MINIMUM INHIBITORY CONCENTRATION (MIC) ACTIVITY OF ACACIA CAESIA AND ACALYPHA FRUTICOSA

8.1. Preparation of Samples

As in antimicrobial studies, the methanolic and ethyl acetate extracts of leaf, stem bark and root parts of both the study species responded well, for MIC activity studies these extracts of respective plant parts were used against the selective bacteria and fungi. Each such solid extract obtained was collected aseptically in sterile microvials and stored at refrigerated temperature. The crude extracts were further diluted in sterile tubes by using the respective solvent. The concentrations of the extracts were 50, 100, 200, 400, 800 and 1000 μg/mL for bacteria and 1250, 1500, 1750 and 2000 μg/mL for fungi.

8.2. Antibacterial activity

8.2.1. Test culture used

 Cultures of the bacteria which were inhibited well viz., Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Serratia sp. were selected for MIC study.

8.2.2. Preparation of test culture

 The bacterial strains (1%) were inoculated aseptically into sterile nutrient broth and incubated at 37 ± 0.5°C for 18 hours. All the enriched cultures were optimized their population load by adjusting 1 mL of each culture to 1 OD at 360nm and further it was seeded into sterile nutrient agar medium to measure $10^6$ CFU (Colony forming unit) /mL.

8.2.3. MIC Assay

 The minimum inhibitory concentration was determined by the macro broth dilution methods (NCCLS, 1993; Okeke et al., 2001) at various concentrations (50, 100, 200, 400, 800 and 1000 μg/mL). The preparation of sterile broth inoculated with 1 mL of $10^6$ CFU of bacteria uniformly. The nutrient agar medium supplemented with various
concentrations of *Acacia caesia* and *Acalypha fruticosa* extracts and one mL of respective test organism was inoculated and incubated at 37°C for 24 hrs. After the incubation, the number of colonies formed was counted. Controls were maintained by adding the same quantity of solvent in the test culture (negative control) and test culture without solvent treated as positive control. Triplicates were maintained for all experiments.

8.3. Antifungal activity

8.3.1. Test culture used

The well inhibited fungal species *viz.*., *Rhizopus* sp., *Fusarium solani*, *Aspergillus niger* and *Mucor rouxii* were selected for MIC studies.

8.3.2. Preparation of test culture

Sterile potato dextrose broth was prepared for the fungal cultures and these cultures were seeded into the broth and incubated for three days. After incubation, these cultures were swabbed onto the sterile potato dextrose agar (PDA) medium under sterile condition. The plates were incubated for 3 days at room temperature. These cultures were used for assaying antifungal activity of various extracts of *Acacia caesia* and *Acalypha fruticosa*.

8.3.3. MIC Assay

For antifungal activities, the samples were evaluated through quantitative method described by Hirasawa *et al.*, 1999. MIC assay was carried out by determining the per cent inhibition of mycelial growth (PIMG) by the crude alcoholic extracts of *Acacia caesia* and *Acalypha fruticosa*. The crude extracts were added to PDA medium at the concentrations of 1250, 1500, 1750 and 2000 μg/mL separately. Agar discs (5 mm) were taken from 10 days old cultures of the above mentioned four human pathogenic fungi and placed in the center of the Petri plates separately. For controls, same size of agar discs of four fungi were placed in the same way on a fresh PDA plate separately which served as a positive control, and negative control was maintained by adding the same quantity of solvent in the test culture and incubated at 25°C for 3 days. Inhibitory activity was assessed by measuring the growth of mycelium on the treated.
media. The diameter (mm) of colony was measured after third day and documented. All pairings of cultures were carried out in three replicates.

8.4. Results

MIC assay of the bacterial and fungal cultures was carried out. The results obtained were as follows: The level of inhibition of colony growth of bacteria was observed to be varied across the extracts of plant parts and alcoholic solvents used (Tables 43 – 44 and Plates XV - XVI). Among the three extracts used (leaf, stem bark and root), the stem bark extract of both the the plant species showed better activity against all the bacterial cultures tested. Among the four bacterial cultures used, *Pseudomonas aeruginosa* was effectively suppressed by all the extracts of both the study plants. However, *Bacillus subtilis* and *Serratia* sp. showed little susceptibility against the low concentration of extracts used in the assay. Generally, *E. coli* showed resistance against all the extracts till 800 and 400 μg/mL for *Acacia caesia* and *Acalypha fruticosa* respectively. However, the extracts of all parts of *Acacia caesia* at higher concentration (800 μg/mL,) totally controlled the growth of *E. coli*.

As in bacteria, the inhibitory effect of plant extracts was also varied widely across the fungi tested (Tables 45 – 46 and Plates XVII - XVIII). Of the plants parts, root extracts showed significant level of inhibitory effect only after 1750 and 1500 μg/mL for *Acacia caesia* and *Acalypha fruticosa* respectively when tested against filamentous fungi. Methanol extract of leaf and ethyl acetate extract of stem bark of both *Acacia caesia* and *Acalypha fruticosa* species showed considerable inhibitory effect against the tested fungal cultures in the ascending series of *Mucor rouxii*, *Fusarium* sp., *Aspergillus niger* and *Rhizopus* sp. However, stem bark extract of *Acacia caesia* at 1500 μg/mL had shown less inhibitory effect over the fungi, *Rhizopus* sp. and *Aspergillus niger*.

8.5. Discussion

From the study it is known that the methanolic extract of leaf part of *Acacia caesia* and *Acalypha fruticosa* respectively at 800 and 400 μg/mL controlled the colonial growth of all the bacteria. Similarly, the stem bark and root parts of *Acacia caesia* and *Acalypha fruticosa* respectively at 800 and 400 μg/mL totally controlled the

69
bacterial colony growth. For antifungal activity, the methanolic leaf extract and ethyl acetate stem bark extract of *Acacia caesia* at 1750 μg/mL controlled the total mycelial growth of all the fungi tested. The ethyl acetate root extract of *Acacia caesia* at 2000 μg/mL is found to be optimum for checking the growth of all tested fungal species. On the other hand, for the other study species, *Acalypha fruticosa*, the minimum inhibitory concentration of methanolic leaf and ethyl acetate stem bark and root extract was determined to be 1750 μg/mL.

Generally, the methanol extract was more active than other extracts. This may be attributed to the presence of soluble phenolics and polyphenolics compounds (Kowalski and Kedzia, 2007; Shihabudeen *et al*., 2010; Canales *et al*., 2011). From our earlier studies, it is reported that these two study species also contained phenolic compounds in large amount (Anandakumar *et al*., 2009; Sathishkumar *et al*., 2009) which may be served as antimicrobial agents. The inhibitory effect of the extracts of *Acacia caesia* and *Acalypha fruticosa* against the pathogenic bacterial strains *viz*., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia* sp., and fungal species *viz*., *Rhizopus* sp., *Fusarium solani*, *Aspergillus niger* and *Mucor rouxii* can introduce the plants as potential candidates for drug development for the treatment of ailments caused by these pathogens.