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Exploring the antibacterial and anti-biofilm properties of *Rosmarinus officinalis* extracts: A natural strategy against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Background: Antimicrobial resistance (AMR), particularly methicillin-resistant *Staphylococcus aureus* (MRSA), has posed a significant challenge to global health care. The increasing ineffectiveness of conventional antibiotics has driven the need for alternative antimicrobial agents. *Rosmarinus officinalis* L. (rosemary) is known for its diverse biological properties, including antibacterial and anti-biofilm activities.

Aim: This study aimed to investigate and compare the antibacterial and anti-biofilm effects of different *R. officinalis L*. solvent extracts against MRSA.

Methods: Antibacterial efficacy was determined using the well-diffusion method, followed by the assessment of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against 10 MRSA strains. The antibiofilm potential was analyzed using a crystal violet assay. In addition, cytotoxicity was evaluated using an MTT assay on HCT-116 colorectal carcinoma cells.

Results: Methanol demonstrated the highest antibacterial activity among the tested extracts. The MIC values ranged between 0.108 and 0.320 mg/ml, with corresponding MBC results confirming strong bactericidal effects. The anti-biofilm analysis indicated that the ethyl acetate extract exhibited the greatest inhibition of MRSA biofilm formation, followed by the dichloromethane and methanol extracts.

Conclusion: *Rosmarinus officinalis L*. (rosemary) extracts, particularly those extracted with methanol and ethyl acetate, exhibited strong antibacterial and anti-biofilm effects against MRSA.

Keywords: Antibiofilm, Antibacterial, MRSA, Antibiotic resistance, Rosemary.

Introduction

Antimicrobial resistance (AMR) is a natural phenomenon, but its uncontrolled spread has become a pressing public health crisis primarily driven by the overuse of antibiotics. The emergence of multidrug-resistant bacteria represents a significant global health challenge, with *Staphylococcus aureus (S. aureus)* being a prominent human pathogen responsible for a wide range of infections (Collignon and Beggs, 2019). This bacterium is the most frequently isolated bacterial pathogen in humans, contributing to serious conditions such as skin and soft tissue infections, *pneumonia*, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis. One of the most common MDR microorganisms is methicillin-resistant *Staphylococcus aureus* (MRSA). At present, over 1.27 million deaths are attributed to infections caused by AMR, and approximately 5,000,000 deaths are associated with MDR infections (O'Neill, 2016; Salam *et al.*, 2023).

MRSA strains are resistant to all available penicillins and other β-lactam antibiotics. The prevalence of MRSA infections has surged in various countries over the past few decades, fueled by epidemics not only in humans but also in animals, including pets and livestock. Methicillin resistance in *S. aureus* has been documented to exceed 20% in all WHO regions, with some areas surpassing 80% (Organization, 2014). The World Health Organization has released a list of priority MDR pathogens contributing to increasing morbidity and mortality worldwide as targets for discovering and developing novel antimicrobial drugs (De Oliveira *et*

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al., 2020). MRSA is a prevalent cause of healthcare-associated infections, including surgical site infections, bloodstream infections, and pneumonia. As a priority MDR pathogen, MRSA is a Gram-positive bacterium that leads to higher rates of morbidity and mortality than methicillin-sensitive *S. aureus*, posing significant challenges in both community and healthcare settings (Larsen *et al.*, 2022).

Considering the alarming increase in the prevalence of MDR pathogens, urgent efforts are required to discover and develop new natural bioactive compounds as alternatives to antibiotics. The plant kingdom has fulfilled human needs by providing a diverse array of medicinal compounds since ancient times. It remains one of the richest sources of bioactive substances with pharmaceutical potential. Research into naturally occurring antibiotic compounds from these plants has been conducted for over a century, and more than 200,000 natural products have been documented across various plant species. Plants are a rich source of various biologically active secondary metabolites, including antibacterial, antifungal, and anticancer agents, and are widely used in traditional medicine worldwide (Dmour *et al.*, 2024).

Bioactive compounds derived from medicinal plants are recognized for their minimal side effects and their ability to suppress pathogen growth through diverse mechanisms, setting them apart from conventional synthetic antibiotics. The composition and abundance of these natural metabolites differ across plant species, influencing their biological properties and therapeutic potential. The abundance and diversity of these valuable compounds make plants an excellent source of antibacterial agents in the pharmaceutical industry. About 25%–28% of modern drugs are derived from the diverse secondary metabolites of higher plants (Anand *et al.*, 2019).

Rosmarinus officinalis L. (rosemary) (Lamiaceae) is a globally distributed fragrant evergreen shrub with needle-like leaves. Traditionally, it has served as a stimulant and pain reliever and is employed to address inflammatory conditions and physical and mental fatigue. Recent research highlights the diverse biological activities of rosemary, including hepatoprotective (Mohamed *et al.*, 2022), antifungal (Yuan *et al.*, 2024b), insecticidal, antioxidant(Luță *et al.*, 2023), and antibacterial effects (Soliman *et al.*, 2024).

The rising incidence of MRSA infections calls for new treatment approaches, with plant-derived extracts such as those from *R. officinalis* showing promise because of their antibacterial and anti-biofilm properties. Phytochemical profiling is essential for identifying and measuring bioactive compounds, such as carnosic acid, rosmarinic acid, and flavonoids, which drive these effects (Manilal *et al.*, 2020). This profiling clarifies how rosemary extracts can disrupt MRSA biofilm formation and increase antibiotic effectiveness by linking the presence and levels of these compounds with bioactivity (Khashei *et al.*, 2025). For example, rosmarinic acid has been shown to reduce *S. aureus* biofilm adhesion by 40%–60% through quorum-sensing disruption (Khashei *et al.*, 2025). Novel therapeutic strategies are required for *S. aureus* infections, with plant-derived extracts such as those from rosemary showing promise due to their antibacterial and anti-biofilm properties (Kabotso *et al.*, 2024). Phytochemical profiling is crucial for identifying and quantifying the bioactive compounds responsible for these effects, such as carnosic acid, rosmarinic acid, and flavonoids (Khashei *et al.*, 2025). By correlating the presence and concentration of these compounds with bioactivity, such profiling elucidates the mechanisms underlying the ability of rosemary extracts to disrupt MRSA biofilm formation and enhance antibiotic susceptibility. For instance, rosmarinic acid inhibits *S. aureus* biofilm adhesion by 40%–60% through quorum-sensing disruption (Ivanov *et al.*, 2022).

Additionally, *R. officinalis* is a widely used plant known for its ease of cultivation and cost-effectiveness. Additionally, research has confirmed that *R. officinalis* extract is both nontoxic and antimutagenic at commonly used concentrations, further supporting its potential for safe therapeutic applications (Lemonica *et al.*, 1996). Therefore, this study aimed to evaluate and compare the antibacterial and antibiofilm activities of *R. officinalis* L. extracts obtained from different solvents against MRSA.

Materials and Methods

Plant collection and extraction

The whole rosemary plant (Fig. 1) was chopped into small pieces and air-dried in the dark at room temperature (23°C ± 2°C) before being ground into a powder using a grinder. The powdered material (50 g) was macerated with 100 ml of methanol, ethyl acetate, and hexane solvents for 7 days in an Erlenmeyer flask at room temperature. Subsequently, the mixture was filtered through Whatman filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The resulting extract was weighed and stored in a refrigerator at 4°C until use in the antimicrobial assay.

Antibacterial activity

Ten *S. aureus* isolates, including MRSA, were collected from clinical, environmental, and health care worker sources at Salahaddin General Hospital, Iraq, and identified using standard microbiological methods (Wayne, 2017).

Ten MRSA strains were used in the study, as shown in Table 1. The standard strain of *S. aureus* American Type Culture Collection (ATCC 25923) was used as the reference organism. All strains were cultured on MSA at 37°C.

The antibacterial activity of various solvent extracts against isolated MRSA was assessed using the well-

Table 1. List of bacterial strains used in the antibacterial assessment.

No. of the clinical isolates	Accession number
MRSA 1	IHN2A
MRSA 2	IHN3A
MRSA 3	IHN4A
MRSA 4	IHN6A
MRSA 5	IHN8A
MRSA 6	IHN10A
MRSA 7	IHN15A
MRSA 8	IHS2A
MRSA 9	IHS3A
MRSA 10	IHE2A

diffusion method (Bacon *et al.*, 2017). As a first step, 6-mm diameter wells were embedded in Muller–Hinton agar. The overnight MRSA strains were cultured on the media after they had been standardized to 0.5 McFarland standards. Then, 50 µl of the extract (50 mg/ml) was poured into the wells. After that, the plates were incubated at 37°C for 24 hours, and the diameter of the growth inhibition zone (mm) was measured. According to Alsohaili and Al-fawwaz (Rojas *et al.*, 2006), antibacterial activity was expressed as the percentage of relative inhibition zone diameter with respect to the standard antibiotic amoxicillin (AML) and calculated using the following formula:

$$\% \text{ RIZD} = [(IZD_{\text{sample}} - IZD_{\text{negative control}})/ IZD_{\text{standard antibiotic}}] \times 100 \quad (1)$$

MIC and MBC determination

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined following a previous study (Al Assi *et al.*, 2023). Finally, the lowest concentration at which no bacterial growth was observed was recorded as the MIC. Furthermore, MBC was determined by adding 10 µl of culture to an extract-free MHA medium. MBC is defined as the lowest concentration at which no bacterial cells grow on an extract-free medium (Chen *et al.*, 2024). For both MIC and MBC, sodium benzyl penicillin was used as a positive control.

Antibiofilm activity of R. officinalis L

Antibiofilm efficacy was determined following Qaralleh *et al.* (2021). The antibiofilm efficacy was examined using a crystal violet assay. After a 24-hour incubation period at 37◦C, crystal violet was introduced into each well, and after 15 minutes of incubation at 25ºC, the contents were removed and subsequently cleaned using tap water. Then, ethanol was added and left for 15 minutes, and the optical density was recorded at 590 nm. The inhibition percentage was calculated using the following formula:

Percentage of biofilm formation inhibition (% inhibition) = (O.D of control—O.D of sample)/(O.D of control) ×100% (1)

Cytotoxic effect of R. officinalis L

The cytotoxic effects of different rosemary solvent extracts were assessed using the MTT assay. The MTT assay was used to determine the cytotoxic activity of the rosemary extracts. In brief, HCT-116 CRC cells were seeded into 96-well plates and allowed to adhere. Cells were exposed to a range of extract concentrations (31.25–1,000 g/ml) for either 48 or 72 hours at 37°C. Finally, an MTT solution was added to each well and incubated for 4 hours to form formazan crystals. After replacing the medium with dimethyl sulfoxide (DMSO), the plates were gently agitated until all crystals had been dissolved. The human umbilical vein endothelial cells cell line served as a standard normal cell line to evaluate extract selectivity (Hansen and Brunner, 1998).

The extent of MTT reduction was assessed by measuring absorbance at 570 nm with a microplate reader, and cell viability was represented as the percentage of treated cells compared with that of untreated controls.

$$\% \text{ Cell viability} = (A/C) \times 100\%$$

Where: A = absorbance of the test extract, C = absorbance of the control.

Similarly, IC_{50} is defined as the concentration of a chemical that results in a 50% reduction in cell viability, determined using a logistic regression nonlinear function.

Statistical analysis

All statistical analyses were conducted using the GraphPad Prism 8.4.3 software. Each experiment was performed in triplicate with three independent repetitions to ensure accuracy and reliability. All values are expressed as mean ± SD (n = 3).

Ethical approval

Not needed for this study.

Results

Effects of various solvents on the extraction yield

The percentage yields derived from the finely powdered rosemary, which were obtained through the maceration method using various polarity solvents, were calculated, and the results are presented in Figure 2. The final weights of the rosemary extracts were 2.5, 2.19, 1.08, and 0.50 g for dichloromethane, methanol, ethyl acetate, and n-hexane extract, respectively. The results showed that the dichloromethane extract of rosemary had the highest yield at 5%, followed by the methanol extract at 4.32% and the ethyl acetate extract at 2.16%. Conversely, the n-hexane extract had the lowest yield of 1%.

Fig. 1. *Rosmarinus officinalis* L.

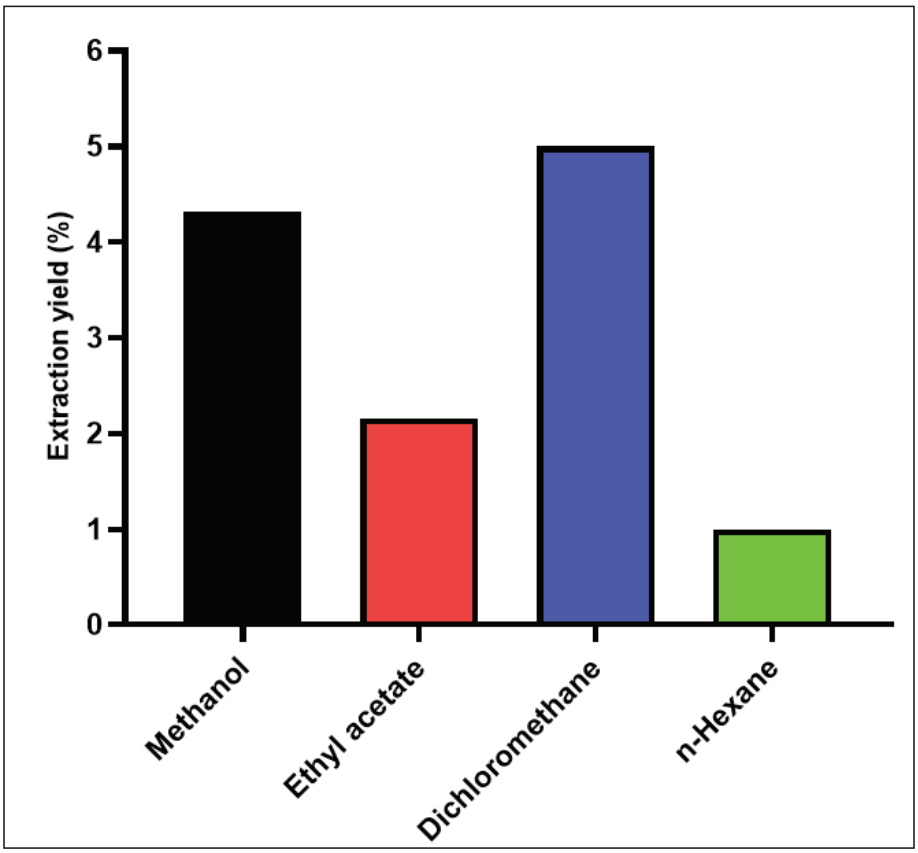

Fig. 2. Yield percentage of different solvent extracts of *R. officinalis* L.

Antibacterial activity determination using the well diffusion method

A well diffusion assay was conducted to assess the inhibitory effects of *R. officinalis* L. on MRSA. Inhibition zones were observed for crude extracts (methanol, n-hexane, ethyl acetate, and dichloromethane) of

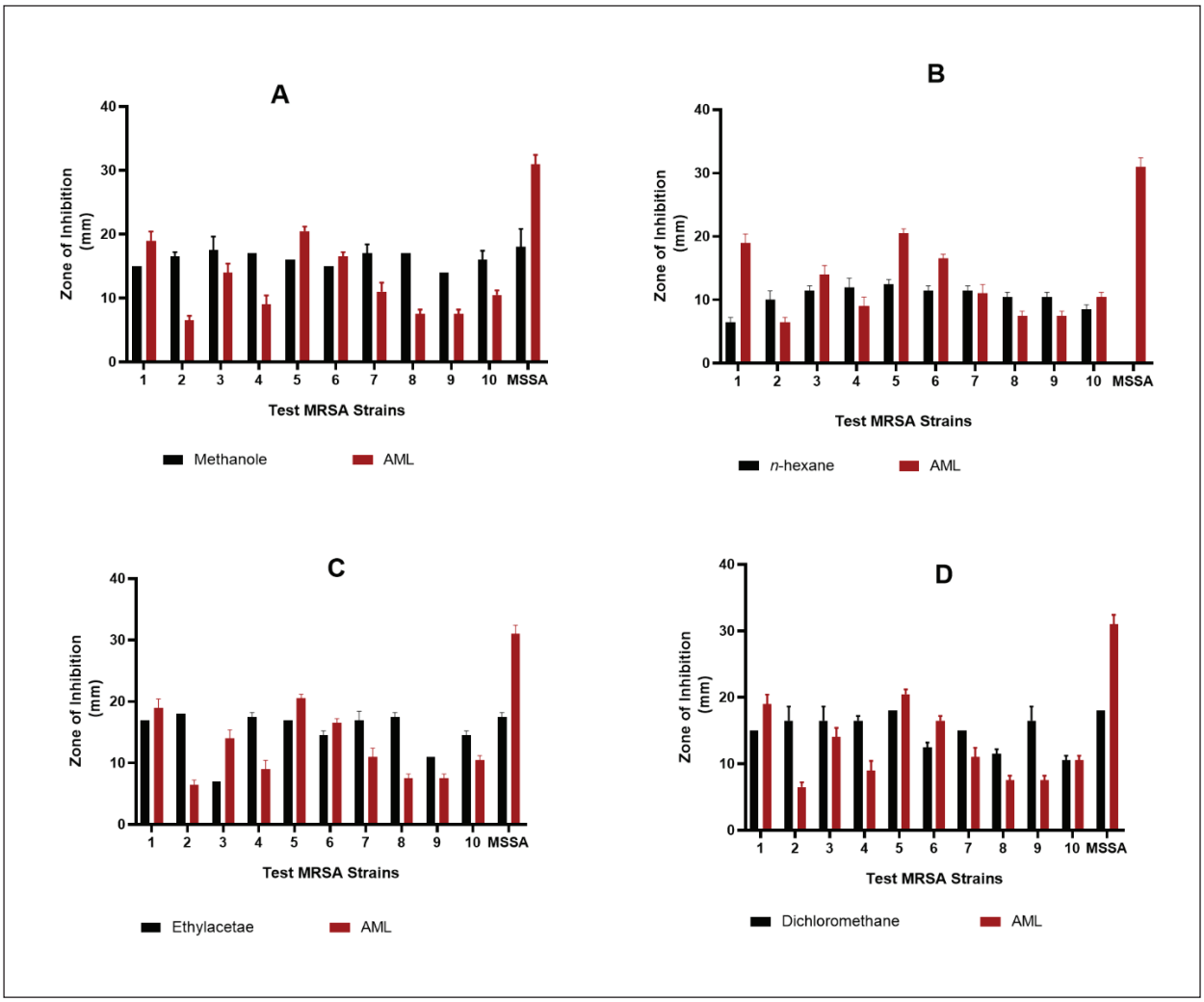

Fig. 3. Inhibitory zones (mm) of different solvent extracts of *R. officinalis* L against MRSA and MSSA, and comparable with the activities of AML (positive controls): (A) methanol extract, (B) n-hexane extract, (C) ethyl acetate extract, and (D) dichloromethane extract. All values are expressed as mean±SD (n = 3).

R. officinalis L. against MRSA (Figs. 3 and 4). The standard antibiotic, AML, produced inhibition zones ranging from 6.00 to 22 mm against MRSA strains and 32 mm against MSSA (ATCC). Similar susceptibility patterns were observed for MSSA (ATCC 25923) and the corresponding plant extracts.

Among the different extracts, all solvents demonstrated significant inhibition, with all proving bactericidal against MRSA strains. Interestingly, the plant extracts' inhibitory effect surpassed the zone of inhibition caused by the standard antibiotic against MRSA strains 2, 7, 8, and 9. The methanolic extract showed the largest inhibition zones, ranging from 14 to 18 mm, whereas the ethyl acetate and dichloromethane extracts produced zones between 7 and 18 mm and 11.5 to 18 mm, respectively. The n-hexane extract exhibited the smallest inhibition zones, ranging from 6.5 to 12.5 mm. Notably, the n-hexane extract of *R. officinalis* L showed higher activity against MRSA strains 2, 4, 7, 8, and 9 than AML. The methanol extract demonstrated strong inhibition against MRSA strains 2, 3, 4, 7, 8, 9, and 10, outperforming AML. The ethyl acetate extract was most effective against MRSA strains 2, 4, 7, 8, 9, and 10, whereas the dichloromethane extract exhibited strong activity against MRSA strains 2, 3, 4, 7, 8, and 9. The antibacterial activity of the extracts was ranked as follows: methanol >ethyl acetate >dichloromethane >n-hexane. The negative control (80% DMSO) showed no antibacterial activity.

Evaluation of the minimum inhibitory and minimum bactericidal concentrations of R. officinalis L rxtracts

Antibacterial activities of various plant extracts and benzyl penicillin sodium against MRSA and MSSA. The MIC and MBC for each strain were determined, as detailed in Table 2.

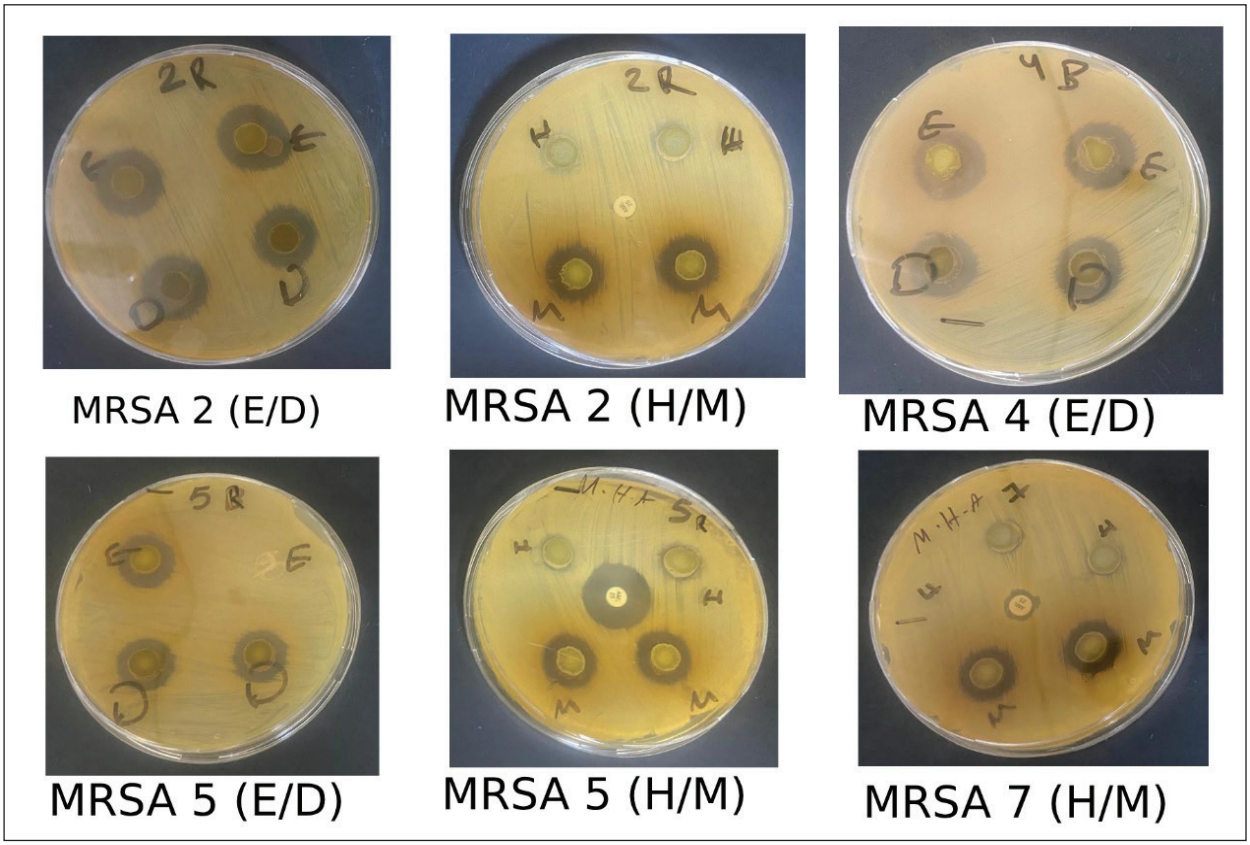

Fig. 4. Inhibition zone (mm) of different extracts of *R. officinalis* L. against MRSA isolates. E: ethyl acetate extract; D: dichloromethane extract; M: methanol extract; H: hexane extract.

Table 2. MIC and MBC of extracts of *R. officinalis* L in different solvents against MRSA isolates.

Test strains	He extract		Di extract		Eth extract		I extract		Benzyl penicillin sodium	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
MRSA 1	0.320	≥2.9	0.320	2.9	0.320	≥2.9	0.108	≥2.9	0.320	2.9
MRSA 2	0.320	2.9	0.108	2.9	0.108	0.970	0.108	0.970	0.970	2.9
MRSA 3	0.320	≥2.9	0.320	2.9	0.320	2.9	0.320	0.320	0.970	2.9
MRSA 4	0.320	2.9	0.108	≥2.9	0.108	2.9	0.108	2.9	0.970	2.9
MRSA 5	0.320	2.9	0.108	2.9	0.320	2.9	0.108	2.9	0.108	0.320
MRSA 6	0.108	≥2.9	0.108	≥2.9	0.108	2.9	0.108	2.9	0.320	0.970
MRSA 7	0.108	≥2.9	0.108	≥2.9	0.108	2.9	0.108	0.970	0.320	2.9
MRSA 8	0.320	≥2.9	0.320	2.9	0.108	2.9	0.108	0.970	0.320	2.9
MRSA 9	0.320	≥2.9	0.320	≥2.9	0.320	2.9	0.108	0.970	0.970	2.9
MRSA 10	0.320	2.9	0.320	2.9	0.320	2.9	0.108	0.970	0.320	0.970
Mean *n* = (10)	0.320 (0.108–0.320)		0.320 (0.108–0.320)		0.320 (0.108–0.320)		0.108 (0.108–0.320)		0.320 (0.108–0.970)	
MSSA	0.970	2.9	0.320	2.9	0.320	2.9	0.970	0.970	0.320	2.9

The lowest MIC for all extracts (ethyl acetate, n-hexane, methanol, and dichloromethane) against MRSA was 0.108 mg/ml. For MSSA, the MIC values for the dichloromethane and ethyl acetate extracts were

Table 3. Cytotoxic effects of different *R. officinalis* L. extracts on HCT-116 and normal cell line (HUVEC).

Cell name	Ethylacetate	Dichloromethane	Methanol	n-hexane	Positive standard (5-fluorouracil)
HCT-116	114.21 ± 13.35	141.93 ± 9.55	147.79 ± 1.61	178.74 ± 8.39	4.3 ± 0.22
HUVEC	>500 >550 >520 >530 __				
Selectivity Index	>4.38 > 3.87 > 3.52 > 2.97 __				

0.320 mg/ml, while the MIC values for the n-hexane and methanol extracts were 0.970 mg/ml (Table 2). The MIC values for all extracts ranged from 0.108 to 0.320 mg/ml, with MBC values varying from 2.9 mg/ml to ≥2.9 mg/ml for n-hexane and dichloromethane and from 0.970 mg/ml to ≥2.9 mg/ml for ethyl acetate and methanol. Benzyl penicillin sodium showed relatively high MBC values for most MRSA strains, indicating limited bactericidal effects. The MIC for benzyl penicillin was between 0.108 and 0.970 mg/ml for MRSA strains, but the MBC was often 2.9 mg/ml, indicating significant resistance.

In addition, the MIC values for different extracts from the same plant varied among individual MRSA strains. For instance, MRSA strains 6 and 7, which were inhibited by 0.108 mg/ml n-hexane extract of *R. officinalis*, exhibited comparable inhibition with other *R. officinalis* extracts, including dichloromethane, ethyl acetate, and methanol extracts.

In contrast, benzyl penicillin sodium required a higher MIC of 0.320 mg/ml to inhibit these strains. Higher MIC values (0.320 mg/ml) were needed for the ethyl acetate, dichloromethane, and n-hexane extracts to inhibit MSSA (ATCC 25923), while the methanol extract exhibited inhibition at a lower MIC of 0.108 mg/ml.

Anti-biofilm activity

The crude extracts of *R. officinalis* L. (methanol, ethyl acetate, dichloromethane, and n-hexane) were evaluated for their ability to inhibit biofilm formation in 10 MRSA strains across a concentration range of 2.9–0.036 mg/ml. All *R. officinalis* L. extracts exhibited dose-dependent inhibition of biofilm formation across the tested bacterial strains (Fig. 5).

In MRSA 1, all extracts displayed significant inhibition, with the ethyl acetate extract showing the greatest effect. At the highest concentration (2.9 mg/ml), the biofilm inhibition percentages against MRSA 1 were as follows: ethyl acetate (63.90%), dichloromethane (60.99%), methanol (54.69%), and n-hexane (22.55%).

For MRSA 2, the ethyl acetate extract again showed the strongest inhibitory effect with 56.53% biofilm inhibition (Fig. 4a), followed by the methanol and dichloromethane extracts, which demonstrated inhibition rates of 47.74% and 47.05%, respectively. The n-hexane extract exhibited the lowest biofilm inhibition (30.23%).

With the exception of MRSA 6, 9, and 10, the ethyl acetate extract exhibited the most significant biofilm inhibitory effect against MRSA strains 1, 2, 3, 4, 5, 7, and 8, with inhibition rates ranging from 56.05% to 67.61% (Fig. 5). The dichloromethane extract showed inhibition levels between 47.09% and 60.99%, followed by the methanol extract, which had biofilm inhibition rates from 44.27% to 55.91%. The n-hexane extract exhibited the least biofilm inhibitory effect, with rates ranging from 22.55% to 56.03%.

Cytotoxicity of rosemary extracts against the HTC-116 cell line

The MTT assay was performed to evaluate the cytotoxic effects of various *R. officinalis* L. extracts on HCT-116 cells relative to the HUVEC line. As illustrated in Figure 6, all evaluated extracts (methanol, ethyl acetate, dichloromethane, and n-hexane) exhibited dose-dependent anticancer effects after 48 hours of treatment, with the results presented as a percentage of inhibition. Table 3 summarizes the cytotoxic effects (IC_{50} values) of these extracts on HCT-116 and HUVEC lines. The findings revealed significant differences among the extracts, with the ethyl acetate fraction exhibiting the greatest effect (IC_{50} = 114.21 ± 13.35 µg/ml), followed by the dichloromethane extract (IC_{50} = 141.93 ± 9.55 µg/ml), methanol extract (IC_{50} = 147.79 ± 1.61 µg/ml), and n-hexane extract (IC_{50} = 178.74 ± 8.39 µg/ml) in comparison to the HUVEC cell line. 5-Fluorouracil served as the positive control, while the HUVEC cell line was utilized as a normal cell model to assess the selective activity of the extracts on different cell lines.

Discussion

With the rise of antimicrobial resistance worldwide, this study seeks to explore plant-based alternatives for preventing oral infections. Research has highlighted the strong antimicrobial properties of *R. officinalis* extracts against both aerobic and anaerobic bacteria. Studies have demonstrated that *R. officinalis* effectively inhibits the growth of *Escherichia coli* (Sienkiewicz *et al.*, 2013), *S. aureus* (Manilal *et al.*, 2020), *Bacillus cereus* (Amaral *et al.*, 2019), *Clostridium perfringens* (Radaelli *et al.*, 2016), and multiple *Salmonella species* (Kim *et al.*, 2011). These findings underscore its potential as a natural antimicrobial agent.

Recent research has highlighted the therapeutic potential of rosemary, focusing on its phytochemical composition and biological activities. Additionally, rosemary has demonstrated significant neuroprotective effects, positively influencing mood, memory, learning,

Fig. 5. Antibiofilm inhibition effect of different concentrations (ranging from 2.9 to 0.036 mg/ml) of *R. officinalis* L crude extracts on biofilm-producing bacteria. All values are expressed as mean ± SD (n = 3).

pain management, anxiety, and sleep (Rahbardar and Hosseinzadeh, 2020). The diverse medicinal applications of rosemary are supported by a growing body of evidence, paving the way for its broader use in health and wellness (Li Pomi *et al.*, 2023). Studies have emphasized its antioxidant, anti-inflammatory, and antimicrobial properties, attributed to bioactive compounds (Andrade *et al.*, 2018).

MRSA poses a significant global health threat due to its resistance to most antibiotics. In recent decades, its prevalence has risen sharply, affecting both humans and animals. Methicillin resistance in *S. aureus* exceeds 20% in all WHO regions, with some areas reporting rates above 80%. This growing resistance has sparked interest in exploring natural alternatives, such as Rosemary, for their potential antibacterial and anti-biofilm properties. Researchers are increasingly investigating plant-based compounds as effective solutions against resistant pathogens such as MRSA (AlSheikh *et al.*, 2020).

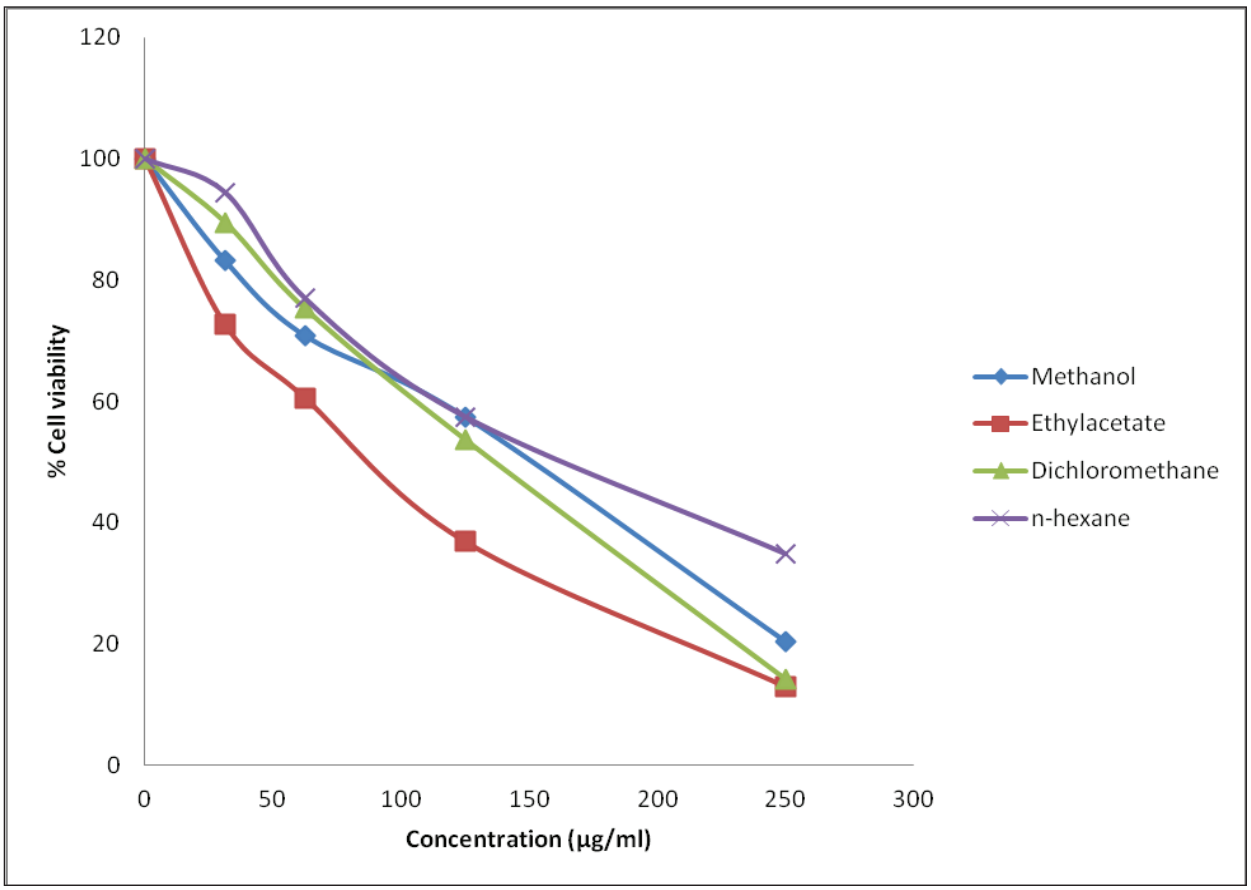

Fig. 6. Cytotoxic effect of different *extracts of Rosmarinus officinalis L. against the* HCT-116 cell line. All values are expressed as mean ± SD (n = 3).

In this study, the dichloromethane extract produced the highest yield, followed by methanol and ethyl acetate, whereas the n-hexane extract produced the lowest yield. The varying polarities of the bioactive compounds found in *R. officinalis*, which determine their solubility in different solvents, likely influence the differences in extraction yields of different extracts (Saghir *et al.*, 2016; Ali *et al.*, 2019).

This study revealed that rosemary extracts exhibit significant antibacterial activity against MRSA. The methanolic extract showed the lowest MIC value of 0.108 mg/ml, followed by ethyl acetate and dichloromethane, with MIC values ranging from 0.108 to 0.320 mg/ml. The MBC values ranged between 0.970 mg/ml and ≥2.9 mg/ml. These results indicate that the methanolic extract is the most potent, a finding that aligns with previous studies (Al Assi *et al.*, 2023) where methanol-based plant extracts exhibited strong antibacterial efficacy against MDR bacteria (Al Assi *et al.*, 2023).

Similar studies have reported comparable MIC and MBC values for plant extracts with antimicrobial properties. Similar narrow MIC ranges for plant extracts against MRSA (0.1–0.5 mg/ml) have been reported in earlier studies (Qaralleh *et al.,* 2021), supporting the plausibility of our results. Therefore, we believe that the consistency of the values reflects robust antimicrobial activity rather than methodological shortcomings.

Additionally, Soliman *et al.* (2024) reported that rosemary essential oils exhibited MIC values of 0.125 mg/ml against Gram-positive bacteria, supporting our findings that rosemary has potent antibacterial properties (Soliman *et al.*, 2024). Moreover, Bacon *et al.* (2017) demonstrated that different solvent extracts impact antibacterial efficiency, with methanol extracts often being more effective due to their ability to dissolve a wide range of bioactive compounds (Bacon *et al.*, 2017).

Our results indicate that the plant extracts exhibit comparable or superior antibacterial effects against MRSA compared to conventional antibiotics.

The biofilm-inhibitory effect of rosemary extracts was concentration-dependent, with ethyl acetate extract showing the highest inhibition (56.05%–67.61%), followed by dichloromethane (47.09%–60.99%) and methanol (44.27%–55.91%). The n-hexane extract exhibited the lowest biofilm inhibition rates (22.55%–56.03%). These findings are consistent with those of

previous reports that highlight the anti-biofilm potential of plant-derived compounds. For instance, Qaralleh *et al.* (2021) demonstrated that plant extracts could inhibit biofilm formation by up to 70% in *S. aureus*, similar to the inhibition rates observed in this study (Qaralleh *et al.*, 2021).

Additionally, Luță *et al.* (2023) reported that plant-based bioactive compounds, such as those found in rosemary and thyme, could effectively disrupt biofilm structures through mechanisms such as quorum-sensing inhibition. These mechanisms may explain the anti-biofilm efficacy observed in our study (Luță *et al.*, 2023). The strong antibiofilm activity of the ethyl acetate extract may be attributed to the presence of specific secondary metabolites that interfere with bacterial adhesion and biofilm formation.

The mechanisms by which natural compounds, such as terpenes and phenolics, exhibit anti-biofilm and anti-quorum-sensing effects against *Pseudomonas aeruginosa* likely involve multiple synergistic molecular and cellular targets (Al-Maddboly *et al.*, 2025). For instance, terpenes, exemplified by components in essential oils, are known for their lipophilic nature, which enables them to intercalate into bacterial cell membranes. This intercalation can lead to membrane disruption, increasing permeability, altering membrane potential, and ultimately causing intracellular contents leakage, thereby impairing bacterial viability and metabolic activity essential for biofilm formation. Such membrane damage can also compromise efflux pumps, enhancing the efficacy of co-administered antibiotics (Elfaky, 2024; Shariati *et al.*, 2024). This intercalation can lead to membrane disruption, increasing permeability, altering membrane potential, and ultimately causing intracellular contents leakage, thereby impairing bacterial viability and metabolic activity essential for biofilm formation (Elfaky, 2024). Such membrane damage can also compromise efflux pumps, enhancing the efficacy of co-administered antibiotics (Elfaky, 2024).

The cytotoxicity assay demonstrated that rosemary extracts exert dose-dependent anticancer activity against the HCT-116 colorectal carcinoma cell line. The ethyl acetate extract exhibited the highest cytotoxic effect (IC_{50} = 114.21 µg/ml), followed by dichloromethane (IC_{50} = 141.93 µg/ml), methanol (IC_{50} = 147.79 µg/ml), and n-hexane (IC_{50} = 178.74 µg/ml). These values are consistent with the findings of Yuan *et al.* (2024), who reported that rosemary extracts exhibited IC_{50} values ranging from 100 to 150 µg/ml against HCT-116 (Yuan *et al.*, 2024a).

The selectivity of the extracts was confirmed by their minimal cytotoxic effect on human umbilical vein endothelial cells. Similar studies have found that rosemary-derived compounds exert selective cytotoxicity against cancer cells while sparing normal cells (Alghonmeen *et al.*, 2024). These findings reinforce the growing interest in plant-derived compounds as potential anticancer agents. The effectiveness of medicinal plant extracts differs across various plant species and geographical locations, as reported in multiple studies. These variations can be attributed to several factors, including differences in climate conditions, soil composition, plant age, and the specific stage of the vegetation cycle. These elements collectively influence the quality, concentration, and chemical composition of the extracted compounds, leading to diverse biological activities in different regions (Angioni *et al.*, 2006; Al Assi *et al.*, 2023).

The ethyl acetate extract demonstrated significant antibacterial and anti-biofilm activity against MRSA, while also exhibiting potent effects against the HCT-116 cancer cell line. The consistency of these findings reinforces the potential of the extract as a powerful antibacterial agent with promising therapeutic applications.

Previous studies have reported comparable results, demonstrating that the ethyl acetate fraction of *Rosmarinus officinalis* is a rich source of polyphenolic compounds with exceptional antioxidant properties. Furthermore, it has shown enhanced effectiveness against dermal fibroblast cell cultures compared with isolated phytochemicals, underscoring its potential for therapeutic and dermatological applications. This potent activity could be attributed to the presence of bioactive compounds, such as caffeic acid, rosmarin, rosmarinic acid methyl ester, luteolin, apigenin, and hispidulin (Hyun *et al.*, 2015).

Conclusion

This study provides strong evidence supporting the antibacterial, antibiofilm, and anticancer potential of rosemary extracts. The observed MIC and MBC values highlight the effectiveness of the plant extracts against MRSA, with methanolic extracts showing the highest antibacterial activity. The anti-biofilm activity of rosemary further supports its potential as a natural alternative for combating bacterial infections. Future studies should focus on elucidating the biofilm-disrupting mechanisms of *R. officinalis* and conducting in vivo evaluations to validate these findings. Furthermore, comprehensive phytochemical profiling using techniques such as HPLC and GC-MS will be essential to identify the active constituents and correlate them with the observed biological activities.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Authors' contribution

MA, EG, EM, LS, and SM conceptualized and designed the proposal. MA, EG, EM, and LS collected the required data. MA, HG, EM, MJ, and SM analyzed the collected data and wrote the manuscript. All authors have revised and approved the final manuscript.

Data availability

All generated and analyzed data are included in this research article.

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