여러가지 차로부터 카테킨 유도체의 분리

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Separation of Catechin Compounds

from Different Teas

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1. INTRODUCTION

Tea(*Camellia sinensis*), which is one of the most popular beverages consumed in the world, is processed in various ways, including non-fermented, semi-fermented and fermented [1]. Three types of tea are produced from the leaves - green teas, black teas, and oolong teas. While there are about 3000 variations of tea, all are derived from the *Camellia Sinensis*. Some herbal infusions, such as chamomile, are often referred to incorrectly as tea. These infusions normally contain leaves, flowers, and other dried ingredients from other plants.

Recent studies have shown that tea confers great beneficial effects to the health of consumers, including the effects of reduction of cholesterol, depression of hypertension, anti-oxidation, anti-microbial, protection against cardiovascular disease and cancer [2]. The main components consisted in green tea are polysaccharides, flavonoids, vitamins B, C, E, R-amino butyric acid, catechin compounds and fluoride. Among them, catechin compounds have been of focus for the strong sulfated effect and anti-cancer function. The pharmaceutical activities of the components have been studied [3-5]. The main catechin compounds found in various tea are (-) epigallocatechin(EGC), (+) catechin(+ C), (-) epicatechin(EC), (-) epigallocatechin gallate(EGCG), (-) epicatechin gallate(ECG) and other compounds. These catechin compounds have been proven to have a variety of physiological functions, such as these affecting duodenum, colon, skin, lung, breast, esophageal, pancreatic and prostate cancer [6-8]. EGCG exhibits stronger sulfated effects 20 of 30, and 2 to 4 times higher than vitamins C, E, and BHA or BHT,

respectively. When often used as sulfated agents in general cosmetics. Sulfated agents protect the vital cells by combining the free radicals before they react with the other vital cells. Basini et. al demonstrate that EGCG from green tea can negatively affect reproductive performances in swine by inhibiting granulosa cell proliferation. EGCG did not bind and intercalate with the dsDNA, suggesting that it must inhibit the enzymes by interacting with them directly. The same results were obtained with other tea catechin derivatives. Therefore, we advise that the use of EGCG as alternative for antibiotics in feeding supplementation should be carefully considered.

2. EXPERRIMENTAL

2-1 Chemicals

The Korean green tea used in this experiment was cultivated at Bosung (Chongnam, Korea). The Chinese green and another teas were purchased from Yanji market (Jilin, China). The standard chemicals of (-)epigallocatechin(EGC), (+) Catechin, (-)epicatechin (EC), (-)epigallocatechin gallate(EGCG), (-)epicatechin gallate(ECG) were purchased from Sigma(ST Louis, MO, USA). Acetonitrile and chloroform were analytical grade and from Duksan Co.(HPLC grade, Korea). Acetic acid(AA) was purchased from Duksan Chemical Co. LTD.(Ansan, Korea). Twice distilled water was filtered by decompressing pump(Division of Millipore, Waters, USA) and filter(FH-0.45 µm).

2-2 Instrumentation

The instruments used in this study were as follows: a 426 HPLC pump(Alltech Co., Korea), a 486 detector(M 7200 Absorbance Detector, Young-In Scientific Co., Korea), a Reodyne injection valve(20 μ l sample loop). The column used in this experiment was commercially available from RS-Tech(0.46×25 cm, C₁₈, 5 μ m, RS-Tech Co., Daejeon, Korea). The mobile phase applied was the binary system of A(water/acetic acid, 100/0.1, vol.%) and B(acetonitrile/acetic acid, 100/0.1, vol.%) from 90:10(A:B vol.%) to 70:30(A:B vol.%) in a linear gradient time of 30min at a flow rate of 1ml/min. The injection volume was 1 μ l and UV wavelength was set at 280 nm. Chromate(ver. 3.0 Interface Eng., Korea) connected to a PC was used as a data acquisition system. The extraction was concentrated with a rotary evaporator(Resona Technics, Switzerland).

3. RESULT AND DISCUSSIONS

Catechin compounds, abundant mainly in green tea, have chemo-preventive effects against carcinogenesis. In analytical separation, the optimum mobile phase for isolating EGCG from the extract consisted of 0.1% acetic acid in water/ ethyl acetate/acetonitrile, 87/1/12 vol.% with 1 ml/min of the mobile phase flow rate. Catechin compounds such as EGC, + C, EC, EGCG, and ECG migrated into the ethyl acetate layer and were collected. The retention times of ECG, + C, EC, EGCG, and EGC were 11.54min, 13.44min, 18.16min, 18.81min and 25.78min, respectively. Figure 1 shows the chromatogram of the extract of catechin compounds from Chinese green tea.

Compared to the Flower tea and Oolong tea, a sight amount of catechin compounds was extracted

from these teas. Similar amounts of ECG, + C, EC, and ECG was extracted from Flower and Oolong teas. But, the amount of EGCG from Oolong tea was more than of that from Flower tea above 3.5 times. All the catechin compounds in Flower and Oolong teas were greatly reduced in comparison with Korean and Chinese green teas. Nevertheless, in this case the very little contents of the catechin compounds were observed.

The amounts of the investigated catechin compounds in various teas calculated by calibration curve eq uation were listed in Table 1. The catechins in green tea make up a large percentage of the total amount of polyphenols. Certain catechins, especially epigallocatechin gallate(EGCG) are believedt o provide the most protection. Normally, the amounts of polyphenols, catechins, and EGCG has included 3 7~56%, 30~42%, and 10~13% of green tea solids, respectively . In this study, the total amounts of five catechin compounds and EGCG from Chinese green tea were 24.99% and 14.95%, res pectively. The total amounts of five catechin compounds and EGCG from Korean green tea were 11.64% a nd 6.14%, respectively. The full content of the catechin compounds from Chinese green tea was 249.92 mg/ g, so it was 2.15 times of that from Korean tea. The amount of the EGCG from Chinese green tea was as 14 9.53 mg/g, so it was 2.44 times of that from Chinese tea.

4. CONCLUSIONS

Follow the order of catechin compounds from high to low; Chinese green tea is first, and Korean gree n tea, Longjing, Oolong, Tieguanyin, Flower, Red, Jasmine, Indian black, Puerh, and Bamboo tea will have this order, respectively. It was suggested that many kinds of the catechin co mpounds was lost during manufacturing processes.

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Teas	(+) C	EC	ECG	EGC	EGCG	Total
Bamboo tea	3.27	4 85	4 22	1.68	0.20	14.22
Flower tea	3.55	5.85	5.87	3.19	3.50	21.97
Green tea (Chinese)	22.15	15.40	45.73	17.10	149.54	249.92
Green tea (Korean)	8.03	6.78	30.66	9.61	61.35	116.43
Indian black tea	3.40	4.86	3.77	2.75	0.21	14.99
Jasmine tea	3.39	4.68	4.26	2.78	0.93	16.03
Longjing tea	6.55	7.73	16.37	4.64	32.67	67.96
Oolong tea	3.54	6.55	7.34	4.26	12.36	34.05
Pu-erh tea	3.36	4.72	3.50	2.72	0.47	14.77
Red tea	4.23	4.68	3.95	2.79	0.83	16.48
Tieguanvin tea	3.79	5.98	6.51	4.12	10.46	30.86

Table 1. Amount of some catechin compounds from different teas



Figure 1. Chromatogram of the extraction of catechin compounds from Chinese green tea