CHEMISTRY AND BIOACTIVITY OF ENDEMIC PLANT GENUS *SCHUMACHERIA* AND VINCRISTINE AND VINBLASTINE FROM AN ENDOPHYTIC FUNGUS OF *CATHARANTHUS ROSEUS*

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The first part of the thesis describes the chemistry and the bioactivity of three species of the endemic genus *Schumacheria* namely, *S. castaneifolia, S. angustifolia* and *S. alnifolia.*

Hexane, dichloromethane and methanol extracts of the plant parts of *Schumacheria* were subjected to, antioxidant assay using DPPH (1,1-diphenyl-2-picrylhydrazine) stable radical, cytotoxic assay using brine shrimps (*Artemia salina*) and antimicrobial assays against *Staphylococcus aureus* (NCTC 8532), *Escherichia coli* (NCTC 10418) and *Aspergillus niger*. The extracts of *S. castaneifolia* flowers exhibited high antibacterial, antioxidant and cytotoxic activities. The dichloromethane and methanol extracts of *S. alnifolia* exhibited antifungal susceptibility against *A. niger*. The methanol extracts of *S. alnifolia* stem-bark and leaves showed antioxidant activity higher than that of α-tocopherol. The total polyphenol content, expressed as the gallic acid equivalent, was determined using Folin-Ciocaltue method. The methanol extracts of *S. alnifolia* stem-bark and leaf showed the highest polyphenol content closely followed by the methanol extracts of *S. castaneifolia* flowers.

The extracts of different plant parts of *Schumacheria* were subjected to several chromatographic fractionations and fifteen compounds were isolated. The structure elucidation and the bioactivity determinations were carried out and found; taraxerol, betulinaldehyde, betulinic acid, β-sitosterol, 3-O-α-L-arabinosyloleanolic acid and β-sitosterol-3-O-β-D-glucopyranoside were present in all three species. The extracts of *S. angustifolia* and *S. alnifolia* gave betulin. Betulonic acid, (6β)-Hydroxy-3-oxolup-20(29)-en-28-oic acid, sorbifolin and epicatechin were only found in the extracts of *S. castaneifolia* and kaempferol, 7-O-methylkaempferol, catechin and gallocatechin were isolated from the extracts of *S. angustifolia.*

(6β)-Hydroxy-3-oxolup-20(29)-en-28-oic acid exhibited antibacterial activity with minimum inhibitory concentration (MIC) value at 50 ppm against both *S. aureus* and *E. coli*. 3-O-α-L-Arabinosyloleanolic acid showed antibacterial activity (MIC, 75 ppm) and toxicity to brine shrimps (LC_{50}, 7.6 ± 0.6 ppm).

The HPLC quantification of catechin and epicatechin of the methanol extract of flowers of *S. castaneifolia* gave 2.3 ± 0.0 and 9.2 ± 0.1 mg, respectively, in 1.0 g of dry flowers.
The genus *Dillenia* was found to be closely related to the genus *Schumacheria* from the chemotaxonomy data based on the presence of oleanene-type triterpenoids.

The second part of the thesis describes the isolation of vinca alkaloids producing endophytic fungus, *Botryosphaeria laricina* (CRS1) from *Catharanthus roseus* and evaluation of factors that affect the production of vinca alkaloids in *B. laricina* (CRS1). Eight endophytic fungi from the fresh aerial parts of *C. roseus* were isolated and one of them, CRS1, produced vinca alkaloids. DNA sequencing of CRS1 gave a 100 % match with the GenBank accession number, KC509580.1, which is related to the *Botryosphaeria laricina* strain JAS6.

Growth of the fungus by fermentation using the CZ medium in the presence of the fresh plant extract of *C. roseus* (7.5 ml) in the dark for 20 days gave catharanthine (3.2 mg), catharanthinic acid (0.1 mg), N-demethylvinblastine (0.4 mg), vinblastine (2.8 mg) and vincristine (2.4 mg). Growth in the absence of the fresh plant extract of *C. roseus* (7.5 ml) or with the preheated (80 °C, for 15 min) plant extract (7.5 ml) did not produce vinca alkaloids.

The plant extract was fractionated by dialysis membranes through various molecular weight (MW) cutoff ranges. Vinca alkaloid production was observed with fractions above 20 kDa MW if dialyzed in buffer at 4 °C. The same fraction dialyzed without buffer at room temperature lacked production of vincristine indicating the sensitivity of enzymes in the biosynthetic pathway. Growth of *B. laricina* (CRS1) in the presence of light:dark (12:12 h), fructose (30.0 g l^{-1}), glucose (30.0 g l^{-1}), Cu^{2+} (0.1 mM) ions or L-tryptophan (0.1 %) and succinic acid (1 %), did not promote the production of vinca alkaloids.

In conclusion, the endophytic fungus *B. laricina* (CRS1) which has the ability to produce catharanthine was found to require some enzymes larger than 20 kDa MW present in the plant extract to enable the production of two vinca alkaloids vinblastine and vincristine.