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Determination of Phenolic Compounds from Various Extracts of Green Tea by HPLC

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Catechins and flavonol glycosides were extracted from green tea with methanol, ethanol and water. Crude extracts derived from green tea were then fractionated using ethyl acetate. After investigation of tea extracts, the following four catechins and three flavonol glycosides were well separated and determined with HPLC analysis *i.e.*, (-)-epigallocatechingallate, (-)-epigallocatechin, (-)- epicatechingallate, (-)-epicatechin, quercetin-3-rhamnosylglucoside, kaempferol-3-rhamnosylglucoside and quercetin-3-glucoside. The content of catechins and flavonol glycosides in green tea extracts varied from 4.27 to 65.46 and 0.06 to 1.66 mg g^{-1} dry weight tea, respectively, depending on the solvent used. Water was confirmed to be most effective solvent for extracting catechins and flavonol glycosides from green tea.

Key Words: Green tea, Extraction, Catechins, Flavonol glycosides, HPLC.

INTRODUCTION

Green tea (*Camellia sinensis*) is an excellent source of polyphenol antioxidants[1]. Catechins are the most abundant polyphenols in the leaves of tea[2], which constitute up to 30 % of the tea solids by weight[3,4]. The major catechins in green tea are (-)-epigallocatechingallate (EGCG), (-)-epigallocatechin (EGC), (-)- epicatechingallate (ECG), (-)- epicatechin (EC)[5-7]. Although catechins are the dominant phenolic compounds, various flavonols (up to 4 %) and flavones (in traces) are also present in the tea leaves[8]. The main flavonols in tea are conjugates of quercetin and kaempferol with conjugating moiety varying from mono- to di- and triglycosides[9]. Tea polyphenols have various biological activities including antifungal, anti-inflammation, antimutagenic, antioxidative, anticarcinogenic, antitumor effects, lowering of plasma cholesterol and triglyceride levels and reduction of blood pressure and platelet aggregation in several systems[10]. Today, in addition to their traditional use for making tea, the leaves of *Camellia sinensis* are industrially processed. Extracts are used in dietary supplements and are added to an increasing range of products, such as beverages, nutrition bars, ice cream and even topical skin creams[11]. The use of tea extracts in edible oil systems[12-14] and cooked muscle foods[15,16] have been reduced the rate of peroxide accumulation, which improves product stability. All analytical methods for quantifying the biologically active compounds present in tea leaves involve extraction, separation and analysis[17]. Various extraction conditions and analysis methods have been used, resulting in a wide variation in the measured concentrations of tea compounds[8,18]. Sharma *et al.*[19] observed qualitative and quantitative differences in tea catechins and xanthine alkaloids extracted with different solvents *e.g.*, acetonitrile, water, methanol, aqueous methanol and acetone. On the other hand, information on the simultaneous analysis of green tea flavonol glycosides and catechins in the literature is lacking or poor. Therefore, the objectives of this study were to (1) determine phenolic compounds (catechins and flavonol glycosides) of various extracts derived from green tea by using methanol, ethanol and water and their ethyl acetate fractions; and (2) evaluate the effect of solvents on the catechins and flavonol glycosides contents.

EXPERIMENTAL

Green tea sample was purchased from a local market in Ankara-Turkey. Tea leaves were ground using a coffee grinder and sieved to obtain 150-300 μm particle size and stored at +4 ºC until used.

EC, EGC, EGCG and ECG were purchased from Sigma (St, Louis, Mo. USA). Kaempferol-3-rhamnosylglucoside (K3RG) was from Chromadex (Santa Ana, ABD). Quercetin-3-rhamnosylglucoside (Q3RG) was from Wako (Pure Chem. Co., Osaka-Japan). Quercetin-3-glucoside (Q3G), orthophosphoric acid, high performance liquid chromatogram (HPLC) grade acetonitrile and analytical grade ethanol (min 99.8 %) and methanol (min 99.9 %) were purchased from Fluka-Riedel-de Haën (BioChemica Fluka Cheme GmbH Buchs-Switzerland). Ethyl acetate (min 99.8 %) was from Merck (Darmstadt-Germany).

Extraction: Extraction was made according to the method as explained by in Erol *et al.*[20].

Fractionation: Fractionation was made according to Erol *et al.*[20] with some modification. The crude dry extracts were dissolved in 10 mL of distilled water. The water solution was partitioned with ethyl acetate in the ratio of 1:1 (v/v). The solvent was chosen as this is in common use to fractionate tea components[21,22]. The liquid-liquid extraction was carried out two times for 10 min by shaking on orbital shaker using a separating funnel and the organic phases were pooled. The combined ethyl acetate phases were dried with anhydrous sodium sulphate and filtered. The ethyl acetate phase was then evaporated under vacuum to dryness. The dry residue was re-dissolved in 50 % ethanol in water.

Determination of tea phenolic compounds: Content of tea phenolic compounds was determined by the previously validated HPLC method described by Turkmen and Velioglu[23] with some modification. The two mobile phases used for gradient HPLC elution were (A) 0.1 % *ortho*phosphoric acid in water (w/v) and (B) acetonitrile. The flow rate was 1 mL min^{-1}. The gradient elution profile was 8 % B (isocratic) for 10 min, B was gradually increased to 18 % at 57 min, to 24 % at 78 min and back to 8 % until 88 min. The column was re-equilibrated with the initial conditions for 15 min before the next injection. The injection volume was 20 µL. Each sample was filtered through a 0.45 µm PTFE membrane filter (Macherey-Nagel-Düren,Germany). Chromatographic peaks in the samples were identified by comparing their retention times and UV spectra with those of their reference standards and by co-chromatography with added standards. Quantification was performed from the peak area of each component and its corresponding calibration curve.

Preparation of standard solutions: Stock standard solutions were prepared dissolving in 80 % methanol to a concentration of 0.5-1.0 g L^{-1} and kept protected from light at -20 ºC for up to 2 months. Each stock solution was then used for the preparation of the diluted solutions (0.25-200 mg L^{-1}) for the calibration curves. Working standard solutions were injected into HPLC and peak areas were obtained. Calibration curves were prepared by plotting concentration *versus* area.

Statistical analysis: Statistical analysis was conducted with SPSS for Windows (ver.10.1) and experimental results were expressed as means ± standard errors of triplicate measurements. One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to test any significant differences among various treatments. Values of $p < 0.05$ were considered as significantly different ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Determination of phenolic compounds of greem tea: A typical HPLC profile of an extract from green tea is shown in Fig. 1. Phenolic compounds were well separated and four catechins (EGCG, EGC, ECG and EC) and three flavonol glycosides (Q3RG, K3RG and Q3G) were determined. EGCG was the most abundant catechin component in all tea extracts (Table-1), which is in agreement with the results from green tea[8,17,18,24-28]. But, the order of other three catechins and the flavonol glycosides varied depending on solvent used.

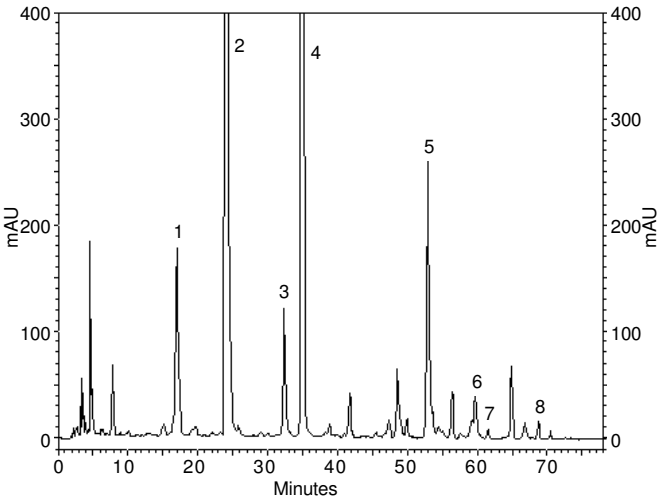

Fig. 1. HPLC profile of methanol extract from green tea at 270 nm, where 1-EGC, 2-caffeine, 3-EC, 4-EGCG, 5-ECG, 6- Q3RG, 7-Q3G, 8-K3RG

Effect of solvents on phenolic compounds of green tea: Tables 1 and 2 show the content of catechins and flavonol glycosides in the green tea extracts obtained using various solvents. Total catechins content of green tea ranged from 4.27 to 65.46 mg g^{-1} dry weight tea depending on extracting solvent used. This is in accordance with the findings by Friedman *et al.*[17]. They found that total levels of catechins for the 24 green teas extracted with 80 % ethanol and water ranged from 4.9-118.5 and from 3.4-83.1 mg g^{-1} dry tea, respectively. Unachukwu *et al.*[18], reported that total catechin content for green tea methanol and water extracts ranged from 32.23-141.24 and from 21.38-228.20 mg g^{-1} dry tea, respectively, which agrees partially with our results. Total flavonol glycosides of green tea ranged from 0.06 to 1.66 mg g^{-1} dry weight tea. However, Wang and Helliwell[29] found that the contents of flavonols (myricetin, quercetin and kaempferol) on a dry

TABLE-1
TOTAL AND INDIVIDUAL CATECHIN CONTENTS (mg/g DRY WEIGHT TEA) OF VARIOUS FRACTIONS OF DIFFERENT SOLVENT EXTRACTS FROM GREEN TEA

Phenolic fraction	Solvent	Catechins				Total catechins
		EGC	EC	EGCG	ECG	
Crude	Water	24.58 ± 1.44[a*]	7.08 ± 0.46[a]	29.38 ± 2.07[a]	4.42 ± 0.34[a]	65.46 ± 4.16[a]
	Methanol	17.71 ± 0.47[b]	5.42 ± 0.05[b]	25.95 ± 1.40[a]	4.54 ± 0.27[a]	53.63 ± 1.87[b]
	Ethanol	2.97 ± 0.13[c]	0.88 ± 0.02[c]	3.65 ± 0.24[b]	0.64 ± 0.03[b]	8.14 ± 0.42[c]
Ethyl acetate	Water	4.51 ± 0.09[a]	3.07 ± 0.15[a]	20.84 ± 0.86[a]	3.79 ± 0.18[a]	32.22 ± 1.14[a]
	Methanol	2.76 ± 0.13[b]	1.98 ± 0.17[b]	16.06 ± 0.59[b]	3.80 ± 0.19[a]	24.60 ± 0.92[b]
	Ethanol	0.44 ± 0.02[c]	0.36 ± 0.04[c]	2.82 ± 0.18[c]	0.65 ± 0.02[b]	4.27 ± 0.18[c]

*Different superscripts in the same column for each fraction indicate significant difference ($p < 0.05$).

TABLE-2
TOTAL AND INDIVIDUAL FLAVONOL GLYCOSIDE CONTENTS (mg /g DRY WEIGHT TEA) OF VARIOUS FRACTIONS OF DIFFERENT SOLVENT EXTRACTS FROM GREEN TEA

Phenolic fraction	Solvent	Flavonol glycosides			Total flavonol glycosides
		Q3RG	Q3G	K3RG	
Crude	Water	0.88 ± 0.08[a*]	0.28 ± 0.02[a]	0.50 ± 0.02[a]	1.66 ± 0.12[a]
	Methanol	0.47 ± 0.00[b]	0.20 ± 0.01[b]	0.26 ± 0.00[b]	0.93 ± 0.01[b]
	Ethanol	0.06 ± 0.01[c]	0.04 ± 0.00[c]	0.09 ± 0.00[c]	0.19 ± 0.01[c]
Ethyl acetate	Water	0.13 ± 0.00[a]	0.18 ± 0.00[a]	0.14 ± 0.00[a]	0.45 ± 0.01[a]
	Methanol	0.08 ± 0.01[b]	0.12 ± 0.01[b]	0.12 ± 0.01[b]	0.31 ± 0.02[b]
	Ethanol	0.01 ± 0.00[c]	0.02 ± 0.00[c]	0.03 ± 0.00[c]	0.06 ± 0.00[c]

*Different superscripts in the same column for each fraction indicate significant difference ($p < 0.05$).

weight base in green tea leaves ranged from 0.83 to 1.59, 1.79 to 4.05 and 1.56 to 3.31 mg/g for myricetin, quercetin and kaempferol, respectively, which is higher than the results from this study. Similarly, Perva-Uzunalic *et al.*[30] found that three flavonols, myricetin, quercetin and kaempferol, contributed 5.2 mg of flavonols per g of dry green tea leaves. The high levels of flavonols found by these studies is possibly due not only to higher extraction yield for these compounds because their extraction solvent was 60 % ethanol but also to total amount of non-glycosylated forms (aglycones) of the flavonols.

According to Tables 1 and 2, the contents of total catechins and flavonol glycosides of crude extracts were higher than those of ethyl acetate fractions. For both crude and ethyl acetate fractions, water extracts had the highest level of total catechins and flavonol glycosides followed by methanolic and ethanolic extracts. Our results confirmed previously published data[8] that extraction efficiency of green tea phenolics is highly dependent on the time of extraction and the solvents used. Similar findings were reported for green tea catechins[22]. Variations in the contents of these compounds from tea extracts can be attributed to the change in relative polarity of solvents used. The increase in the solvent polarity could enhance the solubility of tea polyphenol in the mixture[31].

Conclusion

Four catechins (EGCG, EGC, ECG and EC) and three flavonol glycosides (Q3RG, K3RG and Q3G) were determined simultaneously using HPLC. Total contents of catechins and flavonol glycosides analyzed from green tea varied significantly depending on the extracting solvent. Crude extracts from green tea had higher phenolic compounds than ethyl acetate fractions. The most efficient solvent for catechins and flavonol glycosides from green tea was found to be water in this extraction conditions.

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