INSILICO AND INVITRO STUDY OF MIMOSA PUDICA AND THE BACTERICIDAL ACTIVITY OF ITS PHYTOCOMPOUND MIMOSINE AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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Abstract

Many pathogenic bacteria has developed resistance to all classes of antibiotics and emerged to cause life threatening outbreaks. Hence, there is a need to develop new therapeutic agents from plants. *Mimosa pudica* L. is one of the highly significant plants known from ages for its many notable therapeutic applications. The objective of the study is to screen for phytocompounds such as flavonoids which includes Galangin, Quercetin, Luteolin and alkaloid Mimosine in the plant extract and to test for the bactericidal effect of the active purified compound against MRSA isolated in clinic. *Insilico* docking studies were performed initially to evaluate the drug-likeness and efficacy of the compounds. Docking studies revealed that the active compounds of *Mimosa pudica*, mimosine showed best dock score of 115.26. Hence, the active phytocompound-Mimosine of the plant was separated by TLC using ethylacetate:ethanol (8:2) and then the compound is purified by column chromatographic technique followed by HPLC technique to detect the presence of compound of interest which resulted a peak at 280 nm with a Rt of 20 min. The fractions F3, F4 and F5 were found to have pure mimosine of concentration 1.041, 0.871, 0.606 mg/ml . The purified Mimosine was characterized by FTIR. The purified phytocompound-Mimosine was also assayed for its bactericidal activity against 9 strains of MRSA at different concentrations ranging from 100, 150 and 200 µg/ml. The strains were sensitive to Mimosine with the maximum zone of inhibition (ZOI) of 25.5 mm, 21 mm, 35 mm, 35mm was observed at 200 µg/ml for 4th , 5th , 8th and 9th strain respectively. The resistant strain were tested again with the 300 µg/ml of Mimosine for 1st , 2nd , 3rd , 6th and 7th strain showed maximum ZOI of 22mm, 23mm, 25mm, 23.5mm, 24.5mm, 23mm respectively. The strains were tested for vancomycin also. The results showed that the vancomycin resistant MRSA could be treated with a concentration >200 µg/ml of mimosine.

Keywords: *Mimosa pudica,* antibacterial activity, Methicillin resistant *Staphylococcus aureus.*

1. Introduction

Worldwide, more than hundreds of plants are used as traditional medicine for the treatment of bacterial infections and other diseases [1]. Although many have been treated by conventional pharmaceutical approaches, there is a growing interest in the use of natural products by the general public.

According to the World Health Organization, 80% of Asian and African population still depends on traditional medicine for primary health care. Globally, India has been acknowledged as a major resourceful area in traditional medicine. The primary benefits of using plant derived medicine are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment. Many commercially

proven drugs used in modern medicine are from traditional medical plants, with ethnobotanical and ethnomedical knowledge [2].

Antimicrobial substances present in tissues of higher plants have long been regarded as important factors in the resistance of higher plants to various bacteria. Hence, researchers have always felt the need for scientific screening of the plants, which may help the pharmacologists and phytochemists. In drug discovery, random screening as a tool in identifying new biologically active molecules has been the most productive. As Infectious diseases are the leading cause of high mortality rate, the diseases due to the antibiotic resistance microbes have become a global concern in the current scenario. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Infections due to multidrug resistance pathogens are routinely complicated to deal with because of a relatively limited choice of antimicrobial agents. Almost one-half of all death in tropical countries is due to infectious agents [3]. Ever since it was first discovered by Sir Alexander Ogston in 1880, *Staphylococcus aureus (S. aureus)* has been regarded as a serious threat to human health, capable of causing a multitude of infections. The rise of antibiotic-resistant strains in the 1960s and 1970s, particularly methicillin-resistant *S. aureus* (MRSA) has created additional therapeutic challenges. MRSA isolates have been associated with nosocomial infections and rapidly developed resistance to multiple drug classes [4].

Today, MRSA-multidrug resistant strains are found worldwide. Study of early isolates of MRSA showed that a key genetic component responsible for resistance, *mecA,* is not native to the *S. aureus* genome. To contain this scenario, there is an urgent need for new anti-MRSA compounds Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and also for discovering the actual significance of folkloric remedies [5]. *In vitro* and epidemiologic studies suggest that consumption of foods rich in phenolic compounds might significantly decrease the risk of some health problems because of their antioxidant, anti-mutagenic, anti-inflammatory and antibacterial properties [6]. Although the antimicrobial activity of extracts from *Mimosa pudica* species has been reported [5], [7], [8], studies with the individual compounds present in that extracts are scarce. Therefore, In the present study *Mimosa pudica* (Fabaceae)(*M. pudica*) is characterized and its effectiveness as antibacterial agent is studied. The objective of the study is to evaluate the phytocompounds present in the plant against the MRSA using *insilico* approach followed by the *invitro* study which involves in isolation, characterization and purification of the active phytochemical compound – Mimosine and testing its bactericidal activity against *Staphylococcus aureus.*

2. Materials and method

INSILICO DOCKING STUDIES

Ligands selected for the study:

The 3D structure of Galangin, Catechin, Luteolin, Quercetin and Mimosine were retrieved from PRODRG server. This server uses the software jmol in which the 2-D structures (NCBI- PubChem compound database) of the compounds were obtained. The resulted sequences were saved as PDB format. With the PDF format, the 3D structure was obtained using discovery studio. The 3-D structure of Linezolid was retrieved from Drug Bank database and was used as a control for the study.The mobile genetic element for antibiotic resistance, mecA gene encoding Penicillin binding protein 2A was used as target receptor protein. The three dimensional structure of the PBP2A [9] (PDB ID: 1VQQ) with chains A and B was taken from Protein Data Bank – RCSB site for this study.

Drug likeliness based on lipinski's rule :

Drug likeliness property of the selected ligands (Mimosine, Galangin, Catechin, Luteolin and Quercetin) were screened based on the Lipinski's rule of five in order to find the best lead compounds [10].

Molecular docking study of using accelrys discovery studio 2.5:

Molecular docking protocols are predominantly used to understand the binding affinities for number of ligands [11]. The phyto ligands were docked with receptors of target PBP2A protein by using Accelrys Discovery Studio 2.5. It is the best automated software tool that carries out automated docking of ligand to their macromolecular receptors. The results of the docking studies were summarized based on their Docking Scores of ligands (Mimosine, Galangin, Catechin, Luteolin and Quercetin) against PBP2A receptors.

***INVITRO* STUDIES**

Extraction of phytochemical compounds :

The leaves of Mimosa pudica were collected from Kanchipuram district. Fresh leaves of *Mimosa pudica* were collected and washed with tap water 2-3 times and finally with distilled water to remove dust particles. The leaves were shade dried and pulverized well in an electric blender to provide fine powder and used for extraction. The finely powdered leaves (100g) of *Mimosa pudica* was extracted to exhaustion in a soxhlet apparatus at 50ºC with 300 ml of 80% methanol. The methanolic extract was filtered through a cotton plug, followed by Whatmann filter paper No.1 .The plant extract was evaporated by incubating at 50ºC until the concentrated extract was obtained.

Purification of the phytocompound-Mimosine :

Separation and identification of the bioactive compound- mimosine from *Mimosa pudica* extract using thin layer chromatography:

Various solvents (benzene:choloroform (80:20), chloroform:methanol:acetic acid (18:1:1), hexane:ethyl acetate (5:5), hexane:ethyl acetate (8:2), ethylacetate:ethanol (8:2)) with different ratios were prepared and used as appropriate mobile phase. Pre-coated TLC silica plates were used as stationary phase and in which about 10µl of crude plant sample was loaded above the base line and it was compared with the standard mimosine. The individual fractions from the developed plate was then visualized by staining technique. Dragendroff's reagent was sprayed onto the plate thus obtaining visible spots on the plate, the *Rf* value of each spot was compared with the standard mimosine [12].

Purification By Column Chromatographic Method:

The crude extract of Mimosa pudica (7 g) was subjected to column chromatography and was separated into its component fractions. Silica gel (60-120 mesh) was used as the stationary phase while solvent of increasing polarity was used as the mobile phase . The slurry was prepared by mixing 20 g of silica gel and 350 ml of ethylacetate and ethanol was used as eluent and the crude extract was introduced into the solvent layer above the silica gel in the packed column. The following ratios of solvent combinations was sequentially used in the elution; ethylacetate: ethanol 100:0, 80:20, 60:40, 40: 60, 20: 80 and 0:100. The eluent was collected as a series of fractions of 10 ml in test tubes. The composition of the eluent flow was analysed by spectrometer. The fractions showing peaks at 282 nm for the desired compound Mimosine and were pooled together for rechromatogram. The aliquots obtained were again tested by TLC chromatogram and the Rf values were determined to confirm the presence of mimosine. Further purification was done by HPLC (Yonglin SP930D HPLC System) method [13].

Qualitative analysis of the compound by High Performance Liquid Chromatography:

The HPLC analysis was carried out with Yonglin SP930D system (Yonglin, Anyang, Korea), using a Merck C-18 column (4x250 mm, Merck Co.). All the modules were controlled by PC with interface and HPLC System Manager Window-based software. The C-18 column was used for purification. A simple HPLC gradient elution method, which efficiently separates various phytochemicals was used. Three mobile phases, Solvent A (Water: Acetic Acid 99:1 v/v), Solvent B (Acetonitrile: Acetic Acid 99:1 v/v) and Solvent C (Methanol) were used as eluents. The prepared mobile phases A, B and C (80: 12: 8) were allowed to pass through the column for 10 minutes. After the sample injection, the separation was performed using gradient elution (0-30 min) with a flow rate of 0.5 ml/min and a column temperature of 25ºC. The peak for the standard and samples were recorded and compared [14].

Characterisation of phytocompound – Mimosine by Fourier Transform Infrared Spectroscopy:

The principle of FTIR is based on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A molecule that is

exposed to infrared rays absorbs infrared energy at frequencies which are characteristic to that molecule. The purified compound Mimosine was characterized using FTIR using ATR method on a Nicolet Magna IR-560 Spectrometer with 1 cm-1 resolution in the 400-4000 cm-1 region. It is to identify the presence of certain functional groups in a molecule. Also, unique collection of absorption bands to confirm the identity of a pure compound or to detect the presence of specific impurities. The resulting FTIR spectral pattern is then analyzed and matched with known signatures of identified materials in the FTIR library [15].

Bacterial inoculum preparation:

Clinical isolates of Methicillin-Resistant *Staphylococcus aureus* isolated from hospitals around chengalpet, Tamilnadu were used. In this study, nine strains of MRSA and one standard strain of *Staphylococcus aureus* were subjected for studying the antibacterial efficacy of the extracted compound Mimosine. The bacterial cultures were maintained and stored in Mannitol salt agar at 4ºC for further studies.

Antibacterial activity by Agar Well Diffusion Method:

The overnight culture of MRSA strains and std. *S. aureus* strains were inoculated uniformly on Muller Hinton Agar in triplicates by spread plate technique. Wells were made in the plates and 100µl of mimosine extract in Ethylacetate:ethanol of various concentrations (50, 100, 200 µg/ml) was loaded in to the wells. 100 µl of Ethylacetate:ethanol was added to one well as negative control. The plates were incubated at 37ºC for overnight. The zone of inhibition for test and standard strains were measured and analysed for triplicates [16].

3. Results

Molecular Docking

To identify agents with anti-MRSA activity, the selected phytochemicals(Mimosine, Quercetin, Luteolin, Calechin And Galangin) were analysed by docking studies. The evaluation of potential interactions in active sites and the docking images showed clear interactions between the drug and the MRSA (PBP2A) receptors. All the 5 ligand molecules had dock score greater than 50, of which Mimosine had the maximum docking score of 115.26 followed by Quercetin compound- 62.95 as dock score, Luteolin compound-58.54 as dock score , Catechin compound-57.6 as dock score and Galangin compound-51.33 as dock score. The Values of docking results are shown in Table 1 and Table 2. The results were obtained using DS LigandFit and DS LigandScore of Discovery Studio 2.5.5.

Table 1: Dock scores of ligands against PBP2A receptors of *MRSA* from DS ligandfit and DS ligandscore

Ligands	Lig Score 1	Lig Score 2	-PLP1	-PLP2	-JAIN	-PMF	Dock Score	IE
Mimosine	**2.25**	**1.23**	**17.74**	**15.81**	**-0.27**	**28.51**	**115.26**	**-1.411**
Quercetin	3.95	3.64	53	54.01	0.01	100.53	62.95	-1.736
Luteolin	3.2	3.58	49.31	47.68	-0.27	91.59	58.54	-2.061
Catechin	5.37	4.38	56.88	60.11	2.35	110.53	57.6	-2.184
Galangin	2.41	3.11	30.11	30.93	-0.47	48.31	51.33	-3.549

Table 2: Dock scores of standard antibiotic linezolid against PBP2A receptors of *MRSA* from DS ligandfit and DS ligandscore

Ligands	Lig Score 1	Lig Score 2	-PLP1	-PLP 2	-JAIN	-PMF	Dock Score	IE
Linezolid	4.85	3.8	49.3	49.82	1.71	69.18	38.74	-4.28

The docking score of mimosine was found to be more when compared to linezolid which is the drug of choice for treating MRSA.

Thin Layer Chromatography

In this study different polar and non polar solvents such as ethanol, chloroform, ethyl acetate, hexane, benzene and acetic acid in different combinations were used from which

intermediate polar solvent system ethylacetate and ethanol [17], in the ratio of 8:2 resulted in better chromatographic separation of compounds and the spots were visualized by dragendroff's reagent and then the Rf values of the separated analytes were compared with standard mimosine- Rf value of 0.569 and confirmed the presence of mimosine compound in the plant extract.

Column Chromatography

Twelve eluent fractions were collected by column chromatography (figure 1) and later these fractions were analysed by spectrophotometer. The UV absorption spectrum of each sample were recorded at 200-400 nm to ensure a reliable identification of the compounds. Out of twelve fractions, five fractions (7, 8, 9, 10 and 11) showed maximum absorption peak at 282 nm indicating the presence of mimosine in those fractions. The eluent fractions showed peaks at 282 nm were selected and those fractions were pooled together for rechromatogram. Again five eluent fractions (F1, F2, F3, F4 and F5) were collected (figure 2). The UV absorption spectrum of each sample at 200-400 nm showed a reliable identification of the pure compoud mimosine with the single peak of maximum absorption at 282 nm.

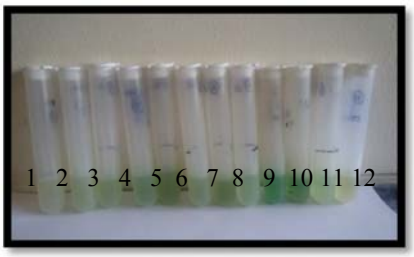


Figure 1: Chromatographic fractions collected

Elution of the extract was done with solvent system of gradually increasing polarity using ethylacetate and ethanol. The 12 eluent fractions were collected in aliquots and was subjected to spectrometric analysis

Figure 2: Re-Chromatographic fractions collected

The rechromatography of the pooled eluent samples collected as five fractions.

Estimation of mimosine

The mimosine contents were estimated quantitatively by spectrophotometric method using ninhydrin reagent in all the samples collected during column chromatography. The standard curve for the Mimosine was ploted using the optical density values obtained from the working standard solutions (S1, S2, S3, S4 and S5) at 280 nm (Figure 3) from which the unknown concentration of mimosine was shown in Table 3.

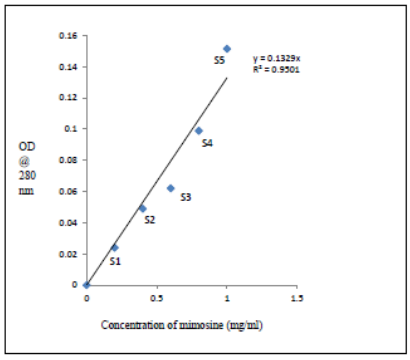


Figure 3: Standard plot for the Estimation of Mimosine

The standard graph was plotted from which the unknown concentration of the Mimosine was determined by comparing with the standard Mimosine concentration from the calibration curve.

Concentration of mimosine (mg/ml)		OD @ 280 nm
BLANK	0	0.000
S1	0.2	0.022
S2	0.4	0.048
S3	0.6	0.062
S4	0.8	0.099
S5	1.0	0.1515
F1	0.287	0.038
F2	0.606	0.080
F3	**1.041**	**0.1375**
F4	0.871	0.115
F5	0.606	0.0805

y=0.132 x

Table 3: Determination of unknown concentration of mimosine

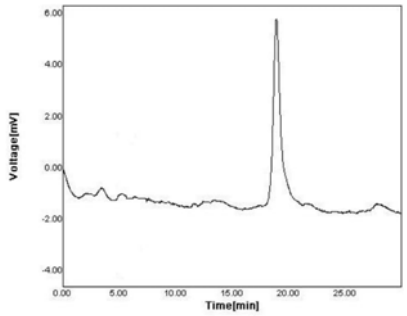
The unknown concentration of the mimosine in all 5 fractions F1, F2, F3, F4 and F5 were determined by spectrometric method and it was found to be 0.287mg/ml, 0.606 mg/ml, 1.041 mg/ml, 0.871 mg/ml and 0.606 mg/ml respectively.

These samples were confirmed by thin layer chromatography for the presence of mimosine

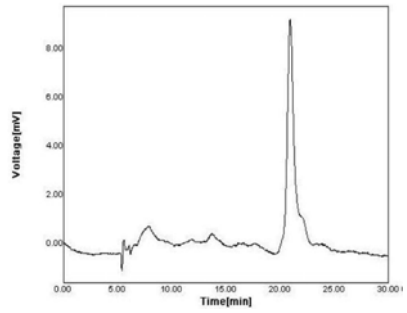
with a standard mimosine and the three rechromatogram samples F3, F4 and F5 were found to have mimosine . These samples were pooled for futher HPLC purification.

High Performance Liquid Chromatography studies of the purified Mimosine

The purified Mimosine fraction was analysed on the HPLC system to confirm the purity of the compound. The HPLC analysis showed a single peak at 280nm of Rt at 20 min (figure 4b) which was similar to that of the standard Mimosine (figure 4a) which confirmed the presence of pure mimosine. This sample was further characterized by FTIR in order to determine the functional groups.



4(a)



4(b)

Figure 4 - (a), (b) : HPLC chromatogram of Standard Mimosine and the purified Mimosine fraction from Mimosa pudica extract at 280 nm

HPLC chromatogram of the standard Mimosine and the purified Mimosine was recorded (a) Mimosine standard peak with Rt at 20 min., (b) Mimosine in the purified fraction showed a peak at 280nm and Rt at 20 min and the presence of Mimosine is confirmed.

FTIR analysis

FTIR spectroscopic analysis result revealed the existence of various chemical constituents with the presence of different functional groups in the crude extract of Mimosa pudica (figure 5) , Standard Mimosine and the purified extract Mimosine (figure 6 - a, b) respectively.

The FT-IR spectrum of the crude extract of the leaves of mimosa pudica was acquired in the range 4000-450 cm-1 ,as this FTIR spectroscopic analysis result revealed the existence of various chemical constituents with the presence of different functional groups in the crude extract of Mimosa pudica (Figure 5) . A broad O-H stretching vibration is obtained for intermolecular hydrogen bonding in the range from 3500-3000 cm-1 which indicates the presence of alcohol,phenols and amine groups. Strong absorption peaks were obtained in the range 2000-450 cm-1 is due to aromatic ring stretching of cyclic compound and aliphatic asymmetric C-H stretching vibration . It confirms the presence of various phytochemicals like, volatile essential oil , flavonoids , tannins , alkaloids and essential oils.

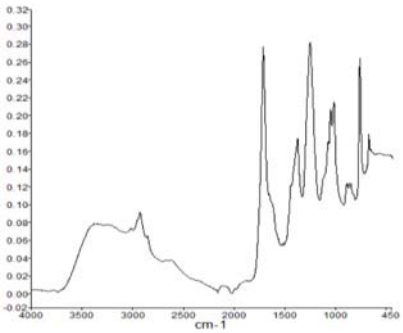
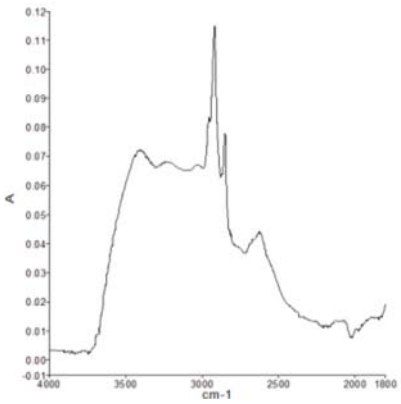
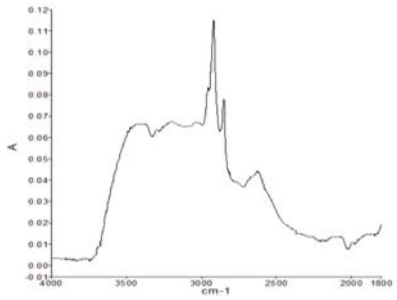


Figure 5: FT-IR spectrum of the crude extract

The spectrum shows the presence of various phytochemicals like, volatile essential oil, flavonoids, tannins, alkaloids and essential oils



6(a)



6(b)

Figure 6-(a), (b) : FT-IR spectrum of standard mimosine and the purified mimosine

FTIR Spectrum of the Standard mimosine (figure (a) and purified phytocompound – Mimosine (figure (b) was compared and it is observed that both the absorption spectrum resulted in a strong absorption peak in the range 3000-2500 cm-1 is due to the stretching vibration of carboxylic acids, amines, ketone and alcohol indicates the presence alkaloid phytocompound compound-mimosine.

The FTIR analysis of the pure compound Mimosine Figure 6- a, b showed a strong absorption peak in the range 3000-2500 cm-1 due to the presence of carboxylic acids, amines, ketone and alcohol functional groups.

Antibacterial assay of the phytocompound-mimosine

In the agar well diffusion method all the strains showed increasing zone of inhibition with the increasing concentration of mimosine of 100, 150 and 200 µg/ml (Table 5). Among all strains, the samples 4, 5, 8 and 9 showed larger zone of inhibition at the concentration 200 µg/ml than the positive control vancomycin. Also among 9 strains, two strains sample 2 and 7 were found to be resistant to Vancomycin antibiotic but are susceptible to mimosine at 200 µg/ml concentration. The negative control well with ethylacetate : ethanol showed no inhibition and hence it infers that inhibition is due to Mimosine.

S. No	Strain No.	Zone of inhibition (mm) formed for different concentration of mimosine (µg/ml)												Zone of inhibition for Vancomycin (30 µg/ml)
		100 µg/ml				150 µg/ml				200 µg/ml				
		Avg	I	II	III	Avg	I	II	III	Avg	I	II	III	
1.	Sample 1	9	9	7	11	13	12	13	14	15	15	15	15	17 (S)
2.	Sample 2	11	13	11	9	13	13	13	13	15	14	15	16	13 (R)
3.	Sample 3	11	11	12	10	15	15	15	15	17	17	17	17	21.3 (S)
4.	Sample 4	13.3	14	13	13	17	18	16	17	25.5	25	25	26	23 (S)
5.	Sample 5	11	12	11	10	15	15	15	15	21	21	21	21	19 (S)
6.	Sample 6	11.3	11	11	12	13	13	14	12	15.5	15	15	16	21 (S)
7.	Sample 7	11.3	11	12	11	13	13	14	12	13	13	13	13	13 (R)
8.	Sample 8	25	25	25	25	31	32	31	30	35	36	35	34	28.3 (S)
9.	Sample 9	27	27	26	28	33.3	33	33	34	35	37	33	35	21 (S)
10.	Std. *S. aureus* MTCC 3160	19	19	19	19	23.3	23	24	23	27	27	27	27	21 (S)

Table 5 Zone of Inhibition for mimosine compound against strains of MRSA by agar diffusion method.

S – sensitive to vancomycin
R – resistant to vancomycin

The plates were incubated at 37°C for overnight. The zone of inhibition for test and standard strains were measured for triplicates. The zone of inhibition of the phytocompound against MRSA and Std. Strains indicates the drug activity.

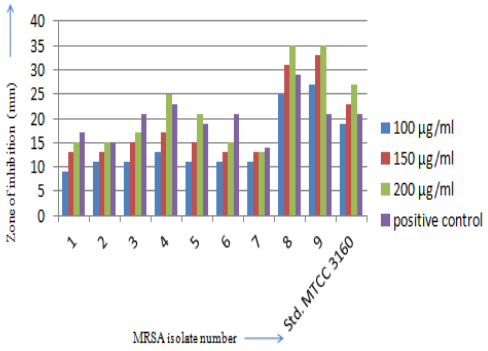


Figure 7 : Graphical representation of the zone of inhibition for the Mimosine against 9 strains of MRSA and Std. *Staphylococcus aureus* MTCC 3160

Graph shows zone of inhibition formed for different concentrations (50, 100, 200µg/ml) of Mimosine against 9 MRSA strains and one std. *S.aureus* strain where in which the isolate number 8 and 9 showed a maximum zone of inhibition among all the ten strains.

Optimization of the concentration of mimosine

Among 9 strains, the strains which resulted in a Zone of inhibition (11-15 mm) were tested against higher concentration of 300, 400, 500 µg/ml of Mimosine. Hence Optimization of the concentration of mimosine was done to check for its antibacterial effect against MRSA isolate number 1, 2, 3, 6 and 7 . The MRSA isolate 1, 2, 3, 6 and 7 showed minimum zone of inhibition at the concentration 200 µg/ml of mimosine was found to show a maximum zone of inhibition at the concentration 300 µg/ml than the positive control vancomycin. The zone of inhibition (mm) for different strains were tabulated (Table 6).

S.No	Strain No.	Zone of inhibition (mm) formed for different concentration of mimosine (µg/ml) – mean of triplicates												Positive control Vancomycin disc 30 µg/ml
		300 µg/ml				400 µg/ml				500 µg/ml				
		Avg	I	II	III	Avg	I	II	III	Avg	I	II	III	
1.	MRSA 1	22	22	22	22	24	24	23	25	26.3	26	26	27	17
2.	MRSA 2	23	23	22	24	26.3	26	26	27	27.3	27	27	28	13
3.	MRSA 3	25	26	24	25	27	27	27	27	29	25	30	32	21.3
4.	MRSA 6	23.3	23	23	24	25.3	25	25	26	27	27	26	28	21
5.	MRSA 7	23	23	25	21	27	27	27	27	29	28	29	30	13

Table 6 Zone of Inhibition formed for mimosine against MRSA isolate number 1, 2, 3, 6 and 7.

The vancomycin resistant organisms (Figure 8) showed increased bactericidal activity with the concentration of 300, 400 and 500 µg/ml of Mimosine. Among which the concentration 300 µg/ml of Mimosine showed enhanced activity for vancomycin resistant organisms (Figure 9). Hence vancomycin resistant micro-organisms could be used treated with concentration >200 µg/ml of Mimosine.

Figure 8 : Vancomycin sensitive organism

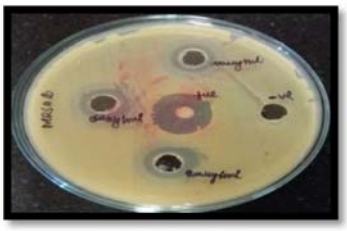


Figure 9 : Vancomycin resistant organism

Figure 10: Graphical representation of zone of inhibition formed for the Mimosine against the MRSA isolate number 1, 2, 3, 6 and 7.

Graph shows zone of inhibition formed for different concentrations (300, 400, 500 µg/ml) of Mimosine against the MRSA isolate number 1, 2, 3, 6 and 7. where in which these MRSA isolates 1, 2, 3, 6 and 7 showed maximum zone of inhibition at the concentration 300 µg/ml than the positive control vancomycin.

4. Discussion

Methicillin Resistant *Staphylococcus aureus* is a versatile pathogen causing life threatening infections in humans [18]. Multiple antibiotic resistant of these strains make it more resistant to treatment. Several plants and herbs species are used traditionally to treat several diseases. Naturally occurring phytocomponents in plants such as flavonoids, alkaloids, steroids, tannins, saponins, etc. have potential antimicrobial and antiviral properties. *Mimosa pudica* which is called as sensitive plant have been used for decades for treating various alignments. The plant has been studied for its anti asthmatic, antimicrobial, antimalarial, aphrodisiac, analgesic, hypolipidemic and antidepressant properties [5], [6], [7].

In previous studies it was reported that Mimosine levels are high in *Leucaena*, but it was not easy to isolate pure Mimosine. The determination of Mimosine via extracting solvents and analytical instruments and spectrophotometrics was conducted However, it is complicated to separate Mimosine from other amino acids in *Leucaena*, the cost of Mimosine pure compound is also rather high [19], [20], hence in this study Mimosine was isolated from the plant *Mimosa pudica* and purified by simple lab techniques at low cost comparatively which involves the identification of the mimosine compound by carrying out analytical TLC, separation and purification of the compound by column chromatography followed by HPLC . In another study by [21] it was reported that the purity of the Mimosine compound was checked by TLC using silica gel G as an adsorbent, Benzene/Ethanol/Ammonia (BEA) as eluent. In this study different polar and non polar solvents such as ethanol, chloroform, ethyl acetate, hexane, benzene and acetic acid in different combinations were used. The intermediate polar solvent system ethylacetate and ethanol as studied by [17], in the volume ratio (8:2), resulted in better chromatographic separation of compounds. The Rf values of the separated analytes were calculated and in which the compound (1) Rf value (0.506) was found to be closer to the standard mimosine Rf value (0.569) thus showing the presence of mimosine compound in the plant extract . Hence the separation and purification of the compound Mimosine, a moderate polar compound using ethylacetate:ethanol solvent had better affinity towards the solvent on increasing polarity during separation [22].

Using simple column chromatography with silica gel to purify mimosine more effectively followed by HPLC analysis gave better results. Whereas [23] used ion exchange resin chromatography and the mimosine concentration of the fractions were determined using HPLC and spectrophotometric method.

[14] investigated the herbicidal activity of the mimosine exerted stronger herbicidal activities with 80-90% inhibition and antifungal studies with 10-40% fungal activity against *R.solani and S.dellfinii* was observed. [7] studied antibacterial activity of the plant extract Mimosa pudica against three organisms namely, *Aspergillus fumigatus, Citrobacter divergens* and *Klebsiella pneumonia* and found maximum zone of inhibition was obtained for *Aspergillus fumigatus* and *Klebsiella pneumonia* at a concentration of 200µg/200µl while *Citrobacter divergens* showed resistance against *Mimosa pudica* extract at all concentration. [24] reported significant antibacterial activity of extract of *Mimosa pudica* against *Staphylococcus* strains (12 mm) which would offers the way to research on this plant against clinical isolates of MRSA. Whereas in this study all the MRSA strains showed zone of inhibition more than 15mm at the concentration 200 µg/ml of Mimosine. Antibacterial activity of the purified phytocompound-mimosine was assayed against 9 isolates of MRSA in which Out of all strains 4^{th} , 5^{th} , 8^{th} and 9^{th} MRSA strain showed larger zone of inhibition (25.5 mm, 21 mm, 35 mm and 35mm) respectively at the concentration 200 µg/ml for vancomycin sensitive MRSA strains. Further the remaining isolates were tested and found that the MRSA isolates 1^{st} , 2^{nd} , 3^{rd} , 6^{th} and 7^{th} showed maximum zone of inhibition (22mm, 23mm, 25mm, 23.5mm, 24.5mm, 23mm) respectively at the concentration 300 µg/ml for vancomycin resistant MRSA. All the vancomycin resistant organisms showed increased bactericidal activity with the concentration of 300, 400 and 500 µg/ml of Mimosine. Among which the concentration 300 µg/ml of Mimosine showed enhanced activity for vancomycin resistant organisms. Hence vancomycin resistant micro-organisms could be used treated with concentration 300 µg/ml of Mimosine. The antibiotic Vancomycin was used as positive control and ethylacetate:ethanol (8:2) as negative control for all the assays performed. Out of 9 strains 2 strains was found to be resistant to Vancomycin and no zone of inhibition was found for negative control in all the strains. Hence this infers that the antimicrobial activity is purely based on the purified phytocompound –Mimosine and no inhibitory activity of ethylacetate and ethanol was found out. Hence the mimosine could be effectively used as an antimicrobial compound against MRSA and also could be used to treat Vancomycin Resistant MRSA.

5. Conclusion

Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions. Given the alarming incidence of antibiotic resistance of MRSA, Insilico analysis showed the effectiveness of herbal compounds present in *Mimosa pudica* and Mimosine could be a best inhibitor against PBP2a of MRSA and further evaluated the antimicrobial activity of purified phytocompound-Mimosine of the *Mimosa pudica* plant based on their inhibition of the growth of MRSA. The Mimosine was effectively purified using various chromatographic techniques and it was tested against 9 clinical isolates of MRSA, it showed maximum inhibitory activity against nine strains of MRSA when compared with vancomycin which is an existing antibiotic currently used against MRSA infections. These findings suggest that the purified phytocompound-Mimosine from the *Mimosa pudica* leaves appears to hold promise as an natural antibacterial agent in the treatment of infection with *S.aureus* including MRSA and should be investigated further in appropriate *invivo* models.

6. References

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