HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHIC SEPARATION AND ANTIBACTERIAL ACTIVITY OF PROANTHOCYANIDINS FROM TEA LEAVES

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S. K. HETTIHEWA

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S. K. Hettihewa

Department of Chemistry

University of Peradeniya

Peradeniya

Sri Lanka

ABSTRACT

The thesis consists of two parts, Part I and Part II. Part I deals with the extraction, purification, and separation of proanthocyanidins from blister blight infected tea flush caused by *Exobasidium vexans* using Sephadex LH-20 and High Speed Counter current Chromatography (HSCCC) as an increase of galloylated proanthocyanidins after the infection has been reported.

The freeze dried acetone extract of the leaves from tea cultivar TRI 2025 was partially purified on Sephadex LH-20. Two methanolic extract fractions (F_1, F_2) and two acetone extract fractions (F_3, F_4) were obtained. F_4 fraction was subjected to further separation using HSCCC as it was the most active fraction, which had the highest yield of proanthocyanidins.

HSCCC resulted in five fractions PA_1-PA_5 with the solvent system hexane: ethyl acetate: methanol: water (1:5:1:5).Thin layer chromatography (TLC) was performed on these fractions, developed with the solvent system, ethyl acetate: water: formic acid (90: 05: 05) and visualized under dimethylaminocinnamaldehyde (DMACA) spray reagent. Retention factor values (R_f) of PA_1-PA_5 were recorded as 0.26, 0.35. 0.51, 0.69, 0.76 and 0.79.

The purity of the each proanthocyanidin fraction was evaluated by high-performance liquid chromatography (HPLC). Only PA_1-PA_3 and PA_5 were homogeneous fractions. Comparison of ^1H NMR spectral data of the proanthocyanidins isolated with those previously reported indicated highly complicated structures. ESI-MS suggested that PA_1 could be a trimeric digallate or a tetrameric flavan–3-ol. ESI-MS suggested that PA_2 may be a trimeric or dimeric proanthocyanidin while PA_3 was a dimeric digallate proanthocyanidins. These results indicate that galloylated proanthocyanidins are present in blister blight infected tea leaves.

Part II deals with the screening of the proanthocyanidins isolated from blister blight infected tea flush against selected human pathogens as i) the antibacterial activity of the catechins free proanthocyanidins from tea flush against selected human pathogens has not yet been reported and ii) the antibacterial properties would be increased due to the gallolylation of the compounds has also been reported.

Methanolic fractions (F_1 and F_2) and acetone fractions (F_3 and F_4) obtained from Sephadex LH-20 column were screened against NCTC and ATCC reference strains of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. Susceptibility tests were performed by the Muller Hinton Agar (MHA) well diffusion method and minimum inhibitory concentrations (MIC) of homogeneous proanthocyanidin fractions (PA_1-PA_3) were carried out by an agar double dilution method.

Pseudomonas aeruginosa and *Escherichia coli* were inhibited by neither of fractions F_1 - F_4 at any of the concentrations between 1-10 mg/ml. Acetone extract fractions F_3 and F_4 showed antibacterial activity against *Staphylococcus aureus* while F_4 demonstrated promising antibacterial activity even at lower concentrations. MICs of the purified fractions of poanthocyanidins were found to vary between 512 ppm-1024 ppm. 13 out of 25 *Staphylococcus aureus* strains showed that MIC value of PA_1 was at 1024 ppm while 19 out of 25 *Staphylococcus aureus* strains showed that MIC value of PA_3 was at 512 ppm. It can be concluded that those results suggest strong antibacterial activity of galloylated proanthocyanidins from tea leaves.