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Chemistry and bioactivity studies of some Sri Lankan flora

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Sri Lankan flora consists of over 3360 species of flowering plants and over a quarter of these species i.e. 830 are considered unique to the country. Out of these, 230 plants are found to be "rare". Even though different types of bioactivities have been detected in Sri Lankan plants, a large portion of Sri Lankan flora is yet to be explored. Hence the present study was carried out to evaluate some of the biological activities of several plant species including a few endemics which are under the threat of extinction. These plants were tested for antibacterial and antifungal activities followed by the isolation of pure compounds and structure elucidation. In addition, antioxidant activity and allelopathic activity were also detected.

Several *Calophyllum* species, *Garcinia xanthochymus*, *Hypericum mysorense* and few other plant species were collected from different parts of the country, air dried, ground to a powder and extracted with organic solvents. The prepared extracts were then subjected to preliminary screening for antibacterial and antifungal activity.

Thirty nine crude plant extracts were tested for antibacterial activity against both Gram positive and Gram negative organisms *Staphylococcus aureus* (NCTC 6571), *Enterococci faecalis* (NCTC 12697), *Pseudomonas aeruginosa* (NCTC 10662), *Klebsiella* PW *and Escherichia coli* (NCTC 10418) control strains using the Disk Diffusion method. The results indicated that none of the crude extracts were active against the Gram negative bacteria used in the study. Considerable activity against *E. faecalis* was observed only in three extracts while sixteen crude extracts were active against *S. aureus*. The active extracts were then subjected to further antibacterial screening against 17 Methicillin Resistant *S. aureus* (MRSA) strains isolated from hospitals. As MRSA have acquired resistance to virtually all of the antibiotics in clinical use and search for new antibiotic lead compounds from plants is of great interest. Our AntiMRSA studies showed that seven extracts were active against all the MRSA strains. Some of these active crude extracts had given a MIC values of 33.33 $\mu g\ mL^{-1}$ or less for several MRSA strains, which indicate the possibility of the presence of strong antibacterial compound/s. It is reasonable to assume the MIC value of such a compound to be much smaller and may be comparable with those of the currently used antibiotics.

Antifungal activity of forty four crude plant extracts was tested against plant pathogens *Aspergillus* and *Cladosporium*, using the disk diffusion method and TLC bioautography method respectively. The results indicated that only the methanol extract of root of *Calophyllum thwaitesii*, had shown activity against both fungi while eight crude extracts were found to be active against *Cladosporium* only. In addition, thirty six crude extracts were tested for antifungal activity against 20 strains of human pathogenic *Candida* using the disk diffusion method. However only the methanol extract of root stem of *C. thwaitesii* had shown positive results.

According to the preliminary investigations, the methanol extract of the root stem of *C. thwaitesii* has shown both antibacterial and antifungal activity. Therefore, emphasis was given to the isolation of biologically active compounds from the above plant extract and it was achieved by the activity guided fractionation.

Gravity column chromatography of the methanol extract of the root stem of *C. thwaitseii* yielded thirteen fractions. Of those, 5 fractions were found to be active against *Cladosporium*, and 3 were active against *Aspergillus*. Further fractionation was carried out for one of the active fraction (30 g) using hexane, ethyl acetate (EtOAc) and methanol which yielded 23 sub fractions. Further fractionation of the above sub fractions was carried out using PTLC and HPLC and were re-tested for antifungal activity. Due to their higher and close polarities, purification was found to be very difficult even with the help of HPLC. After purification, yellow crystalline products were obtained and identification of above compounds was carried out based on physical properties (spectroscopic data) and some chemical conversions. These pure isolates were tested for their antimicrobial activities in a similar manner to that of the preliminary investigation of the crude plant extracts.

In this investigation, the methanol extract of the root stem of *C. thwaitesii* has been shown to contain seven xanthones including 1,7- dihydroxyxanthone, which have previously been isolated from the bark and the timber of *C. thwaitseii*. For the first time 1-hydroxy-5-methoxyxanthone, 1-methoxy-5-hydroxyxanthone, 1,6-dihydroxy-5-methoxyxanthone, 1-hydroxy-5,6-dimethoxyxanthone, 1-hydroxy-7-methoxyxanthone and 1,5-dihydroxy-6-methoxyxanthone were isolated from *C. thwaitseii*. This is the first time methylated xanthones have been reported from *C. thwaitesii* even though some of the above methylated xanthones are reported from other *Calophyllum* species.

In addition to the above extract, the root inner bark of *C. thwaitesii* was successively extracted with cold n-hexane, CH_2Cl_2, EtOAc and methanol. The cold hexane extract (5 g) was subjected to column chromatography with hexane and CH_2Cl_2. Elution of

the column with 50% hexane in CH_2Cl_2 yielded bright yellow needles which was identified as Thwaitesixanthone, a well known xanthone present in *C. thwaitesii* and in several other *Calophyllum* species. This compound was also subjected to antimicrobial activity studies, but did not show positive results.

Even though the crude extract of *C. thwaitesii* root stem, had shown activity against *S. aureus* NCTC 6571, *E. feacalis* and MRSA strains at a concentration of 200 µg/disk, none of the pure compounds have shown antibacterial activity against any organism at the same concentration. This may be attributed to a synergetic effect of the compounds present in the crude extract. The results of the experiment carried out to evaluate the possible synergistic effect indicated that there was some sort of synergistic effect between these compounds for some MRSA strains. However the width of the inhibition zone was comparatively small. The sub fractions from which the compounds were isolated showed clear inhibition zones and it might be due to the presence of other minor compounds which were not detected or lost in the isolation procedure.

Antifungal activity studies of the pure compounds revealed that four compounds (1-hydroxy-5-methoxyxanthone, 1-methoxy-5-hydroxyxanthone, 1,6-dihydroxy-5-methoxyxanthone and 1-hydroxy-5,6-dimethoxyxanthone) were active against the two plant pathogenic fungi. MICs of these compounds were found to be in the range of 50- 200 µg / spot. None of the xanthones were active against any of the *Candida* strains and this might suggest the possibility of the inhibition of fungal enzymes such as cutinases that are present in the plant pathogenic fungi.

Qualitative and quantitative analysis of antioxidant activity of the above xanthones were carried out using the free radical reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH). The qualitative analysis indicated that, some of the xanthones were found to posses radical scavenging properties. However the spectrophotometric analysis followed by the calculation of EC_{50} values indicated that the pure compounds do not posses significant activity. Even the mixture of the above xanthones did not show significant activity, thus indicating no synergistic effect. However, the crude extract of the root stem of *C. thwaitesii* exhibited a good activity with a EC_{50} value of 57.9 which is even better than the EC_{50} value of the positive control, ascorbic acid (EC_{50} = 85.7) . The very high activity observed in the crude extract could be due to the presence of antioxidants such as phenolic acids, flavonoids, isoflavonoids, quinines etc. in addition to the presence of xanthones in the crude extract.

Apart from the above study, allelopathic activity of some plant extracts was detected using the 'Lettuce seed germination assay' which is widely used in the detection of allelochemicals, throughout the world. In this study the normal 'Lettuce seed germination assay' was slightly modified and sixteen crude plant extracts were screened. Preliminary observations suggested that *Cardiospermum halicacabum* shows seed germination inhibitory activity which might be due to the presence of allelochemicals that could be used as a source of natural herbicides in the future.

The present study could be used as an initial step in the search for new pharmaceuticals, fungicides and herbicides from plant secondary metabolites. Also the chemical analysis of these plants could be helpful in finding chemotaxonomic relationships between different plant families, plant genera and also between different species of a same genus. In addition, it is important to evaluate the hidden biological activities of Sri Lankan endemics before these plants disappear from the country.

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