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Original Article

In vitro Antibacterial and *in silico* Evaluation of *Ficus benghalensis* Methanolic Bark Extract, as Potential Anti-MRSA Agent

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA), known to be highly resistant bacterium, leading to dreadful infections with limited treatment options. **Objectives:** The current study mainly focused to investigate the anti-staphylococcal activity of *Ficus benghalensis* methanolic bark (FBMB) extract against Methicillin-resistant *Staphylococcus aureus* (MRSA). **Materials and Methods:** The assessment of *Ficus benghalensis* methanolic bark (FBMB) extract was performed by agar well diffusion method and broth dilution method. Further, the pathogenic factors such as formation of biofilm, hemolysis, adhesion and staphyloxanthin production were evaluated to confirm antibiofilm and anti-adhesion activity. Cytoplasmic leakage analysis was also carried out to confirm the antibacterial activity of the extract. **Results:** From agar well diffusion method, it was observed that even at low dosage (1.6 mg/ml) showed the zone of inhibition which is significantly as dose dependent manner. 50 mg/ml extract showed Minimum inhibitory concentration (MIC) and 100 mg/ml extract showed minimum bactericidal concentration (MBC). The inhibitory activity of extracts was observed information of biofilm, hemolytic activity and production of staphyloxanthin. Oxidative survival resulted in decreased colonies of Methicillin-resistant *Staphylococcus aureus* (MRSA) and cytoplasmic leakage elevation was observed with the treatment. Further, *in silico* analysis also confirms the binding and interaction of the active molecules of FBMB extract, such as Beta-sitosterol, Lupeol Acetate and Alpha-amyrin against the MSCRAMMs family of MRSA using AutoDock Vina, Accelrys BIOVIA and pyMol tools and furthermore these molecules have better pharmacokinetic profile as based on Lipinski's rule of five violation. **Conclusion:** *Ficus benghalensis* methanolic bark extract, can be used as an drug candidate for antibiotic-resistant infection caused by Methicillin-resistant *Staphylococcus aureus* (MRSA).

Keywords: MRSA, *Ficus benghalensis*, Bark extract, Anti-biofilm, Anti-virulence, Anti-bacterial, Membrane stability, *in silico.*

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INTRODUCTION

Nosocomial and hospital acquired infection is increasing in alarming rate worldwide. Most of the bacteria's have developed resistance towards antibiotics that contains beta lactam ring such as Penicillin and its derivatives. Vast usage of antibiotics is the root cause for the development of resistance in many pathogens. The most known groups that have developed resistance are; Methicillin resistance *Staphylococcus aureus*, Extended spectrum beta lactamase *Escherichia coli* (ESBLEC) and *Pseudomonas aeruginosa*.[1] Among them MRSA is a highly life-threatening nosocomial pathogen. The spread of *Staphylococcus aureus* infection, is in rise due to its ability of excessive adhesion towards host and its evading mechanism from host immune system. On the flipside, a plenty of resistance bacterial strains were identified, which possess a threat to the current antimicrobial treatments.[2] So alternative antimicrobial drugs to deal with these pathogens is inexorable. Millions of people have been using the plant derived products and drugs to treat various range infections which has resulted in the global shift towards the use of Phyto medication rather than costly chemically synthesized drugs and compounds which leads to many side effects.[3]

Ficus benghalensis belongs to family Moraceae, common name is Banyan tree, Fig tree. The tree is a fast growing, grow up to 30 meters, with spreading branches and many aerial roots.[4] Some natural compounds derived from bark extract of *Ficus benghalensis* are glucoside, 20 tetratriaconthene-2-one, meso inositol, 6-heptatriacontane-10-one, pentariacontan-5-one

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and β–sitosterol-α-D-glucose.[5] *F. benghalensis* water and methanolic extracts exhibits immunomodulatory properties and boost phagocytic potential of Peripheral Blood Mononuclear Cells (PBMCs).[6] The different parts of the tree have various pharmacological properties such as antimicrobial, anti-diabetic, antioxidant, antiseptic and anti-gonorrhoea.[7]

The studies show the wide range of properties of various parts of the Fig tree, whereas there were very limited studies that have reported its antibacterial activity of *Ficus benghalensis* bark extract against Methicillin resisted *Staphylococcus aureus*. So, in this context, our research work aimed to study the antibacterial activity of *Ficus benghalensis* methanolic bark (FBMB) extract against MRSA focused on its ability to form biofilms, virulence parameters and membrane stability and also *in silico* analysis of active molecules namely Beta-sitosterol, Lupeol Acetate, Alpha-amyrin acetate from the FBMB extract has been chosen for the analysis of molecular docking study against the MSCRAMMs family of MRSA, which is an adhesion protein mainly responsible for the attachment of bacteria to host and confer the virulence. Furthermore, the properties of these molecules to be formulated as drug has also been analysed computationally.

MATERIALS AND METHODS

Preparation of FBMB extract

Ficus benghalensis bark was collected and dried. After complete drying, the dried bark sample was immersed in methanol (200g/l) for 48 hr with stirring, following by filtered using Whatman filter paper No.1. The filtrate was kept for drying at 40°C for 24 hr and stored at refrigeration until use.

Agar well Diffusion

Antibacterial activity of FBMB extract was studied against MRSA by Agar well diffusion method as described by Gonelimali *et al*., 2018.[8] Overnight culture of MRSA (OD 600nm = 0.4-0.6) was swabbed in MHA plate. Samples were prepared in the different concentration ranging from 1.6 mg /ml to 200 mg/ml (two-fold concentration). Each well with 8mm diameter received 100 µl of sample. The plates were incubated at 37°C for 24 hr. The plates were observed for the zone of inhibition of MRSA and the zone was measured. DMSO, the diluent served as negative control whereas the standard antibiotic disc Chloramphenicol (30 mcg/disc) served as positive control. The results were recorded.

MIC and MBC determination

The MIC of FBMB extract was studied by Broth micro dilution method as per CLSI guidelines.[9] Overnight culture of MRSA was normalized to 0.1 – 0.4 OD at 600 nm and was used for the assay. Extract prepared at various concentration in the range between 200 – 0.1 mg/ml through serially dilution in two-fold manner using Muller Hinton Broth (MHB). 100 µL of normalized inoculum was added to equal volume of serially diluted extract in 96 well plate. Positive and negative controls such as culture and plain media were maintained respectively for comparison. The 96 well plate was incubated at 37°C. The resulting turbidity was observed after 24 hr and the OD was measured at 600 nm. Minimum turbidity with lowest concentration of FBMB fixed as MIC value.

The 50 µl of sample from MIC assay was cultured in MHA plate by spread plate method. Plates were incubated. The complete inhibition of the growth of MRSA was fixed as MBC of FBMB.

Time kill assay

MRSA growth Inhibition was studied with respect to time intervals. The overnight culture was normalized to 0.1-0.4 at OD 600 nm. Treated and untreated cultures in MHB were incubated at 37°C in 120 rpm orbital shaker. The initial OD_{600} nm was observed at the time of incubation. Small volume of sample withdrawn at 4, 8, 12 and 24 hr and OD at 600 nm was measured, as well as cultured in MHA agar plates. The plates were incubated for 24 hr. The colony forming unit was counted and recorded.

Biofilm assay

The Biofilm formation capacity of MRSA with extract was performed by the method of Plyuta *et al*., (2013)[10] with slight modifications. Briefly, 150 µl of overnight culture was taken with various concentration of extract (25, 50 and 75 mg/ml) in 96 well flat bottom plate. The plate was incubated for 18 to 24 hr at 37°C then, washed with PBS thrice and kept 15 min at room temperature (RT) followed by 0.5% of 200 µl crystal violet stain added and kept for 5 min at RT. Plates washed three times with distilled water. After drying, 95% of 200 µl ethanol added to solubilize the stains and the OD was recorded at 570 nm. Plain broth (negative control) and inoculum alone (positive control).

Hemolysis Activity

Effect of α hemolysin of MRSA was carried out by the method followed in Zohra and Fowzia, 2014,[11] with slight changes. Human Blood was collected freshly which was centrifuged to remove platelets and buffy coats. The erythrocytes collected was diluted with PBS (1:10 ratio) to obtained final concentration of 3%. The extract was taken at different concentration (25, 50 and 75 mg/ml) with erythrocytes and inoculum which was incubated at 37°C for 1 hr followed by centrifugation at 1500 rpm for 10 min. The supernatant was read at 543 nm. Positive control 0.1% triton X-100, erythrocytes with PBS negative control. Hemolysis percentage calculated as,

Hydrophobicity Assay

Cell surface hydrophobicity was determined by the protocol followed in Silva *et al*., 2017.[12] Treated (25, 50 and 75 mg/mL) and untreated MRSA culture was incubated for 24 hr and harvested

$$\text{Hemolysis percentage} = \frac{\text{OD of test sample} - \text{OD of negative control}}{\text{OD of Triton X-100} - \text{OD of negative control}} \times 100$$

the cell pellets. The pellet was washed with PBS and the initial $OD_{600}$was adjusted to 0.3 (Ai). 200 µl of Toluene was mixed with adjusted cell suspension and absorbance was recorded for top layer at 600 nm (Af). HPBI (hydrophobicity index) was calculated by

HPBI (hydrophobicity index) = (Ai–Af)/Ai X 100%

Qualitative and quantitative of Staphyloxanthin

The reduction in staphyloxanthin production capability of MRSA with FBMB treatment was assessed by the method performed by Zang *et al.*,2018[13] with minor modifications. MRSA culture was incubated with presence and absence of extract for 24 hr. The cell pellet was collected by centrifugation and was washed with PBS twice. Pellets resuspended in 200 µl methanol. The mixture was heated at 55°C for 30 min followed by centrifugation at 16,600 g for 10 min. The process was repeated thrice to increase the yield and OD was measured at 465 nm using spectrophotometer (Thermo Fisher Scientific, US).

Oxidative stress survival assay

Survival ability of MRSA with Hydrogen Peroxide (H_2O_2) was studied using the method followed by Liu *et al.*, 2005.[14] The treated and untreated MRSA culture was incubated for 24 hr. The cells were harvested by centrifugation and washed with sterile saline. The cell pellet was resuspended in PBS and the OD_{600} was adjusted to 0.150 and incubated with 1.5% of H_2O_2 at 37°C under 150rpm for 60 min. Then, plated in MHA. Cell survival percentage was observed by colony-forming units (CFU)/ml.

Membrane stability

The impact of FBMB extract on the outer membrane of the MRSA was investigated by SDS treatment. Log phase culture of MRSA was mixed with PBS-buffer containing 25, 50 and 75mg/ml of extract respectively and incubated for 30 min followed by centrifugation. The pellets was mixed in the equal volume of PBS. SDS (0.15%) was added to both treated and untreated cells. Optical density was measured at 565 nm every 2 min up to 10 min.[15]

Determination of extracellular protein leakage

Protein estimation was carried out using a BCA kit (Himedia). Briefly, MRSA culture were treated with various concentration of extract and untreated kept as control. The samples were withdrawn every 1 hr and the supernatant was collected by centrifugation at 6000 rpm for 15 min. 200 µl of the supernatant added with 1ml of the Bradford reagent. The OD was read at 595 nm after 10 min of incubation in the dark. Standard graph was plotted by using BSA as a standard protein.[16]

Nuclei acid analysis

Treated (25, 50 and 75 mg/ml) and untreated MRSA culture taken in respective broth. The initial OD at 260 nm was recorded. Sample drawn for every one hour for analysis up to 5 hr. The supernatant obtained by centrifugation. The OD was recorded at 260 nm using UV-spectrophotometer (Thermo Fisher Scientific, US).[17]

Molecular Docking Study

Molecular docking

AutoDock Vina was used to analyze the binding of Beta-sitosterol, Lupeol Acetate, Alpha-amyrin against the MSCRAMM complex. The ligand structures were obtained from PubChem and protein structure was obtained from Protein Data Bank (PDB). Energy minimization, molecule preparation of all the molecules and grid selection were done using MGL autodock tools.[18] All the docking studies were done with standard grid dimensions of 60x60x60 and with 0.375Å spacing. For the molecular docking, an exhaustiveness factor of 8 and energy difference of 4 kcal/mol was set. Accelrys BIOVIA Discovery Studio version 2017 R^2 and pyMOL was used to visualise the binding of ligands with protein.

Prediction of Activity Spectra for Substances

The PASS parameters are useful in recognizing the capability of any chemical molecule to be formulated as a drug. Lipinski's Rule of Five: The drug-likeliness of all the active molecules based on their physio-chemical properties can be assessed through Lipinski's Rule of Five. This is performed using the molinspiration online tool (https://www.molinspiration.com/). Drug likeness and Toxicity: The basic parameter for every drug is its toxicity to the host. Hence, a drug-likeliness score and toxicity properties of a chemical compound can give a clear idea on its ability to be used as an oral drug by humans. ORISIS Data warrior software was used to compute various toxicity parameters such as mutagen, carcinogen, reproductive effect and irritant.[19]

Bioactivity Score

This indicates the overall potential of the compound to selectively act as a drug and confer is purpose in the host system without disturbing normal functions of the host. Molinspiration was used to predict the bioactivity score along with the interaction of the compounds with various human functions.

ADMET

Adsorption, distribution, metabolism, excretion, and toxicity (ADMET) is a vital pharmacokinetic parameter to consider the drug's metabolic efficiency. Properties like skin permeability, lipophilicity, and ability to be absorbed by tissues, metabolic

inhibition, etc, are considered in this study. SwissADME online tool (http://www.swissadme.ch/index.php) is used to calculate all these parameters.

Statistical Analysis

The data obtained were subjected to statical analysis using GraphPad Prism version 5.1. The results were expressed as mean ± standard deviation and analysed by one-way analysis of variance (ANOVA) with Tukey's multiple *t*-test. Triplicates performed for all the assays.

RESULTS

Agar well diffusion

Agar well diffusion, a primary antibacterial assay was performed to evaluate the effect of FBMB extract against MRSA. 4 mm zone of inhibition obtained at low dosage of extract (1.6 mg/mL) which was increasing in a dose dependent manner shown in Figure 1 A – C and Table 1. Positive control – Chloramphenicol (30 mcg/disc) showed 10 mm zone of inhibition.

MIC and MBC determination

Microdilution method, a secondary level of antibacterial assay was carried out to investigate the lower range of extract to inhibit the growth of MRSA by 50%. 50 mg/mL of extract exhibited 49.98% of inhibition whereas 75 mg/mL showed 69.92%. In higher concentration, ie. 100 mg/mL, there was complete inhibition in the growth of MRSA and this was considered as minimum bactericidal concentration. The results are illustrated in Figure 2 and Figure 3 A - E.

Time kill assay

The survival ability of MRSA at various concentrations of the extract was investigated with respect to time intervals. With an increase in the time interval, the optical density was decreased gradually in a dose dependent manner as represented in Figure 4. Also, a reduction in viable cell count was observed with an increase in dose with respect to time as shown in Table 2. The untreated culture showed consistent growth.

Biofilm inhibition

Inhibition of Biofilm was assessed by the crystal violet staining method. The results revealed that the extract has the capability to distort the biofilm, which indirectly has an impact on the attachment of bacterial cells. The percentage of MRSA survival was 75.30, 52.09, and 25.41 on treating the cells with extract concentrations of 25, 50, and 75 mg/mL respectively. The biofilm formation decreased consistently compared to the control which showed 100% survival after 24 hr. The results obtained was showed in Figure 5.

Hemolysis assay

To study the virulence factor of MRSA, hemolysis assay was performed. The obtained results are shown in Figure 6. The hemolysis was performed with treated and untreated cultures of MRSA. Treated cultures showed decreasing activity of hemolysis compared to untreated. The hemolysis percentage decreased significantly to 90.39, 44.1, and 14.79 at 25, 50, and 75 mg/mL of extract respectively. The control showed complete lysis.

Surface hydrophobicity

One of the important properties of the bacteria is to adhere to the surface to form the biofilm and to evaluate this property, surface hydrophobicity was studied. The results are depicted in Figure 7. The treated culture exhibited a moderate decrease in the percentage of hydrophobicity in a dose dependent manner compared to the untreated culture showed 100% hydrophobicity.

Staphyloxanthin assay

Bacterial resistance to ROS produced by the immune system was studied using the staphyloxanthin assay. The MRSA treated with extract showed significant decrease compared to control.

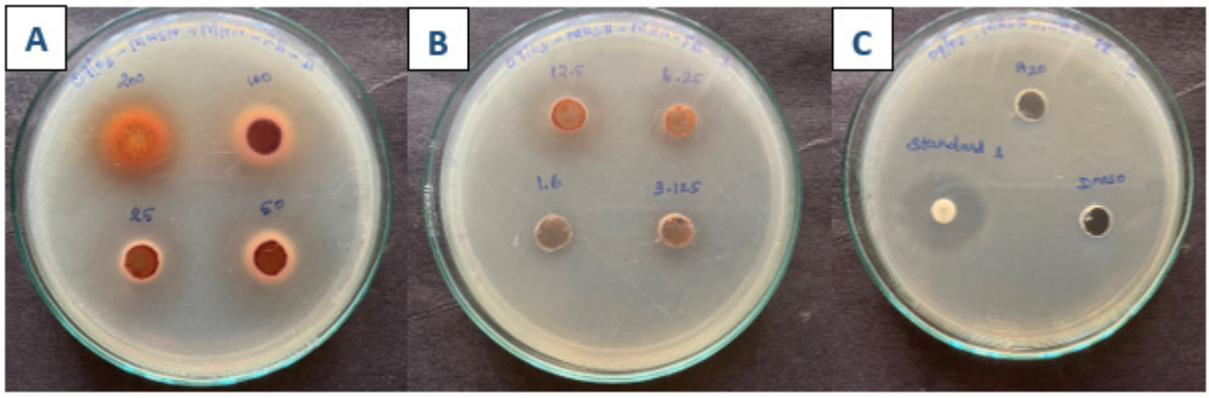


Figure 1: Agar well diffusion of FBMB against MRSA.

The Figure Shows the Zone of inhibition of FBMB against MRSA. A - 200, 100, 50 and 25 mg/mL of FBMB extract, B - 12.5, 6.25, 3.125 and 1.6 mg/mL of FBMB extract, C - sterilized water, DMSO diluent, Chloramphenicol standard (30 mcg/disc).

Table 1: Agar well diffusion assay of FBMB extract against MRSA.

Dose (mg/mL)	Zone of Inhibition (in mm)
Water	-
DMSO	-
Chloramphenicol	10
1.6	04
3.125	05
6.25	07
12.5	08
25	09
50	10
100	10
200	14

The zone of inhibition of MRSA with FBMB extract at different concentrations in mg/mL, Chloramphenicol disc 30 mcg/disc used as standard.

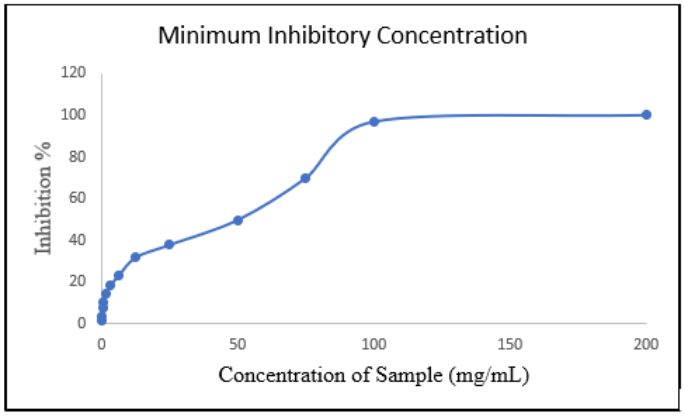


Figure 2: Minimum inhibitory concentration.

The Figure shows the minimum inhibitory concentration of FBMB against MRSA in the range between 0 - 200 mg/mL. 49.98% of inhibition obtained at 50 mg/mL resulted in MIC value. The percentage of inhibition increased in dose dependent manner.

The staphyloxanthin quantification reduced significantly with an increase in dosage range as shown in Figure 8.

Hydrogen Peroxide survival assay

The survival ability of MRSA in oxidative stress was studied by the hydrogen peroxide method. The results are depicted in Figure 9 A-D. A higher number of colonies was observed in untreated control which decreased gradually in treated cultures as the concentration of the extract increased which confirms the susceptibility of MRSA when treated with the extract.

Membrane stability and leakage analysis

The MRSA treated with the extract was subjected to membrane stability and membrane leakage analysis. The results of the membrane stability assay confirm that the FBMB extract has a strong impact on cell wall damage and intercellular leakage which is shown in Figure 10. Protein and nucleic acid were estimated in supernatant which indicates the cytoplasmic leakage. The samples were analyzed every 1 hr. The results are shown in Figure 11 and Figure 12 respectively. The results revealed that there was an increased concentration of protein and nucleic acids found in the supernatant with respect to time in a dose dependent manner. The disintegration of cell wall leads to reduced viability which is shown in the time kill assay.

Molecular Docking

The poses from molecular docking were visualized through Accelrys BIOVIA Discovery Studio and pyMOL. Molecular docking reveals that Beta-sitosterol, Lupeol Acetate, Alpha-amyrin binds with MSCRAMMs with excellent binding energy of -8.6, -7.4, -7.8 kcal/mol respectively. The 2D and 3D images with binding sites are given in Table 3.

Prediction of Activity Spectra for Substances

Lipinski's Rule of Five: According to Lipinski's rule of five, any chemical compound that violates more than 1 physiochemical aspect is not fit to be formulated as a drug. All 3 molecules have only one Lipinski violation, which means all the molecules pass the Lipinski rule of five. Drug likeliness and toxicity: According to the drug likeliness and toxicity data obtained from ORISIS Data warrior, none of the molecule possess any mutagenic or cancer-causing characteristic and shows a very good drug-likeliness score.

Bioactivity Score

Bioactivity scores for each compound are calculated for various activities such as ligand for GPCR, Ion channel modulator, Nuclease receptor ligand, kinase, protease, and enzyme inhibitor. Bioactivity score greater than 0.000 is considered to be bioactive, score in the range between -0.50 and 0.00 are said to be moderately active, whereas scores below or equal to -0.50 is biologically inactive. All the compounds prove to be active in more than one parameter discussed in the study.

ADMET

The properties analyzed using SwissADME gives evident result that all the 3 active ingredients can be metabolized by the human body. The lower the log k *p* value is, the less permeant it is to the skin. Lupeol acetate seems to be the least permeant and Beta sitosterol the most permeant to skin. Consensus log *p* value is the median value of all parameters that indicate the drug's ability to reach the target site. All the compounds have a log p value greater than one, which means all are lipophilic and can be absorbed well. Moreover, none of the compounds were either permeable to blood brain barrier or served as a substrate to P-glycoprotein. Also, no compounds acted as inhibitor to any cytochrome class. All these confirm that Beta-sitosterol, Lupeol Acetate, Alpha-amyrin has excellent metabolic properties and do

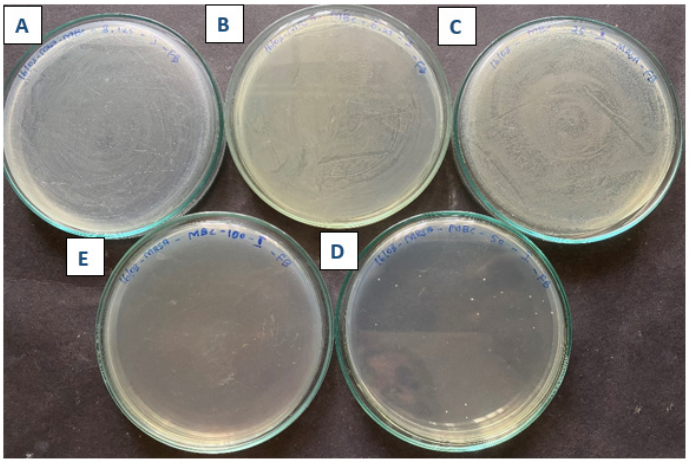


Figure 3: Minimum bactericidal concentration.

The Figure shows the TSA Plates. A, B, C, D and E represents 3.165, 6.25, 25, 50 and 100 mg/mL of FBMB extract respectively. Plates A, B, and C indicate the lawn culture of MRSA whereas plate D showed the countable colonies, Plate E - there was no growth observed which is fixed as MBC concentration.

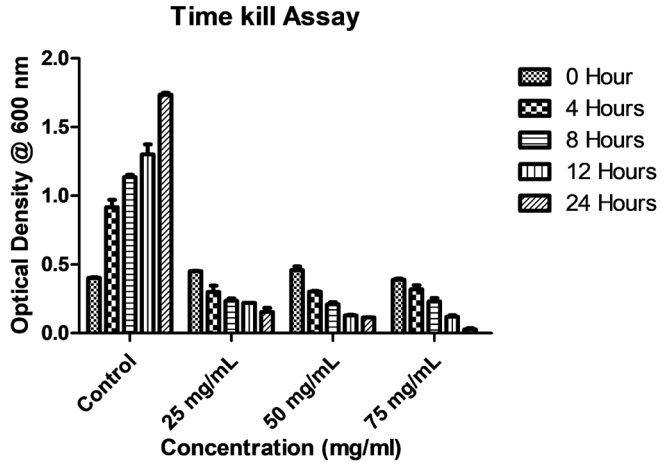


Figure 4: Time kill assay.

The Figure shows the impact of FBMB extract on the growth of MRSA depending on time indicated as OD at 600 nm. The control showed a gradual increase along with time, whereas the treated groups with various concentrations (25, 50 and 75 mg/ml) showed a significant reduction in a dose dependent manner. Data are expressed as mean ±SD. Treated groups showed high significance compared to the control group (p<0.0001).

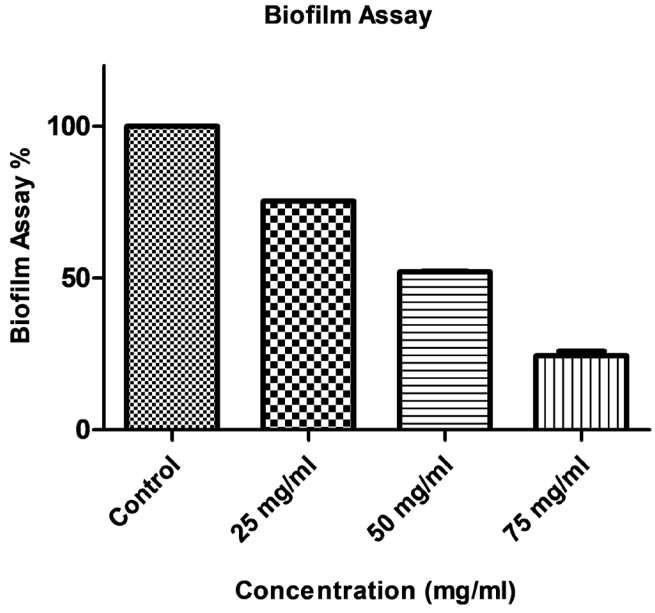


Figure 5: Biofilm inhibition.

The Figure shows the inhibition of biofilm. The treated groups with various concentrations (25, 50 and 75 mg/ml) showed a decreased biofilm formation percentage with increasing sample concentration compared to control whereas control showed ideal biofilm formation. Data are expressed as mean ±SD. Treated groups showed high significance compared to the control group.

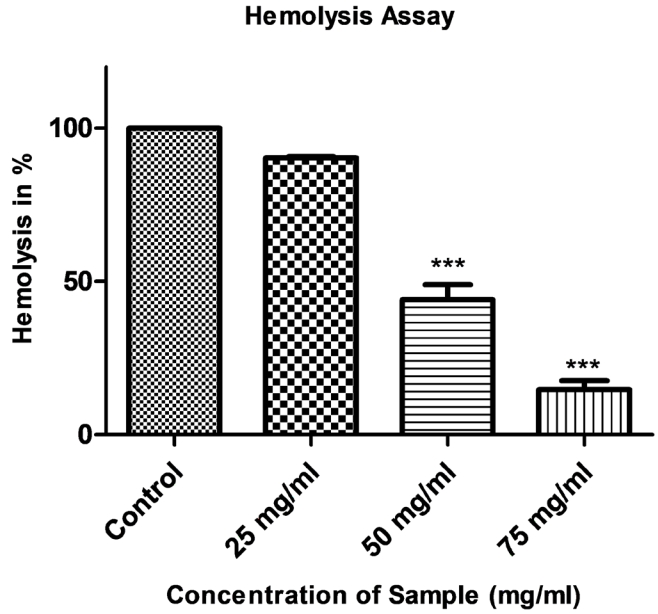


Figure 6: Hemolytic Activity.

Hemolysis percentage is shown in figure. The Control group showed complete lysis. The treated groups showed a significant decrease in lysis percentage with increase in dosage concentration. Data are expressed as mean ±SD. Treated groups showed high significance compared to the control group. ***(p<0.0001) as compared to the control group.

Table 2: Time-kill assay - Colony-forming unit.

	0 Hr	4 Hr	8 Hr	12 Hr	24 Hr
Control	70 x 10^4	TNTC	TNTC	TNTC	TNTC
25 mg/mL	50 x 10^4	47 x 10^4	6.0 x 10^4	2.3 x 10^4	1.3 x 10^3
50 mg/mL	70 x 10^4	45 x10^4	7.7 x 10^4	4.5 x 10^4	5.0 x 10^3
75 mg/mL	45 x 10^4	12 x 10^4	2.6 x 10^4	1.0 x 10^3	5.0 x 10^2

The table showed the number of colonies of MRSA treated with extract of various concentrations (25, 50 and 75 mg/mL) at different periods such as 0, 4, 8, 12 and 24 hr. The result showed the decrease in colony count at various time interval in a dose-dependent manner.

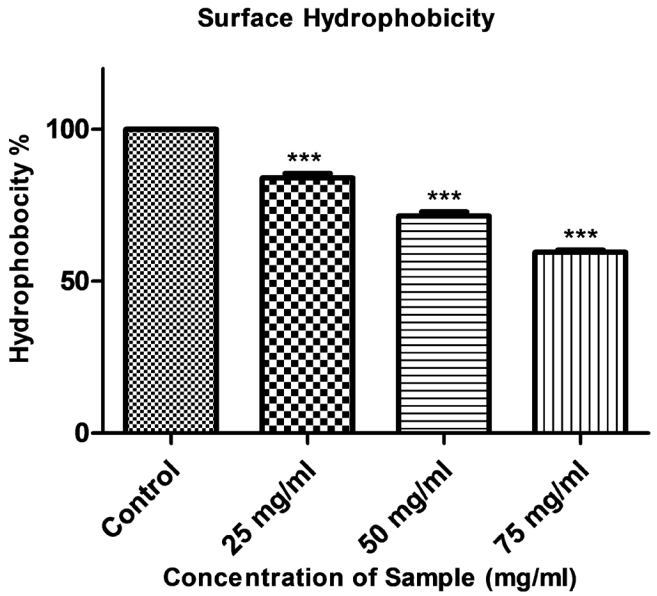


Figure 7: Surface Hydrophobicity.

FBMB extract effect on Surface hydrophobicity is depicted in the figure. the control group showed a high percentage of hydrophobicity index which was reduced moderately in the treated groups based on the increased sample concentration. Data are expressed as mean ±SD. Treated groups showed high significance compared to the control group. ***(p<0.0001) as compared to the control group.

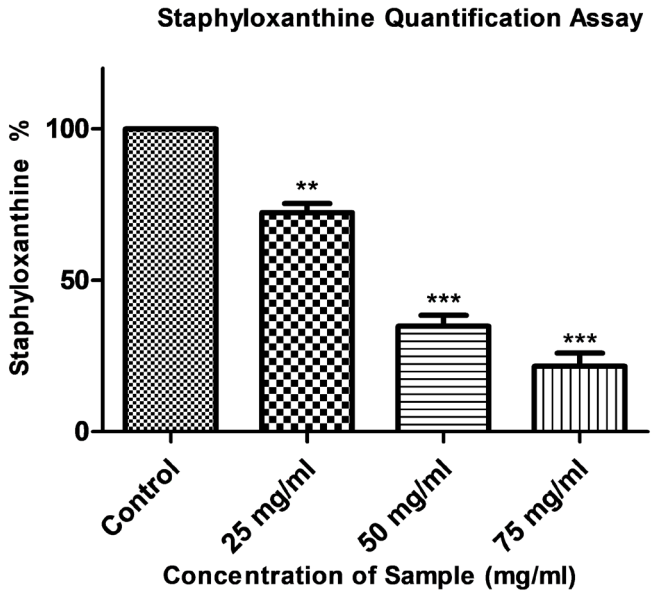


Figure 8: Staphyloxanthin Quantification Assay.

Staphyloxanthin production was analyzed. Figure 8 depicted the production percentage of staphyloxanthin in treated and non-treated groups. The percentage was decreased in a dose dependent manner compared to the control. Data are expressed as mean ±SD. Treated groups showed high significance compared to the control group. ***(p<0.0001) as compared to the control group.

not disturb the normal functioning of the host system. The results of *in silico* analysis was given in Table 3.

DISCUSSION

Multidrug resistance (MDR) bacterium cause extremely life-threatening infections worldwide. Methicillin resistance *Staphylococcus aureus* is one of the major causative agents of nosocomial diseases. So, there is an emergence for finding and promoting new natural therapeutic drug compounds.[1] Herbal medicines have gained global attention because they exhibit potent anti-virulence properties that can combat MDR bacterium.[20] *F. bengalensis,* known as the banyan tree possess high medicinal properties and is used to treat vaginal complaints, skin allergies, inflammations, fever, and gonorrhea, and also serves as antidiarrheal, anti-dysenteric, hemostatic, and anti-hemorrhoidal agent.[4]

Previous studies with aqueous and alcoholic extract of bark of *F. bengalensis* showed significant antimicrobial activity. Limited studies were carried out on *F. bengalensis* bark extract against MRSA. Based on these considerations, we investigated the effect of methanolic bark extract of *F. bengalensis* against MRSA.

In Agar well diffusion, the zone of inhibition increased with extract concentration. The results obtained confirmed that methanolic bark extract possesses antibacterial activity against MRSA. The zone of inhibition obtained in this study was 14 mm at 100 mg/ml whereas the earlier studies on *S. aureus* reported 12 mm and 13.5 mm zone of inhibition when treated with 1mg/mL of aqueous extract and 0.1 mg/mL of FBMB respectively. The increased concentration of extract is required to combat resistant bacterium.[4,21] Sarminto *et al.*, 2011[22] stated that the zone of inhibition for methicillin sensitive strains were twice as large as those for MRSA based on the result obtained as MSSA -14.33 mm and MRSA - 7.67 zone of inhibition respectively at

Table 3: Molecular docking of Bioactive molecules of FBMB extract against surface protein of MRSA.

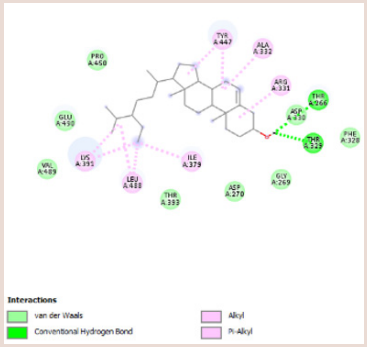
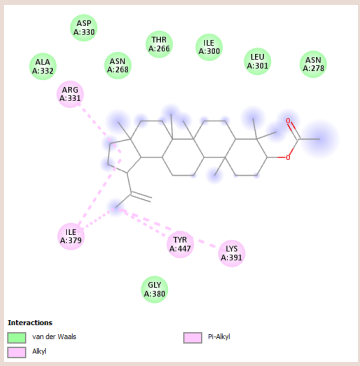
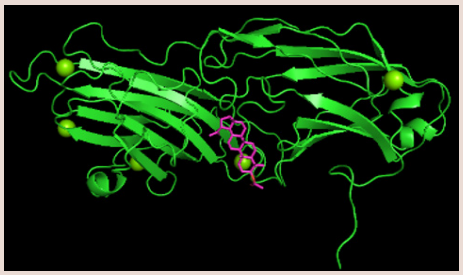
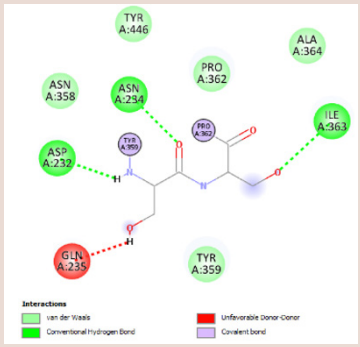
Ligand	BE (kcal/mol)	Interacting Amino Acids	2D structure	3D Structure
Beta-sitosterol Pubchem Id- 222284 MF- $C_{29}H_{50}O$ MW- 414.7	-8.6	LYS391, LEU488, ILE379, THR266, THR329, ASP330, ARG331, ALA332, TYR447		
Lupeol Acetate Pubchem Id- 92157 MF- $C_{32}H_{52}O_2$ MW- 468.8	-7.9	ARG,331, ILE379, GLY380, TYR447, LYS391		
Alpha-Amyrin Acetate Pubchem Id- 92842 MF- $C_{32}H_{52}O_2$ MW- 468.8	- 8.1	ASP232, ASN234, TYR359, PRO362, ILE363, GLN235		

Table shows the interactions of bioactive molecules such as Beta-sitosterol, Lupeol Acetate and Alpha-amyrin acetate with surface protein MSCRAMMs of MRSA predicted using Bio info tools such as AutoDock Vina, Accelrys BIOVIA Discovery Studio and pyMOL. 2D and 3D structures shows the interaction between ligand and amino acids.

100% of plant extract. These results suggest that the nature of the solvents influences the solubility of phytochemical compounds. Methanolic extracts had better phytochemical recovery and hence was preferred in this study.[23,24]

Microdilution method was performed to evaluate the minimum concentration of extract that inhibit half of the population of MRSA. The results showed the MIC at 50 mg/mL and MBC - the bactericidal concentration as 100 mg/mL. This inhibitory activity may be due to the presence of various phytochemicals present in the extract. As stated in the aforementioned paragraph, the polarity of solvent has an impact on the extractability of the phenolic.[25,26] Flavonoids and saponins are also known to play a vital role in the antibacterial effect stated by Ikrame *et al*., 2019.[27]

The survival ability of treated and untreated MRSA culture was analysed by time kill assay. The results revealed that there was a reduction of viable cell count which implies the growth inhibition activity of extract. Compared to the untreated culture, the optical density values were reduced in a dose dependent manner in treated culture with respect to time. The results obtained clearly indicates that the extract has strong antibacterial activity by limiting the cellular metabolism, synthesis of primary metabolites and replication in the lag phase itself.[15]

MRSA is well-known to form biofilms. The formation of biofilm is a major virulence feature for host-pathogenic interaction to cause infection. Biofilm provides a defence system for bacteria from phagocytosis.[28] In this study, inhibition of biofilm was

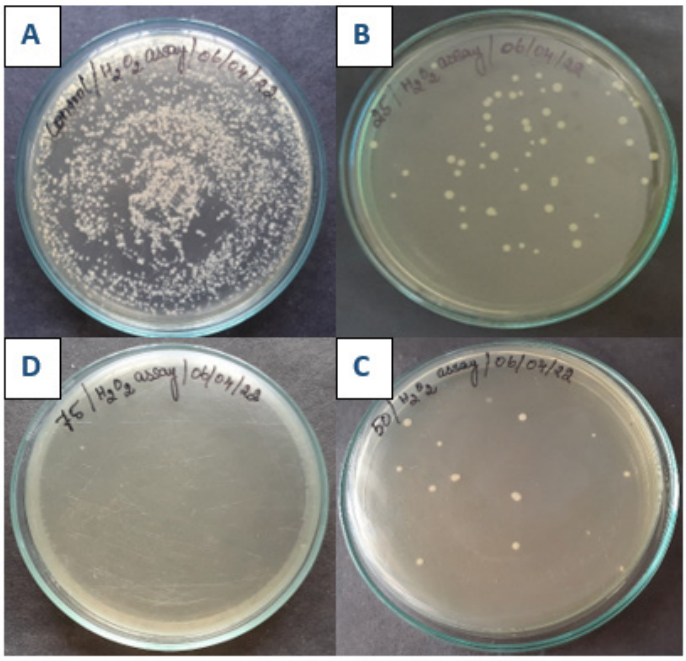


Figure 9: Hydrogen Peroxide resistance.

The Figure shows the colonies of MRSA with Hydrogen Peroxide conditions. The treated and untreated cultures were subjected to oxidative stress and the figure showed a decreased colony-forming unit with the increase in dose concentration. The control showed high CFU.

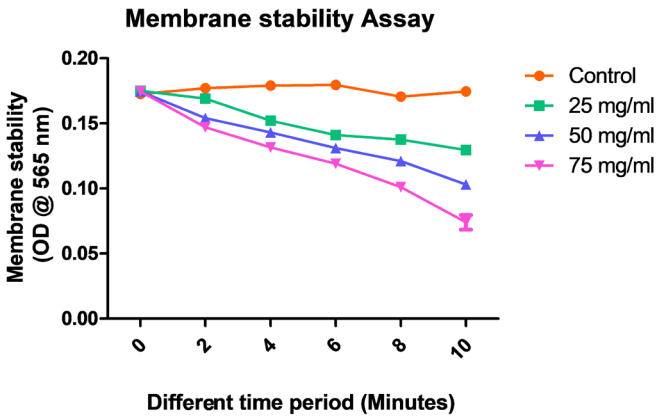


Figure 10: Membrane Stability.

Figure illustrated the membrane stability of MRSA treated with FBMB. The treated groups showed a decrease in membrane stability depending on time intervals whereas the control maintained the consistent membrane stability. Data are expressed as mean ±SD. Treated groups showed high significance compared to the control group (p<0.0001).

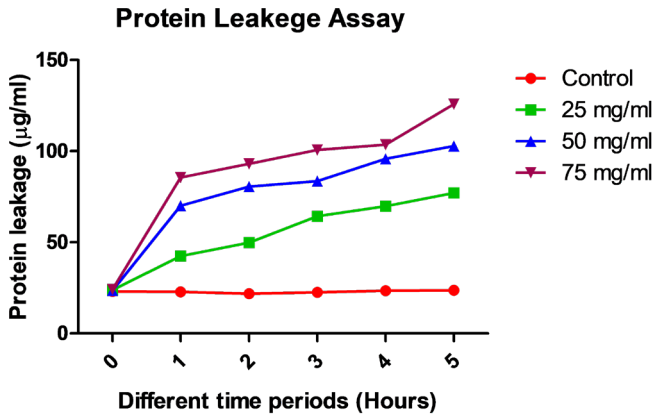


Figure 11: Protein Leakage assay.

Cytoplasmic leakage was studied. The availability of protein in the supernatant was evaluated with respect to time. The Figure showed that there was the increase in protein concentration in a dose dependent manner with time intervals. Control showed less concentration of protein. Data are expressed as mean ±SD. Treated groups showed high significance compared to the control group (p<0.0001).

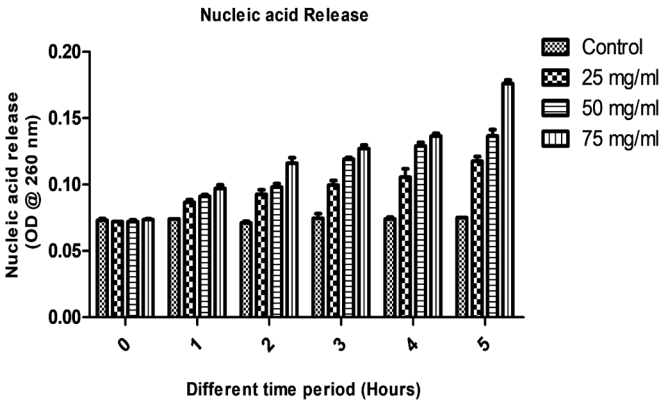


Figure 12: Nucleic acid Release assay.

The Figure shows the significant increase of nucleic acid release in the treated group with an increase in sample concentration whereas the control group showed a very less amount of nucleic acid. Data are expressed as mean ±SD. Treated groups showed high significance compared to the control group (p<0.0001).

investigated. Quantitative examination of biofilm indicates that the treated culture is incompetent to form a biofilm with an increase in dosage. The bark of *F. bengalensis* primarily has phenols, terpenes, flavonoids, terpenoids, and many phytoconstituents.[29] Various literatures stated that phytochemicals exhibit various mode of actions towards antibacterial activity; one among them is to inhibit biofilm formation to prevent cell to cell adhesion. It can be concluded that the phenolic and flavonoids of bark extract may be the compounds that inhibits biofilms formation.

The percentage of hemolysis was determined by the ability of α-hemolysin toxin produced by MRSA to lyse RBCs. The treated cultures showed decreased hemolytic activity compared to the control. This result indicates that the extract disrupts α-hemolysin production by controlling molecular mechanisms.[30]

Surface hydrophobicity is one of the major virulence factors of MRSA which helps bacteria to adhere with host tissue and leads to the colonization. The hydrophobicity index of the treated culture was evaluated. The results show that there was a moderate decrease in hydrophobicity index in a dose dependent manner. The decreased hydrophobicity corroborates the biofilm formation as hydrophobicity is directly proportional to the formation of biofilm.[31,32]

Staphyloxanthin-yellow carotenoid pigment, act as a defender of MRSA from phagocytosis. It also acts as a foremost radical scavenger that protects MRSA from oxidative stress created by human neutrophil cells.[13,33] In this study, we investigated the ability of the extract to disrupt the synthesis of staphyloxanthin. The results showed a decrease in the percentage of staphyloxanthin in a dose dependent manner which revealed that the extract has reduced the synthesis of staphyloxanthin. The reduction in staphyloxanthin makes MRSA more susceptible to the reactive oxygen species leading to the killing of bacteria. The survival ability of MRSA in oxidative stress was also investigated in this study. The treated cultures were more susceptible to H_2O_2 conditions this furthermore substantiates the effect of FBMB extract on staphyloxanthin production. The results showed a decreased number of colony-forming units. The loss of staphyloxanthin failed to protect cells from ROS leading to inhibition of biofilm formation. This may be due to the presence of flavonoids as stated in earlier studies.[34] Staphyloxanthin is located in the inner cell wall membrane which provides structural integrity, but the reduction in percentage of staphyloxanthin makes the bacteria more susceptible to the membrane-targeted drugs.[20,35] We investigated the cytoplasmic leakage in the treated and untreated cultures. Protein and nucleic acid content were increased with respect to incubation time and concentration of extract, this indicates membrane damage in MRSA. Cytoplasmic leakage is indicative of irreversible membrane damage. Some phenolic compounds have activity towards bacterial membrane causing intracellular leakage. Phenolic compounds are easily permeable and cause a loss in selective permeability of cytoplasmic membrane resulting in leakage of proteins, nucleic acids, and ions like potassium and phosphate. This leakage elevates osmotic pressure and induces the complete disruption of the cell wall leading to cell death.[36] Phenolics and other phytochemicals have different modes of action towards bacteria which include; cell wall damage, decrease in hydrophobicity, inhibition of biofilm formation, and inhibition of Staphylococcal protein A, the major anchoring receptor which helps to attach with host tissue for colonization.[37]

To identify the anti-virulence activity of *F. benghalensis* bark, an *in silico* molecular docking study using 3 active ingredients from the methanolic extract of the bark was conducted, namely Beta-sitosterol, Lupeol Acetate and Alpha-amyrin acetate. They were tested for binding affinity against MSCRAMMs, an adhesion protein which plays a major role in the virulence of MRSA. The molecular docking analysis using AutoDock Vina showed that Beta-sitosterol, Lupeol Acetate, Alpha-amyrin acetate interact well with MSCRAMMs with maximum binding energies -8.6, -7.9, -8.1 kcal/mol respectively. The results when visualized in Discovery studio and pyMOL tools, showed various kinds of bonds shared by the 3 ligands and MSCRAMMs. The protein, MSCRAMMs shares 2 polar bonds with Beta-sitosterol through hydrogen bonding, both with threonine at 329th and 266th positions. It also shares a polar bond with alpha-amyrin acetate in the form of hydrogen bonding with Aspartic acid, Asparagine, and Isoleucine at 232, 234, 363 positions respectively. However, MSCRAMMs does not share any polar bond with Lupeol acetate yet forms a stable complex through other non-polar interactions which includes van der Waals, pi-alkyl and alkyl interactions. The other 2 active ingredients also form non-polar interactions with the protein apart from the hydrogen bonding, making the complex more stable. The strong binding affinity and multiple interactions with the target suggests that there is a formation of stable complex and thus renders all 3 active compounds as potential inhibitor of MSCRAMMs. Furthermore, various physiological, physio-chemical, and pharmacokinetic properties were tested for the compounds' likeliness to be used as a drug. According to Lipinski's rule of five, there must not be more than 1 violation of parameters for oral drugs. All the 3 active ingredients satisfy this criterion with only one violation each, which is higher cLog*P* value. None of the compounds showed any mutagenic, carcinogenic, or irritant property in the drug likeliness done using Data Warrior software. The bioactivity score is a major indicator of the compound's potential to be available in the target site and confer its action. As evident from the table, Beta-sitosterol and Lupeol acetate are able to confer bioactivity by acting against GPCR, as an Ion channel modulator, nuclease receptor ligand and inhibitor for protease and enzymes except kinase. Whereas Alpha-amyrin acetate lacks the ability to act as an Ion channel modulator. The ADMET properties calculated using SwissADME suggests that none of the active compounds has any metabolic effect in the host system as it does not pass through the BBB. Cytochromes are important metabolic enzymes that help in biotransformation of xenobiotics to protect tissues. Surprisingly, none of the molecules acts as inhibitors to any cytochromes, which means they can pass through the host system without inducing any toxicity. All the compounds are also resistant to being eliminated from the system as they do not act as the ATP dependent bioavailability protein pump permeability-glycoprotein (P-gp), which eliminates medicines from the system. The study proves that apart from its anti-microbial activity, the bark of *F. benghalensis* also has active compounds with anti-virulence activity which acts by reducing the adhesion of major cell wall proteins of MRSA. Furthermore, all three active molecules also prove to be equally good to be synthesised as a oral drug that can confer its activity at the target site without affect the host metabolism or showing any toxicity.

In conclusion, our study reveals that the methanolic bark extract of *Ficus benghalensis* exhibited significant anti-staphylococcal activity through various mechanisms. The plant may be an active therapeutic agent for MRSA in the future and further molecular level investigations are necessary to understand specific mechanisms.

CONCLUSION

Collectively, it is concluded that *Ficus benghalensis* methanolic bark (FBMB) possesses antibacterial activity against methicillin-resistant *Staphylococcus aureus*. The anti-bacterial activity of FBMB extract may due to the presence of a rich phytochemical profile. These results explore the ability of FBMB extract makes an impact on physiological, as well as biological factors of MRSA leading to the decrease in the formation of biofilm, production of α hemolysin, surface hydrophobicity, and also in the production of staphyloxanthin. Also, it reduces the survival ability of MRSA by damaging the outer membrane. Moreover, *in silico* analysis also confirms the interaction of FBMB active molecules with MSCRAMM complex of MRSA and possesses preferable pharmacokinetic profile which can be used as therapeutic agent in future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FBMB: *Ficus benghalensis* methanolic bark; **MRSA:** Methicillin resistant *Staphylococcus aureus*; **MIC:** Minimum inhibitory concentration; **MBC:** Minimum bactericidal concentration; **OD:** Optical density; **MHA:** Muller Hinton Agar; **RPM:** Rotation per minute; **CFU:** Colony forming unit; **SDS:** Sodium Dodecyl Sulphate; **MDR:** Multidrug resistance; **ROS:** Reactive Oxygen species.

SUMMARY

Most of the bacteria related to nosocomial infection have developed resistance towards antibiotics. The most known nosocomial pathogen is Methicillin resistant *Staphylococcus aureus*, an infection with increasing morbidity and mortality.

Methanolic bark extract of *Ficus benghalensis*, showed antibacterial activity against methicillin resistant *Staphylococcus aureus* Antibacterial activity was confirmed by agar well diffusion method, MIC and MBC and time kill assay through inhibition of bacterial growth exerted by the extract.

Anti-virulence activity against MRSA was confirmed by decreasing in biofilm formation, hemolysis, and staphyloxanthin production and also by membrane damage studies including protein and nucleic acid estimation.

Anti-staphylococcal activity of FBMB extract further confirmed with *in silico* analysis. The bioactive molecules of FBMB such as Beta-sitosterol, Lupeol Acetate and Alpha-amyrin acetate showed high binding affinity against MSCRAMMs, an adhesion protein which plays a major role in the virulence of MRSA

REFERENCES

1. Bataineh SMB, Tarazi YH, Ahmad WA. Antibacterial efficacy of some medicinal plants on multidrug resistance bacteria and their toxicity on eukaryotic cells. Appl Sci. 2021;11(18):8479. doi: 10.3390/app11188479.
2. Kipre BG, Guessennd NK, Koné MW, Gbonon V, Coulibaly JK, Dosso M. Antibacterial activity of the stem bark of *Tieghemella heckelii* Pierre ex. A Chev against methicillin-resistant *Staphylococcus aureus*. BMC Complement Altern Med. 2017;17(1):170. doi: 10.1186/s12906-017-1681-8, PMID 28347339.
3. Aliya S. A, Prameela Y. Antibacterial Act Methanolic Extract Unripe *Carica papaya* Linn. Fruit. 2016;31:19-22.
4. Manimozhi DM SS, KGS. Effect of different extracts of stem bark of *Ficus* sp. On multidrug resistant pathogenic bacteria. Int J Pharm Sci Res. 2012;3(7):2122-9.
5. Subramanian PM, Misra GS. Chemical constituents of *Ficus bengalensis* (part II) - PubMed. Pol J Pharmacol Pharm. 1978;30(4):559-62. PMID 740556.
6. Gabhe S, Tatke P, Khan T. Evaluation of the immunomodulatory activity of the methanol extract of *Ficus benghalensis* roots in rats. Indian J Pharmacol. 2006;38(4):271. doi: 10.4103/0253-7613.27024.
7. Alimuddin S, Hemlata R, Patel NM. Evaluation of antimicrobial activity of stem bark of *Ficus bengalensis* Linn. Collected from different geographical regions. Pharmacogn J. 2010;2(7):178-80. doi: 10.1016/S0975-3575(10)80088-4.
8. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, et al. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Front Microbiol. 2018;9:1639. doi: 10.3389/fmicb.2018.01639, PMID 30087662.
9. Wayne P. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—ninth edition. In: CLSI, CLSI, editors. 2012;2012. Available from: https://www.researchgate.net/file.PostFileLoader.html?id=564ceedf5e9d97daf08b45a2&assetKey=AS%3A297254750572544%401447882463055.
10. Plyuta V, Zaitseva J, Lobakova E, Zagoskina N, Kuznetsov A, Khmel I. Effect of plant phenolic compounds on biofilm formation by *Pseudomonas aeruginosa*. APMIS. 2013;121(11):1073-81. doi: 10.1111/apm.12083, PMID 23594262.
11. Zohra M, Fawzia A. Hemolytic activity of different herbal extracts used in Algeria. Int J Pharm Sci Res. 2014;5(8):495-500.
12. Silva LN, Da Hora GCA, Soares TA, Bojer MS, Ingmer H, Macedo AJ, *et al*. Myricetin protects *Galleria mellonella* against *Staphylococcus aureus* infection and inhibits multiple virulence factors. Sci Rep. 2017;7(1):1-6.
13. Zhang J, Suo Y, Zhang D, Jin F, Zhao H, Shi C. Genetic and virulent difference between pigmented and non-pigmented *Staphylococcus aureus*. Front Microbiol. 2018;9(APR):598. doi: 10.3389/fmicb.2018.00598, PMID 29666612.
14. Liu GY, Essex A, Buchanan JT, Datta V, Hoffman HM, Bastian JF, *et al*. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. J Exp Med. 2005;202(2):209-15. doi: 10.1084/jem.20050846, PMID 16009720.
15. Reddy LS, Nisha MM, Joice M, Shilpa PN. Antimicrobial activity of zinc oxide (ZnO) nanoparticle against *Klebsiella pneumoniae*. Pharm Biol. 2014;52(11):1388-97. doi: 10.3109/13880209.2014.893001, PMID 25026353.
16. Song X, Liu T, Wang L, Liu L, Li X, Wu X. Antibacterial effects and mechanism of mandarin (*Citrus reticulata* L.) essential oil against *Staphylococcus aureus*. Molecules. 2020;25(21). doi: 10.3390/molecules25214956, PMID 33114746.
17. Cai R, Miao M, Yue T, Zhang Y, Cui L, Wang Z, *et al*. Antibacterial activity and mechanism of cinnamic acid and chlorogenic acid against *Alicyclobacillus acidoterrestris* vegetative cells in apple juice. Int J Food Sci Technol. 2019;54(5):1697-705. doi: 10.1111/ijfs.14051.
18. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61. doi: 10.1002/jcc.21334, PMID 19499576.

19. Sander T, Freyss J, Von Korff M, Rufener C. DataWarrior: An open-source program for chemistry aware data visualization and analysis. J Chem Inf Model. 2015;55(2):460-73. doi: 10.1021/ci500588j, PMID 25558886.
20. Valliammai A, Selvaraj A, Muthuramalingam P, Priya A, Ramesh M, Pandian SK. Staphyloxanthin inhibitory potential of thymol impairs antioxidant fitness, enhances neutrophil mediated killing and alters membrane fluidity of methicillin resistant *Staphylococcus aureus*. Biomed Pharmacother. 2021;141:111933. doi: 10.1016/j.biopha.2021.111933, PMID 34328107.
21. Gayathri M, Kannabiran K. Antimicrobial activity of *Hemidesmus indicus, Ficus bengalensis* and *Pterocarpus marsupium* roxb. Indian J Pharm Sci. 2009;71(5):578-81. doi: 10.4103/0250-474X.58182, PMID 20502584.
22. Sarmiento WC, Maramba CC, Liza M, Gonzales M. An *in vitro* Study on the antibacterial Effect of Neem (*Azadirachta indica*) Leaf Extract on methicillin-sensitive and methicillin-Resistant *Staphylococcus aureus*. PIDSP J. 2011;12(1):40-5.
23. Koona SJ, Subba Rao B. *In vitro* evaluation of antibacterial activity of crude extracts of *Ficus benghalensis* Linn., the banyan tree leaves. Indian J Nat Prod Resour. 2012;3(2):281-4.
24. Uma B, Prabhakar K, Rajendran S. Ethnobot Leaf. *In vitro* Antimicrobial Activity and Phytochemical Analysis of *Ficus religiosa* L. and *Ficus bengalensis* L. against Diarrhoeal Enterotoxigenic *E. coli*. 2009;13(1):472-4://opensiuc.
25. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem. 2007;105(3):1126-34. doi: 10.1016/j.foodchem.2007.02.010.
26. Trabelsi N, Megdiche W, Ksouri R, Falleh H, Oueslati S, Soumaya B, *et al*. Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. LWT Food Sci Technol. 2010;43(4):632-9. doi: 10.1016/j.lwt.2009.11.003.
27. Zeouk I, Balouiri M, Bekhti K. Antistaphylococcal activity and phytochemical analysis of crude extracts of five medicinal plants used in the center of morocco against dermatitis. Int J Microbiol. 2019;2019:1803102. doi: 10.1155/2019/1803102, PMID 31781227.
28. Zhang L, Ismail MM, Rocchetti G, Fayek NM, Lucini L, Saber FR. The untargeted phytochemical profile of three Meliaceae species related to *in vitro* cytotoxicity and anti-virulence activity against MRSA isolates. Molecules. 2022;27(2). doi: 10.3390/molecules27020435, PMID 35056761.
29. Murugesu S, Selamat J, Perumal V. Phytochemistry, pharmacological properties, and recent applications of *Ficus benghalensis* and *Ficus religiosa*. Plants (Basel). 2021;10(12). doi: 10.3390/plants10122749, PMID 34961220.
30. Qiu J, Niu X, Dong J, Wang D, Wang J, Li H, *et al*. Baicalin protects mice from *Staphylococcus aureus* pneumonia via inhibition of the cytolytic activity of-hemolysin. J Infect Dis. 2012;206(2):292-301. doi: 10.1093/infdis/jis336, PMID 22551812.
31. Kouidhi B, Zmantar T, Hentati H, Bakhrouf A. Cell surface hydrophobicity, biofilm formation, adhesives properties and molecular detection of adhesins genes in *Staphylococcus aureus* associated to dental caries. Microb Pathog. 2010;49:(1-7).
32. Gowrishankar S, Kamaladevi A, Ayyanar KS, Balamurugan K, Pandian SK. *Bacillus amyloliquefaciens* secreted cyclic dipeptide – cyclo(l-leucyl-l-prolyl) inhibits biofilm and virulence production in methicillin-resistant *Staphylococcus aureus*. RSC Adv. 2015;5(116):95788-804. doi: 10.1039/C5RA11641D.
33. Dong PT, Mohammad H, Hui J, Leanse LG, Li J, Liang L, *et al*. Photolysis of staphyloxanthin in methicillin-resistant *Staphylococcus aureus* potentiates killing by reactive oxygen species. Adv Sci (Weinh). 2019;6(11):1900030. doi: 10.1002/advs.201900030, PMID 31179216.
34. Lee JH, Park JH, Cho MH, Lee J. Flavone reduces the production of virulence factors, staphyloxanthin and α-hemolysin, in *Staphylococcus aureus*. Curr Microbiol. 2012;65(6):726-32. doi: 10.1007/s00284-012-0229-x, PMID 22965624.
35. Clauditz A, Resch A, Wieland KP, Peschel A, Götz F. Staphyloxanthin plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. Infect Immun. 2006;74(8):4950-3. doi: 10.1128/IAI.00204-06, PMID 16861688.
36. Borges A, Ferreira C, Saavedra MJ, Simões M. Antibacterial activity and mode of action of ferulic and Gallic acids against pathogenic bacteria. Microb Drug Resist. 2013;19(4):256-65. doi: 10.1089/mdr.2012.0244, PMID 23480526.
37. Lu L, Hu W, Tian Z, Yuan D, Yi G, Zhou Y, *et al*. Developing natural products as potential anti-biofilm agents. Chin Med. 2019;14:11. doi: 10.1186/s13020-019-0232-2, PMID 30936939.

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