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Original Article

Phytochemical Profiling and Antibiofilm Efficacy of *Myrtus communis* Extract Against Multidrug-Resistant Pathogens: A Sustainable Biotherapeutic Approach Aligned with Circular Economy Principles

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Abstract

The increasing prevalence of multidrug-resistant (MDR) bacteria and their ability to form persistent biofilms represent major challenges in the treatment of chronic and recurrent infections, highlighting the need for effective natural antimicrobial agents. This study evaluated the phytochemical composition and antibacterial and antibiofilm activities of methanolic leaf extract of *Myrtus communis* against clinical MDR isolates of *Staphylococcus aureus* and *Klebsiella pneumoniae*. The extract was prepared by maceration with methanol and characterized using gas chromatography–mass spectrometry (GC–MS). Antibacterial activity was assessed using agar well diffusion and resazurin-based broth microdilution assays to determine the minimum inhibitory concentration (MIC). Biofilm formation and inhibition were evaluated using the crystal violet microplate assay. GC–MS analysis identified 35 phytochemical compounds, with tert-butylhydroquinone (26.34%), a hypoxanthine-related compound (10.10%), and 5-hydroxymethylfurfural (8.76%) as major constituents. The extract demonstrated antibacterial activity against both MDR isolates, with *S. aureus* showing greater susceptibility (MIC 3.125 mg/mL) than *K. pneumoniae* (MIC 6.25 mg/mL). Both isolates exhibited strong biofilm formation before treatment. Following exposure to the extract, biofilm biomass decreased significantly from 0.1290 ± 0.0026 to 0.0287 ± 0.0015 in *S. aureus* ($P = 0.0002$) and from 0.1287 ± 0.0091 to 0.0293 ± 0.0038 in *K. pneumoniae* ($P = 0.0027$). These findings demonstrate that *M. communis* possesses considerable antibacterial and antibiofilm activities against clinically relevant MDR bacteria, supporting its potential as a natural source for developing alternative antimicrobial strategies.

Keywords: *Myrtus communis*; multidrug-resistant bacteria; biofilm inhibition; phytochemicals; *Staphylococcus aureus*; *Klebsiella pneumoniae*.

1. Introduction

Multidrug-resistant (MDR) bacteria have emerged as a major public health challenge, significantly contributing to morbidity and mortality in modern healthcare and imposing a significant economic toll on healthcare systems around the world [1]. These pathogens are also exacerbated by their ability to form biofilms, which are tightly constructed communities of microorganisms surrounded by an extracellular matrix that they produce. Biofilm formation creates several advantages for bacteria, including the ability to persist in the presence of antibiotics and to evade host immune system responses, which allows for chronic and recurrent

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infections in both hospital and community settings [2,3]. *Staphylococcus aureus* and *Klebsiella pneumoniae* are two clinically important biofilm-forming bacteria that have gained resistance to multiple classes of antimicrobials, such as β-lactams and fluoroquinolones, that are used in routine clinical treatment [4,5].

Evolutionary development of synthetic antibiotics in turn has encouraged the interest in medicinal plants as a valuable source of new antimicrobial compounds. Myrtus communis L. Red and opposite-leafed leaves are commonly known as common myrtle (Myrtus communis L.), a shrub perennial which has been used for a long time in traditional medicine for the treatment of infectious and inflammatory diseases, and for wound repair [6]. It is mainly attributed to its therapeutic properties due to the presence of a wide range of secondary metabolites, such as volatile oils, tannins, terpenoids, flavonoids, and phenolic compounds, among which are those with potent antimicrobial, antioxidant, and antibiofilm properties [7].

The characterization of plant-derived bioactive molecules has been greatly enhanced by advances in phytochemical research. Recently, modern analytical platforms such as gas chromatography coupled with mass spectrometry (GC–MS) have been used to extensively profile extracts of M. communis and to identify several compounds with known bioactivity, like eucalyptol, maltol, theobromine, and other metabolites [8]. The antimicrobial properties of these phytochemicals can be mediated by several mechanisms, including disruption of the integrity of bacterial cell walls, inhibition of quorum sensing signaling pathways, inhibition of EPS production, and eventually the weakening of the structure and formation of biofilms [9].

Botanical antimicrobials offer a promising alternative to conventional drugs from a sustainability point of view due to their renewable, biodegradable, and in general, low environmental impact. The use of these also supports circular economy approaches by ensuring efficient use of plant resources such as agricultural residues and processing by-products to produce high-value therapeutic products [10,11]. This helps to foster eco-friendly health care, diminish reliance on synthetic antimicrobials, and create more environmentally friendly pharmaceutical technologies.

Therefore, the current study aimed to determine the phytochemical profile of methanolic extract of M. communis leaves and its antibacterial and antibiofilm efficacy against clinically relevant multidrug-resistant (MDR) isolates of S. aureus and K. pneumoniae. The study aims to deliver more evidence for the use of M. communis as a natural source of a sustainable antimicrobial agent for biofilm-associated infections and help the development of sustainable therapeutic strategies in line with public health objectives and the circular economy.

2. Methodology

2.1. Plant Collection and Identification

Myrtus communis leaves were collected in Ramadi, Anbar, from the western region of Iraq. The identification of the plant was performed by Dr. Mohammed Abdul and deposited in the laboratory of Bioactive Substances.

2.2. Extraction of Active Compounds with Some Modification

Initially, leaves by-products were kept in good condition (dark and dry with proper temperature) and dried thoroughly. The usable parts of the plant were powdered in a mill containing a sieve with a pore size of 200 micrometers. Then, 200 grams of each plant powder was dissolved in 1000 mL of absolute methanol [12]. In this study, the maceration method was used to prepare plant extracts. Then, extracts were dried by use of a rotary evaporator (Heidolph, Germany) under vacuum. The extracts were kept in the refrigerator until the time of the experiment.

2.3. Antibacterial Activity of Natural Products

In the current study, the well-agar diffusion method was used to determine the zone of inhibition for myrtus products. Muller-Hinton agar was prepared and allowed to sterilize for 15 minutes at 120 lbs, and was poured into disinfected plates and left aside to

solidify. Wells were created using a cork borer. Later, the test organisms were swabbed into them. The natural products and neem extract with different concentrations were loaded, and the plates were incubated at 37 °C for 24 hours. After the incubation time, the zone of inhibition was measured. The presence of antimicrobial activity is indicated by the absence of bacterial growth directly below the test sample [13].

2.4. Determination of Minimum Inhibitor Concentration (MIC)

The minimum inhibitory concentration (MIC) of myrtle was evaluated by the Resazurin Microtiter-plate Assay (REMA) with some modifications. In aseptic conditions, 100 µl of Mueller-Hinton broth (MHB) was added to all wells of microtiter plates. Then, 100 µl of myrtle was separately transferred into the first row of the 96-well plates. Serial dilutions were performed by pipetting 100 µl of the material test from the first well to the next well in serially decreasing concentrations (1/2,1/4,1/8,1/16,1/32,1/64 and 1/128). 10µl of bacterial suspension containing 1.5×10 CFU/ml was added to each well. They were wrapped loosely with Parafilm and incubated for 18-24 h at 37°C. After incubation, 10 µl of resazurin solution (Alamar blue) was added to each well, and the plate was re-incubated for 18-24 hr for the observation of color change. The results were analyzed visually by observing the changes in the color of resazurin, with changes from purple to pink or red being recorded as positive. The lowest concentration with no change in resazurin color was taken as the MIC value [14].

2.5. Evaluation of Biofilm Formation and Antibiofilm Activity of Myrtus communis Extract Against Multidrug-Resistant Bacteria

Multidrug-resistant (MDR) bacterial isolates were tested for the capacity to form biofilm using a standard microtiter plate crystal violet assay for bacterial adherence and biofilm biomass, which is widely accepted and reproducible [15]. Gram-negative *Klebsiella pneumoniae* and Gram-positive *Staphylococcus aureus* were cultured overnight at 37 °C in trypticase soy broth (TSB) with 2% glucose to enhance the production of biofilms [16]. After incubation, 2 µL of each of the bacterial suspensions was transferred to 198 µL of fresh TSB-glucose medium in sterile, flat-bottom 96-well microtiter plates. The sterile broth was used for the negative control wells.

The plates were kept statically for 24 hours at 37 °C. After incubation, the planktonic cells were removed by aspiration, and the wells were washed gently three times with sterile phosphate-buffered saline (PBS, pH 7.2) to wash away non-adherent cells. The plates were turned upside down and allowed to dry in the air at room temperature. The biofilm was stained with 0.1% crystal violet (CV), which was then added to each well and incubated for 15 minutes. The stained biofilms were thoroughly washed with sterile distilled water to remove excess stain and then dried. For quantification of the biomass of the biofilm, 200 µL of 5% isopropanol in 1% hydrochloric acid (HCl) was added to solubilize the CV, and optical density (OD) was measured at 630 nm by a microplate reader [17].

The ability of the bacteria to form a biofilm was classified according to Stepanović et al. [18] as follows: ODc<OD ≤ 2×ODc (weak), 2×ODc<OD ≤ 4×ODc (moderate), and OD> 4×ODc (strong biofilm former), where ODc is the mean OD of the negative control wells plus 3 standard deviations.

Sub-inhibitory concentrations (determined by broth dilution MIC assays) of Myrtus communis extract were added to the biofilm formation phase of the assay to assess the antibiofilm activity. The extract was added to the bacterial suspension and then incubated under the same conditions as the untreated controls. Biofilm biomass was quantified after treatment with the same CV assay. The significant decrease in OD values with respect to untreated groups suggests that M. communis phytoconstituents, particularly polyphenols and terpenoids, have an effective inhibitory or disruptive effect on the development of biofilm formation, as reported earlier [19,20].

This method enabled a strong and quantitative assessment of the intrinsic biofilm-forming capacity

of MDR pathogens and the antibiofilm activity of extract from Myrtus communis, substantiating its potential as a natural agent for alternative measures aimed at controlling infections.

2.6. Analysis of Research Data

Statistical analysis was performed using GraphPad Prism software (version 8.0). Chi-square and paired t-tests were utilized, with a significance threshold of $p < 0.05$.

3. Results

3.1. GC-MS profile of Myrtus

The GC–MS analysis gives the chromatographic area percentage data, which is a semi-quantitative estimation of the relative distribution of phytochemical constituents in the methanolic extract of Myrtus communis. Thirty-five compounds were identified with a chemically diverse profile, showing a variety of concentrations of bioactive metabolites.

Of the detected constituents, tert-butylhydroquinone (TBHQ) was the major compound in the chromatogram with 26.34% of the chromatographic area. The significant amount of this antioxidant compound may contribute greatly to the biological activities of the extract, such as its antioxidant activity and antimicrobial potential.

The second most abundant component was the purine-related compound 2,6-dihydroxy-7-methylpurine (10.10%), suggesting that there is a significant amount of purine derivatives present in the extract. Previously, these compounds were linked to various biological activities such as antimicrobial and anti-inflammatory activities. The other major component was 5-hydroxymethylfurfural (HMF) with a concentration of 8.76% of the total profile. This compound is known for its antioxidant and antimicrobial properties and is also a common product of thermal processing or dehydration of plant carbohydrates.

A relatively high abundance of 2,3-difluorobenzamide (6.70%) was also observed. This compound has been reported in a number of chemical contexts but may be present in the extract due to interactions during extraction and/or processing of the sample. Other constituents found at intermediate concentrations were oxalic acid derivatives, butyl 1-menthyl ester (4.54%) and hexyl 1-menthyl ester (2.47%), which may affect the physicochemical properties of the extract and its biological action owing to their lipophilic properties. The presence of a number of compounds with known pharmacological activity was detected in moderate quantities. These consisted of maltol (3.25%), theobromine (2.38%), decamethylcobaltocene (2.22%) and eucalyptol (1.95%). These metabolites, when taken together, have been claimed to have a variety of biological activities, such as antioxidant, bronchodilatory, antimicrobial, and flavour-enhancing properties, which enhance the therapeutic profile of the extract.

Other constituents were also measured at lower levels and are also biologically significant. These comprised a 2H-indazole derivative (2.91%), a quinizarin-related anthraquinone (1.20%), and androsterone-related compounds (0.43%). Although these metabolites are relatively scarce, they can contribute to the extract with specific pharmacological effects such as cytotoxic, hormone-like, or other special mechanisms.

The extract was additionally enriched with many trace compounds, with a presence of less than 1% of the total chromatographic area, including vaccinic acid (0.41%), paraxanthine (0.70%), and 4-vinylguaiacol (0.54%). Each is expressed singly at low concentrations, but may have synergistic or complementary interactions that boost the biological activity of the phytochemical blend.

In general, the GC–MS profile confirms that Myrtus communis is chemically heterogenic with a high concentration of antioxidants, phenols, purine derivatives, and other metabolically active substances. The presence of these different phytochemicals may be responsible for the wide range of pharmacological activities exhibited by the extract, including antimicrobial and antioxidant effects, and could make it a promising source of plant-based therapeutic compounds.

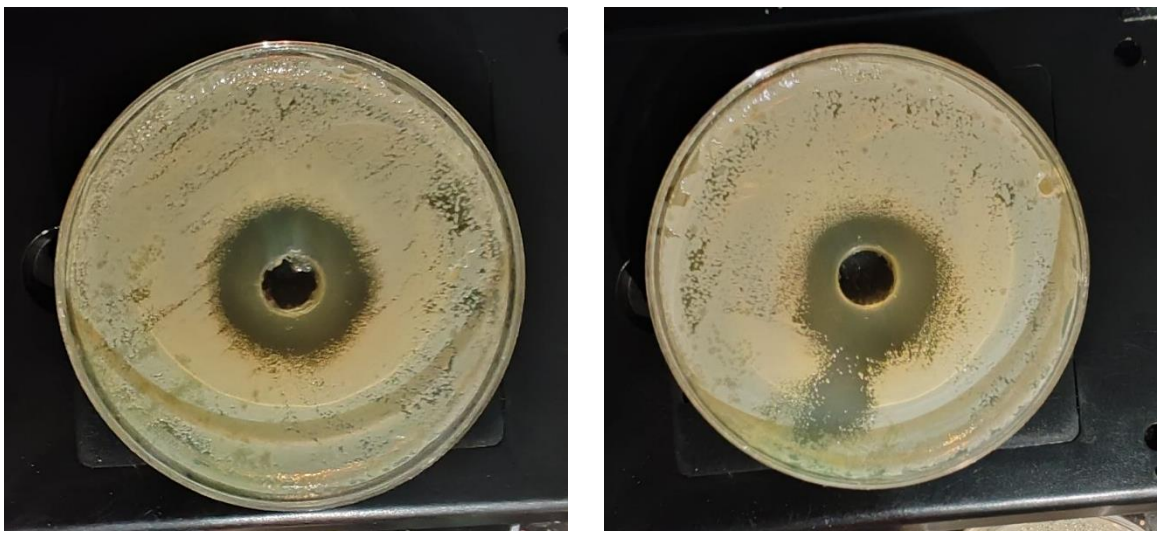
Table 1. GC–MS Identified Compounds of *Myrtus communis*

Pk#	RT (min)	Area (%)	Compound Name (IUPAC / Library)	Common Name	CAS#
1	4.403	1.95	Eucalyptol	1,8-Cineole	470-82-6
2	5.761	0.86	m-Fluoroanisole	–	456-49-5
3	6.566	3.25	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Maltol	28564-83-2
4	6.999	1.18	α-Terpineol	Terpineol	98-55-5
5	8.228	8.76	5-Hydroxymethylfurfural	HMF	67-47-0
6	9.397	0.55	3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate	Linalyl acetate (isomer)	80-26-2
7	9.604	0.97	Butylphosphonic acid, dodecyl pentyl ester	–	1000322-86-2
8	10.548	0.54	2-Methoxy-4-vinylphenol	4-Vinylguaiacol	7786-61-0
9	11.197	0.42	Bicyclo[2.2.2]octan-1-ol, 2-methyl	–	1000258-29-6
10	12.201	10.10	2,6-Dihydroxy-7-methylpurine	Hypoxanthine derivative	552-62-5
11	12.513	26.34	t-Butylhydroquinone	TBHQ	1948-33-0
12	12.885	1.42	2,4-Diethoxy-5-methoxy-1-(2-propenyl)benzene	–	1000122-72-0
13	13.049	1.61	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	Syringaldehyde	134-96-3
14	13.214	2.11	Benzene, 1,2,3-trimethoxy-5-methyl	–	6443-69-2
15	14.122	0.70	1,7-Dimethylxanthine	Paraxanthine	611-59-6
16	14.451	1.86	Acetic acid, 2-(2-methoxyphenyl)hydrazide	–	79984-63-7
17	14.564	1.15	4-Hydroxy-2-mercapto pteridine	–	52023-48-0
18	14.668	2.38	Theobromine	–	83-67-0
19	14.875	2.87	3-Methyl-4-(3-methyl-5-isoxazolylidene)-5-isoxazolone	–	1518-01-0
20	15.040	6.70	2,3-Difluorobenzamide	–	18355-75-4
21	15.204	4.54	Oxalic acid, butyl 1-menthyl ester	–	1000314-97-2
22	15.352	2.47	Oxalic acid, hexyl 1-menthyl ester	–	1000314-97-4
23	15.473	0.68	3-[p-Chlorophenyl]furazan	–	10349-08-3
24	16.200	0.77	2,2-Dimethyl-4-octenal	–	30390-60-4
25	16.892	0.42	2H-Quinolizin-1-ol, octahydro-	–	10447-20-8
26	18.649	1.18	n-Hexadecanoic acid	Palmitic acid	57-10-3
27	20.943	0.41	cis-Vaccenic acid	Vaccenic acid	506-17-2

28	22.795	1.20	1-Hydroxy-4-(p-toluidino)anthraquinone	Quinizarin derivative	81-48-1
29	23.219	4.23	4Bh,9bh,14bh-5,10,15-trioxa-14c-azabenzonaphthacene	–	1000327-58-9
30	23.513	0.43	Androstan-1-one, (5α)	Androsterone derivative	1755-29-9
31	23.626	2.91	2H-Indazole, 4,6-dinitro-2-(4-nitrophenyl)-	–	1000277-66-5
32	23.860	2.22	Cobaltocene, decamethyl-	Decamethylcobaltocene	74507-62-3
33	23.998	1.28	Isophthalic acid, 2-formylphenyl nonyl ester	–	1000344-61-7
34	24.483	1.02	2H-1,3-Dithiolo[4,5-c]coumarine derivative	–	135056-17-6
35	29.399	0.51	Terephthalic acid, phenyl undecyl ester	–	1000324-06-9

3.2. Antibacterial Activity of *Myrtus communis* Against Multidrug-Resistant Pathogens

The antibacterial activity of the extract from Myrtus communis was tested at 100 mg/mL against multidrug-resistant clinical isolates of *Klebsiella pneumoniae* and *Staphylococcus aureus* using the agar well diffusion assay. As observed in Figure 1, the inhibitory activity of the extract against both bacterial species was observed, with formation of distinct inhibition zones around the wells.



A **B**

Figure 1. Representative agar well diffusion assay demonstrating the antibacterial activity of *Myrtus communis* extract (100 mg/mL) against multidrug-resistant pathogens: (A) *Klebsiella pneumoniae* and (B) *Staphylococcus aureus*.

3.3. Minimum Inhibitory Concentration (MIC) of *Myrtus communis*

The minimum inhibitory concentration (MIC) values of Myrtus communis extract for multidrug-resistant Staphylococcus aureus and Klebsiella pneumoniae were obtained by the resazurin-based broth microdilution method. The bacterial growth was reflected by the change in colour of the medium from blue/purple to pink, whereas the absence of colour change showed complete inhibition of bacterial proliferation, as shown in Figure 2.

The MIC of the extract from Myrtus communis against Staphylococcus aureus was 3.125mg/mL, which shows that the extract has completely inhibited bacterial growth. The MIC for Klebsiella pneumoniae was 6.25 mg/mL, indicating that the extract needs to be two times more concentrated to inhibit the growth of this organism.

The results showed that the extract of Myrtus communis (MCE) was found to have antibacterial activity against the multidrug-resistant pathogens, with the best result against Staphylococcus aureus compared to Klebsiella pneumoniae under the experimental conditions used.

3.4. Biofilm-Forming Capacity of the Selected Bacterial Isolates

Bacterial isolates were first tested for biofilm formation by the microtiter plate method before their antibiofilm activity was tested with Myrtus communis extract. Only those isolates that produced robust biofilm formation in (Figure 3) were chosen for further studies to assess the effect of the extract against MDR pathogens that have strong biofilm-forming capacity.

From the biofilm classification criteria, it is observed that both *Staphylococcus aureus* and *Klebsiella pneumoniae* are strong biofilm-producing organisms with a high biofilm biomass as compared to the cut-off value. Therefore, all the following antimicrobial and anti-biofilm studies were conducted with these isolates.

The results of choosing strong biofilm-forming isolates provide a solid experimental model to assess the inhibitory activity of Myrtus communis against mature biofilms of MDD bacteria.

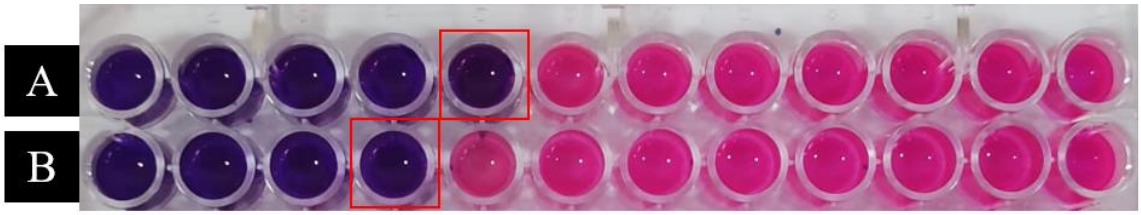


Figure 2. Determination of the minimum inhibitory concentration (MIC) of *Myrtus communis* extract against multidrug-resistant bacteria using the resazurin broth microdilution assay. (A) *Staphylococcus aureus* and (B) *Klebsiella pneumoniae*. Red boxes denote the MIC for each bacterial isolate.

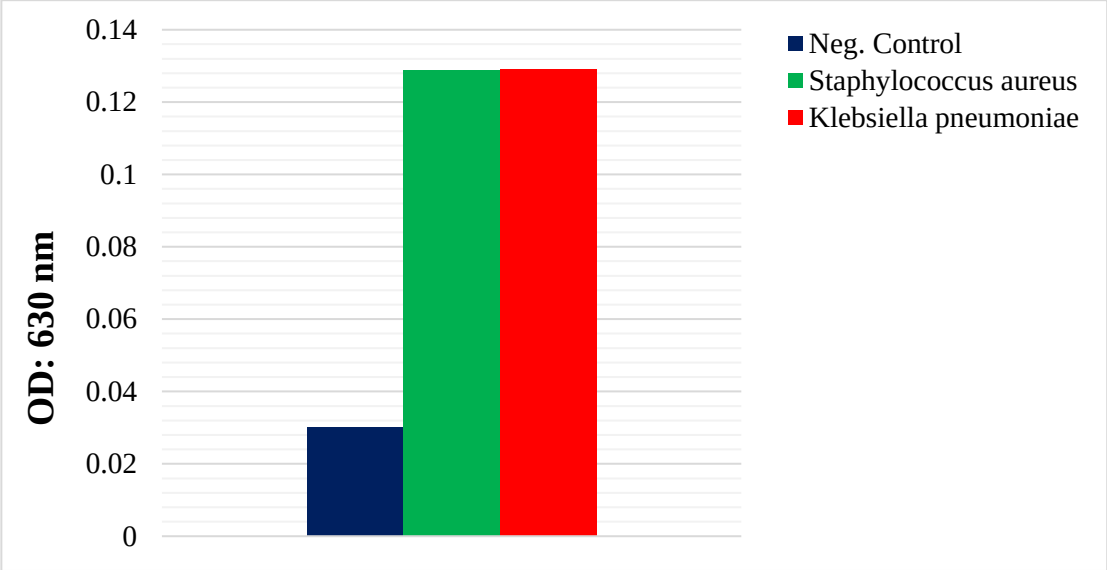


Figure 3. Quantitative assessment of biofilm-forming capacity of multidrug-resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* isolates using the microtiter plate assay at OD_{630} nm.

3.5. Antibiofilm Activity of *Myrtus communis* Against Multidrug-Resistant Pathogens

The anti-biofilm property of Myrtus communis extract was assessed against multidrug-resistant Staphylococcus aureus and Klebsiella pneumoniae by the crystal violet microtiter plate assay. The optical density at 630 nm (OD_{630}) of the biofilms was measured before and after treatment with the plant extract to quantify the amount of biofilm biomass.

As shown in Table 2 and Figure 4, the treatment with the Myrtus communis extract led to a significant decrease in biofilm biomass in both bacterial species. The mean biofilm biomass was significantly reduced from 0.1290 ± 0.002646 before treatment to 0.02873 ± 0.001528 after treatment (P = 0.0002), indicating a highly significant decrease in biofilm formation.

Likewise, Klebsiella pneumoniae had a substantial decrease in biofilm biomass when treated with the extract, with the mean optical density of the biofloc reduced from 0.1287 ± 0.009074 before the extract to 0.02933 ± 0.003786 after the extract (P = 0.0027). The results showed significant inhibitory effects of the extract on biofilm development in this species.

Myrtus communis overall proved to be a potent antibiofilm agent against the two MDR pathogens, resulting in a significant reduction in biofilm biomass. The inhibitory effect was observed in both Gram-positive and Gram-negative bacteria and, under experimental conditions, Staphylococcus aureus was slightly more susceptible than Klebsiella pneumoniae.

Table 2: Treatment of biofilm-forming MDR bacteria, Gram-negative bacteria (*K. pneumonia*) and Gram-positive bacteria (*S. aureus*), with *Myrtus communis*

MDR bacteria	Before Treatment **M ± SD**	After Treatment with *Myrtus communis* **M ± SD**	*p-Value*
Gram-negative bacteria (*K. pneumonia*)	0.1287± 0.009074	0.02933± 0.003786	0.0027**
Gram-positive bacteria (*S. aureus*)	0.1290±0.002646	0.02873±0.001528	0.0002***

M: Mean; SD: Std. Deviation; ***: highly significant.

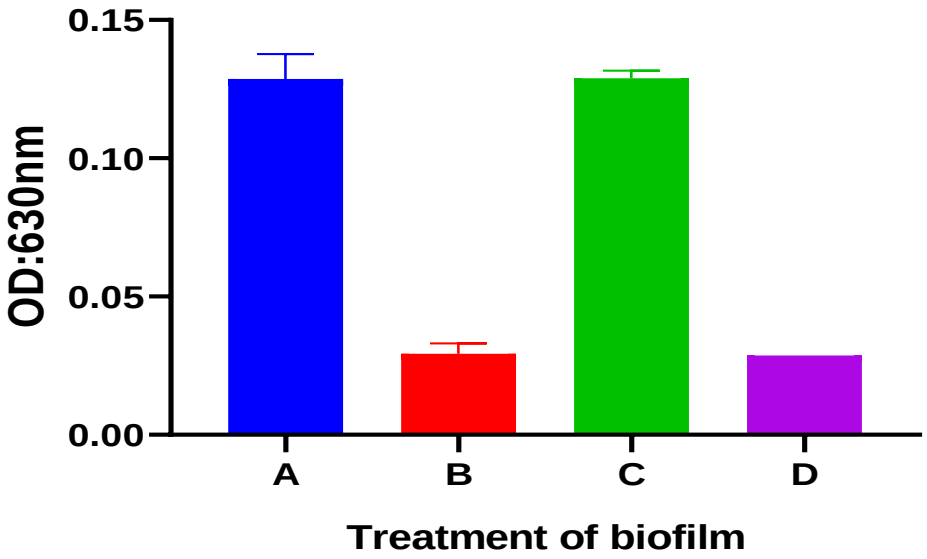


Figure 4. Effect of Myrtus communis extract on biofilm formation by multidrug-resistant Staphylococcus aureus and Klebsiella pneumoniae. (A) Untreated S. aureus; (B) S. aureus treated with Myrtus communis extract; (C) untreated K. pneumoniae; and (D) K. pneumoniae treated with Myrtus communis extract. Biofilm biomass was quantified using the crystal violet assay at OD_{630} nm.

4. Discussion

Multidrug-resistant (MDR) bacteria are one of the greatest challenges facing healthcare worldwide, especially if they have more than one resistance mechanism and have made conventional antimicrobials very ineffective. Apart from antimicrobial resistance, these microorganisms have the ability to form biofilms, which can further hinder treatment by shielding bacterial cells from host immune responses and preventing antibiotics from reaching the bacteria. With this background, medicinal plants with biologically active phytochemicals have become the subject of great interest, as they are also able to inhibit bacterial growth and biofilm formation. The present study revealed significant antimicrobial activity against MDR S. aureus and K. pneumoniae, with remarkable antibiofilm activity of M. communis, which could be considered a potential source of natural antimicrobials with novel compounds. The results obtained agree with the previous studies, which found that M. communis has a wide spectrum of

antimicrobial activity due to the complex phytochemical profile [21,22].

The phytochemical constituents identified in the methanolic extract of M. communis by GC–MS analysis showed that the extract is rich in a wide variety of bioactive secondary metabolites, with 35 constituents. The main compounds detected were tert-butylhydroquinone (TBHQ), a hypoxanthine derivative, 5-hydroxymethylfurfural (HMF), 2,3-difluorobenzamide, maltol, eucalyptol, α-terpineol, and theobromine. The diversity observed indicates that the biological activity observed is not due to the activity of one metabolite alone but rather due to the interaction of several metabolites. This diversity of phytochemicals has been previously reported in M. communis, in which phenolic compounds, flavonoids, terpenoids, and oxygenated monoterpenes were identified as being the main responsible for the pharmacological properties of the plant [23]. Another justification for the strong biological activity of the present extract is the predominance of antioxidant compounds, which have been linked to impaired bacterial metabolism and sensitivity of microbial cells to antimicrobial agents, as a result of oxidative stress modulation.

Eucalyptol (1,8-cineole) has been recently found as one of the compounds identified to be very effective in disrupting the bacterial cytoplasmic membranes, causing a leakage of bacterial intracellular constituents, and, resulting in bacterial death [24]. Similarly, α-terpineol has shown strong antimicrobial properties by disrupting the cell membrane phospholipids and inhibiting the respiratory enzymes [25]. Maltol and hydroxymethylfurfural have also been reported to show antioxidant and antimicrobial activity that could inhibit bacterial metabolism and decrease oxidative damage [26]. While some compounds were found in relatively small quantities, many studies have demonstrated that the minor phytochemicals often have synergistic interactions with the major constituent, resulting in a significantly enhanced antimicrobial activity compared to the major constituents alone [27]. Thus, the biological effects observed in the present study may be due to the synergistic effect of these phytochemicals altogether.

The agar well diffusion assay showed inhibitory activity of M. communis extract against both MDR S. aureus and K. pneumoniae, which proved its wide-spectrum antibacterial activity. In addition, the minimum inhibitory concentration (MIC) assay revealed that the isolate of K. pneumoniae was about twice as resistant as the Gram-positive S. aureus, with S. aureus being completely inhibited at 3.125 mg/mL, and K. pneumoniae at 6.25 mg/mL. The results are consistent with earlier studies that have shown that bacteria belonging to the Gram-positive category are more susceptible than Gram-negative bacteria to plant phytochemicals [28,29].

These differences in susceptibility can be largely accounted for by the structural differences between the two bacteria. The peptidoglycan layer of Gram-positive bacteria is thick but permeable, allowing relatively easy diffusion of hydrophobic phytochemicals into the cytoplasmic membrane. In contrast, Gram-negative bacteria have an extra outer membrane containing lipopolysaccharides (LPS), which serves as a formidable barrier to penetration of many antimicrobial compounds [30]. In addition, MDR K. pneumonia harbours several intrinsic resistance mechanisms, such as production of β-lactamases, production of capsule-associated resistance, reduced membrane permeability, and multiple multidrug efflux pumps, which act together and result in resistance to antimicrobial agent(s) [31]. Therefore, the obtained MICs of K. pneumoniae were in a reasonable biological range, and it is similar to previous investigations reporting the activity of medicinal plant extracts against MDR pathogens.

The other significant result obtained from the present study was the high level of biofilm formation of both MDR isolates before treatment. This

observation is clinically important, as one of the major virulence factors that leads to persistent infections, recurrent disease, chronic inflammation, and higher resistance to antimicrobial therapy is the formation of biofilm. Biofilms form a highly structured extracellular polymeric substance (EPS) matrix that shields bacterial cells from the effects of antibiotics, oxidative stress, phagocytosis, and environmental stressors and enables horizontal gene transfer and the spread of antimicrobial resistance genes [2,32]. Thus, the use of strong biofilm producers as an experimental model was used to analyse the genuine antibiofilm activity of M. communis.

The M. communis extract was highly effective in significantly reducing biofilm biomass in both bacterial species. In S. aureus, biofilm biomass decreased from 0.1290 ± 0.0026 to 0.0287 ± 0.0015 ($P = 0.0002$), whereas K. pneumoniae exhibited a reduction from 0.1287 ± 0.0091 to 0.0293 ± 0.0038 ($P = 0.0027$), demonstrating potent antibiofilm activity against both Gram-positive and Gram-negative pathogens. The results show that the extract is not simply a growth inhibitor of planktonic bacteria, but also an inhibitor of the formation of biofilms. In the same way, M. communis extracts and other medicinal plants with a high phenolic content have been shown to reduce biofilm biomass to a significant degree by the inhibition of bacterial adhesion, down-regulation of extracellular polysaccharide production, and disruption of the mature biofilm architecture [33,34].

A number of molecular mechanisms may account for the antibiofilm activity found during this study. The polyphenols, flavonoids, and terpenoids have been demonstrated to interfere with quorum sensing systems that mediate bacterial cell-to-cell communication, resulting in a decrease in expression of bacterial genes related to adhesion, EPS production, virulence factor secretion, and biofilm maturation [35]. Furthermore, the phytochemicals can directly affect the bacterial membrane, inhibit ATP production, disrupt the permeability of bacterial membranes, and cause oxidative stress, which collectively leads to decreased viability of bacteria in biofilm [36]. Other studies have previously revealed that phenolics in plants disrupt the structural integrity of EPS, as they inhibit its extracellular protein, polysaccharide, and extracellular DNA production, with a resulting weakening of mature biofilms and increased sensitivity to antimicrobial agents [37].

Interestingly, S. aureus showed slightly more susceptibility to antibiofilm treatment than K. pneumoniae, which was similar to the results of MIC results achieved in the present study. This difference could also be because of the presence of the Gram-negative outer membrane and the thick capsular polysaccharide layer found in K. pneumoniae that both make the cell surface of bacteria and biofilms more resistant to penetration by phytochemicals [38]. Furthermore, K. pneumoniae has highly efficient quorum sensing networks and capsule-associated virulence determinants that add to enhanced stability and persistence of biofilm [39]. Even with these protective mechanisms, M. communis extract still had a highly significant reduction in biofilm biomass, suggesting that phytochemical constituents of this extract appeared to be able to overcome multiple bacterial defense mechanisms.

5. Conclusion

In the present study, it has been revealed that methanolic extract of Myrtus communis exhibited strong antibacterial and antibiofilm activities against multidrug-resistant (MDR) strains of Staphylococcus aureus and Klebsiella pneumoniae bacteria. GC–MS analysis detected a high number of different phytochemicals (35), including TBHQ, a hypoxanthine derivative, 5-hydroxymethylfurfural, maltol, eucalyptol, and α-terpineol as major constituents. The antimicrobial activity of these bioactive compounds is probably the result of various complementary and synergistic

mechanisms. This extract showed good antibacterial activity against both MDR bacteria, with S. aureus being more susceptible than K. pneumonia with lower MIC values. Additionally, both bacterial species showed a highly significant decrease in biofilm biomass after treatment with Myrtus communis, indicating its efficacy in inhibiting the growth of biofilm in clinically important Gram-positive and Gram-negative pathogens. Results indicate that the extract may be affecting more than just the growth of the bacteria, but also important processes associated with the formation and maintenance of a biofilm.

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