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**SEPARATION OF TEA FLAVAN-3-OLS BY HIGH-SPEED
COUNTER-CURRENT CHROMATOGRAPHY AND
ANTIOXIDANT AND ANTIFUNGAL ACTIVITY STUDIES**

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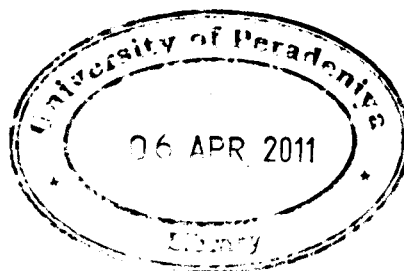
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SEPARATION OF TEA FLAVAN-3-OLS BY HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHY AND ANTIOXIDANT AND ANTIFUNGAL ACTIVITY STUDIES

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ABSTRACT

Theaflavins (TFs) in black tea and proanthocyanidins (PAs) found in fresh tea leaves are oligomeric and polymeric flavan-3-ols. Theaflavins (TFs) are orange coloured pigments found in black tea and are important compounds that determine the quality of black tea.

Theaflavin (TF), theaflavin-3-gallate (TF3MG), theaflavin-3'-gallate (TF3'MG) and theaflavin-3, 3'-digallate (TFDG) are the four major TFs found in black tea. The present study was carried out to determine whether high-speed counter-current chromatography (HSCCC) could be used to obtain pure reference samples of the four major TFs, Theaflavin (TF), theaflavin-3-gallate (TF3MG), theaflavin-3'-gallate (TF3'MG) and theaflavin-3,3'-digallate (TFDG). An *iso*-butyl methyl ketone (IBMK) extract of theaflavins prepared from black tea TRI dust no 1 sample was fractionated by HSCCC, without a preliminary clean-up step. The use of different combinations of n-hexane-EtOAc-MeOH-water only was investigated as the solvent system for HSCCC. The solvent systems n-hexane-EtOAc-MeOH-water (1:4:1:4 and 1:5:1:5) were selected as these were observed to be the most effective for separation of TFs. It was found that the two theaflavin monogallates, theaflavin-3-gallate, theaflavin-3'-gallate were not separated while theaflavin and theaflavin-3, 3'-digallate were obtained in high purity

after a single HSCCC run. Previous workers had achieved these same results after either using a preliminary clean up procedure or by separating standard samples of TFs.

Comparative studies of antioxidant activity performed with the IBMK theaflavin extract of black tea and the extract from one cup of black tea suggested that TFs in black tea contribute to the high antioxidant properties of black tea.

PAs found in tea flush have been implicated in the resistance of tea cultivars to infection by the fungus *Exobasidium vexans*, an obligatory biotrophic fungus which causes blister blight leaf disease of tea. An aqueous acetone extract of proanthocyanidins prepared from healthy tea leaves was partially purified using Sephadex LH-20 chromatography. The crude proanthocyanidin extract obtained was fractionated with high-speed counter-current chromatography (HSCCC) using the solvent system n-hexane-EtOAc-MeOH-water (1:5:1:5) which was found to result in the best separation. Five proanthocyanidins and two other flavanoids were separated in high purity by a single HSCCC run. NMR spectroscopy and comparison of data with those reported in the literature were used to establish their structures. The proanthocyanidins were found to be epigallocatechin gallate-(4 β →6)-epigallocatechingallate (46), epicatechingallate-(4 β →6)-epigallocatechingallate (47), epigallocatechingallate-(4 β →6)-epicatechingallate (48), epifazelechin-(4 β →6)-epigallocatechin gallate (49) and epicatechingallate-(4 β →6)-epicatechingallate (50). The two flavanoids were epigallocatechindigallate (45) and epicatechin digallate (51).

Pure proanthocyanidin and catechins isolated were used to determine their fungitoxicity against *E. vexans*. The spore fall technique showed that PAs and catechins from tea leaves inhibited spore germination of *E. vexans* on laboratory culture media. The crude proanthocyanidin and catechin extracts showed 100% inhibition of spore germination at 250 ppm. More than 95% inhibition was shown by the pure proanthocyanidin samples (PA1-PA4) that were isolated, while the pure catechin samples epigallocatechin gallate (EGCG) and epigallocatechin digallate (EGCDG) showed 100% inhibition at 128 ppm. Fungitoxic activity of pure catechin and proanthocyanidins against *E. vexans* has not been reported previously.