High-Speed Counter-Current Chromatography for the Isolation of Proanthocyanidins from Tea Flush

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Introduction

Monomeric flavan-3-ols (catechins) and oligomeric proanthocyanidins are important constituents of tea (*Camellia sinensis*) flush. Proanthocyanidins (PA's) are formed by the formation of inter-flavan links between monomeric catechins such as (+)-catechin and (-)-epicatechin. PA's are widely distributed plant defence compounds, and have a general toxicity towards fungi, yeast and bacteria (Dixon *et al.*, 2004). It has been postulated that the composition and nature of PA's may be related to the increased resistance of some tea cultivars to infection of tea flush by the fungus *Exabasidium vexans* (Punyasiri *et al.*, 2005), that causes Blister Blight leaf disease, the most serious leaf disease of tea in Sri Lanka. Tea is cultivated for its leaf, and tea flush is particularly susceptible to attack by *E. vexans* which attacks young and succulent tea leaves and stems.

High-speed counter-current chromatography (HSCCC) is a support-free liquid-liquid partition method that has been used for the separation of diverse groups of natural products. It is particularly useful for the fractionation of labile polyphenolic compounds which may undergo either spontaneous oxidation or oxidation by polyphenol oxidase. Pure samples were required for the assays of fungitoxic activity of PA's towards *E. vexans*. A method involving HSCCC was developed for the separation of PA's from tea flush. The present study resulted in the separation of five pure proanthocyanidin samples after a single HSCCC run.

Materials and methods

Collection of plant materials

Fresh tea shoots (two leaves and a bud) were collected from the experimental tea garden at the Tea Research Institute in Talawakelle.

Extraction of proanthocyanidins

An extract of PA's in aqueous 70% acetone was prepared from 1 kg of fresh tea leaves according to the procedure described previously (Tammer *et al.*, 1994) and purified by solvent partition and chromatography on Sephadex LH-20. The effluent from the column was concentrated on a rotar vapor and freeze dried to give the PA extract.

HSCCC - Solvent systems for HSCCC

Four different two phase solvent systems containing different ratios of hexane, EtOAc, MeOH and water were prepared. The two phase solvent system Hex (1). EtOAc (5). MeOH (1). H_2O (5) was selected for the separation of the PA extract on the basis of the partition coefficient and settling time.

HSCCC Model CCC-100 Pharma–Tech Research Corporation was used for the separation. The PA extract (200 mg) was injected into the HSCCC through the injection port and samples were collected at an elution speed of 2.0 ml/min.

High-Performance liquid Chromatography (HPLC)

The fractions collected were monitored by HPLC. HPLC instrumentation consisted of Waters Alliance 2690XE Separation module coupled to a Waters 996 photodiode array detector (PDA) and Waters Millennium 32 data system were used along with a Luna-5 µm Phenyl-Hexyl column, 4.6 mm × 250 mm (Phenomenx Inc, USA) with a guard column made of the same material. A linear gradient program of mobile phase A (9% acetonitrile containing 2% acetic acid) and mobile phase B (80% aqueous acetonitrile) was used for the analysis. Flow rate was 1.00 ml/min and the column oven temperature was maintained at 35 °C.

Results and discussion

Stationary phase retention of the solvent system [(Volume of the Stationary phase in the HSCCC column/Total volume of the HSCCC column) × 100%] selected was found to be 60-65% and gave a good separation. A single HSCCC run resulted in the separation of six fractions PA-1, PA-2, FL-X, PA-3, PA- 4 and PA-5. HPLC of the fractions indicated that PA-2, FL-X, PA-3, PA-4 and PA-5 proanthocyanidin fractions separated were very pure (> 95%) while PA -1 was a mixed fraction (Figure 1). HPLC retention times (given in minutes, within parentheses) indicated that PA-1 (24.856), PA-2 (25.519), PA-3 (26.498), PA-4 (27.315) and PA-5 (26.669) were proanthocyanidins. The nature of the FL-X (23.921), fraction has not been established as yet. The samples isolated are to be used to assess fungi toxicity. The five PA fractions are sufficiently pure for structure elucidation using spectroscopic methods of analysis and for fungi toxic assays.

Conclusions

HSCCC is a fast and efficient method for the separation of proanthocyanidins from tea flush.

Acknowledgments

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References

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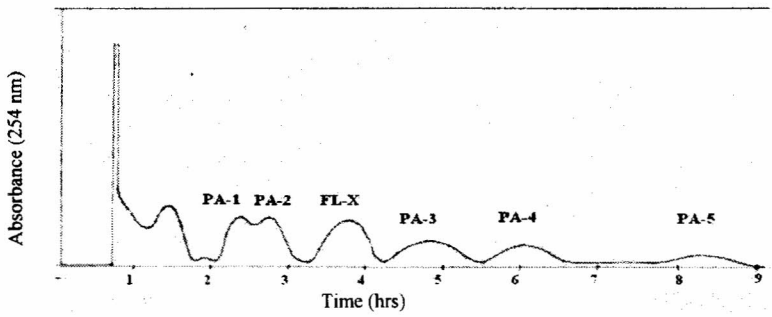

Figure 1. HSCCC of crude extract of proanthocyanidin