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Antibacterial and antibiofilm properties of methanolic and ethanolic extracts of medicinal plant *Rhazya stricta* against methicillin-resistant *Staphylococcus aureus*

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Abstract

MRSA **(**Methicillin-resistant bacteria *Staphylococcus aureus*) is the main causative agent of chronic infections associated with biofilms in humans, which are responsible for serious healthcare problems in the world. Biofilm-associated infections are challenging to manage with traditional antibiotic treatments due to the protective nature of a surrounding extracellular matrix. In this research, 16S rDNA of thirty *S. aureus* strains (isolated from King Faisal Hospital) was sequenced to identify the isolates, and the results showed 98–100% identity with comparable *S. aureus* from the NCBI database. The isolates were then placed in the NCBI GenBank and assigned the accession numbers from OP363093 to OP363122. Then, we screened ethanolic and methanolic extracts of *Rhazya stricta* leaves against clinical MRSA isolates to measure their growth inhibition property using MIC and disk diffusion methods. In addition, these extracts were used to measure the degree to which crystal violet inhibited biofilms. HPLC testing revealed 19 constituents including stilbene (resveratrol), quinol, 11 phenolic acids, and 6 flavonoids, and showed that there are differences between the extracts in the element's number and their amounts. Both extracts showed antimicrobial properties against pathogenic microbes such as MRSA isolates. Extracts from *R. stricta* displayed potent inhibitory action on biofilms, with inhibition rates extending from 71.5% to 99% and from 26.2% to 98.9% for the ethanolic and methanolic extracts, respectively. *R. stricta* leaf extracts revealed potent antimicrobial as well as anti-biofilm activities of MRSA isolates and might be a distinct substitute for the prevention and therapy of the pathogen MRSA**.**

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Introduction

The most prevalent bacterium responsible for nosocomial infections and a major issue affecting public health is staphylococcus (Wojtyczka *et al.*, 2014). This bacterium regularly colonizes the human body, enhancing the chance of nosocomial infection, specifically in hospitalized and immunocompromised patients (Wojtyczka *et al.*, 2014). MRSA (Methicillin-resistant *Staphylococcus aureus*) strains, owing to their multi-drug resistance, present a significant therapeutic challenge for hospitalized patients due to their multi-drug resistance character.

Among the bacteria that form biofilms, *S. aureus* is frequently responsible for infections. *S. aureus* biofilms are made up of components such as surface proteins, exopolysaccharides, functional amyloids, and extracellular DNA (Taglialegna *et al.*, 2018; Karygianni *et al.*, 2020). Biofilm formation by MRSA strains is considered a general virulence component that is responsible for nearly 80% of all human infections (Piechota *et al.*, 2018). In biofilm, bacteria present an important source of ecological, industrial, and sanitary problems, and are safeguarded against immune defense systems, and physical and chemical agents such as antibiotics making their treatments increasingly difficult (Gilbert *et al.*, 2002; Nassima *et al.*, 2019). Hence, to combat biofilm forming bacteria, researchers have concentrated on antibiofilm molecules, among them, natural substances derived from plants.

Plant-based products made from natural ingredients have seen extensive application in folkloric medicine, which possesses biological properties activities (Macé *et al.*, 2017). Evidently, plant-based phenolic components such as phenolic acids or flavonoids display antimicrobial and antibiofilm properties against *S. aureus* and different pathogens (Miklasińska-Majdanik *et al.*, 2018; Nassima *et al.*, 2019).

Economically, the drug-producing plant *Rhazya stricta* has acquired significant utility (Albeshri *et al.*, 2021). *R. stricta* and its byproducts are conventionally employed in numerous Asian nations and Saudi Arabia to handle a wide range of sicknesses, including cancer, skin disorders, rheumatism, hypertension, sore throats, syphilis, parasite infections, fever and, inflammatory problems (Baeshen *et al.*, 2015). According to some investigations, several phytochemical components, including flavonoids, alkaloids, volatile bases, and triterpenes, have been found in various parts of *R. stricta*, and they could possess biological activities, including antibacterial properties (Marwat *et al.*, 2012; Bukhari *et al.*, 2017; Albeshri *et al.*, 2021). Additionally, *R. stricta* extracts (from fruits and leaves) have shown antimicrobial activity against MRSA and a variety of multidrug-resistant human pathogens (Sultana *et al.*, 2010; Raziuddin *et al.*, 2018; Garoy *et al.*, 2019). Nevertheless, *R. stricta* antibiofilm capabilities have not yet been explored.

Consequently, the main idea of this study is to analyze the antimicrobial and antibiofilm properties associated with clinical MRSA isolates using the methanolic and ethanolic extracts derived from *R. stricta*.

Materials and methods

Bacterial isolates

Bacterial strains were provided from King Faisal Hospital, Taif, Saudi Arabia. Firstly, *S. aureus* was morphological identified and confirmed as previously reported using 16S rDNA (Alsanie, 2020). Then, consistent with the BSAC (British Soc. for Antimicrobial Chemotherapy), The Vitek 2 system was used to identify the methicillin resistance characteristics. The 30 isolates were declared methicillin-resistant at MIC (minimum inhibitory concentration) breakpoint of oxacillin (>2 mg/l) and cefoxitin (>4 mg/l) (Garoy *et al.*, 2019).

The 16S rDNA gene sequencing

All *S. aureus* isolates' genomic DNA was extracted following the manufacturer's instructions using a DNA extraction kit (Gena Bioscience, Germany). The 16S rDNA gene was employed to amplify a single DNA fragment (1465 bp) for each isolate, using

protocols previously reported (Alsanie *et al.*, 2018). The fragments were then sequenced in DNA Analyzer 3146 (Applied Biosystems, USA) after being punctuated using the QIA quick PCR purification kit (QIAGEN, Valencia, CA, USA). DNASTAR software was used to edit and assemble the sequencing texts (Laser gene, Madison, WI, USA). BLAST searches were conducted using the NCBI service (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Plant

R. stricta was selected for its traditional medicine uses to treat chronic rheumatism, syphilis, body pain, inflammatory conditions, and many diseases (Albeshri *et al.*, 2021). The antimicrobial activity of *R. stricta* was reported in literature (Raziuddin *et al.*, 2018); however, its antibiofilm activity was not explored.

Plant leaves collection and extraction procedure

Leaves of the *R. stricta* plant were harvested in September 2020 from their native location (Al-Hada) in Taif Governorate, Saudi Arabia. The fresh leaves of the plant were air-dried, powdered, and then extracted in 100 mL quantities of 95%. After centrifuging each extract for 15 min at 7000 rpm, Whatman filter paper (No. 1) was used three times to clear the pure filtrate. Extracts (pellets) were dissolved in a 1% dimethyl sulfoxide after the filtrate was filtered through a Buchner funnel employing an evaporator at 30 °C (Andrews and Howe, 2011). Experiments and HPLC analysis were conducted using the extracts.

HPLC analysis

The detection of phenolic compounds for the extracts was conducted utilizing Agilent 1260 infinity HPLC Series (outfitted with a quaternary pump). Kinetex® 5 µm EVO C18 (100 mm × 4.6 mm) was utilized as the column (at 30 °C) (Matilla-Cuenca *et al.*, 2020). Three different solvents, including (A) HPLC grade water 0.2% and H_3PO_4 (v/v), (B) acetonitrile, and (C) methanol, were used to create a linear elution gradient that allowed for the separation. Next, a 20 µL volume was administered. An AVWD detector with a 284 nm wavelength was utilized to detect flavonoids and phenols.

Antibacterial activity of R. stricta extracts

Disc diffusion

Extracts of *R. stricta* leaves were tested for antimicrobial properties in triplicate utilizing the agar disc diffusion technique (Beigomi *et al.*, 2021; Xie *et al.*, 2021). MRSA cells were grown for 24h at 37°C. Subsequently, using a DENSIMAT, the MRSA suspension was made in saline water and set to 0.5 McFarland turbidity criteria. Inhibitory activity was evaluated according to the zone of inhibition (ZI) as previously described (Bhawna *et al.*, 2015; Lagha *et al.*, 2019). Three sets of MIC and MBC assays were performed on a microtiter plate (96-well) (Bagamboula *et al.*, 2004).

Biofilm analysis

Biofilm formation was assessed as described previously (Qin *et al.*, 2013; Oulkheir *et al.*, 2017). MRSA strains were tested for their potential to get biofilm on microtiter plates (polystyrene, U-bottomed 96-well, Nunc, Roskilde, Denmark) using a crystal violet (Qin *et al.*, 2013; Oulkheir *et al.*, 2017). Biofilm inhibition was determined as described previously (Qin *et al.*, 2013; Oulkheir *et al.*, 2017).

Using MICs concentrations, the ability of *R. stricta* leaf ethanolic and methanolic extracts to inhibit MRSA isolates' biofilm formation was evaluated. The negative control well contains TSB and sterile water. Crystal violet test was used to determine the biofilm. The following formula was used to compute the percentage of biofilm inhibition (Chaieb *et al.*, 2007). The analyses were performed thrice.

% Inhibition = 100 – {(OD570 sample)/(OD570 control) × 100}

Statistical analysis

Using SPSS 20, the Pearson's simple linear correlation coefficient (r) and its significance (P) were examined.

Results

Molecular genotyping of MRSA isolates according to 16S-rRNA gene

All *S. aureus* isolates had their 16S rRNA genes amplified and sequenced, and the individual

fragments were then aligned and compared with the NCBI database's collection of 16S rRNA sequences for other *S. aureus* isolates. The NCBI GenBank has the isolates of *S. aureus* sequences, with accession numbers ranging from OP363093 to OP363122. The partial 16S rRNA sequences are closely related to other sequences in the NCBI database, according to the BLAST findings. *S. aureus* isolates and closely related bacteria from the NCBI database showed a similarity matrix of 98%-100% with zero E vale. For example, *S. aureus* STU-1, STU-3, STU-4, STU-7, STU-10, STU-12, STU-14, STU-17, STU-21, STU-23 and STU-26 isolates with has low similarity to *S. aureus* MK775201 strain. *S. aureus* STU-11, STU-13 and STU-22 isolates with accession numbers OP363103, OP363105 and OP363114 have moderate similarity to *S. aureus* strain MW175695. While, *S. aureus* STU-5, STU-19, STU-24 and STU-25 isolate with accession numbers OP363097, OP363111, OP363116 and OP363117 have high similarity with *S. aureus* strain MZ919968 with about 100 % similarity (Table 1, Fig. 1).

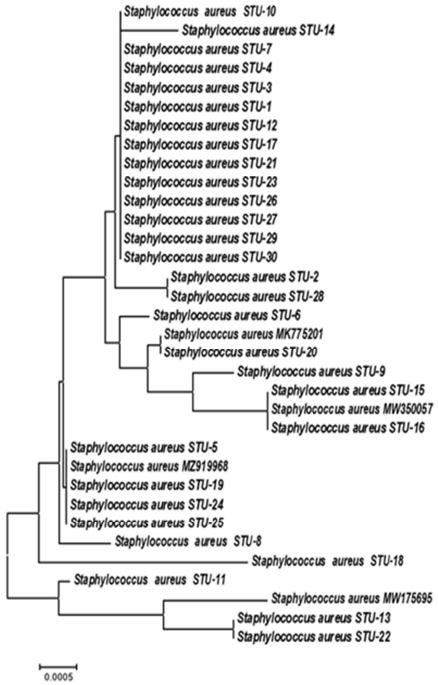

Fig. 1. Neighbour-Joining phylogeny based on 16S rDNA gene sequences of *Staphylococcus aureus* isolated from Taif Hospital region, Saudi Arabia

Chemical composition of R. stricta leaves extracts

The chemical compositions of *R. stricta* methanolic and ethanolic extracts are shown in Fig. 2.

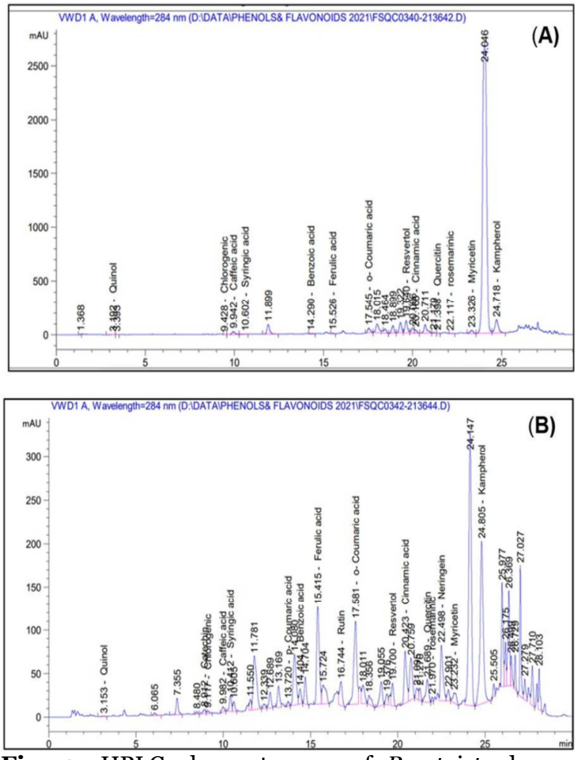

Fig. 2. HPLC chromatogram of *R. stricta* leaves extracts. A: ethanolic extract; B: methanolic extract.

Table 1. Summary of identified *Staphylococcus aureus* following BLAST search at NCBI.

Sample	Identified species	Query	E-value	Identity	Accession
1	*S. aureus*	100	0.0	100	OP363093
2	*S. aureus*	100	0.0	99	OP363094
3	*S. aureus*	100	0.0	98	OP363095
4	*S. aureus*	99	0.0	100	OP363096
5	*S. aureus*	99	0.0	100	OP363097
6	*S. aureus*	100	0.0	100	OP363098
7	*S. aureus*	100	0.0	100	OP363099
8	*S. aureus*	100	0.0	100	OP363100
9	*S. aureus*	100	0.0	100	OP363101
10	*S. aureus*	100	0.0	100	OP363102
11	*S. aureus*	100	0.0	100	OP363103
12	*S. aureus*	100	0.0	100	OP363104
13	*S. aureus*	100	0.0	100	OP363105
14	*S. aureus*	100	0.0	100	OP363106
15	*S. aureus*	100	0.0	100	OP363107
16	*S. aureus*	99	0.0	100	OP363108
17	*S. aureus*	99	0.0	100	OP363109
18	*S. aureus*	99	0.0	100	OP363110
19	*S. aureus*	98	0.0	100	OP363111
20	*S. aureus*	97	0.0	100	OP363112
21	*S. aureus*	100	0.0	100	OP363113
22	*S. aureus*	99	0.0	100	OP363114
23	*S. aureus*	99	0.0	100	OP363115
24	*S. aureus*	99	0.0	100	OP363116
25	*S. aureus*	99	0.0	100	OP363117
26	*S. aureus*	99	0.0	100	OP363118
27	*S. aureus*	99	0.0	100	OP363119
28	*S. aureus*	100	0.0	100	OP363120
29	*S. aureus*	99	0.0	100	OP363121
30	*S. aureus*	98	0.0	100	OP363122

Table 2. *R. stricta* leaf extract's antibacterial effectiveness against MRSA isolates.

R. stricta extract	(+ + + +) *n* (%)	(+ + +) *n* (%)	(+ +) *n* (%)	(+) *n* (%)	(-) *n* (%)
Ethanolic extract	5 (16.67%)	13 (43.33%)	5 (16.67%)	7 (23.33%)	-
Methanolic extract	4 (13.33%)	15 (50.00%)	6 (20.00%)	5 (16.67%)	-

There are five types of inhibitory actions: complete (+ + + +), partial (+ +), slight (+), and no inhibitory (-). The number of isolates is represented by the letter n.

HPLC analysis revealed 19 components in these extracts, divided into 6 flavonoids and 11 phenolic acids, stilbene (resveratrol), and quinol. In total, 17 components were found in each extract with quantities of 15292.89 mg/kg and 33050.65 mg/kg for the methanolic and ethanolic extracts, respectively, which indicates that the ethanolic extract was deficient in phenolic components compared to the methanolic extract.

The results showed differences between the two extracts in terms of the number of components and their amounts. The main constituents of the methanolic extract are resveratrol, ferulic acid, benzoic acid, rutin, neringein, quercitin and kaempferol. Those of the ethanolic extract are resveratrol, quinol, p- coumaric acid, rutin, benzoic acid, myricetin, quercitin and kaempferol. In *R. stricta* extracts, flavonoids are more abundant (11104.27 mg/kg and 21357.93 mg/kg for methanolic and ethanolic extract, respectively) in comparison to other phenolic compounds (4188.62 mg/kg and 11692.72 mg/kg for ethanolic and methanolic extract respectively).

Antibacterial activity of R. stricta extracts against MRSA

Disc diffusion

Both ethanolic and methanolic extracts of *R. stricta* leaves were tested for their ability to prevent the development of MRSA isolates (Table 2). Firstly, the disc diffusion method revealed that both extracts possess activity on all isolates despite the differences in the types of inhibitory actions. Comparatively, the methanolic extract of *R. stricta* exhibited a strong inhibitory action on 13.33% of the isolates, whereas the ethanolic extract displayed a strong inhibitory activity on 16.67% of the strains. According to table 2, the ethanolic extract was effective against MRSA isolates compared to the methanolic extract.

MICs and MBCs

The methanolic and ethanolic extracts from the leaves of *R. stricta* were evaluated for their antibacterial properties using the MBCs and MICs for the 30 MRSA isolates. For the ethanolic extract, the MICs extended from 0.122 mg/ml to 0.970 mg/ml, while the MBCs extended from 0.224 mg/ml to 1.9 mg/ml. Concerning the methanolic extract of *R. stricta* leaves, the MIC values extended from 0.224 mg/ml to 1.9 mg/ml, while the MBC values extended from 0.448 mg/ml to 3.9 mg/ml. Consequently, the ethanolic extract showed the highest growth inhibition activity against MRSA isolates than to the methanolic extract.

Table 3. Biofilm formation properties of MRSA isolates.

Isolates	OD570 ± SD	Biofilm phenotype
1	0.5925±0.081	LGP
2	1.1945±0.902	HP
3	0.875±0.319	LGP
4	0.4575±0.109	LGP
5	1.2515±0.286	HP
6	1.598±0.818	HP
7	0.5445±0.350	LGP
8	0.633±0.158	LGP
9	0.810±0.025	LGP
10	0.539±0.041	LGP
11	0.607±0.226	LGP
12	0.4965±0.444	LGP
13	1.0145±0.006	HP
14	0.6475±0.115	LGP
15	0.336±0.014	LGP
16	0.139±0.179	LGP
17	0.0385±0.007	N
18	0.0055±0.006	N
19	0.040±0.011	N
20	0.014±0.011	N
21	0.001±0.008	N
22	0.0075±0.002	N
23	0.042±0.004	N
24	0.0295±0.026	N
25	0.5675±0.092	LGP
26	0.8125±0.259	LGP
27	1.300±0.988	HP
28	0.5885±0.294	LGP
29	1.3155±0.437	HP
30	1.8435±0.180	HP

Biofilm formation on polystyrene surface

The ability of MRSA isolates to develop biofilm on polystyrene surfaces was examined (Table 3). Results showed that 73.33% of bacterial strains were able to form biofilms, and they were distributed as follows: 50% were low-grade positive with OD570 values ranging from 0.139 to 0.875, while 23.33% produced highly positive biofilms with OD570 values ranging from 1.014 to 1.843. However, 23.33% of the strains were unable to produce biofilm.

Biofilm inhibition

The ability of methanolic and ethanolic extracts of *R. stricta* to inhibit biofilm formation by MRSA isolates is summarized in Table 4. The isolates that exhibited biofilm formation potential were selected for this experiment. A total of 22 strains were used, which were recorded as low grade and high-grade positive biofilm, and both extracts showed strong biofilm inhibitory activity.

Antibiofilm activity testing revealed that the ethanolic extract possess a strong biofilm inhibition activity on all the isolates (22 strains) with percentage of inhibition extended from 71.5% to 99%. Results demonstrated that six of the seven isolates (85.71%) were passed from highly positive to biofilm negative. In addition, 14 low-grade positive isolates (93.33%) become biofilm negative.

Table 4. Potential antibiofilm characteristics of ethanolic and methanolic extracts of *R. stricta* leaves against isolates of MRSA.

		R. stricta Inhibition			
MRSA	Control	Ethanolic extract	(%)	Methanolic extract	(%)
Isolates	OD570±SD	OD570±SD		extract OD570±SD	
1	0.592±0.081	0.036±0.006*	93.8	0.069±0.073*	88.3
2	1.194±0.902	0.025±0.021***	97.8	0.095±0.103***	92
3	0.875±0.319	0.028±0.009*	96.7	0.034±0.011*	96.1
4	0.457±0.109	0.067±0.019*	85.2	0.192±0.146	57.9
5	1.251±0.286	0.033±0.031***	97.3	0.049±0.057***	96.0
6	1.598±0.818	0.089±0.024***	94.3	0.023±0.002***	98.5
7	0.544±0.350	0.017±0.014*	96.8	0.132±0.106	75.7
8	0.633±0.158	0.058±0.024*	90.8	0.011±0.089*	98.2
9	0.810±0.025	0.072±0.046*	91.1	0.265±0.063	67.2
10	0.539±0.041	0.08±0.066*	85.1	0.237±0.230	56.0
11	0.607±0.226	0.072±0.079*	88.1	0.604±0.253	0
12	0.496±0.444	0.01±0.016*	97.9	0.194±0.260	60.9
13	1.014±0.006	0.124±0.132**	87.7	0.206±0.097**	79.6
14	0.647±0.115	0.01±0.001*	98.4	0.477±0.385	26.2
15	0.336±0.014	0.01±0.001*	96.8	0.19±0.111	43.4
16	0.139±0.179	0.039±0.078*	71.5	0.02±0.039*	85.6
25	0.567±0.092	0.028±0.014*	94.9	0.057±0.073*	89.9
26	0.812±0.259	0.046±0.007*	94.2	0.123±0.134	84.8
27	1.30±0.988	0.013±0.012***	99	0.026±0.017***	97.9
28	0.588±0.294	0.145±0.101	75.2	0.256±0.193	56.4
29	1.315±0.437	0.052±0.011***	96	0.067±0.079***	94.8
30	1.843±0.180	0.040±0.026***	97.8	0.019±0.004***	98.9

* Isolates became biofilm negative after changing from low-grade positive. ** Isolates became low-grade positive after changing from highly positive. *** Isolates became negative after changing from highly positive.

Concerning the methanolic extract, we observed a great biofilm inhibitory potential in 95.45% of the isolates (21 strains), with the percentage of inhibition ranging from 26.2% to 98.9%. The same result as for the seven highly positive biofilm isolates under the ethanolic extract was obtained after treatment with the methanolic extract. However, 5 low-grade positive isolates (33.33%) became biofilm negative.

Despite the significant reduction in the volume of biofilm, the isolate 28 maintained its original biofilm phenotype after treatment with the two extracts. In comparison, the isolate 11 ability to form biofilm was unaffected by the methanolic extract (Table 4).The MIC and antibiofilm activity of methanolic and ethanolic extracts of *R. stricta* leaves showed no significant relationship.

Discussion

Modern molecular methods based on DNA sequencing techniques are becoming more widely used for research purposes. Therefore, an alternative approach for the molecular identification of different bacteria, including *Staphylococcus*, was 16S rRNA gene sequencing (Hassan *et al.*, 2023). 16S rRNA gene sequencing is useful because the 16S rRNA gene is present in all bacteria and facilitates accurate identification of bacteria at the genus and species level (Alsanie *et al.*, 2020). Therefore, sequencing is a practical tool for preparing many microorganisms, particularly those isolated from natural environments or from other animals, and the cost and time required to obtain the results is a limiting thing to the use of sequencing, but with time, the cost and time of sequencing results continue to decrease with the occurrence of new discoveries. Our findings, which are consistent with earlier research, demonstrated the resemblance between *S. aureus* isolates recovered from hospitals and those found in GenBank, suggesting that sequencing may be a more sensitive method of identification than morphological and microscopic analysis depending on culture (Hassan *et al.*, 2023).

MRSA is the most common health problem in most countries of the world. These bacteria are among the most important types of pathogenic bacteria that are resistant to many antibiotics used around the world. The danger of these bacteria is due to the possibility of infection being easily transmitted from one person to another through skin contact or through the use of contaminated medical equipment (Cushnie *et al.*, 2011). MRSA infection is commonly related with significant mortality and morbidity (Zhao *et al.*, 2015). Thereby, the development of novel therapeutic agents and particularly natural products against MRSA is of great importance.

Recently, natural plant constituents have emerged as potential alternatives to antibiotics due to scientists' interest in searching for alternatives to antibiotics. *R. stricta* is a plant commonly used in ancient folk medicine in India and East Asia. In this report, the possible antimicrobial property of ethanolic and methanolic *R. stricta* leaf extracts against clinical isolates of MRSA was studied using growth reduction assays. Analysis revealed that both extracts had strong growth activity against MRSA which is in agreement with the results of Bukhari *et al.* (2017) and Beigomi *et al.* (2021), which showed that the ethanolic and methanolic extract of *R. stricta* possessed potent antimicrobial property against *Staphylococcus aureus*.

The great activity of *R. stricta* leaf extracts, as demonstrated by this research, could possibly be attributed to the phenolic and flavonoid components found in these extracts (Andrews *et al.*, 2011; Macé *et al.*, 2017; Alsanie *et al.*, 2018). Studies have demonstrated that flavonoids such as quercitin (Beigomi *et al.* 2021), kaempherol (Xie *et al.*, 2021) and catechin (Bhawna *et al.*, 2015) significantly inhibited the growth of MRSA clinical isolates. Flavonoids, which are more abundant in these extracts, are responsible for inhibition of the nucleic acid synthesis (Lagha *et al.*, 2019), also damage the cytoplasmic membrane by altering its function (Bagamboula *et al.*, 2004), inhibit energy metabolism by altering the outer and cytoplasmic membranes, and prevent bacteria from receiving enough energy to grow (Qin *et al.*, 2013). Additionally, the aggregator effect on whole bacterial cells and the inhibition of cell membrane synthesis has been also documented (Oulkheir *et al.*, 2017). Several findings have revealed the antibacterial characteristics of phenolic acids especially caffeic acid, coumaric acid, ferulic acid and chlorogenic acid which have anti-staphylococcal activities (Chaieb *et al.*, 2007; Cushnie *et al.*, 2011; Hassan *et al.*, 2023). Phenolic acids destroy the S. aureus cell wall, leading to cell explosion and leakage of cellular cytoplasmic components (Cushnie *et al.*, 2011; Zhao *et al.*, 2015; Hassan *et al.*, 2023). In addition, scientists found that the high growth inhibition of MRSA is due to the synergistic activity of some flavonoids extracted from *R. stricta* leaves.

The ethanolic extract of R. stricta leaves was found to be more efficient against MRSA strains compared to

the methanolic extract, although the percentage of flavonoids and phenols was the lowest in the ethanolic extract. This may be due to the effect of quinol and chlorogenic acid, which are not present in the methanolic extract, or to myricetin and pumaric acid, that have a higher concentration in the ethanolic extract. Consequently, Kepa *et al.* (2018) demonstrated that myricetin showed the most important antimicrobial property among all flavonoids, and its broad activity was demonstrated against MRSA as well as many pathogenic bacteria tested (Lima *et al.*, 2016; Borges *et al.*, 2013). Moreover, myricetin showed inhibitory activity on the Escherichia coli DnaB helicase which plays an important role in DNA replication (Xie *et al.*, 2017). Coumaric acid also possesses an effective growth inhibition activity against *Staphylococcus aureus* and several bacterial pathogens. This phenolic acid causes destruction the cytoplasmic membrane of bacteria and inhibits cellular functions by controlling the bacterial genome (Park *et al.*, 2016). Regarding quinol, Ma *et al.* (2019) reported that quinol showed antimicrobial activity similar to MRSA. Which depends on the mechanism of destroying the bacterial membrane and cell wall, increasing selective permeability and affecting gene expression. On the other hand, studies have shown that chlorogenic acid lacks a significant amount of anti-*S. staphylococcal* activity (Griep *et al.*, 2007). The effect of chlorogenic acid as an antidote to many different types of pathogenic bacteria has also been documented (Zaixiang *et al.*, 2012).

MRSA isolates were tested for their capability to produce biofilms on the surface of polystyrene, and the results revealed that 23.33% of the isolates produced biofilms at a high rate while 50% produced biofilms at a moderate rate. This result indicated the high ability of MRSA strains to produce biofilms, confirming the fact that *S. aureus* is the most infectious bacteria associated with biofilms. 65% of hospital infections and 80% of microbial infections are due to biofilms, which appear to be an important virulence factor (Lagha *et al.*, 2019; Hassan *et al.*, 2023). Biofilms are associated with many disease manifestations such as nasal skin colonization, soft tissue, endocarditis, and urinary tract infections and other staphylococcal illness (Wang *et al.*, 2015). Biofilms also play major role in the field of urology due to their primarily responsibility for the survival of bacteria in the genitourinary system (Griep *et al.*, 2007; Zaixiang *et al.*, 2012). The presence of biofilms makes bacteria more attached to medical equipment and chronic infections and makes treating bacteria difficult due to their resistance to antibiotics and resistance to phagocytosis processes (Hassan *et al.*, 2023). Therefore, it is crucial to find novel therapeutic approaches to limit biofilm growth.

R. stricta leaves were evaluated for their capability to reduce biofilm developed by MRSA clinical isolates. Antibiofilm analysis demonstrated that both of plant extracts possessed strong biofilm inhibitory property up to a 99% reduction in the quantity of biofilm produced. This activity is mainly due to flavonoids, as a major compound, as well as to other phenolic components identified in the extracts. This work confirmed the finding of Hassan *et al.* (2023) and Lagha *et al.* (2019), which have shown that flavonoids are responsible for reducing bacterial adhesion and biofilm formation as well as inhibiting the quorum-sensing signal receptors TraR and RhlR. Furthermore, a decrease in the quantity of biofilm could be considered as an alteration of MRSA virulence which is in accordance with the finding of Miklasińska-Majdanik *et al.* (2018), who reported that flavonoids reduce bacterial virulence factors. Moreover, Hassan *et al.* (2023), demonstrated that the activity of the autoinducer-2 responsible for cell-to-cell communication is suppressed by flavonoids such quercetin, kaempferol, naringenin, and apigenin, which reduces the synthesis of biofilm. In this investigation, the ethanolic extract also shown biofilm inhibitory property more than the methanolic extract, which suggests that the components involved in growth inhibition and those related to biofilm inhibition are the same. Among them, myricetin that shown reduction of biofilm formation of *S. aureus* (Lagha *et al.* 2019). Additionally, Matilla-Cuenca *et al.* (2020) revealed that this flavonoid inhibits

efficiently the staphylococcal biofilm matrix by targeting Bap- like amyloids. Myricetin demonstrated also curli-dependent E. coli biofilm formation inhibition (Lagha *et al*. 2019).

Conclusion

R. stricta is widely used in traditional medicine and the present study proves that phenolic compounds identified in ethanolic and methanolic extracts of medicinal plant *R. stricta* leaves constitute a promising source of effective antibacterial and antibiofilm components against MRSA. The antimicrobial potential of *R. stricta* phenolic compounds as a natural product open a wide range of possibilities for new antibacterial therapy. The application of combined therapy with antibiotics can improve their effectiveness and reduce drug dosage. However, further complementary investigations should be conducted to isolate and identify the bioactive molecules responsible for the antibacterial and antibiofilm activities of each extract. In addition, in vivo tests and clinical trials will be necessary to define the usefulness of these molecules in the clinical area.

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