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# INVESTIGATION OF ANTI-QUORUM SENSING AND BIOFILM INHIBITION ACTIVITIES OF MEDICINAL PLANTS AGAINST ANTIBIOTIC RESISTANT MICROBIAL BIOFILMS

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**ABSTRACT**
Biofilm is a complex microbial community highly resistant to antimicrobials. Multidrug-resistance bacteria commonly use cell-to-cell communication that leads to biofilm formation as one of the mechanisms for developing resistance. Quorum sensing inhibition is an effective approach for the prevention of biofilm formation. In the present study the Antimicrobial, Antibiotic and Antibiofilm activities of three hydroalcoholic leaf extracts were evaluated against isolated microorganisms. Preliminary screening by diffusion well assay showed the antimicrobial activity. Anti-quorum sensing activities of hydroalcoholic extracts of medicinal plants *Carica papaya, Tamarindusindica* and *Mimosa pudica* against quorum sensing mediated virulence factors such as Motility, Biofilm production, Phycocyanin production, Enzyme activity and Proteolytic activity has been carried out. Among the plants *Carica papaya* revealed maximum inhibition.

**KEYWORDS**: Antimicrobial, Antibiotic, Antibiofilm, *Carica papaya, Tamarindus indica, Mimosa pudica*, Biofilm.

## INTRODUCTION

Biofilm, a group of micro-organisms where the cells stick to each other on a surface (Hall-Stoodley *et al.,* 2004). It is commonly found in industrial places, hotels, waste water channels, bathrooms, labs, hospital settings. It can be formed as floating mats on surface of liquid and occur on both living and non-living surfaces (Costerton *et al.,* 1999). The biofilm formation is based on the expression of specific genes (Sauer *et al.,* 2004). After gene expression and adherence of cells to the surface, the microorganisms undergo certain changes (Rajan *et al.,* 2002). The steps involved in biofilm formation are (a) attachment initially to a surface (b) formation of micro-colony (c) three dimensional structure formation (d) biofilm formation, maturation and detachment (Rivera *et al.,* 2001).

Quorum sensing (QS) is a mechanism of bacterial cell-cell communication that relies on the production, release, and group-wide detection of extracellular signal molecules called auto inducers (AIs). Quorum sensing (QS) enables populations of bacteria, where a change in gene expression leads to biofilm formation, bioluminescence and secreting various virulence factors (Luo *et al.,* 2016). The research has recently focused on controlling the bacterial diseases by developing antipathogenic agents on inhibiting quorum sensing (Chan *et al.,* 2011). Both gram-negative and gram-positive bacteria are known to have QS mechanism (Papenfort *et al.,* 2016).

There are various mechanisms for increasing the antibiotic resistance in biofilms such as physical barrier which is formed by extracellular polymeric substance (EPS) (Costerton *et al.,* 1999). Based upon the structure of dormant and slow growing bacteria they are inert towards antibiotics (Lynch *et al.,* 2008).The genes expressed in biofilms are resistant to some antibiotics (Mah *et al.,* 2001). Most medicinal plants have application in antimicrobial and antioxidant effects (Wallace, 2004). The antimicrobial efficacy of the herbal plants is mediated by quorum sensing inhibition. Herbal Plants have also evolved to produce antiquorum sensing compounds which are used to quench the quorum sensing signals in the pathogens (Lowry *et al.,* 1951). Papaya (*Carica papaya*) is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya leaves are well known in traditional system of medicine. Each part of papaya tree possesses economic value when it is grown on a commercial scale (Krishna *et al.,* 2008). The *Carica papaya* possesses antimicrobial, antioxidant, and anti-inflammatory activities. It is well known for healing chronic ulcer (Shivananda Nayak *et al.,* 2007). Papaya leaf-extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5,7-dimethoxy coumarin, caffeic acid, kaempferol, quercetin, and chlorogenic acid (Romasi *et al.,* 2011). Tamarind (*Tamarindus indica)* is a medium-sized tree belonging to the Caesalpinaceae family. Tamarind has been used for centuries as a medicinal plant (Escalona *et al.,* 1995). The leaves have a proven hepatoprotective activity associated with the presence of polyhydroxylated compounds, with flavonolicnature (Jouyex *et al.,* 1995). Flavonoid and other polyphenols are metabolites which is also found in tamarind leaves (Chitra Arya, 1999).Touch me not (*Mimosapudica)* a famous ornamental plant commonly known as sleeping grass, sensitive plant, humble plant, shy plant. The closure of leaves takes place through certain stimuli like light, vibration, wounds, wind, touch, heat, and cold. It has been used to treat high blood pressure, menorrhagia, and leucorrhea (Volkov *et al.,* 2010).

## MATERIALS AND METHODS

### Collection of Plant Samples

The plant leaves of *Mimosa pudica, Tamarindus indica* and*Carica papaya* were collected from Madras Christian college, Tambaram, Chennai, Tamilnadu, India and stored in sterile container. The plant leaves were washed with distilled water. Further the plant leaves were subjected to shade dried for 48hrs, blended and sealed in a polythene bag.

### Collection of microorganisms

20 mL of sewage water was collected from M. E. S road, Tambaram, Chennai, Tamilnadu, India and stored in the plastic container at 37ºC.

### Preparation of extracts

The dried plant leaves were soaked in 2% hydroalcohol with continuous agitation at 150rpm for 24 hrs. Further, filtration of the soaked leaf solution was carried out using Whatman filter and the filtrate was considered as plant extract.

### Phytochemical analysis of the extracts

Phytochemical screening was done to detect the presence of bioactive agents. After the addition of specific reagents to the test solutions, the tests were detected by visual observation of colour change or by precipitate formation. The qualitative analysis was done with standard procedure described in Phytochemical methods (Harborne, 1973), medicinal plants and traditional medicine in Africa (Sofowara, 1993), Pharmacognosy (Trease and Evans, 1989).The phytochemical analysis of leaf extract for the determination of alkaloids, carbohydrates, glycosides, steroids, tannins, phenolic compounds, saponins, proteins, amino acids, terpenoids, flavanoids were carried out.

## QUALITATIVE ANALYSIS

### Test for alkaloids

The alkaloids were determined by using Wagner's test. The filtrates of leaf extracts were taken in 1mL and Wagner's reagent of 2mL was added.

### Test for carbohydrates

The carbohydrates were determined by using Fehling's test. 1 mL of leaf extracts was taken and it was boiled in water bath for 15 min. 1mL of Fehling reagent A and B were added.

### Test for glycosides

The Glycosides were determined by using Born trager's test. 3mL of chloroform was added to 2 mL of leaf extracts. It was agitated for a minute and 10% of 1mL ammonia solution was added.

### Test for saponins

The Saponins weredetermined by using Foam test. 1 mL of leaf extracts was dissolved in 2 mL of distilled water. It was agitated for a minute.

### Test for proteins

The Proteins were determined by using Biurette's test.1 mL of copper sulphate solution was added to 2 mL of leaf extract. Then 1 mL of ethanol and 3 Potassium hydroxide pellets were added.

### Test for amino acids

The Amino acids were determined by using ninhydrin's test. Two drops of ninhydrin solution was added to 2 mL of leaf extracts.

### Test for phenolic compounds

The phenolic compounds were determined by using Ferric chloride test. 1 mL ofleaf extracts was dissolved in 2 mL of distilled water and 2 drops of ferric chloride solution was added.

### Test for terpenoids

1 mL of chloroform was added to 2mL of leaf extracts and mixed thoroughly. Then 1mL of concentrated hydro-sulphuric acid was added.

### Test for steroids

2 µL of chloroform was added into 2mL of leaf extracts. Then few drops of acetic acid and concentrated hydrosulphuric acid were added.

### Test for flavonoids

1 mL of leaf extracts was taken in a test tube and 2 mL of ammonia solution was added to the leaf extracts.

### Test for tannins

1mL of leaf extracts was added to 2 mL of distilled water and 2 drops of ferric chloride solution was added.

## QUANTITATIVE ANALYSIS

### Determination of total phenolic content

1mL of Folin's reagent and disodium carbonate were added to 0.5 mL of plant extracts. It was incubated for 90 min at room temperature. The absorbance values were measured at 750 nm using UV Spectrophotometer.

### Determination of total flavonoid content

0.3 mL of sodium nitrite and aluminium chloride was added to 0.5 mL of plant extracts. It was incubated for 6 min at room temperature. Then 2 mL of sodium hydroxide was added. The absorbance values were measured at 510 nmusing UV Spectrophotometer.

### Determination of antioxidant assay

2 mL of DPPH (2, 2 diphenyl 1, Picrylhydrazyl) solution was added to leaf extracts of different concentrations (20 µL, 40µL, 60 µL, 80 µL, 100 µL). The absorbance values were measured at 517 nm using UV Spectrophotometer.

### Isolation of microorganisms

1g of biofilm sample was taken in a test tubes and it was dissolved in 10 mL of distilled water. Further the serial dilution was carried out for the concentration of $10^{-3}$and $10^{-4}$. 1mL of the 3$^{rd}$ and 4$^{th}$ fold serial dilutions was spread on the nutrient agar plates. The plates were incubated at 37ºC for 24 hours.

## BIOFILM SCREENING ASSAY

### Congored agar assay

150 mL of congored agar was prepared and spread into the petriplates.The isolated bacterial colonies were inoculated into the Congored agar medium and incubated at 37ºC for 24hrs.

### Tube method

10 mL of Trypticase soy broth was added to 1% of glucose. The isolated bacterial colonies were inoculated into the Trypticase soy broth and incubated at 37ºC for24 hrs. Further the tubes were decanted and washed with phosphate buffer saline at pH 7.3. The tubes were dried for 10 mins. 0.1% of crystal violet stains were added to the test tubes and incubated at 37ºC for 15 min. The excess stains were washed with deionized water bath.The test tubes were dried in inverted position for 15 mins.

## DETERMINATION OF ANTIBACTERIAL ACTIVITY

### Preparation of culture medium

100mL of distilled water was boiled in a round bottom flask0 .2g of yeast, 1.5g of agar, 0.5g of NaCl and peptone were added into the flask and stirred continuously. The flask with the medium was placed in polythene bags. Further the medium was sterilized at a pressure of 15 lbs at 121°C for 20 min in an autoclave. The pH of the nutrient agar medium was adjusted to 7.2.

### Well diffusion method

The antibacterial activity of *Carica papaya*, *Tamarindus indica* and *Mimosa pudica* leaf extracts were determined using agar well diffusion method. The isolated bacterial strains (strain 1, strain 2, strain 3) were swabbed on three nutrient agar plates respectively. Then four wells were punched on each agar plate. 100µL of different plant leaf extracts were added in 3 wells and 20 µL of 0.1 g Gentamicin were added to the remaining well. The petriplates were incubated for 24hrs.

### Determination of antibiotic activity

100 mL of nutrient agar medium was prepared and poured into 3 petriplates. The isolated bacterial strains (strain 1, strain 2, strain 3) were swabbed on three nutrient agar plates respectively. Then five wells were punched on each agar plate.20 µL of Ampicillin, Amoxcillin, Pencillin, Tetracyclin, Chloramphenicol were added toeachwells. The petriplates were incubated for 24 hrs.

## ANTIQUORUM SENSING ACTIVITY

### Biofilm inhibition assay

150 µL of Nutrient broth, 10µL of inoculum and different concentrations (20µL, 40µL, 60 µL, 80 µL and 100 µL) of leaf extracts were loaded in 96 well plate and incubated for 24hrs. The plate was washed with 0.2 mL of Phosphate buffer saline. In each plates 0.2mL of sodium acetate and 0.1% of crystal violet stains were added. The solutions in the 96 well plates were discarded and the wells were washed with ethanol. The micro titre plate was incubated for15 min and the absorbance was measured at 595 nm.

### Cell adhesion

150 µL of Bovine serum albumin, 10 µl of inoculum and different concentrations (20 µL, 40 µL, 60 µL, 80 µL and 100 µL) of the plant leaves extracts were loaded in 96 well plate and incubated for 24 hrs. The plates were washed with 0.2 mL of Phosphate buffer saline and 50 µl of crystal violet was added in plates. The solutions in the plates were discarded and washed with ethanol. 0.2% of Triton X-100 was added into the wells and the absorbance were measured at 570 nm.

### Phycocyanin activity

500 µL of inoculum and plant leaf extract of different concentrations (20 µL, 40 µL, 60 µL, 80 µL and 100 µL) were added into the tubes and incubated at 37ºC for24 hrs. Then the tubes were centrifuged at 4000 rpm. 1.5mL of hydrochloric acid was added to the 5 mL of Supernatant which was taken from the centrifuged tubes.The absorbance was measured at 520 nm.

### Enzyme activity

0.25 mL of Casein and 0.5 mL of inoculum were added to the test tubes. Then the tubes were incubated at 25ºC for 15 min. 1.2 mL of Trichloro acetic acid was added to the tubes and incubated at 37ºC for 15 min. Then the tubes were centrifuged at 8000 rpm for 5 min. 1.4 mL of NaOH was added to 1.2 mL of supernatant which was taken from the centrifuged tubes and the absorbance were measured at 440 nm.

### Motility

500 µl of leaf extracts of *Carica papaya* and 250µL of culture were mixed in eppendorftube. The tube was kept in room temperature for 30min.Further the culture was inoculated in Luria broth and incubated for 24hrs at 37ºC. 1% of 2 drops crystal violet solution was added to the tube.

## RESULTS AND DISSCUSION

### Qualitative analysis

Phytochemical analysis was carried out using the selected plants leaves using the hydroalcoholic extracts and the results were given in the table

(+) – presence of phytochemicals

(-) – Absence of phytochemicals

The Qualitative analysis for *Carica papaya*, *Tamarindus indica* and *Mimosa pudica* were performed and the presence of alkaloids, carbohydrates, glycosides, protein, amino acids, steroids, tannins, phenolic compounds, saponins, terpenoids and flavonoids were detected in the hydroalcoholic extracts. The negative results were recorded for the glycosides, protein and amino acids. The steroids were found in the *Carica papaya* and carbohydrates were found in *Tamarindus indica.*

**TABLE 1.**Qualitative Analysis

| | | Hydroalcoholic extract | | |
|---|---|---|---|---|
| S.No. | Phytochemicals | *Tamarindusindica* | *Carica papaya* | *Mimosa pudica* |
| 1 | Alkaloids | + | + | + |
| 2 | Carbohydrates | + | - | - |
| 3 | Glycosides | - | - | - |
| 4 | Steroids | - | + | - |
| 5 | Tannins | + | + | + |
| 6 | Phenolic compound | + | + | + |
| 7 | Saponins | + | + | + |
| 8 | Protein | - | - | - |
| 9 | Amino acids | - | - | - |
| 10 | Terpenoids | + | + | + |
| 11 | Flavonoids | + | + | + |

The higher percentage of scavenging activity of *Tamarindusindica* (100µL) at 570 nm was found as 78.9 %.The *Tamarindusindica*showed higher scavenging activity than other leaf extracts ranged from 40.11±0.03% (MouriceMbunde, 2013).

**QUANTITATIVE ANALYSIS**

In quantitative analysis the total phenolic content and total flavonoid were determined by Folin's reagent and aluminium chloride assay. The absorbance of the mixture was noted at 750 and 510 nm using UV spectrophotometer respectively.

**TABLE 2.** Quantitative Analysis

| Hydroalcoholic Extract | Phenol | Flavanoid |
|---|---|---|
| *Tamarindusindica* | 3.426 | 0.566 |
| *Carica papaya* | 1.183 | 0.394 |
| *Mimosa pudica* | 3.534 | 0.354 |

From table 2, The highest absorbance value of phenol content 3.534 was found in *Mimosa pudica.* The highest absorbance value of flavanoid content 0.566 was found in *Tamarindusindica.*

**ANTIOXIDANT ACTIVITY**

**TABLE 3(a).** Antioxidant activity of *Tamarindusindica*

| S.No. | Concentration | Absorbance At 570nm | % scavenging Activity |
|---|---|---|---|
| 1 | CONTROL | 0.931 | - |
| 2 | S1 (20µL) | 0.311 | 66.6 |
| 3 | S2 (40µL) | 0.255 | 72.6 |
| 4 | S3 (60µL) | 0.213 | 77.1 |
| 5 | S4 (80µL) | 0.201 | 78.4 |
| 6 | S5 (100µL) | 0.196 | 78.9 |

The highest percentage of scavenging activity of *Tamarindus indica* (100 µL) at 517 nm was found as 78.9%. The *Tamarindus indica* showed higher scavenging activity than other leaf extracts ranged from 40.11±0.03% (Mourice Mbunde, 2013).

**TABLE 3(b).** Antioxidant activity of *Carica papaya*

| S.No. | Concentration | Absorbance At 570 nm | % scavenging Activity |
|---|---|---|---|
| 1 | Control | 0.931 | - |
| 2 | S1(20µl) | 0.839 | 9.88 |
| 3 | S2(40µl) | 0.806 | 13.42 |
| 4 | S3(60µl) | 0.769 | 17.4 |
| 5 | S4(80µl) | 0.529 | 43.17 |
| 6 | S5(100µl) | 0.465 | 50.05 |

The highest percentage of scavenging activity of *Carica papaya* (100 µL) at 517 nm was found as 50.05%. The *Carica papaya* showed higher scavenging activity than other leaf extracts ranged from 73.08±5.11% (Sahirabanu *et al.*, 2018).

**3(c) Antioxidant activity of *Mimosa pudica***

The highest percentage of scavenging activity of M*imosa pudica* (100µL) at 517 nm was found as 65.3 %. The M*imosa pudica*showed higher scavenging activity than other leaf extracts ranged from 22.367±0.512 % (Jing zhang*et al.*, 2011).

**CONGO RED ASSAY**

Fig:1(a) Fig:1(b) Fig:1(c)

**FIGURE: 1** screening of the isolates for biofilm formation by Congo red agar method

The appearance of black colour in the bacterial isolates indicate the biofilm producers, and red colour indicates the non-biofilm producers.Incongored agar method 5 strains produced biofilm and 10 strains were non-biofilm

producers. The rate of positivity of congored agar method for our study was 7.35%. Rate of positivity in congored agar method in our study is higher than that ofMathur*etal.*,(2006)which reported 5.26% biofilm producers by congo red agar method.

**TUBE METHOD**

The biofilm was confirmed by tube method. Ring formation indicates the presence of biofilm and it is obtained from the three bacterial strains.

The rate of biofilms producers using congored agar method was 7.35% whereas 10.25% are moderate biofilm producers by tube method.In tube method 3 strains were found as positive biofilm producers and 12 strains were found as non-biofilm producers. Rate of biofilm producers in our study is higher than that of Bose *et al.,* (2009)which reported 8.25% by tube method

**IDENTIFICATION BASED ON16s rRNASEQUENCE ANALYSIS**

**16S rRNAGENE PARTIAL SEQUENCE (~1171bp)**

CAAGGGGGACAACATTTCGAAAGGAATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCTTCGGACCTTG
CGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCTGTAGCGGGTCT
GAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACA
ATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGA
GGCTACTTTAGATAATACCTAGAGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCG
GTAATACAGAGGGTGCAAGCGTTAATCGGATTTACTGGGCGTAAAGCGCGCGTAGGCGGCTAATTAAGTCAAATGTGA
AATCCCCGAGCTTAACTTGGGAATTGCATTCGATACTGGTTAGCTAGAGTGTGGGAGAGGATGGTAGAATTCCAGGTGT
AGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCTAACACTGACGCTGAGGTGC
GAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGTCTACTAGCCGTTGGGGCCTT
TGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAATGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATA
GTAAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTTACATACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCG
TGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTCCTTATTTGCCAGCGAGTAATGTCGGGAACTTTAAGGA
TACTGCCAGTGACAAACTGGAGGAAGGCGGGGACGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTG

**FIGURE 2:** Gene sequence of *Acinetobacterpittii* (strain AB9)

**TABLE 3(c)** Antioxidant activity of *Mimosa pudica*

| S.No | Concentration | Absorbance at 517 nm | % Scavenging activity |
|---|---|---|---|
| 1 | Control | 0.931 | - |
| 2 | S1 (20 µL) | 0.645 | 30.71 |
| 3 | S2 (40 µL) | 0.579 | 37.8 |
| 4 | S3 (60 µL) | 0.449 | 51.7 |
| 5 | S4 (80 µL) | 0.392 | 57.8 |
| 6 | S5 (100 µL) | 0.323 | 65.3 |

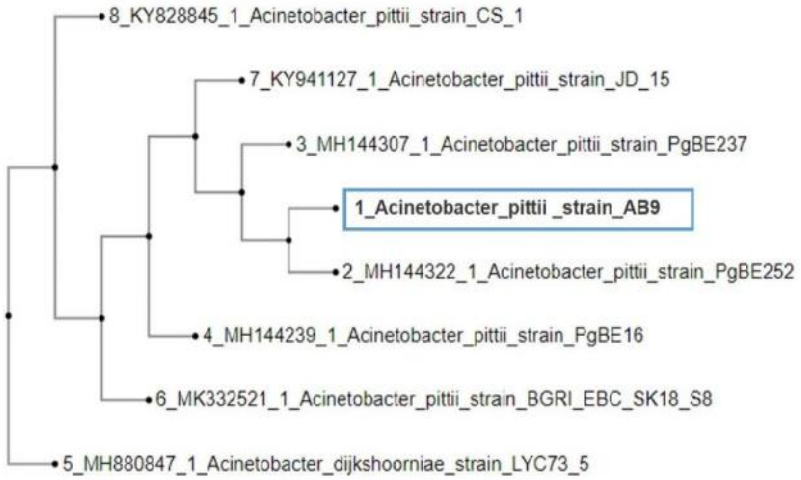


**FIGURE 3:** Phylogenetic tree generated from the 16S rRNA gene sequence

**ANTIBACTERIAL ACTIVITY**

The bacterial species such as *Acinetobacterpittii* (strain AB9) and other two bacterial strains were selected and the antimicrobial activity was carried out using the Agar medium.Theswab technique was followed for testing the microbial activity where the wells were punched. The extract was added at 100 µL concentrations and zone of inhibition was observed during 24-48 hrs.

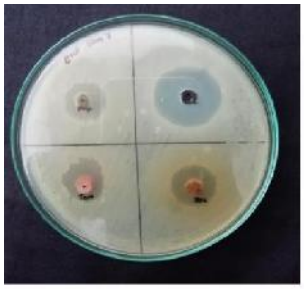
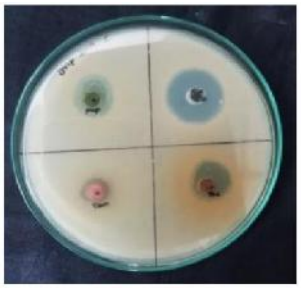
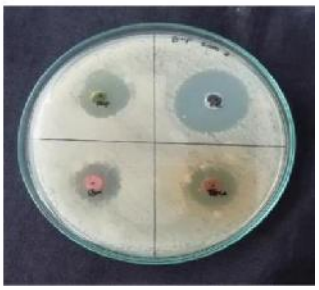

Fig 4(a) Fig 4(b) Fig 4(c)

**FIGURE 4** (a) (b) and (c) shows the Antibacterial activity of isolates using agar well diffusion method

**TABLE 4:** Antibacterial activity of extracts of screened medicinal plants

| | Bacterial | SAMPLE | | | |
|---|---|---|---|---|---|
| S.No | Strains | Control | *Tamarindus indica* | *Carica papaya* | Mimosa pudica |
| 1. | Strain 1 | 12 mm | 9 mm | 10 mm | 9 mm |
| 2. | Strain 2 | 11 mm | 6 mm | 7 mm | 8 mm |
| 3. | Strain 3 | 10 mm | 6 mm | 10 mm | 9 mm |

The zone of inhibition in bacterial culture plates were found to be higher in *Carica papaya* (100 µL) with 10 mm diameter for the bacteria strain 1,for bacterial strain 2 with 7 mm diameter and for *Acinetobacterpittii*(strain AB9) with 10 mm diameter in hydroalcoholic solvents. The antibacterial activity of *Mimosa pudica*extracts on the agar plates varied in water and ethanol solvents ranging from11 to 19 mm (Nguyen Thi Le Thoa*et al.*, 2015).

**Antibiotic activity**

Antibiotic resistance of selected bacterial strains was performed by following Agar well diffusion. The bacterial strains shows no zone of inhibition to chloramphenicol, since the activity of *Tamarindusindica* fruit water extract produced no inhibition of zone to gentamicin (Warda.S.*et al*., 2007).

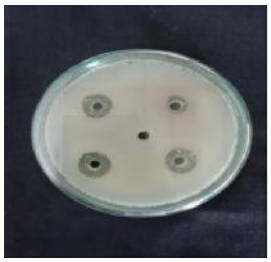
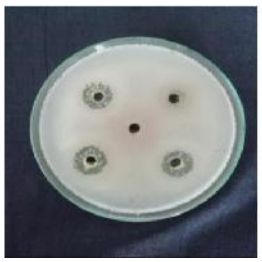


Fig 5(a) Fig 5(b) Fig 5(c)

**FIGURE 5:** (a) (b) and 3(c) Antibiotic activity of isolates using agar well diffusion method

Antibiotic resistance of selected bacterial strains was performed by following agar well diffusion. The *Acinetobacterpitti* (strain AB9) shows zone of nhibition to ampicillin, penicillin, chloramphenicol, amoxicillin and tetracycline.

**QUOROM SENSING ACTIVITY**

**Biofilm inhibition**

The microtiter plate showed that the biofilm inhibition activity, compounds slightly reduce microbial growth without effect on biofilms. Our study shows no formation of biofilm in the microtitre plate whereas in Nathaniel et al., reported the densely formed biofilm within the wells without inhibiting the biofilm forming bacteria.Inhibitory effect of plant extracts against biofilm of *Acinetobacter pittii* (strainAB9)was carried out by microtitre plate crystal violet spectrophotometric assay at different concentration of (20,40,60,80,100)µL.

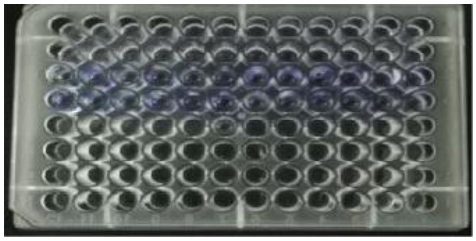

**FIGURE 6:** Anti biofilm activities against gram negative bacteria

**TABLE 5:** Percent inhibition of biofilm formation

| S.No. | Concentration | Absorbance At 595nm | % scavenging Activity |
|---|---|---|---|
| 1. | Control | 0.141 | - |
| 2. | S1(20µl) | 0.104 | 26.2 |
| 3. | S2 (40µl) | 0.081 | 42.5 |
| 4. | S3 (60µl) | 0.064 | 54.6 |
| 5. | S4 (80µl) | 0.040 | 71.6 |
| 6. | S5 (100µl) | 0.034 | 75.8 |

The highest percentage of scavenging activity of Biofilm (100 µl) at 595 nm was found as 75.8%The leaf extract shows the ability to inhibit biofilm formation (100 µl) at 595 nm by 75.8 % .The effect of plant extracts on biofilm formation which revealed all the tested plant extracts reduced significantly. *Eucalyptus globules* and *Aegle marmelos* were reduced the biofilm formation by 99.77%, 99.05% and 95.26% of reduction was recorded in *Ocimumtenuiflorum, Cynodondactylon* and *Azadirachta indica*. (S. KarthickRaja Namasivayam *et al*., 2016).

**CELL ADHESION**

**TABLE 6:** Percent inhibition of cell adhesion

| S.No | Concentration | Absorbance At 570 nm | %scavenging Activity |
|---|---|---|---|
| 1. | Control | 0.284 | - |
| 2. | S1(20µl) | 0.212 | 25.3 |
| 3. | S2 (40µl) | 0.190 | 33 |
| 4. | S3 (60µl) | 0.152 | 46.4 |
| 5. | S4 (80µl) | 0.137 | 51.7 |
| 6. | S5 (100µl) | 0.089 | 68.6 |

The higher percentage of cell adhesion activity was found as 68.6% in (100µl) at 570 nm.

**PHYCOCYANIN**

Phycocyanin activity was measured from culture supernatants and the absorbance value was measured using a spectrophotometer. In our study the inhibitory effects of phycocyanin on the viability and proliferation of cells were determined. Whereas in Shuai *et al.,* shows that phycocyanin supress the colony formation of the cells.

**TABLE**7**:** Percent inhibition of Phycocyanin

| S.No. | Concentration | Absorbance At 520 nm | % scavenging Activity |
|---|---|---|---|
| 1. | Control | 0.296 | - |
| 2. | S1(20µl) | 0.192 | 35.1 |
| 3. | S2 (40µl) | 0.288 | 2.7 |
| 4. | S3 (60µl) | 0.269 | 9.7 |
| 5. | S4 (80µl) | 0.339 | 14.5 |
| 6. | S5 (100µl) | 0.372 | 25.6 |

The higher percentage of phycocyanin inhibition activity was found to be 25.6% in (100 µl) at 520 nm

**Enzyme activity and proteolytic activity**

Another effective way to degrade biofilm is the application of enzymes. Biofilm consists of extra/cellular polymeric substance (EPS, therefore these enzymes have the potential to degrade EPS. Biofilm is mainly composed of bacteria and EPS. When the biofilm is degraded by enzymes which results release of components and planktonic cells which is easily clear by immune systems.

**TABLE 8:** Percent inhibition of enzyme and proteolytic activity

| S.No | Absorbance At 440 nm | % scavenging Activity |
|---|---|---|
| 1. | 0.532 | - |
| 2. | 0.248 | 53.3 |

The higher percentage of enzyme and proteolytic activity was found as 53.3% at 440 nm .

**MOTILITY**

Swarming motility is considered as one of the virulence factor because it is required for biofilm development and antibiotic resistance. The greatest inhibition of swarming motility was achieved.

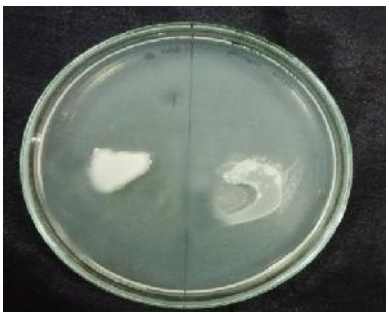

**FIGURE 7:** Swarming motility of Acinetobacterpittii(strain AB9)

Diameter of the stained growth (radius) of testedbacterial strain treated with the plant extracts was found to be less that of control (0.1 cm). Among the plantextractstested,*Eucalyptus globules.* revealed complete inhibition with 0 cm of stained growth followed by *Aeglemarmelos*(1.0 cm) (Al-Haidari *et al*., 2016).

## CONCLUSION

The present work has led to use the traditional medicinal plants to manage bacterial infections. The plant extract possess anti-qourum sensing properties in gram negative bacteria. we have identified three plants namely *Tamarindusindica*, *Carica papaya* and *Mimosa pudica*that afforded hydroalcoholic extract. They contained compounds with antimicrobial, antibiotic and antiquorum sensing activities. Targeting a Quorum sensing system is important for therapeutics and this may be used for effective treatment of biofilms related infections.

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