EVALUATING THE ANTIBACTERIAL ACTIVITY OF FLAVONOID EXTRACTED FROM FICUS BENGALENSIS

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ABSTRACT

Aim: Flavonoids are produced by plants in response to bacterial infection. Plants described in ayurvedic literature namely, Ficus benghalensis, Ficus glomerata possess significant amount of flavonoids as secondary metabolites. Flavonoids gained recent interest because of their broad pharmacological and antibacterial activity. An attempt was taken to extract flavonoids from F.beghalensis and the its antibacterial activity was analysed by appropriate methods. Methods: F.benghalensis was screened for phytoconstituents. The flavonoid was extracted from the plant. The Total Flavonoid content was determined by standard method. The antibacterial activity was determined by disc diffusion assay and Minimum Inhibitory Concentration methods. Results: The results of antibacterial activity revealed that flavonid extract of the plant exhibits good activity against all the gram positive and gram negative bacteria. The result was significant because the extract showed inhibitory activity against Staphylococcus aureus possessing β-lactamase activity. Conclusion: The flavonoid extract showed invitro antibacterial activity against almost all the tested bacteria except Staphylococcus aureus in lower concentrations. Minimal inhibitory concentration of methanolic extract of flavonoid was found to be significantly low for all the five bacterial strains. Our findings suggest that flavonoids have excellent antibacterial activity against several pathogenic bacteria like β-lactamase positive Staphylococcus aureus and gram negative bacteria.

Key words: Antibacterial, Ficus beghalensis, Flavonoid extract, MIC, TFC.

INTRODUCTION

Natural products are a rich source of bioactive compounds used for the treatment of wide range of human ailments.[1]. Even in light of new methodologies for screening large, diversity oriented small molecule libraries, natural products still provide a large number of lead compounds used for developing new drugs [2]. Natural product drugs include aromatic polyketides, polyethers, coumarins, flavonoids, terpenoids, alkaloids and aminoglycosides [3]. In a single species, dozens of different flavonoids may be present. Their increasing use led to the need for standardization and analysis of the natural products.

Flavonoids have been recognised as having a protective effect in plants against microbial invasion by plant pathogens.[4]. Flavonoid rich plant extracts have been used for centuries to treat human diseases. [5]. Isolated flavonoids have been shown to possess a host of important biological activities, including antifungal and antibacterial activities [6, 7, 8]. The potential of naturally occurring flavonoids as anti-infective agents has been recognized [9]. However, reports of activity in the field of antibacterial flavonoids research are widely conflicting, probably owing to inter and intra-assay variation in the susceptibility testing [5]. The present study was aimed at investigating the antibacterial activity of flavonoids isolated from the bark of Ficus benghalensis against five bacterial species.

In recent years, secondary plant metabolites (phytochemicals) previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [10].
Phytochemicals with adequate, antibacterial effect will be used for the treatment of bacterial infections [11]. It is time to examine more closely our natural resources, ie., the plants, which contain compounds of potential medical use. One such compound is flavonoids which appear to play a major role in the successful medical treatments of ancient times and their use has preserved till date [12]. Ventakamaran [13] also claimed that Moraceae family contains phytochemistry related to flavonoids, flavonoids with isoprenoid substituents and stilbenes. Flavonoids are a group of poly phenolic compounds possessing low molecular weight that exhibit a common benzo –r- pyrene structure. They are categorized into various subclass including flavones, flavonols, flavonones, isoflavonones, isoflavonoids, anthocyanidings, and catechins. [14, 15].

Flavonoids are a broad group of secondary metabolites with varied and important roles in plant physiology as well as they have gained recent interest because of their broad pharmacological activity. Putative therapeutic effects of many traditional medicines may be ascribed to the presence of flavonoids [16,17]. Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern [18]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens [19]. Multiple drug resistance has significantly increased in recent years. The existence of enzymes of extended-spectrum β-lactamases producing organisms that are resistant to virtually β-lactam antibiotics have been reported [20]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drugs because of the unmatched availability of chemical diversity.

**Ficus benghalensis**

*Ficus benghalensis* belongs to the family Moraceae, which is commonly known as Banyan tree. *F. benghalensis* are fast growing, evergreen tree found in monsoon and rain forests, grow up to 30 meters, with spreading branches and many aerial roots (figure 1). Leaves, stalked, ovate-corate, 3-nerved entire, when young downy on both sides; petiole with a broad smooth greasy gland at the apex, compressed, downy; Fruit in auxiliary paris, the size of a cherry (figure 3).

External features of the bark: Mature bark is 12-18 mm thick, grey, closely adhered ashy white, light bluish-green or grey patches, slightly curve, thickness varies with the age of the tree. Surface is deeply fissured and rough due to the presence of longitudinal and transverse row of lenticles, mostly circular and prominent, fracture short in outer 2/3 of bark while inner portion shows a fibrous fracture.[21] (figure 2)

**Taxonomic classification of Ficus benghalensis** (22)

- **Kingdom**: Plantae
- **SubKingdom**: Tracheobionta
- **Super division**: Spermatophyta
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Subclass**: Hamamelidae
- **Order**: Urticales
- **Family**: Moraceae
- **Genus**: Ficus
- **Species**: F.benghalensis
Figure 1. *Ficus benghalensis* – Fully grown tree

Figure 2. Dried barks of *Ficus benghalensis*
Chemical constituents of the plant studied

Preliminary phytochemical investigation of root of *F. benghalensis* showed the presence of carbohydrates, flavonoids, amino acids/proteins, steroids, saponins and Tannins [23]. The bark of the *Ficus benghalensis* contains leucopelargonidin -3-0-x-L rhamnoside and leuco cyanidin. 3-0-x-D galactosyl celllobioside, glucoside beta glucoside, 20-tetra tria conthene-2-one, 6- hepatatria contene-10-one, pentatricentan -5-one, beta sitosterol- alpha –D- glucose and mesoinositol [24, 25] and these are shown in figure 4,5 and 6.
Ethnobotany of the plant studied.

Roots of *Ficus benghalensis* shows antihelminthic activity. The extracts also reported to inhibit insulinase activity from liver and kidney. Fruit extracts exhibits anti-tumour activity. [26]. The fruit extracts of *Ficus benghalensis* exhibit antitumor activity and antibacterial activity, but no antifungal activity [27]. *Ficus benghalensis* used in Ayurveda for treatment of diarrhea, piles, teeth and skin disorders. The bark is used in inflammation, swelling at neck, gonorrhea, scabies mouthwash for tooth ache, and for strengthening gums, and steeped freshly burnt bark has been said to cure cases of obstinate hiccup. The latex [28] is used in inflammations and haemorrhages.

**Antibacterial activity of Ficus benghalensis**

In 2007, aqueous and ethanolic extracts of *F. benghalensis* were investigated for antibacterial activity against *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Alcaligenes faecalis* and *Salmonella typhimurium*. The ethanolic extract showed considerable antibacterial activity against *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Bacillus cereus*. It also showed certain antibacterial effects against *A. faecalis* and *S. typhimurium* but it was inactive against *S. aureus*. Aqueous extract of *F. benghalensis* had no antibacterial activity against any of the six bacterial strains investigated. From the results of experiment
it was concluded that ethanolic extract of F. bengalensis has great potential as antimicrobial compound against microorganisms and it can be used for the treatment of infectious diseases caused by resistant microorganisms [28]. Actinomyces viscosus belongs to group of Actinomycetes. It is gram positive, aerobic, non sporing rod shaped bacteria. It is frequently encountered in high proportion of smooth tooth surface and gingiva. Various experiments were performed to check the antibacterial activity of F. bengalensis against A. viscosus. These show that the extract of F.bengalensis bark of 0.08 mg/ml to 0.1 mg/ml have better antibacterial activity [29].

**Antimicrobial activity of Flavonoids**

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol [30]. Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection [31]), it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes [32]. Catechins, the most reduced form of the C3 unit in flavonoid compounds, deserve special mention. These flavonoids have been extensively researched due to their occurrence in oolong green teas. It was noticed some time ago that teas exerted antimicrobial activity and that they contain a mixture of catechin compounds. These compounds inhibited in vitro *Vibrio cholerae* O1, *Streptococcus mutans*, *Shigella* and other bacteria and microorganisms [33]. The catechins inactivated cholera toxin in *Vibrio* and inhibited isolated bacterial glucosyltransferases in *S. mutans* [34].

**MATERIALS AND METHODS**

**Plant material collection:**

The barks of *Ficus benghalensis* were collected from herbal garden of Gloris biomed Research center, Vadapalani. The plants authenticated identification done by Dr. S. Sankaranarayanan, Head, Department of Medicinal Botany, Sairam Siddha Medical College, Tambaram. The voucher specimens were submitted to Presidency College, Department of Botany. The voucher numbers are P.5123.

**Test organisms:**

The screening for antibacterial activity was carried out in vitro condition using *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus*. All bacterial strains were obtained from Madras Medical College, Chennai-600034.

**Preparation of Extracts:**

Aqueous and methanolic extracts of bark of *Ficus benghalensis* were prepared in 20g/200ml.The solvent of organic extract was dried at 60°C protected from light .The dried bark powder stored at 4°C until use.

**Phytochemical analysis of the plant extract**

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, flavonoids, alkaloids and glycosides in accordance with Allan(1976)[35] and Harborne (1998)[36] with little modification.

**I. Test for tannins:** About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

**II. Test for saponin:** About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

**III. Test for flavonoids:** Three methods were used to determine the presence of flavonoids in the plant sample [37,38]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H₂SO₄. A yellow colouration observed in each extract indicated the
The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

IV. Test for terpenoids (Salkowski test): Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H$_2$SO$_4$ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

V. Test for glycosides (Keller-Killani test): Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

VI. Test for Alkaloids

(A) Dragendorffs reagent: 8g of bismuth nitrates Bi (No3)3 5 H2o was dissolve in 20ml of HNo3 and 2.72g of Potassium iodide in 50ml of H2o. These were mixed and allowed to stand for deposition of KNo3 Crystals. The Supernatant was decanted off and made up to 100ml with distilled water. Procedure: To 0.5ml of leaf extract 2ml of HCl was added. To this acidic medium 1ml of dragendorffs reagent was added on, orange or red precipitate produced immediately indicate the presence of alkaloids.

(B) Mayers test: 1.36g of Mercuric chloride was dissolved in 60ml of distilled water and 5g of Potassium iodide in 10ml of water. These two solutions were mixed and diluted to 100 ml with distilled water. Procedure: 1.2 ml of plant extract was taken in a test tube and to this 0.2 ml of dilute HCl and 0.1 ml of Mayers reagent were added. Formation of yellowish Puff coloured precipitate indicates the presence of alkaloid.

Beta lactamase test:

*Staphylococcus aureus* is one of the major pathogens found on the mucous membranes and the skin of around a third of the population. Its extremely adaptable to antibiotic pressure. It was the first bacterium in which penicillin resistance was found MRSA was responsible for 37% of fatal cases of blood poisoning in the UK in 1999, up from 4% in 1991, half of all *S. aureus* infections in the U.S are resistant to penicillin, methicillin, tetracyline and erythromycin.

**Staphylococcal Beta-lactamase Detection Methods**

In order to perform iodometric slide method, one million units penicillin was dissolved in 1 ml sterile distilled water. The solution was divided into portions of 0.15 ml and stored at -20°C until use. On test day, iodine solution was added to penicillin solution and mixed. The mixture was dropped on the slide and bacteria were transferred to the solution. Solution and bacteria were mixed by loop and 4% sterile starch solution was dropped. If the purple color of the solution disappeared, bacteria were considered to be beta-lactamase positive [39]. In iodometric tube method, benzyl penicillin was dissolved in phosphate buffer, which was adjusted to pH 6. 0.1 ml of the solution was taken to microtitration plate. The solution was made cloudy with 3-4 colonies of bacteria. After 30-60 min, 20 µl of sterile 1% starch solution and iodine solution were added. If the color of iodine disappeared in 5 min, the isolate was considered beta-lactamase positive [40].

**Total flavonoid assay:-**

The total flavonoid assay was conducted according to Marninova et al. (2005)[41]. Total flavonoid assay was conducted using aluminum chloride colorimetric method. One milliliter of extract was added with 4ml distilled water in a flask. After that 0.3ml 5% NaNo2 was added. After 5min, 0.3ml of 10% AlCl3 was added. After the sixth minute, 2ml of IM NaoH was added. Then the mixture was diluted to 10ml adding 2.4ml distilled water. The mixture was mixed and the absorbance was measured at 510mm. total flavonoids content was expressed as mg catechin equivalents (CE)/g samples.
Isolation of Flavonoids

100gm air-dried bark of *Ficus benghalensis* was powdered and defatted with (60-80) petroleum ether (750ml). It was then extracted with 750ml – distilled ethanol in soxlet for 16hrs. The extract was filtered and concentrated in a rotary flash evaporator at 60°C. The concentrated ethanolic extract was poured into excess of distilled water with stirring and filtered. The filtrate that comprises water-soluble portion of extract was extracted in liquid – liquid extractor with Petroleum ether (60 - 80), Benzene & Ethyl Acetate. Ethyl Acetate extract was concentrated to a small volume and was kept in a refrigerator for 48 hrs, which yielded, yellow crystals. These crystals were dissolved in ethyl acetate and were tested for the presence of flavonoids [42].

Scheme of procedure adopted for the isolation of Flavonoids:

100 gm of air-dried bark powder

\[\text{Defatted with pet. Either (60-80)}\]

\[\text{Defatted dried powder} \quad \text{Pet. Ether extract}\]

\[\text{Extracted with distilled ethanol}\]

\[\text{Ethanolic extract}\]

\[\text{Poured into excess dist. Water with stirring & filtered}\]

\[\text{Clear aqueous portion (filtrate)}\]

\[\text{Extracted with pet. Ether (60-80)}\]

\[\text{Aqueous fraction} \quad \text{Pet-ether fraction}\]

\[\text{Extracted with benzene}\]

\[\text{Aqueous fraction} \quad \text{Benzene fraction}\]

\[\text{Extracted with ethyl acetate}\]

\[\text{Aqueous fraction} \quad \text{Ethyl acetate fraction}\]

\[\text{Concentrated & kept in refrigerator}\]

\[\text{Residue (Compound F)}\]
Antibacterial activity of flavonoid extracts:

The antibacterial activity was studied using the disc-diffusion Method [39]. Bacteria were grown overnight on Mueller Hinton agar plates, five young colonies were suspended with 5ml of sterile saline (0.9%) and the density of the suspension adjusted to approximately $3 \times 10^8$ colony forming units (CFU). The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar. The medium was allowed to dry for about 3 min before adding a sterile paper disc of 9mm diameter. Each disc was tapped gently down onto the agar to provide uniform contact. Compounds (50µg) were weighed and dissolved in 1ml of 7% acetone. Twenty microlitres of the compounds were introduced on each disc (five replicates) and 7% acetone alone served as a normal control. The plates were incubated at 37°C for 24 h; inhibition zones were measured and calculated.

Minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations of the extracts were determined by dilution method [43]. The strains were grown in Mueller Hinton broth to exponential phase with an $A_{560}$ of 0.8, representing $3.2 \times 10^8$ CFU/ml. Different dilutions of each extracts were prepared to give solutions of 25, 50, 75 and 100 µg/ml. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of $10^6$ CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% acetone used as bacterial control, 4.5 ml of uninoculated MH broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37°C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at 560 nm.

RESULTS

Table 1. Phytochemical screening of bark extract of Ficus benghalensis

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Phytochemical Constituents</th>
<th>Observation</th>
<th>Methanol extract of Ficus benghalensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendorff’s test Orange / red precipitate</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayers test Yellowish precipitation</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>Intense yellow colour</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkali Reagent</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>Green-blue colour</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KellerKialni</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Tannin</td>
<td>Bluish black colouration</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FeCl$_3$ test</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Saponins</td>
<td>Foam</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frothing test</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Terpenoids</td>
<td>Reddish brown at the interface</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salkowski test</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Anthraquinones</td>
<td>Pink colour</td>
<td>-</td>
</tr>
</tbody>
</table>

‘—’ Negative (absent) ‘+’ Positive (present)
Table-2 The Total Flavonoid content of methanol extract of *Ficus benghalensis*

<table>
<thead>
<tr>
<th>EXTRACT</th>
<th>CODE</th>
<th>TOTAL FLAVONOID CONTENT (mg qe/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic bark extract of <em>Ficus benghalensis</em></td>
<td>Bark FB</td>
<td>1.89±0.6</td>
</tr>
</tbody>
</table>

The total flavonoid content (TFC) was determined as mean ± SD for three replicate measurements.

Table-3 Antibacterial activity of flavonoid extract of *Ficus benghalensis* against bacteria. (disc diffusion assay).

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Concentration per disc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 μg/ml</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>9.1±0.56</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>14.03±0.55</td>
</tr>
<tr>
<td><em>Pseudomonas auruginosa</em></td>
<td>12.5±0.45</td>
</tr>
</tbody>
</table>

‘-’ No Activity Zone of inhibition in mm

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD for three replicates.

Table-4 Minimum inhibitory concentration (MIC) of flavonoid extract against bacteria.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 μg/ml</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.700±0.01</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>0.660±0.04</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.560±0.04</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>0.610±0.02</td>
</tr>
<tr>
<td><em>Pseudomonas auruginosa</em></td>
<td>0.500±0.02</td>
</tr>
</tbody>
</table>

Minimal Inhibitory Concentration was determined by measuring the turbidity of the bacterial culture that is the mean of triplicates ± SD of three replicates.

The bacterial strains were tested for their β-lactamase activity. *Staphylococcus aureus* and *Proteus vulgaris* showed positive results for the tests. *Ficus benghalensis* is rich in flavonoids.[44]. The total flavonoid content...
content (TFC) was calculated using catechin equivalent and tabulated in table-1. It was observed that *Ficus benghalensis* contained a very high amount of flavonoids as compared to *Ficus religiosa* in genus *Ficus*.

Flavonoids from *F. benghalensis* extracted by appropriate method. The total flavonoid content (TFC) was calculated and tabulated in table-1. The antibacterial properties of methanol extract of flavonoid was tested against five bacterial strains. Antibacterial activity of flavonoid extract was found out by disc-diffusion assay and Minimum Inhibitory Concentration (MIC). Results for antibacterial activity was calculated by measuring the zone of inhibition. The results for antibacterial activity were shown in table-2. MIC or minimal inhibitory concentration, is the lowest concentration of an antimicrobial compound that will inhibit the growth of the organism being tested [42] . The MIC was calculated to indicate the antibacterial potency of flavonoid extract.

The bacterial suspension was used as positive control and sterile saline added with uninoculated broth as negative control. Minimum inhibitory concentration results revealed that the OD value was higher in the control because bacteria caused turbidity. There was a gradual decrease in the optical density at higher concentration. The results were tabulated (3). The flavonoid extract showed potent antibacterial activity against *E. coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The extract was active against *Staphylococcus aureus* (β-lactamase positive) in higher concentration. However, the zone of inhibition was more than 12 mm. It showed good antibacterial activity.

**DISCUSSION**

With ever increasing momentum in the quest for newer antimicrobial agents, to counteract the spiralling bacterial drug resistance, plants are being increasingly explored in many parts of the world. These plants may offer a new source of potential activity against infective microorganisms [46]. We studied the antibacterial properties of flavonoid extract of *Ficus benghalensis*. This study shows *F. benghalensis* has significant antibacterial activity against most of the tested bacterial pathogens. Of these *S. aureus* and *P. aeruginosa* are important human pathogens with known potential for drug resistance. *P. vulgaris* is again important multi-drug resistant pathogen with potential for producing metallo-β-lactamases and extended spectrum β-lactamases. The MIC for *Proteus vulgaris* and *Bacillus subtilis* were extremely low followed by *Pseudomonas aeruginosa* and *E. coli*. The MIC of *S. aureus* was little high compared to other gram negative bacteria. The number of flavonoids inhibit the growth of *E. coli* and *Plasmodium falciparum* in vivo by targeting specific enzymes of fatty acid biosynthesis [47].

**CONCLUSION**

This article comprised of plant description, phytochemical constitution, total flavonoid content, antibacterial activity (disc diffusion assay, minimum inhibitory concentration) of *Ficus benghalensis* Linn. (moraceae), a medicinal plant found throughout India and also in Bengal. This plant has a great medicinal value as it has been reported to have versatile phytochemical constituents including flavonoids.

Flavonoids showed invivo antimicrobial activity against all the bacterial strains. This is the evaluation of the activity of flavonoids against β-lactamase producing *Staphylococcus aureus* At present, flavonoid-containing preparations are also produced for the treatment of stomach and duodenum disorders [48], as well as glaucoma, hemorrhagic retinopathy, and thyroid gland hyperfunction [49]. Thus, flavonoids may be considered potential therapeutic compounds for infections that may be caused by these pathogenic bacteria in the future. Therefore, further work is underway to identify the bioactive compound. Additionally, the antimicrobial activity of some antibacterials in combination with flavonoids against the pathogenic bacteria may also need to be evaluated for the treatment of infections.

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