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Short communication

Separation of catechin constituents from five tea cultivars using high-speed counter-current chromatography

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Abstract

Catechins were extracted from five different tea (*Camellia sinensis* L.) cultivars. High-speed counter-current chromatography was found to be an efficient method for the separation of seven catechins from the catechin extracts. High-performance liquid chromatography was used to assess the purity of the catechins isolated. Epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and epigallocatechin (EGC) of high purity (91–99%) were isolated in high yield after a single high-speed counter-current chromatography run. The two-phase solvent mixtures used for the separation of the catechin extracts were hexane:ethyl acetate:methanol:water (1:6:1:6 for TRI 2023); (1:7:1:7 for TRI 2025 and TRI 2043); (1:5:1:5 for TRI 3079) and (1:6.5:1:6.5 for TRI 4006). Fresh tea shoots from the tea cultivar TRI 2023 (150 g) gave 440 mg of 96% pure EGCG while TRI 2025 (235 g) gave 347 mg of 99% pure EGCG and 40 mg of 97% ECG, and TRI 3079 (225 g) gave 432 mg of 97% pure EGCG and 32 mg of 96% pure ECG. Tea cultivar TRI 4006 (160 g) gave EGCG (272 mg, 96% pure) and EGC (104 mg, 90% pure). ^{1}H and ^{13}C NMR chemical shifts for catechin gallate (CG), EGC, ECG, EGCG and epigallocatechin 3,5-di-*O*-gallate (EGCDG) in CD_3OD were also recorded.

Keywords: Tea catechins; Flavan-3-ols; High-speed counter-current chromatography separation; Purity by high-performance liquid chromatography; ^{1}H and ^{13}C NMR

1. Introduction

Catechins (flavan-3-ol's) are the predominant components of young vegetative shoots of tea (*Camellia sinensis* L.). Tea catechins, which are responsible for most of the useful biological effects of tea [1–3] are powerful antioxidants that may help to offset chronic diseases such as cancer and cardiovascular diseases. Tea catechins have many other interesting effects including the ability to inhibit the biological activities of cariogenic streptococci such as *Streptococcus mutans* and *Streptococcus sobrinus* [4] and have been reported to be implicated in the reversing of methicillin resistance in the methicillin resistant 'super-bug' of *Staphylococccus aureus* [5]. Epicatechin gallate (ECG) and epicatechingallocatechin gallate (EGCG) from green tea leaves have been reported to exhibit 50% inhibition of the AIDS virus at 0.01–0.02 μg/ml [6]. Ready availability of pure samples of tea catechins would facilitate clinical and other studies that are required to exploit their biological properties. In this paper we report the results of a study carried out on the use of high-speed counter-current chromatography [7] to separate tea catechins. Catechin samples of high purity were isolated after a single high-speed counter-current chromatography run. Methods used previously for the separation of catechins include preparative Sephadex LH-20 column chromatography [8] and/or high-performance liquid chromatography [9]. These methods required large amounts of solvent, were time consuming and the catechin mixtures obtained required further chromatography to obtain catechin samples of high purity. Degenhardt et al. [10] separated green tea catechins using high-speed counter-current chromatography, but the purity of the catechins isolated was not reported. Three catechins of high purity were sepa-

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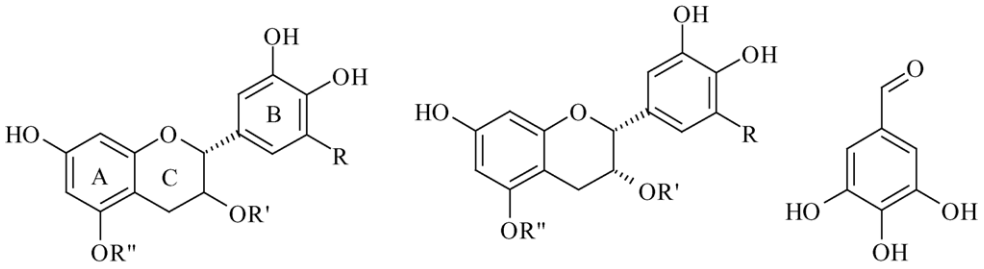

	R	R'	R''
Catechin(**1**)	H	H	H
CG (**2**)	H	G	H

	R	R'	R''
EC (**3**)	H	H	H
EGC (**4**)	OH	H	H
ECG (**5**)	H	G	H
EGCG (**6**)	OH	G	H
EGCDG (**7**)	OH	G	G

Fig. 1. Structure of tea catechins.

rated by Du et al. [11] using counter-current chromatography, while a low speed counter-current chromatographic system using a slowly rotating helical device, was used by Cao et al. [12] for the industrial separation of a crude tea extract.

In the present study catechins were extracted from five different tea cultivars and fractionated using high-speed counter-current chromatography [7]. Seven catechins (Fig. 1) including (−)-catechin (C, **1**), (+)-catechin gallate (CG, **2**), (−)-epicatechin (EC, **3**), (−)-epigallocatechin (EGC, **4**), (−)-epicatechin gallate (ECG, **5**), (−) epigallocatechin gallate (EGCG, **6**) and (−)-epigallocatechin-3,5-di-*O*-gallate (EGCDG,**7**) were isolated after high-speed counter-current chromatography. The identity of the catechins was established using ^{1}H and ^{13}C NMR. A significant result was the isolation of 440 mg of 96% pure epi-gallocatechin gallate (EGCG, **6**) from 800 mg of the ethyl acetate extract from the tea cultivar TRI 2023 after a single high-speed counter-current chromatography run. Similarly the tea cultivar TRI 2025 yielded 99% pure EGCG constituting 347 mg per 800 mg of ethyl acetate extract. The purity of the fractions isolated was estimated high-performance liquid chromatography. EGCG has been associated with many of the important biological effects of tea catechins.

2. Experimental

2.1. Collection of plant material

Fresh tea shoots (two leaves and a bud) of the tea cultivars TRI 2023, TRI 2025, TRI 2043, TRI 3079 and TRI 4006 were collected from the Tea Research Institute sub-station at Hantane, Kandy, Sri Lanka.

2.2. Preparation of catechin extracts

The crude catechin extracts from the shoots of cultivars TRI 2023 (150 g), TRI 2025 (235 g), TRI 2043 (85 g), TRI 3079 (225 g) and TRI 4006 (160 g) were extracted (5 min)with boiling aqueous 70% MeOH (500 ml × 2), concentrated on a rotavapor (40°) and partitioned with an equal volume of light petroleum (40–60). The aqueous layer was partitioned with EtOAc and the EtOAc layer was concentrated on a rotavapor (<40 °C). The sticky residue obtained was dissolved in water and freeze dried to obtain the catechin extracts as pale yellow solids from TRI 2023 (5.0 g), TRI 2025 (4.75 g), TRI 2043 (600 mg), TRI 3079 (6.0 g) and TRI 4006 (3.0 g).

2.3. Solvent system for high-speed counter-current chromatography

Two-phase solvent mixtures containing different ratios of hexane, EtOAc, MeOH and water were prepared (Table 1). Solvent mixtures which gave approximately equal volumes of each phase were used to determine the partition coefficient *K* for each catechin extract according to the procedure described by Ito and Conway [7]. A small amount of the cat-

Table 1
Two-phase solvent systems selected for HSCCC separation of catechin mixture isolated from each tea cultivar

Tea cultivar	Hex	EtOAC	MeOH	H_2O	*K*
TRI 2023	1	6	1	6	1.09
TRI 2025	1	7	1	7	1.00
TRI 2043	1	7	1	7	1.06
TRI 3079	1	5	1	5	1.06
TRI 4006	1	6.5	1	6.5	1.22