Antibacterial activities of various sequential extracts of *Ficus racemosa* stem bark

Faiyaz Ahmed¹, Sharanappa P², Asna Urooj¹,

¹Department of Studies in Food Science and Nutrition, University of Mysore, Mysore - 570 006, India
²Department of Bioscience, University of Mysore, Post Graduate Centre, Hassan, India

Address for correspondence: Dr. Faiyaz Ahmed, Department of Studies in Food Science and Nutrition, University of Mysore, Manasagangotri, Mysore – 570 006, India. E-mail: fayaz_ahmed09@yahoo.co.in

**Abstract**

The present study evaluated the antibacterial activity of sequential extracts of *Ficus racemosa* stem bark against *Staphylococcus aureus* [MTCC 3160], *Bacillus cereus* [MTCC 1306], *Pseudomonas aeruginosa* [MTCC 1034], *Escherichia coli* [MTCC 1089] and *Bacillus subtilis* [MTCC 1133] by disk-diffusion and agar-diffusion methods. In disk-diffusion assay chloroform, acetone and methanol extracts showed moderate antibacterial against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis* compared to the positive control, while petroleum ether extract did not exhibit antibacterial activity against any of the organisms tested. Aqueous extract inhibited only *Bacillus subtilis*, while none of the extracts inhibited *Pseudomonas aeruginosa*. In agar-diffusion assay, both petroleum ether and aqueous extract did not show any inhibitory activity against any of the test organisms, while methanol extract showed moderate activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. Acetone extract showed moderate inhibition of *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* to some extent.

**Keywords:** *Ficus racemosa*, Antibacterial, Sequential extracts, Moraceae, MIC.

**INTRODUCTION**

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. Research on new antimicrobial substances from natural products must therefore be continued. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected on the basis of their ethnomedicinal use.

Different parts of *F. racemosa* have been shown to possess significant antibacterial activity. Mandal et al. evaluated various extracts of *F. racemosa* leaves for antibacterial potential against *Escherichia coli*, *Bacillus pumilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It was found that the petroleum ether extract was most effective against the tested organisms and the effect produced was significant and was compared with chloramphenicol, a known antibiotic. The 50% methylene chloride in hexane flash column fraction of the extract of the leaves of *F. racemosa* effectively inhibited the growth of *Curvularia sp*, *Colletotrichum gloeosporioides*, *Alternaria sp*, *Corynespora cassicola* and *Fusarium sp*.

With this background, the present study evaluated the antibacterial activity of the sequential extracts of *F. racemosa* bark against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* using disk-diffusion and agar-diffusion assay.

**MATERIALS AND METHODS**

**Chemicals and plant material**

*Ficus racemosa* stem bark was collected from Mukkadhally, Chamarajanagar district of Karnataka, India during September 2007, subsequently identified by Dr. Shivprasad Huded, JSS Ayurvedic Medical College, Mysore, and the voucher specimen [BOT-001/2008] was deposited at the
herbarium of Department of Studies in Botany, University of Mysore, Mysore, India. The bark was cut into small pieces, dried \([50^\circ \text{C}}\) and powdered, passed through 60 mesh sieve [BS] and stored in an air tight container at \(4^\circ \text{C}\) till further use. All the other reagents and chemicals used in the study were of extra pure analytical grade.

**Micro-organisms**

The five bacterial strains of Microbial Type Culture Collection [MTCC] namely, *Staphylococcus aureus* [MTCC 3160], *Bacillus cereus* [MTCC 1306], *Pseudomonas aeruginosa* [MTCC 1034], *Escherichia coli* [MTCC 1089] and *Bacillus subtilis* [MTCC 1133] were obtained from the Institute of Microbiological Technology [IMTECH], Chandigarh, India.

**Preparation of extracts**

The bark powder \([100 \text{ g}]\) was extracted sequentially with petroleum ether, chloroform, acetone, methanol and water in a soxhlet extractor by continuous hot percolation to yield sequential petroleum ether extract [FRSPE], sequential chloroform extract [FRSCE], sequential acetone extract [FRSACE], sequential methanol extract [FRSME] and sequential aqueous extract [FRSAE]. Each time before extracting with the next solvent of higher polarity the powdered drug was dried in a hot air oven below \(50^\circ \text{C}\) for 10 min. solvents were evaporated in a rotary vacuum evaporator and the dried extracts were weighed [Bhattacharya & Zaman, 2009].

**Antibacterial activity**

Antibacterial activity of various sequential extracts of *F. racemosa* bark was evaluated by disk-diffusion method employing 24 h cultures of five test organisms including two Gram-positive bacteria; *Staphylococcus aureus* and *Bacillus cereus*, three Gram-negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. The test organisms were inoculated into sterile nutrient agar medium by uniformly mixing 1 mL of inoculum with 20 mL sterile melted nutrient agar cooled to \(48^\circ–50^\circ \text{C}\), in a sterile Petri dish. When the agar solidified, eight holes of uniform diameter \([6 \text{ mm}]\) were made by using a sterile borer. Three volumes of each of the test solutions as well as standard solution [Chloramphenicol] and the blank [respective solvents] were placed in each hole separately under specific condition and the plates were then maintained at room temperature for 2 h to allow the diffusion of the solution into the medium. All the plates were then incubated at \(37^\circ \text{C}\) for 24 h and the zone of inhibition was measured.[5]

**Determination of minimum inhibitory concentration [MIC]**

Minimum inhibitory concentrations of various sequential extracts were determined using agar dilution assay. A loopful of the bacterial culture from the slant is inoculated in the nutrient broth and incubated at \(37^\circ \text{C}\) for 24 h. The fresh broth \([20 \text{ mL}]\) is seeded with 0.25 mL of the 24 h broth cultures and two fold serial dilution method is followed as described below. The test sample was dissolved in water or solvent to obtain \(10 \text{mg/mL}\) solution. A 0.2 mL of the solution of test material is added to 1.8 mL of the seeded broth and this form the first dilution.

1 mL of this dilution is diluted further with 1 mL of the seeded broth to produce the second dilution, and the process is repeated until six such dilutions are obtained. A set of tubers containing only seeded broth is kept as control and suitable solvent controls are also maintained. After incubation for 24 h at \(37^\circ \text{C}\) the last tube with no visible growth of the microorganism is taken to represent the minimum inhibitory concentration of the test sample which is expressed in \(\mu \text{g mL}^{-1}\).

**RESULTS**

The antibacterial activities of different extracts were indicated by the zone of inhibition [Table 1]. In disk diffusion assay FRSCE, FRSACE and FRSME showed moderate antibacterial against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis* compared to the positive control. FRSPE did not exhibit antibacterial activity against any of the organisms tested while, FRAE inhibited only *Bacillus subtilis*. None of the extracts exhibited antibacterial activity against *Pseudomonas aeruginosa*.

In agar-diffusion assay, both FRSPE and FRSAE did not show any inhibitory activity against any of the test organisms, while, FRSME showed moderate activity against *Staphylococcus aureus, Bacillus subtilis, and Escherichia coli*. FRSCE inhibited *Staphylococcus aureus, Bacillus cereus* and FRSCE inhibited *Bacillus subtilis* and *Escherichia coli* to some extent [Table 2].

The minimum inhibitory concentrations of various extracts for different bacteria are presented in Table 3. FRSCE and FRSME showed a MIC value of 100 \(\mu \text{g mL}^{-1}\) against *Escherichia coli*. FRSACE showed a value of 150 \(\mu \text{g mL}^{-1}\) for *Staphylococcus aureus* and *Bacillus cereus*, while FRSME showed a value of 150 \(\mu \text{g mL}^{-1}\) for *Staphylococcus aureus* and *Bacillus subtilis*.

**DISCUSSION**

Due to the continuous emergence of antibiotic-resistant strains there is continual demand for new antibiotics.
In many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives. Various solvent extracts from *F. racemosa* bark showed moderate inhibiting activity on disease causing Gram-negative and Gram-positive bacteria, the most inhibited being *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, and Escherichia coli*. This is particularly interesting from a medical point of view because these microbial agents are responsible for severe opportunistic infections. Our findings on antibacterial activity of *F. racemosa* bark could justify some ethnopharmacological uses such as against diarrhea and dysentery because we demonstrated good activity of this plant against some pathogens of the digestive tract. The antimicrobial activity of FRSACE, FRAME and FRSAE could be attributed to the presence of phenolic substances which is well documented.

These findings are in good agreement with a number of earlier studies, wherein antibacterial potential of *F. racemosa* against different bacterial strains are reported. Nair and Chanda reported that the ethanol extract of the stem bark was effective against *Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Bacillus cereus, Alcaligenes faecalis* and *Salmonella typhimurium* bacterial strains, indicating the scope to discover bioactive natural

### Table 1. Zone of inhibition [mm] for various micro-organisms

<table>
<thead>
<tr>
<th></th>
<th>Staphylococcus aureus</th>
<th>Bacillus cereus</th>
<th>Bacillus subtilis</th>
<th>Pseudomonas aeruginosa</th>
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</thead>
<tbody>
<tr>
<td>FRSPE</td>
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<tr>
<td>FRSME</td>
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<td>-</td>
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<tr>
<td>FRSAE</td>
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<td>6</td>
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<tr>
<td>Control</td>
<td>28</td>
<td>30</td>
<td>28</td>
<td>15</td>
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</tbody>
</table>

* Control: antibiotic disc containing 30 μg of Chloramphenical C

** discs diameter 5 mm, each disc impregnated to contain 4 μl [1 mg mL⁻¹] of solution.

### Table 2. Zone of inhibition [mm] for various micro-organisms

<table>
<thead>
<tr>
<th></th>
<th>Staphylococcus aureus</th>
<th>Bacillus cereus</th>
<th>Bacillus subtilis</th>
<th>Pseudomonas aeruginosa</th>
<th>Escherichia coli</th>
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</thead>
<tbody>
<tr>
<td>FRSPE</td>
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<tr>
<td>Cephalexin</td>
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<td>25</td>
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</tbody>
</table>

** Each well filled with 50 μl [1 mg mL⁻¹] of solution.

### Table 3. Minimum inhibitory concentrations of different *Ficus racemosa* bark extracts against various micro-organisms

<table>
<thead>
<tr>
<th></th>
<th>Staphylococcus aureus</th>
<th>Bacillus cereus</th>
<th>Bacillus subtilis</th>
<th>Pseudomonas aeruginosa</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRSPE</td>
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<tr>
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<td>FRSAE</td>
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** Each well filled with 50 μl [1 mg mL⁻¹] of solution.
products that may serve as leads in the development of new pharmaceuticals in order to address unmet therapeutic needs.\[9\] In another study the same authors reported that the ethanol extract of stem bark exhibited significant antibacterial activity against Pseudomonas aeruginosa, Proteus mirabilis and Bacillus cereus bacterial strains while the aqueous extract inhibited Streptococcus fecalis significantly\[10\] and the methanol extract exhibited significant antibacterial activity against Bacillus subtilis.\[11\]

Other parts of F. racemosa tree have also shown significant antibacterial activity. Mandal et al. evaluated various extracts of F. racemosa leaves for antibacterial potential against Escherichia coli, Basillus pumilis, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus. It was found that the petroleum ether extract was most effective against the tested organisms and the effect produced was significant and was compared with chloramphenicol, a known antibiotic, supporting the use of F. racemosa for treating dysentery and diarrhea in the traditional system of medicine.\[3\] The 50% methylene chloride in hexane flash column fraction of the extract of the leaves of F. racemosa effectively inhibited the growth of Curvularia sp, Colletotrichum gloeosporioides, Alternaria sp, Corynespora cassiicola and Fusarium sp.\[4\]

The reported wound healing property of F. racemosa may also be due to its ability to inhibit the growth of micro-organisms thereby preventing infection and accelerating the process of wound healing. This view can be supported from a study, wherein the ointment prepared from the F. racemosa leaf powder in an 8 mm full-thickness punch wound rat model showed highly significant generation of tissue DNA, RNA, and total protein during healing process in comparison with untreated control rats.\[12\]

From the results of this investigation, it is inferred that F. racemosa stem bark possesses potential antibacterial activity against certain micro-organisms. However, the results are not highly encouraging for the development and utilization of F. racemosa bark extracts as antibiotics, as the antibacterial activity exhibited by them was moderate and not excellent/exceptional. But, it is of interest, rather useful to use F. racemosa bark in infections of gastrointestinal tract caused by the bacterial pathogens, as the anti-diarrheal and gastroprotective activity of the bark is very well established.

ACKNOWLEDGEMENTS

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REFERENCES