes and can be transmitted from hands and nose to wounds. wounds offer an ideal environment for bacterial growth and are more persistent sources of infection compared to surgical wounds, largely due to their larger size and extended hospital stays. Extensive wounds can also lead to immunosuppression[33]. An antibiotic disc containing cefoxitin (30 µg/disc) was used to identify methicillin-resistant isolates of *S. aureus* due to its outstanding sensitivity for recognizing the *mecA* gene [23, 23]. Also Using the conventional PCR, the *mecA* gene's existence was molecularly verified. upon the appearance of a 162 bp band on a 2% agarose gel. wherein PCR was used to demonstrate that 68/82 (82.92%) isolates carried the *mecA* gene.

Table 1: Phenotypic characteristics and Biochemical test results for *S. aureus* isolate.

Gram stain	Fermentation of Mannitol	Catalase Test	Coagulase Test (Slide and Tube)	Oxidase Test	Indol Test
+	+	+	+	-	-

Figure (1) Molecular identification by *nuc* and *mecA* genes.

Antibiotic sensitivity test

Among 82 isolates of *S. aureus*, 68 isolates (82.92%) were MRSA while 14 isolates (17.03%)were MSSA. According to the findings, there was the greatest penicillin resistance (100%), cefoxitin (83%), oxacillin (83%), amoxicillin (76%), Azethromycin(61%), vancomycin (20%)Tetracycline(25%), doxycyillin(28%), sulfamethoxazole(16%), Chloramphenicol(15%), ciprofloxacin(7%), nitrofurantions(27%), gentamycin(0%), cefixime(100%), amikacin (0%) fig(2). Of the isolates, 14(17%) Sensitive isolate and 68(83%) resistant isolates emerged. These results are consistent with those of Kandala et al. (2017), who found that 13.95% of isolates were methicillin-sensitive and 86.04% of isolates were methicillin-resistant[34]. The bacterial isolates' high level of resistance to Penicillin G may result from mutations in Penicillin Binding Proteins, the presence of resistance genes on chromosomes or plasmids, or beta-lactamases that analyze Penicillin[35].

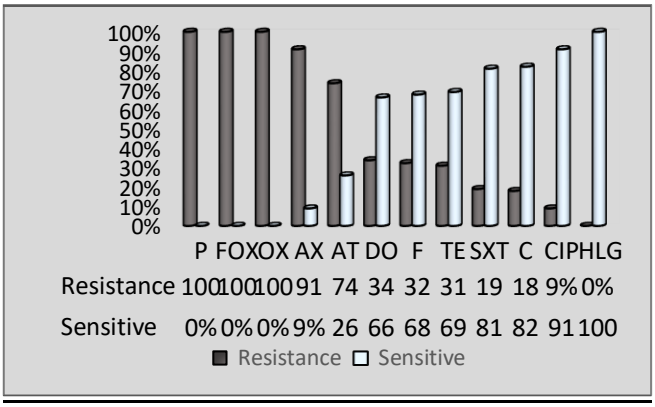


Fig. 2. The percentage of antibiotic susceptibility test of *S. aureus* isolates. Penicillin (P), Cefoxitin (FOX), Oxacillin (OX), Amoxicillin (AX), Azithromycin (AT), Doxycyillin (DO), Nitrofurantoin (F), Tetracycline (TE), Sulfamethoxazole (SXT), Chloramphenicol (C), Ciprofloxacin (CIP), Gentamycin (HLG), Vancomycin(V), Amikacin (AK).

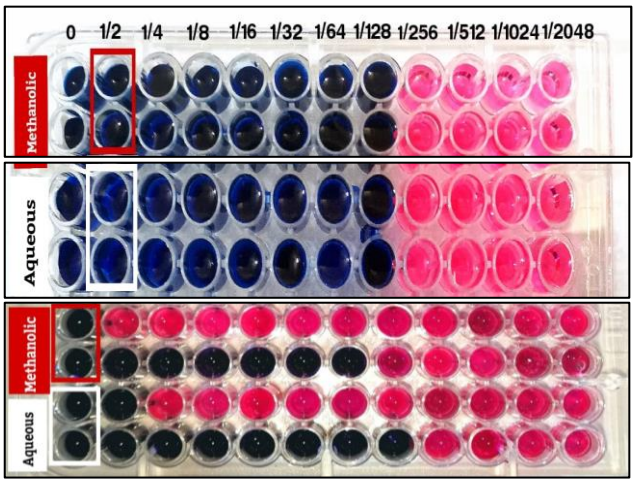
Biofilm formation analysis

Although all MRSA isolates were capable of producing biofilms, only (22) 32% of isolates were able to do so, 43) 63% of isolates were intermediate, and 4% of isolates were weak, the results showed the average biofilm formation of (22) strong isolates was (1.118±0.30).

Determination of Minimum Inhibitor Concentration (MIC)

The MIC results of aquatic and methanolic extract solutions are shown in Figure (3). The present study displayed that the Methanolic and Aquatic extracts of *Myrtus communis* had antibacterial activity on *s.aureus*. The antibacterial action on *S. aureus* was seen at all concentrations of the aqueous extract and methanolic extract of *Myrtus communis*, ranging from 2 mg/ml to 0.0312 mg/ml. with Mansouri [36], was that the alcoholic extract (Ethanol 95%) of leaves of the Iranian *Myrtus communis* has good

growth inhibition activity against *S. aureus* and the ability of the *Myrtus communis* methanolic and aquatic extracts to inhibit both Gram-positive and Gram-negative bacteria. There are no significant differences ($P > 0.05$) between the effect of the aqueous and alcoholic extract of the Myrtle. Contents of Myrtle leaf extracts of highly toxic phenols and polyphenols that inhibit the growth of Gram-positive and negative bacteria enhance its effectiveness. In addition, the presence of other compounds such as α- phinene and 1.4 cineole are effective in inhibiting the growth of bacteria[37]. *Myrtus communis* methanolic and aqueous extractions exhibited antibacterial activity against *S. aureus*. It does not appear essential to provide an alcoholic extract, as this is an expensive and time-consuming procedure, given that the majority of concentrations of *Myrtus communis* aqueous extract were effective against bacteria.



Figure(3) MIC results of aquatic and methanolic extract solution

Inhibitory effect of extract on biofilm formation

A. Inhibition of Initial Cell Attachment

The results showed that the high effect of *Myrtus communis* on biomass attachment, the inhibition percentage exceeds 72% at 2 MIC, and at the MIC, also 0.5 MIC, and 0.25 MIC the inhibition exceeded 50%. Even at 0.125 MIC, and 0.0625 MIC, there was a reduction in biofilm formation but not as much as the reduction of 2 MIC or MIC, as shown in Table.2, figure (4).

Table 2: The impact of varying *Myrtus communis* concentrations on initial cell attachment of *S. aureus.*

	methanolic extract		Aquatic extract		
Concentrations	**Mean%**	**± SD**	**Mean%**	**± SD**	**Sig**
0.0625mg/ml	**42.34**	**± 7.18**	**49.09**	**± 6.68**	***p*>0.05**
0.125mg/ml	**45.18**	**± 7.23**	**51.58**	**± 7.21**	***p*>0.05**
0.25mg/ml	**60.65**	**± 7.47**	**57.00**	**± 7.16**	***p*>0.05**
0.5mg/ml	**63.16**	**± 7.47**	**62.30**	**± 7.23**	***p*>0.05**
1mg/ml	**72.68**	**± 7.64**	**70.96**	**± 7.51**	***p*>0.05**
2mg/ml	**75.13**	**± 7.69**	**73.86**	**± 7.74**	***p*>0.05**

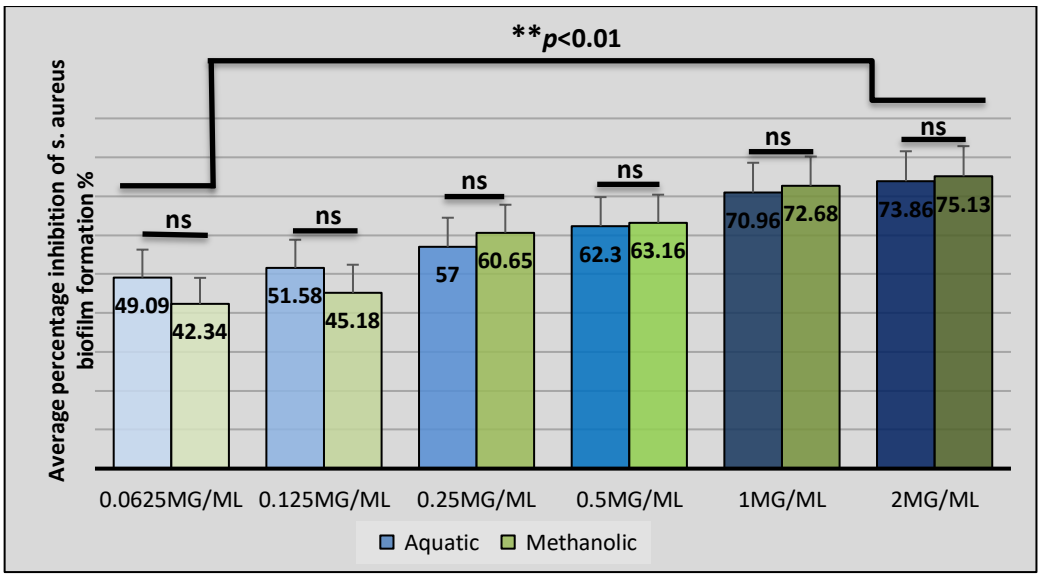


Figure (4) Effect of different concentrations of *Myrtus communis* on initial cell attachment of *S. aureus*, displayed as Percentage inhibition of *S. aureus* biofilm formation (%).

B. Inhibition of preformed biofilm

The *Myrtus communis* were tested against preformed biofilms (24 h) and examined after 8h, 12h, 16h, 20h, and 24h of incubation. We observed that as the incubation period increased, the percentage inhibition of preformed *S. aureus* biofilm also increased. figure (5). The results of the average biofilm formation treated with methanolic and aqueous extracts were detailed in Tables 3 and 4. High anti-biofilm characteristics of natural substances can be categorized into five main types. These include polypeptides, alkaloids, phenolics, essential oils, terpenoids, lectins, and polyacetylenes [38]. The extract of myrtle plant contains polyphenolic compounds that display antibacterial Activity[39]. The results obtained show that myrtle extract possesses antibacterial activity against all the tested (MRSA) isolates with a particularly significant inhibitory effect against the biofilms. Natural anti-biofilm compounds may target the various stages of the complex process that biofilm formation and development entails to stop biofilm development. Some of the well-studied stages of biofilm development include (1) bacterial cell adhesion to an appropriate biotic or abiotic surface, (2) biofilm structure creation, (3) biofilm maturation, and (4) dispersion. Targeting one or both of the first two stages appears to be the best course of action for preventing the formation of biofilms, as they are crucial to the development of biofilms [40]. A class of chemicals is known as phenols. Condensed tannins, one of its seven subclasses, exhibit anti-biofilm action, along with phenolic acids, quinones, flavonoids, flavones, flavonols, tannins, and coumarins[41]. These chemicals interact with biofilm by six primary mechanisms: they connect to proteins, interact with eukaryotic DNA, impede viral fusion, disrupt membranes, and bind to adhesin complex and cell wall[42] ;[43]. The extracts' phenolic content could be a factor in the antibacterial activities that have been found. The primary flavonoids in the myrtle extract appear to have some connection to the antibacterial action that has been shown, particularly against Gram-positive bacteria[44]. Numerous polyphenols have been shown to have anti-biofilm qualities against *S. aureus* in this regard[45,46], even though biofilms may withstand antibiotics far better than planktonic cells [47]. In addition, polyphenols can cause cell death by reducing the concentration of certain necessary metabolites, disrupting the balance ionic strength, or altering the proton gradient. As a potential source of antibacterial agents for the creation of novel antibiotic therapeutics, It is possible to utilize plant polyphenols alone or in combination with the antibiotics that are present.

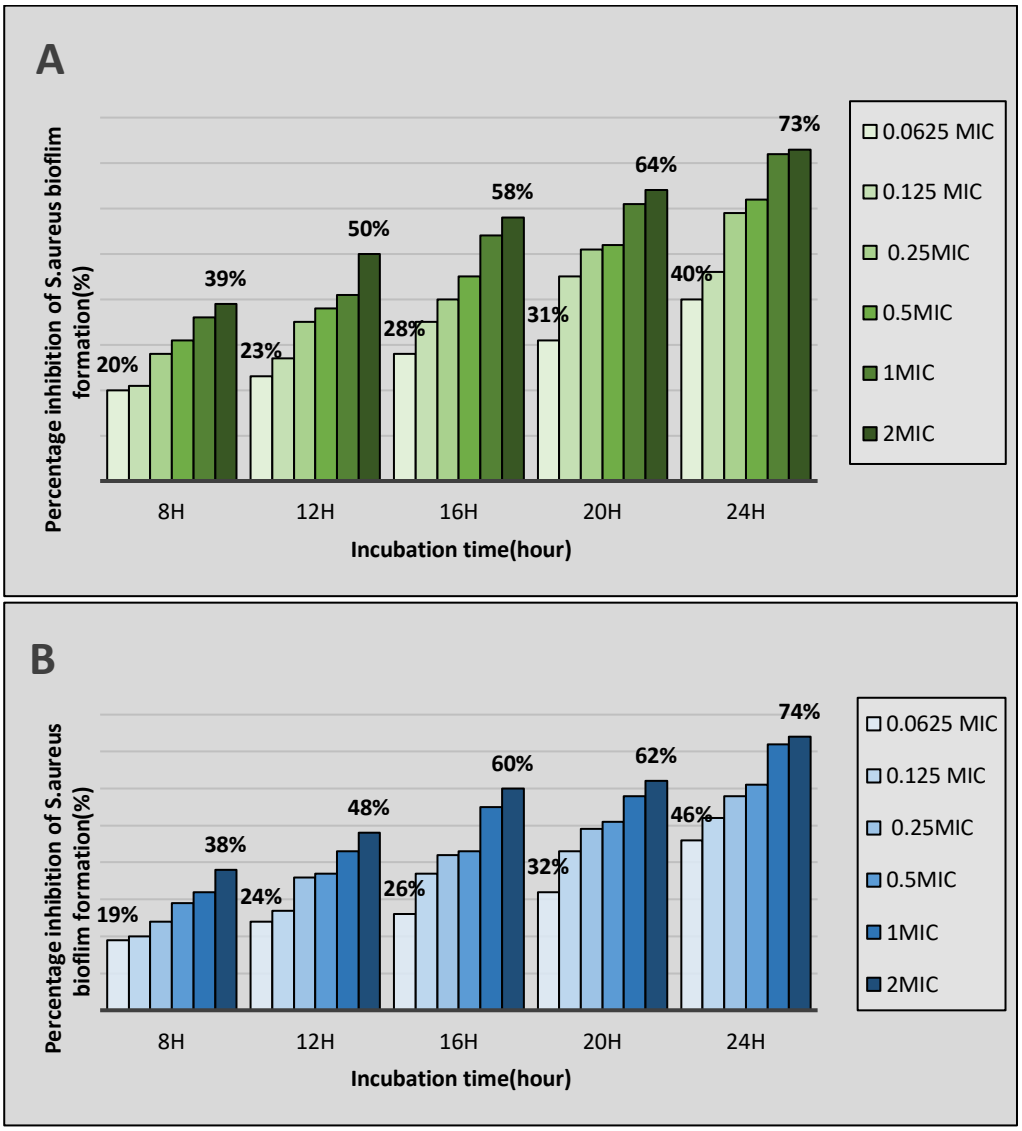


Figure (5) Inhibition percentage of performed *S. aureus* biofilm of *S. aureus* on 24h. (A) methanolic extract of *Myrtus communis*. (B) Aquatic extract of *Myrtus communis*.

Table 3: Mean and standard deviation of biofilm formation with methanolic extract in various concentrations and times.

Concentrations (mg/ml)	8h Mean ± SD	12h Mean ± SD	16h Mean ± SD	20h Mean ± SD	24h Mean ± SD
0.0625 mg/ml	0.898 ± 0.2*	0.863 ± 0.2*	0.811 ± 0.3*	0.773 ± 0.2*	0.667 ± 0.3*
0.125mg/ml	0.887 ± 0.2*	0.812 ± 0.5*	0.725 ± 0.2*	0.618 ± 0.1*	0.599 ± 0.2*
0.25mg/ml	0.811 ± 0.1*	0.723 ± 0.3*	0.674 ± 0.1*	0.543 ± 0.1*	0.460 ± 0.1*
0.5mg/ml	0.775 ± 0.3*	0.698 ± 0.2*	0.614 ± 0.3*	0.539 ± 0.3*	0.423 ± 0.09*
1mg/ml	0.712 ± 0.1*	0.656 ± 0.2*	0.514 ± 0.1*	0.440 ± 0.1*	0.310 ± 0.3*
2mg/ml	0.678 ± 0.1*	0.564 ± 0.2*	0.469 ± 0.1*	0.402 ± 0.4	0.302 ± 0.2*
Control	1.118±0.30				

*There was a significant difference between the average of biofilm formation treated and control groups (P<0.05).

Table 4: Mean and standard deviation of biofilm formation with Aquatic extract in various concentrations and times.

Concentrations (mg/ml)	8h Mean ± SD	12h Mean ± SD	16h Mean ± SD	20h Mean ± SD	24h Mean ± SD
0.0625mg/ml	0.910 ± 0.2*	0.848 ± 0.2*	0.825 ± 0.3*	0.756 ± 0.3*	0.602 ± 0.3*
0.125mg/ml	0.892 ± 0.2*	0.820 ± 0.2*	0.703 ± 0.2*	0.639 ± 0.3*	0.537 ± 0.2*
0.25mg/ml	0.854 ± 0.2*	0.714 ± 0.3*	0.650 ± 0.2*	0.572 ± 0.3*	0.468 ± 0.2*
0.5mg/ml	0.798 ± 0.2*	0.701 ± 0.3*	0.632 ± 0.3*	0.545 ± 0.3*	0.441 ± 0.3*
1mg/ml	0.763 ± 0.2*	0.641 ± 0.3*	0.502 ± 0.3*	0.473 ± 0.2*	0.311 ± 0.3*
2mg/ml	0.689 ± 0.3*	0.583 ± 0.3*	0.452 ± 0.3*	0.430 ± 0.2*	0.296 ± 0.2*
Control	1.118±0.30				

*There was a significant difference between the average of biofilm formation treated and control groups (P<0.05).

Conclusions

In the context of struggling against pathogenic bacteria, using biological techniques, we examined through this study the anti-*S. aureus* activities of methanolic and aquatic extracts of *Myrtus communis*. Our results showed the potent efficacy of both methanolic and aquatic extracts on *S. aureus*. Additionally, we highlight the anti-biofilm properties due to their active compounds. that have exhibited significant antibiofilm activity against MRSA isolates at low-range MIC values. Due to their multitargeted nature, polyphenols' antibiofilm potential has a very diversified mode of action. One of the primary targets of polyphenols appears to be biofilm. The studied extract appears to be a potential low-cost antibacterial agent that comes from a widely available plant. Myrtle extract has the potential to serve as a substitute for traditional antimicrobial treatments, which often use synthetic compounds like parabens, chlorohexidine, phenoxyethanol, and antibiotics that are less safe for human health and more polluting.

Conflict of interests: None

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