

# Evaluation of Anti-biofilm Formation of Methicillin Resistant *Staphylococcus* Species in Clinical Milk Samples of Bovine Mastitis using Selective Medicinal Plants

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## ABSTRACT

*Staphylococcus aureus* is a Gram positive bacterium which is a member of the firmicutes is the most considered causative agent for bovine mastitis in dairy herds worldwide. The Clinical milk samples were collected from the glycerol stock. After subculture, the confirmation of *Staphylococcus* species was performed using Selective agar plate method, Gram staining and Biochemical characterization. Antibiotic susceptibility test was performed for the predominant culture of *Staphylococcus aureus* to determine the organism's resistance, susceptibility or intermediate towards certain antibiotics. The screening for Methicillin Resistance *Staphylococcus aureus* was performed using HI chrome Rapid Methicillin Resistant *Staphylococcus aureus* agar base media. The detection of biofilm forming ability in the isolates was performed using different phenotypic assays. Three different medicinal plants (*Erythrina variegata*, *Cissus quadrangularis* and *Vitex negundo*) were selected for the study and used for the preparation of methanol, Chloroform and aqueous extracts. The extracts were then subjected for the qualitative and quantitative photochemical analysis. The determination of the lowest concentration of the plant extracts in inhibiting the growth of the organism (Antibiofilm formation) by Minimum Inhibitory Concentration and Minimum Bacterial Concentration in MRSA were found to have higher sensitivity to the methanol extracts and resistant to the chloroform and aqueous extracts.

**Key words:** *Staphylococcus aureus*, MRSA, Bovine mastitis, Medicinal plants, biofilm, Minimum inhibitory concentration and Minimum Bacterial concentration.

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## INTRODUCTION

Bovine mastitis is the commonest disease affecting dairy cows which causes inflammation of the mammary gland that is predominantly by a bacterial infection in the udder. The inflammation causes changes in the biochemical composition of milk and the gland tissue that are damaged due to toxins released by the bacteria resulting in reduced milk yield and quality.<sup>[1]</sup>

The microorganisms invade the tissue breast causing an inflammation of the gland.<sup>[2]</sup> Bacteria that are known to cause mastitis include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Brucella melitensis*, *Corynebacterium bovis*, *Mycoplasma species*, *Escherichia coli*, *Klebsiella pneumoniae*.<sup>[3]</sup>

## *Staphylococcus* species prevalence and its ability

*Staphylococcus* species is a highly adaptive and versatile Gram-positive, non-spore forming cocci bacterium that belonging to the family *Micrococcaceae* that are often found as normal human microbiota of the skin and nasal cavity. *Staphylococcus* species is able to cause many superficial phylogenetic (pus-forming) infections of the dermis and underlying tissues as well as serious

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systemic infections.<sup>[4]</sup> *Staphylococcus* species is responsible for causing several types of infections, many of which are related to biofilm production. The biofilm is characterized as a group of bacteria attached to biotic or abiotic surfaces and embedded in a self-synthesized organic polymer matrix.<sup>[5]</sup> Therefore, biofilm formation is a predominant contributor to *Staphylococcus* species pathogenesis and the need for alternative therapies that directly tackle this element is of utmost importance to combat number of diseases.<sup>[6]</sup>

### Role of Medicinal Plants in Bovine Mastitis

The role for the alternative medicine to combat various diseases is significant and proved through various research evidences,<sup>[7]</sup> corticosteroids, cytokines,<sup>[8]</sup> homeopathic formulation, ozone, Ayurveda or any means of traditional herbal medicine etc. To enhance the value of indigenous/traditional medicines, three different medicinal plants with varied therapeutic qualities were selected for the study was *Erythrina variegata*, commonly known as Kalyana Murungai, *Cissus quadrangularis* is commonly called as Pirandai and *Vitex negundo*, commonly known as the Chinese chaste tree.

## MATERIALS AND METHODS

### Morphological and Biochemical Identification of the isolates

The study was conducted in the School of Biotechnology, Dr. G.R. Damodaran College of Science Coimbatore. About 70 samples were retrieved from the glycerol stock of School of Biotechnology, Dr. GRDCS and are labeled as BM<sub>01</sub> to BM<sub>70</sub>. The culture was maintained as slant and sub-cultured at regular intervals. Gram's staining procedure was performed according to the.<sup>[9]</sup> The isolates were subjected to the biochemical tests such as Indole production test, Methyl red test, Proskauer

test, Citrate utilization test, Catalase test, Oxidase test, Hydrogen Sulphide Production test, Nitrate reduction test, SIM test, Gelatin utilization test, Casein hydrolysis, Starch hydrolysis test and Lipase test to identify the genus for the microorganism.<sup>[10]</sup> The collected samples were inoculated in various selective and differential agar media to confirm the presence of *Staphylococcus aureus*. The selective media like Mannitol Salt Hi-Veg Agar, Barid Parker's Agar, DNase and Blood Agar were used for the confirmation of *Staphylococcus aureus* and HI chrome Rapid MRSA Agar for the Methicillin Resistant *Staphylococcus aureus*.

### Multidrug Antibiotic Susceptibility Test

The disc diffusion method was used to perform the antibiotic susceptibility test. Overnight nutrient broth culture of clinical *Staphylococcus aureus* pathogen was prepared and inoculated (swab inoculation) the same onto the sterile Muller-Hinton agar plates with seven general antibiotics such as Methicillin, Vancomycin, Oxacillin, Penicillin, Tetracycline, Gentamycin, and Ciprofloxacin and incubated at 37°C for 24 hr. After incubation, antibiotic activity was determined by measuring the zone of inhibition around the wells in diameters and compared as susceptible, intermediate or resistant to the isolates.<sup>[11]</sup>

### Detection of Biofilm Formation

The production of biofilm depends on the ability of the bacteria to attach to abiotic/biotic surfaces, proliferate, and produce an extracellular matrix, which is mainly formed by Polysaccharide Intercellular Adhesion (PIA), in *Staphylococcus aureus*. PIA is the major component of the extra polysaccharide matrix, also known as slime. The Biofilm formation is detected by four phenotypic methods such as Congo red agar method,<sup>[12]</sup> Modified

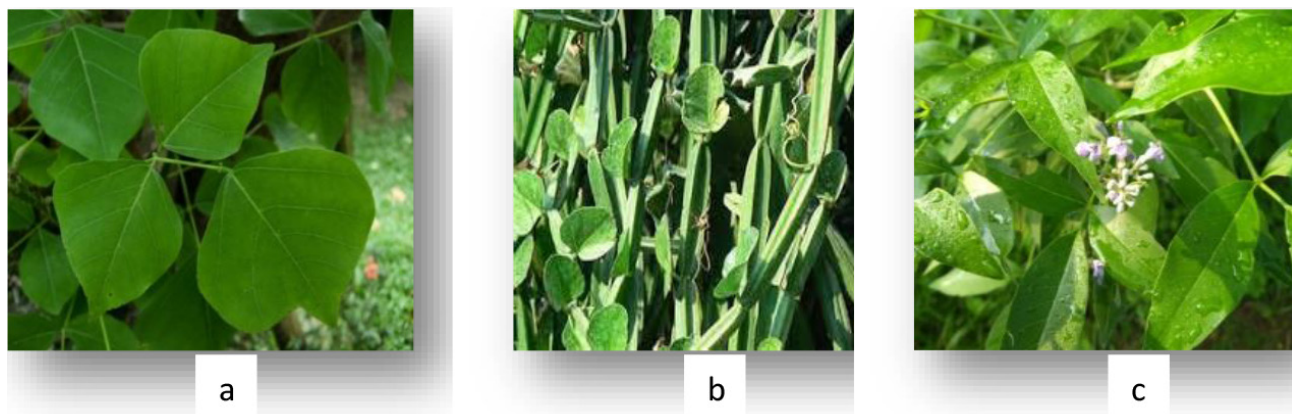


Figure 1: The selected plant species a) *Erythrina variegata* b) *Cissus quadrangularis* c) *Vitex negundo*.

**Table 1: Scientific Classification of the selected medicinal plants.**

Classification	<i>Erythrina variegata</i>	<i>Cissus quadrangularis</i>	<i>Vitex negundo</i>
Kingdom	Plantae	Plantae	Plantae
Phylum	Angiosperms	Angiosperms	Angiosperms
Class	Eudicots	Eudicots	Eudicots
Clade	Rosids	Rosids	Asterids
Order	Fabales	Vitales	Lamiales
Family	Fabaceae	Vitaceae	Lamiaceae
Genus	Erythrina	Cissus	Vitex
Species	<i>E. variegata</i>	<i>C. quadrangularis</i>	<i>V. negundo</i>

Congo Red agar method, Tube Adherent Method,<sup>[13]</sup> Microtiter Plate Method.<sup>[14,15]</sup>

### Phytochemical Analysis of Selective Plant Species

The selected plant species *Erythrina variegata*, *Cissus quadrangularis* and *Vitex negundo*<sup>[16,17,18]</sup> (Table 1, Figure 1) were dried in the shade for three to four weeks. Then, the dry plants are blended to form fine powder then solvent extraction carried out using with Petroleum ether, Chloroform, Acetone and Methanol. The leaf extracts was analysed for the presence of flavonoids, alkaloids, glycosides, phenols, saponins, terpenoid, cardiac glycosides and tannins according to standard methods.<sup>[19-21]</sup>

### Determination of Minimum Inhibitory Concentration (MIC)

The determination of the lowest concentration of the extracts capable of inhibiting the growth of MRSA (i.e. MIC) for each of the three medicinal plant extracts (aqueous, methanol and chloroform) was performed using broth micro dilution test.<sup>[22]</sup> MIC values were evaluated based on positive interpretation for both no visible turbidity and negative culturing.

### Determination of Minimum Bacterial Concentration (MBC)

The determination of the lowest bactericidal concentration of the extracts capable of inhibiting the growth of MRSA (i.e. MBC) for each of the three medicinal plant extracts was performed. MBC values were evaluated based on positive interpretation for both no visible turbidity and negative culturing.

## RESULTS

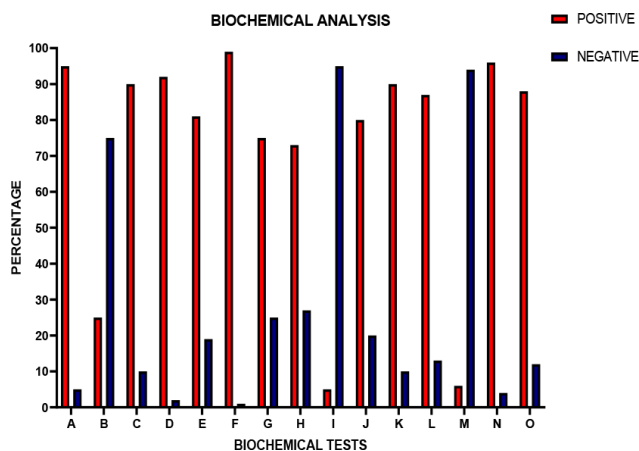
### Isolation, Identification and Confirmation of the *Staphylococcus* cultures

About 70 samples were retrieved from the glycerol stock. The cultures are used for further studies and are

sub cultured, labeled and maintained. Gram staining results are observed as violet as positive and pink colour as negative. 95% of the cultures were appeared as cluster grape like cocci structure and were observed as positive and 5% as negative cocci. Around 15 different Biochemical identification tests were performed for all the 70 *Staphylococcus* cultures. The Biochemical characterization results were observed as, 25% positive and 75% negative for Indole production test, 90% positive and 10% negative for Methyl red test, 92% positive and 8% negative for Vogues – Proskauer test, 81% positive and 19% negative for catalase test, 99% positive and 1% negative for Citrate utilization test, 75% positive and 25% negative for Gelatin hydrolysis test, 73% positive and 27% negative for Casein hydrolysis test, 5% positive and 95% negative for Hydrogen sulphide production test, 80% positive and 20% negative for Carbohydrate test, 90% positive and 10% negative for Nitrate reduction test, 87% positive and 13% negative for Urease test, 6% positive and 94% negative for Glucose utilization test, 96% positive and 4% negative for Oxidase test, 88% positive and 12% negative for Coagulase test (Graph 1).

### Selective and Differential media

Mannitol Salt Agar is specially used to isolate *Staphylococcus aureus* and *Staphylococcus epidermis* due to phenol red indicator the media colour changes are pink for epidermis and yellow for aureus. The results were observed with the high rate of *Staphylococcus aureus* cultures with 90% of the yellow coloured plates followed by *Staphylococcus epidermis* with 10% of the pink coloured



**Graph 1: Graph showing the positive and negative results for Biochemical characterization.**

(A – Indole Production Test, B – Methyl Red Test, C – Vogues – Proskauer Test, D – Catalase Test, E – Citrate Utilization Test, F – Gelatin Hydrolysis Test, G – Casein Hydrolysis Test, H – Hydrogen Sulphide Production Test, I – Carbohydrate Test, J – Nitrate Reduction Test, K – Urease Test, L – Glucose Utilization Test, M – Oxidase Test, N – Coagulase test).

plates. In the Blood Agar (BA) media plates the zone of clearance were observed for the *Staphylococcus aureus* for 85% of the cultures. In the DNase media plates the zone of clearance were observed for the *Staphylococcus aureus* for 80% of the cultures. In the Barid Parker Agar (BPA) media plates the results were observed to be positive for 83% of *Staphylococcus aureus* with black coloured colonies. The HI chrome media were used to confirm the Methicillin Resistant *Staphylococcus aureus* (MRSA) the results were observed as green coloured colonies with 85%. (Table 2 and Figure 2).

### Antibiotic Susceptibility Test

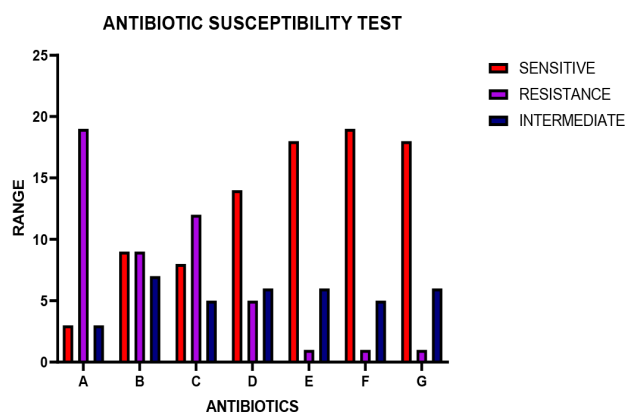
Antibiotic susceptibility test was performed for the predominant culture of *Staphylococcus aureus* to determine the organism's resistance, susceptibility or intermediate towards certain antibiotics. The disc diffusion method is followed here and around 7 antibiotics are used for the test (Table 3, Figure 3 and Graph 2).

### Screening of biofilm formation of Methicillin Resistant *Staphylococcus aureus*

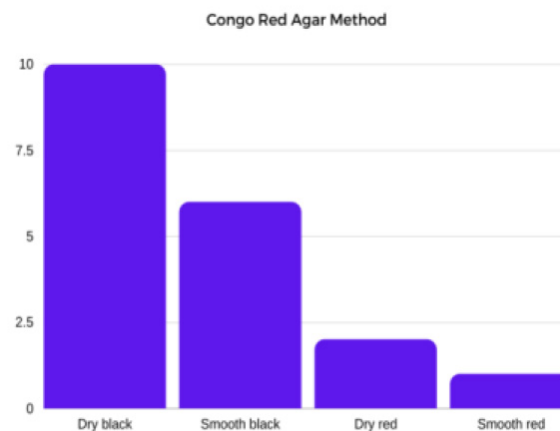
Detection of Biofilm forming capability in the organisms isolated from the mastitis milk samples several types of phenotypic assays were performed which include - Congo red agar, Modified Congo red agar, Test tube method, Modified test tube method and EPS method.

### Congo Red Agar Method

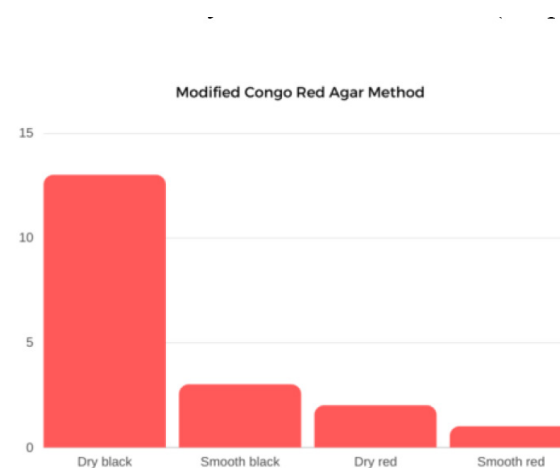
All the MRSA isolates were subjected for biofilm formation and in Modified Congo red agar method, out of 19 Methicillin Resistant *Staphylococcus aureus* strains, 10 were dry black, 6 smooth black, 2 dry red and 1 smooth red (Graph 3).



**Graph 2: Graph showing Multidrug Antibiotic Susceptibility test (A – Methicillin, B – Mupirocin, C – Penicillin, D – Ampicillin, E – Vancomycin, F – Gentamycin, G – Tetracycline).**



**Graph 3: Graph showing biofilm formation in Congo red agar method.**



**Graph 4: Graph showing biofilm formation in Modified Congo red agar method.**

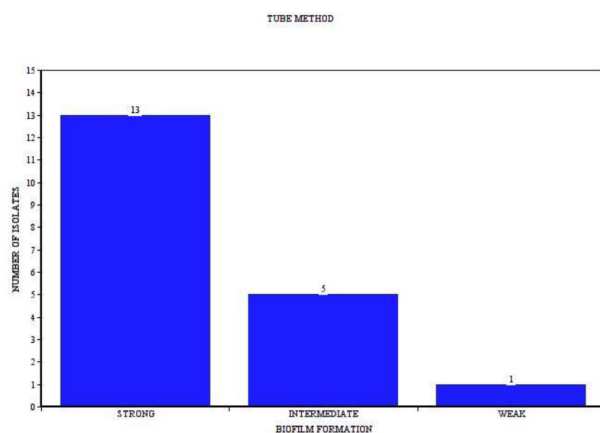
### Modified Congo Red Agar Method

All the MRSA isolates were subjected for biofilm formation and in Modified Congo red agar method, out of 19 Methicillin Resistant *Staphylococcus aureus* strains, 13 were dry black, 3 smooth black, 2 dry red and 1 smooth red (Graph 4).

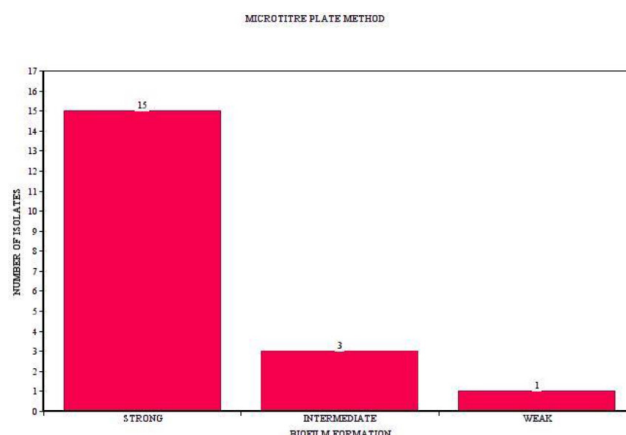
### Test Tube Method

Test tube method is done for checking the biofilm formation on the inner surface of the test tube. The test tube contains the organisms in the broth then after washing them out still the biofilm layer will be present on the inner wall of test tube. It is then found with crystal violet around the layer of the test tube then it is said to be contain the biofilm capacity of the

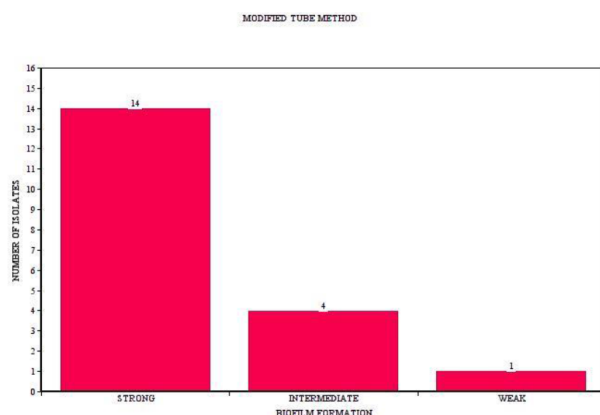




Graph 5: Graph showing biofilm formation in Test tube method.



Graph 7: Graph showing biofilm formation in Microtiter plate method.



Graph 6: Graph showing biofilm formation in Modified Test tube method.

organism. All the MRSA isolates were subjected for biofilm formation and in Test Tube Method, out of 19 Methicillin Resistant *Staphylococcus aureus* strains, 13 were strong, 3 intermediate and 2 weak (Graph 5).

#### Modified Test Tube Method

Modified test tube method is preceded for accounting the biofilm formation on the inner surface of the test tubes. The key difference between test tube and modified test tube method is the composition which contains glucose and sucrose with trypticase soy broth and brain heart infusion broth and found with the formation of ring like structure. All the MRSA isolates were subjected for biofilm formation and in Modified Test Tube Method, out of 19 Methicillin Resistant *Staphylococcus aureus* strains, 14 were strong, 4 intermediate and 1 weak (Graph 6).

#### Microtitre Plate Method

In the microtiter plate method, bacterial cultures were inoculated in the 96 wells microtiter plate and the

overnight incubated culture is washed with phosphate buffer saline (PBS) for three times and the microtiter plate were air dried, the crystal violet was further added to the microtiter plate and kept for 2 min after that the crystal violet have been discarded. Then optical density of each well was measured at 470nm using an ELISA reader. MTP method was reported to have high specificity, sensitivity and positive predictive values. All the MRSA isolates were subjected for biofilm formation and in Microtitre Plate Method, out of 19 Methicillin Resistant *Staphylococcus aureus* strains, 15 were strong, 3 intermediate and 1 weak (Graph 6).

#### Phytochemical Analysis

The selected medicinal plants i.e. *Erythrina variegata*, *Cissus quadrangularis* and *Vitex negundo* were collected, air dried and the dried plants were blended and powered. The extracts have been taken from the plants for phytochemical analysis from the medicinal plants.

#### Anti-biofilm Study

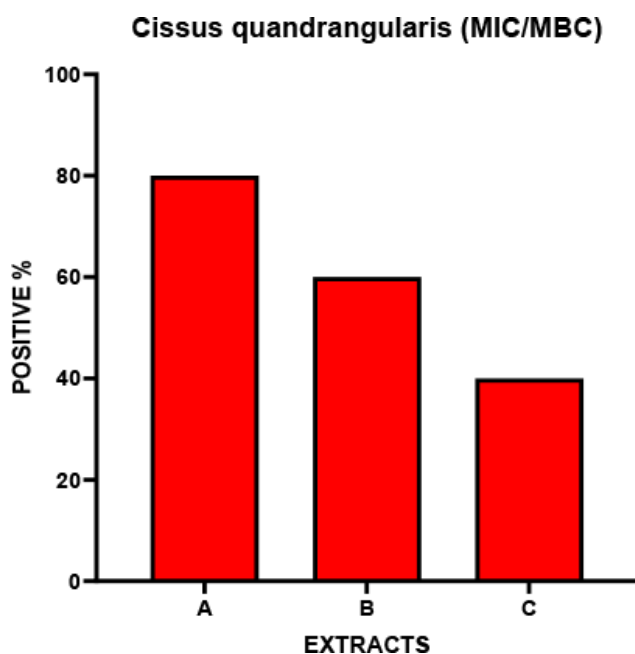
##### Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) for each of the three medicinal plant extracts was performed using disc diffusion method. Mostly the Methanolic extract plants are sensitive, the chloroform and aqueous extract plants are highly resistant to the organism. The comparative study has been done between the plant extracts for the Minimum Inhibitory Concentration and the results were observed as, the Methanolic extracts of the three plants i.e. *Cissus quadrangularis*, *Vitex negundo* and *Erythrina variegata* has reacted with the *Staphylococcus aureus* cultures and shows higher sensitivity. Compared with those the Methanolic extract of *Vitex negundo* shows more sensitive. Followed by the Chloroform and

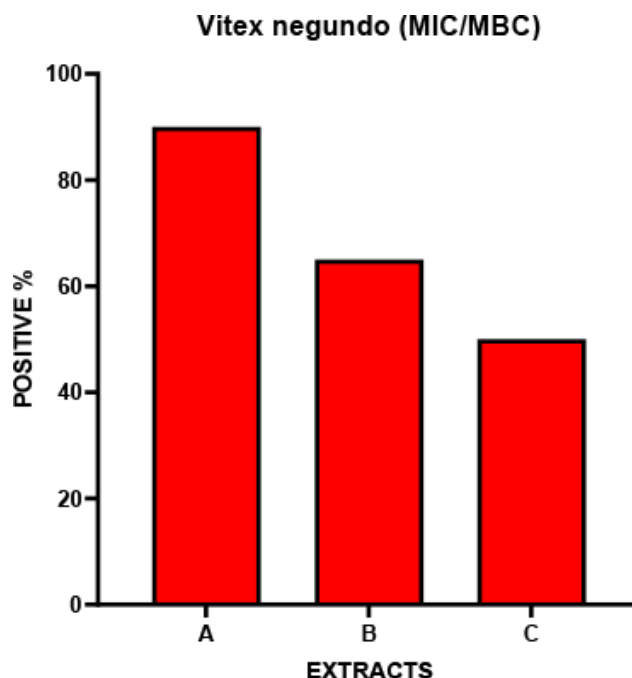
Aqueous extracts shows sensitivity and also resistant to the *Staphylococcus aureus* cultures. The Aqueous extracts shows more resistant rather than the Chloroform extracts. Even though the *Vitex negundo* shows more sensitivity to the cultures other than the two plants. Therefore the Methanolic extract plants were sensitive compared to the Chloroform and Aqueous extract plants.

#### Minimum Bacterial Concentration (MBC)

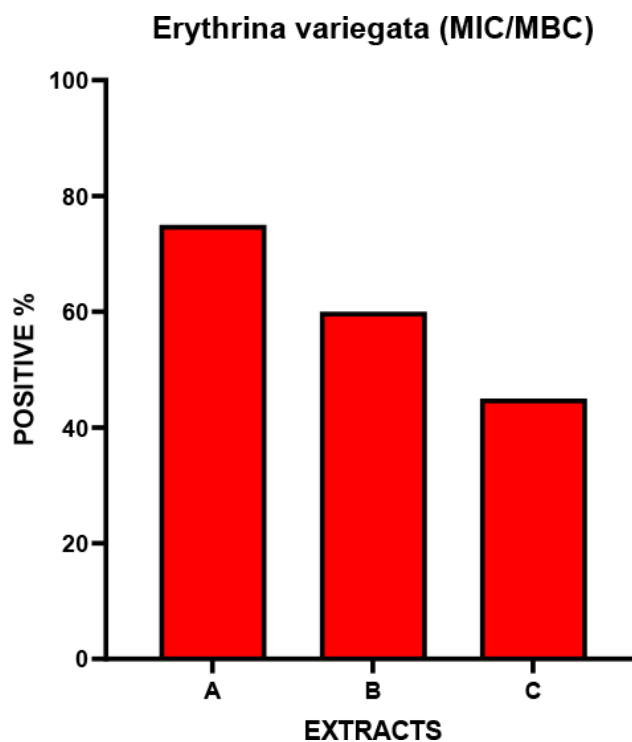
Minimum Bacterial Concentration (MBC) for each of the three medicinal plant extracts was performed using micro dilution method. The Methanolic extract plants are sensitive, the chloroform and aqueous extract plants are mostly resistant to the organism. The comparative study has been done between the plant extracts for the Minimum Bacterial Concentration, the results were observed as, the Methanolic extracts of the three plants i.e. *Cissus quadrangularis*, *Vitex negundo* and *Erythrina variegata* has reacted with the *Staphylococcus aureus* cultures and shows higher sensitivity. Compared with those the Methanolic extract of *Vitex negundo* shows more sensitive. Followed by the chloroform and aqueous extracts shows sensitivity and also resistant to the *Staphylococcus aureus* cultures. The Aqueous extracts shows more resistant rather than the Chloroform extracts. Even though the *Vitex negundo* shows more sensitivity to the cultures other than the two plants. Therefore, the Methanolic extract plants were sensitive compared to the Chloroform and Aqueous extract



Graph 8: Graphical representation of Minimum Bacterial Concentration (MBC) for *Cissus quadrangularis*\* (A – Methanol, B – Chloroform, C – Aqueous).



Graph 8: Graphical representation of Minimum Bacterial Concentration (MBC) for *Vitex negundo*\* (A – Methanol, B – Chloroform, C – Aqueous).



Graph 8: Graphical representation of Minimum Bacterial Concentration (MBC) for *Erythrina variegata*\* (A – Methanol, B – Chloroform, C – Aqueous).

**Table 2: Identification of MRSS using Selective Test**

Selective Test	Number Of Pure Culture
Mannitol Salt Agar	79
Hichrome Rapid MRSA Media	72
Barid Parker Agar	70
DNase Agar	71
Coagulase Test	73

**Table 3: Antibiotic Suscetibility Test Results for Isolated Organisms**

Antibiotics	Sensitive	Resistance	Intermediate
Methicillin	14	30	16
Mupirocin	19	22	19
Penicillin	13	38	9
Ampicillin	13	24	23
Vancomycin	19	25	16
Gentamycin	18	23	21
Tetracycline	15	31	14

plants. All the MRSA isolates were subjected for biofilm formation and in Microtitre Plate Method, out of 19 Methicillin Resistant *Staphylococcus aureus* strains, 15 were strong, 3 intermediate and 1 weak (Graph 8).

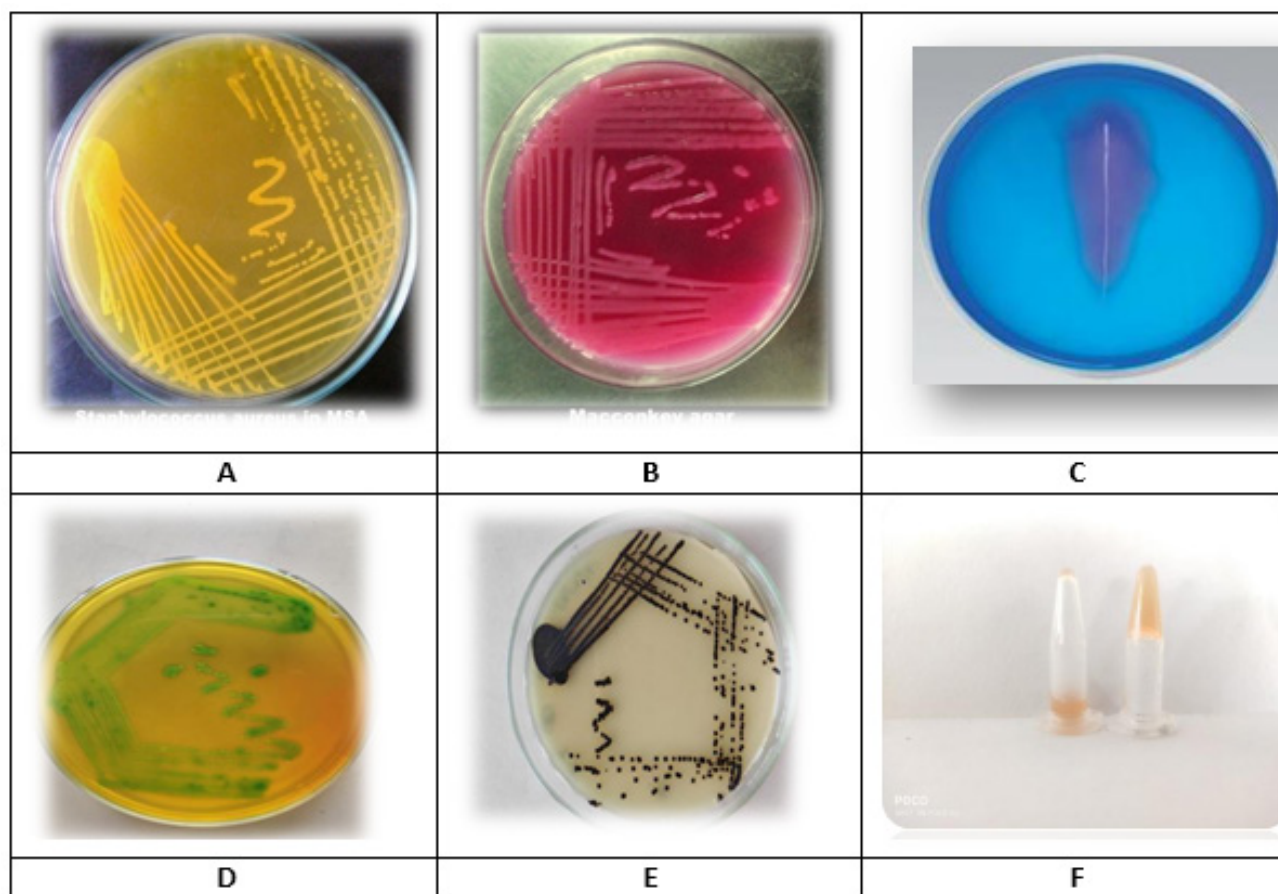
## DISCUSSION

Mastitis in dairy cows is a serious problem and it was an economically devastating disease causing immense economic losses in dairy cows and bio health hazard to human worldwide especially in most of the developing countries and change in quality of milk.<sup>[23,24]</sup> The high prevalence of clinical mastitis in dairy cattle may be due poor hygiene and poor management in rural areas. Isolation of the causative organisms by culturing is considered as the most suitable, accurate and reliable method for identification of the causative agent.<sup>[25,26]</sup> Methicillin Resistant *Staphylococcus species* is a zoonotic pathogen capable of causing a wide variety of syndromes in Bovine mastitis. About 70 samples were randomly selected from the glycerol stock, a total of 70 *Staphylococcus* cultures were recovered, and they were identified by biochemical test and found the isolated bacteria belonging to Methicillin Resistant *Staphylococcus species* as in Table 2 and Figure 2. Many authors agreed with these outcomes. They are regarded as a gold standard method for bacterial culture. The highest prevalence of *Staphylococcus species* could be

due to transmission from teat to teat or cow to cow, possibly via the Milker's hands due to a lack of hygiene. These findings were in agreement with Das *et al.*,<sup>[27]</sup> who considered these microorganisms to be major etiological agents of clinical mastitis worldwide. Other bacteria isolated in this analysis, include CNS, *E. coli*, *Streptococcus spp.*, *Pseudomonas spp.*, and *Klebsiella spp.*, are minor causes of bovine mastitis.<sup>[28]</sup> The other significant incidence rate of *E. coli*, i.e. *Staphylococcus aureus*, could be associated with poor hygienic conditions in the microenvironment; *E. coli* enters the udder through the teat canal and infects it. *Staphylococcus aureus* causes food poisoning and is commonly detected in milk from dairy cows suffering from mastitis.<sup>[29]</sup>

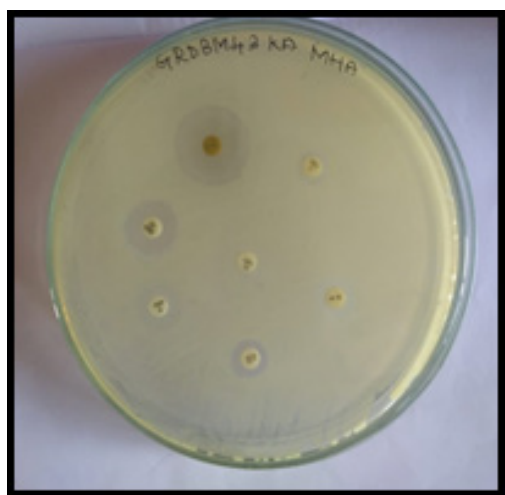
Many authors have recorded an increase in the drug resistance bacteria isolated from bovine mastitis.<sup>[30]</sup> Antibiotics sensitivity test is important to suggest suitable antibacterial treatment to prevent antibiotic resistance, potential health risk for humans. The results in Table 3 and Figures 3 and 4 clearly showed that methicillin, mupirocin, and penicillin were found to be less effective against bacteria isolates from bovine mastitis. This could be due to increased indiscriminate and frequent use of those antibiotics in dairy animals, resulting in the development of antibiotic resistance bacteria, necessitating the development and search for novel sources as antimicrobial agents. In this study, all isolates from the clinical milk samples showed higher biofilm production. This result was in concordance with the study of Rodriguez-Lazaro *et al.*<sup>[31]</sup> Most of the Methicillin Resistant *Staphylococcus species* isolates from bovine mastitis formed biofilms, which was in concordance with a previous report on *Methicillin Resistant Staphylococcus species* isolates from clinical bovine milk samples.<sup>[32]</sup> In the present study, the isolates were biofilm-producing Methicillin Resistant *Staphylococcus species*. Similarly, clinical isolates with the ability to generate biofilm were observed to be more often.<sup>[33]</sup>

Compounds derived from medicinal plants have wide interest in the search for alternative antibacterial agents. They are safe and have long been used in medicine to treat infectious diseases.<sup>[34]</sup> According to the World Health Organization, medicinal plants will be the best source of access to a wide variety of medicines and active ingredients. Therefore, such systems need to be studied to understand their characteristics, safety, and efficiency.<sup>[35]</sup> The aim of the current study was to evaluate the antimicrobial activity of selected some medicinal plant extracts such as *Erythrina variegata*, *Cissus quadrangularis* and *Vitex negundo* against bacteria isolated from bovine mastitis, our results revealed that the plant extracts by different solvent Petroleum ether,



**Figure 2: Selective test for confirmation of staphylococcus species.**

**A-yellow colonies on mannitol salt agar, b-pink colonies on mannitol salt agar,c-zone of clearance on dnase agar,d-green colonies on hi-chrome mrsa agar,e-black colonies on barid parker agar, f-coagulase test.**



**Figure 3: Antibiotic susceptibility test results for isolated organisms**

Chloroform, Acetone and Methanol exhibited a level of antibacterial activity against MRSS isolated from bovine mastitis. Compared with those the Methanolic extract of *Vitex negundo* shows more sensitive. The

Chloroform and Aqueous extracts shows sensitivity and also resistant to the *Staphylococcus aureus* cultures. The Aqueous extracts shows more resistant rather than the Chloroform extracts. Therefore the Methanolic extract plants were sensitive compared to the Chloroform and Aqueous extract plants and this result is in agreement with Sampimona *et al.*<sup>[36]</sup> The most effective antibacterial activity was recorded for *Vitex negundo* showed more sensitivity to the cultures other than the two plants and showed marked level antibacterial activity against Methicillin Resistant *Staphylococcus species* and induce inhibition zone these results were in agreement with Mothana *et al.*<sup>[37]</sup>

Evaluation of antibacterial activity by MIC different plant extracts of *Erythrina variegata*, *Cissus quadrangularis* and *Vitex negundo* with different solvents (Petroleum ether, Chloroform, Acetone and Methanol) against Gram's-positive Methicillin Resistant *Staphylococcus species*. The MIC value of *Vitex negundo* shows more sensitivity to the cultures other than the two plants. Therefore the Methanolic extract plants were sensitive compared



to the Chloroform and Aqueous extract plants which may be due to attributed to the rich plant contents of active components such as alkaloids and flavonoids. The Methanolic extracts of the three plants i.e. *Cissus quadrangularis*, *Vitex negundo* and *Erythrina variegata* has reacted with the *Staphylococcus aureus* cultures and shows higher sensitivity. Compared with those the Methanolic extract of *Vitex negundo* shows more sensitive. Followed by the chloroform and aqueous extracts shows sensitivity and also resistant to the *Staphylococcus aureus* cultures. The Aqueous extracts shows more resistant rather than the Chloroform extracts. Even though the *Vitex negundo* shows more sensitivity to the cultures other than the two plants. Therefore, the Methanolic extract plants were sensitive compared to the Chloroform and Aqueous extract plants. All the MRSA isolates were subjected for biofilm formation and in Microtitre Plate Method, out of 19 Methicillin Resistant *Staphylococcus aureus* strains, 15 were strong, 3 intermediate and 1 weak and these results were in agreement with Sampimona et al. and Sumathi et al.<sup>[38]</sup>

## CONCLUSION

From the present study, Milk samples from bovine mastitis were collected and isolated different organisms and all the isolates were tested for morphological characterization such as Gram's Staining, Biochemical characterization and Antibiotic Susceptibility Test (ABST). The patterns in the isolated pathogens against these antibiotics were susceptible, intermediate and resistant. 85% of the cultures had high biofilm formation, 15% had moderate biofilm formation and no biofilm formation was not seen. Three medicinal plants i.e. *Cissus quadrangularis*, *Vitex negundo* and *Erythrina variegata* were used and the results were more effective for Methanolic extracts followed by Chloroform and Aqueous extracts. From the study, it was concluded that the MRSA was evaluated for anti-biofilm forming ability. Then, three different medicinal plant extracts were used against MRSA isolated from bovine found to have better activity in Methanolic extract than the other with the evaluation of MIC and MBC.

## ACKNOWLEDGEMENT

I would like to thank the management and the Department of Biotechnology, Dr GRD college, Coimbatore, for supporting me throughout this project.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**SIM:** Sulfur, Indole, Motility; **MRSA:** Methicillin Resistant *Staphylococcus aureus*; **MRSS:** Methicillin Resistant *Staphylococcus* species; **PIA:** Polysaccharide Inter-cellular Adhesion; **MIC:** Minimum Inhibitory Concentration; **MBC:** Minimum Bacterial Concentration; **BA:** Blood Agar; **BPA:** Barid Parker Agar; **DNase:** Deoxyribonuclease; **EPS:** Exopolysaccharide; **PBS:** Phosphate buffer saline; **MTP:** Microtitre Plate Method.

## SUMMARY

Infectious bovine mastitis is responsible for serious economic losses in dairy cattle breeding. Information about the damages caused by micro-organisms is of great interest and importance. The purpose of the present study was to report on the evaluating the biofilm forming Methicillin Resistant *Staphylococcus* species and eradication of biofilm using *Cissus quadrangularis*, *Vitex negundo* and *Erythrina variegata*. About 90 samples were randomly selected from the glycerol stock, a total of 70 *Staphylococcus* cultures were recovered, and they were identified by biochemical test and found the isolated bacteria belonging to Methicillin Resistant *Staphylococcus* species. From the study, it was concluded that the MRSA were more effective for Methanolic extracts in all of the plant extracts used against MRSA isolated from bovine found to have better activity in Methanolic extract and found to have better activity in Methanolic extract than the other with the evaluation of MIC and MBC.

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