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Evaluation of antimicrobial properties of some Sri Lankan lower plants using diffusion- and micro-dilution assays

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Natural compounds from lower plants are being explored throughout the globe for beneficial bioactive properties, though reports on such analyses in Sri Lanka are scarce. The objective of the reported study was to compare three standard antimicrobial bioassay techniques, namely disc- and well-diffusion and micro-dilution assays, in order to investigate and establish a suitable alternative procedure to screen extracts of lower plant species for antimicrobial properties as the diffusion-type assays were shown to have some limitations and were unsuccessful in prior experiments on crude extracts of some bryophyte species. Dried and powdered plant materials were soaked in a 1:1 mixture of methanol and dichloromethane for two weeks, and concentrated by completely removing the solvent and water. The screening of crude extracts for antimicrobial activity was done using Kirby-Bauer disk-diffusion assay, well-diffusion assay and a microtitre-plate based micro-dilution assay using resazurin indicator for microbial growth. The extracts were tested against four bacterial species and on the yeast *Candida albicans.* The antibiotics Ciprofloxacin and ketoconazole were used as positive controls for bacterial and fungal bioassays respectively. Mueller-Hinton II and Sabouraud dextrose growth media were used for bacteria and fungi respectively. For diffusion assays, at 1 mg and 5 mg per disc or per well strength, none of the extracts showed any significant inhibition zones. However, the same extracts when screened using the resazurin based micro-dilution method, exhibited different degrees of antibiotic activity against the test organisms, with *Selaginella wightii*, *Homaliodendron flabellatum* and *Pallavicinia lyellii* showing comparatively higher values against the bacterial species, compared to *C. albicans*. Minimum Inhibitory Concentration (MIC) of *S. wightii* and *H. flabellatum* extracts ranged between 0.26 and 1.25 mg cm^{-3}, for the bacterial species, while for the *P. lyellii* extract, it was between 0.2604 and 1.87 mg cm^{-3}. This study revealed that some Sri Lankan lower plant species possess potential antimicrobial properties. The results have also shown that, with regard to lower plant extracts, particularly of bryophytes, the resazurin based broth micro-dilution method could overcome some of the limitations associated with the diffusion-type assays used routinely, and could be effectively used to draw quantitative conclusions by determining the MIC values for natural antimicrobials of lower plants.

Keywords: Antimicrobial, lower plants, micro-dilution, MIC, resazurin

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