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Comparative study of catechin levels from green tea, oolong tea and black tea product with various treatments

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Abstract

Background: Tea is a refreshing drink that contains polyphenol compounds, namely catechins that are used for medicine and cosmetics. This study was to assess the content of catechin compounds in green tea, oolong and black tea products from Indonesia, China and Taiwan.

Methods: Some tea products are brewed at varying temperatures (75 ± 2 ; 85 ± 2 and 95 ± 20 C) and times (5; 10 and 15 minutes). Identification of catechin compounds was carried out using chemical reagents and UV spectrophotometry. The level of catechin in tea products were analyzed by spectrophotometer at 280 nm wavelength.

Results: The results obtained indicate that green tea, oolong tea and black tea contain epigallocatechin-3-gallate (EGCG) compounds according to the color change based on chemical reagents and for UV spectrum analysis which has λ_{max} in the range 268-274 nm. The results of quantitative tests using UV-Vis spectrophotometry showed that the green tea samples gave the highest levels of catechins followed by oolong tea and black tea with brewing temperature at 95 ± 20 °C.

Conclusion: The catechin content of tea obtained from various products varies according to the type of processing method and the brewing temperature. Therefore, this study is expected to provide information related to catechin content to the public and researchers.

Keywords: Green Tea; Oolong Tea; Black Tea; Catechin; UV Spectrophotometry

1. Introduction

Tea is a refreshing drink that has long been recognized and is a favorite of Indonesians. Several chemical compounds in tea can give the impression of color, taste, and aroma that satisfies the drinker. So until now, tea is one of the most popular refreshing drinks. Apart from being a beverage ingredient, tea is also widely used for medicine and cosmetics [1].

Catechins are one of the secondary metabolites contained in tea leaves. Secondary metabolites are produced or synthesized in specific cells and taxonomic groups at a certain level of growth or stress. Catechin compounds also play a role in determining tea products' properties, such as taste, color, and smell. Catechin compounds, in their reaction with caffeine, protein, peptides, copper ions, and cyclodextrins, form several complex compounds closely related to taste and smell. Catechins are found in high concentrations in fresh tea leaves, rock rose leaves, green beans, red grapes,

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strawberries, apples, blackberries, cherry, black grapes, and green tea. The content of catechins will decrease in levels due to the withering process, enzymatic oxidation, grinding, and drying [2–5].

Tea catechins are antimicrobial (bacteria and viruses) [6], antioxidants, anti-radiation, strengthen blood vessels, promote urine secretion, and inhibit cancer cell growth [7–9] and decreased risk of disease metabolic [10,11]. This advantage can provide beneficial effects in the health areas, especially in the pharmaceutical industry. Apart from being an ingredient in tea drinks, it is also widely used for medicine and cosmetics. To obtain high catechins levels, it is necessary to research the effect of genotype and height because several types of plants show that genotype and size significantly affect plants' bioactive content [5, 10, 12].

Green tea is obtained without a fermentation process (enzymatic oxidation), which activates the phenolase enzyme in fresh tea leave [3]. Black tea is obtained through a fermentation process. In this process, most catechins are oxidized to theaflavins and thearubigins, an antioxidant compound that is not as strong as catechins [4]. Oolong tea is semi-fermented. Making and processing oolong tea is between green tea and black tea. Oolong tea is produced through a heating process that is carried out immediately after the leaf rolling process to stop the fermentation process. Therefore oolong tea is called semi-fermented tea [4, 5].

2. Material and methods

2.1. Materials

The materials were used consist of green tea; oolong tea; and black tea were collected from Indonesia, Taiwan and China. The chemicals used are aquadest, acetic acid, iron (III) chloride (FeCl₃), formaldehyde, catechins was purchase from Sigma Aldrich, and filter paper.

2.2. Sample Preparation

The tea samples were obtained from Indonesian and foreign brands. Each tea powder was weighed as much as 5 grams, then brewed with 100 mL aquadest at 75±2°C, then stirred using a magnetic stirrer with a variation of 5 minutes, 10 minutes, and 15 minutes, until the tea extract was obtained, then filtered. The same treatment was carried out at temperatures of 85±2°C and 95±2 °C.

2.3. Identification with Chemical Reagents

Each tea powder was weighed as much as 5 grams, then added with 100 mL of water and boiled for 15 minutes. After that, it is cooled and filtered to obtain the filtrate. Each filtrate was put in into a test tube and then added with 1 (% w/v) FeCl₃ solution. If a green-purple or black color was formed, this result indicated that the sample contained catechin. Furthermore, in the same way, it is carried out using a steasny reagent (formaldehyde 20% and 2: 1 concentrated HCl) added to the test tube containing each sample solution and heated, if an orange, a red precipitate is formed when the positive sample contains gallocatechin.

2.4. UV Spectrophotometric Identification

The sample solution and standard catechin solution (20 ppm) were scanned at a speed of 240 nm/cm over a wave length range of 200-400 nm using a 1 cm cuvette. The sample wavelength was compared with the standard wavelength.

2.5. Preparation of Catechin Standard Solutions

A total of 10 mg of pure catechins, dissolved using 10 mL absolute ethanol solvent in a 10 mL flask to obtain a concentration of 1000 µg/mL catechin solution, then pipette as much as 1 mL of 1000 µg/mL catechin stock solution and dissolved with ethanol p.a. in a 10 mL flask (concentration 100 µg/mL). After that, the catechin solution was dilute to make a serial concentration of 10; 20; 30; 40 and 50µg/mL with pipette from the stock solution (concentration 100 ppm) as much as 0.5; 1; 1.5; 2; and 2.5 mL and diluted with absolute ethanol solvent in a 5 mL flask. The serial concentration of standard curve were measured the absorbance using UV-Vis spectrophotometer at wavelength 280 nm.

2.6. Determination of Catechin Levels of Tea

Each of the steeping solutions of green tea, oolong tea, and black tea were piped as much as 0.5 mL, and the volume was sufficient to 5 mL with ethanol p.a. The absorption of the sample solution was measured using a UV-Vis spectrophotometer at a wavelength of 280 nm.

2.7. Data Analysis

Catechin levels in the sample were calculated based on the linear regression equation ($y = bx \pm a$) of the standard catechin curve. The catechin levels obtained were then statistically analyzed using SPSS version 17.

3. Results and discussion

In this study, three samples were used, namely green tea, oolong tea, and black tea. Each tea was obtained from Indonesia, China, and Taiwan. Several stages were carried out in a comparative study of catechin levels in green tea, oolong tea, and black tea. In the first stage, the process carried out is extraction by brewing using three temperature variations, namely at temperatures of 75 ± 2 , 85 ± 2 , and 95 ± 2 °C, and variations of time, namely 5, 10, and 15 minutes. This process aims to determine the effect of temperature and brewing time required so that the catechins found in tea can be maximally extracted.

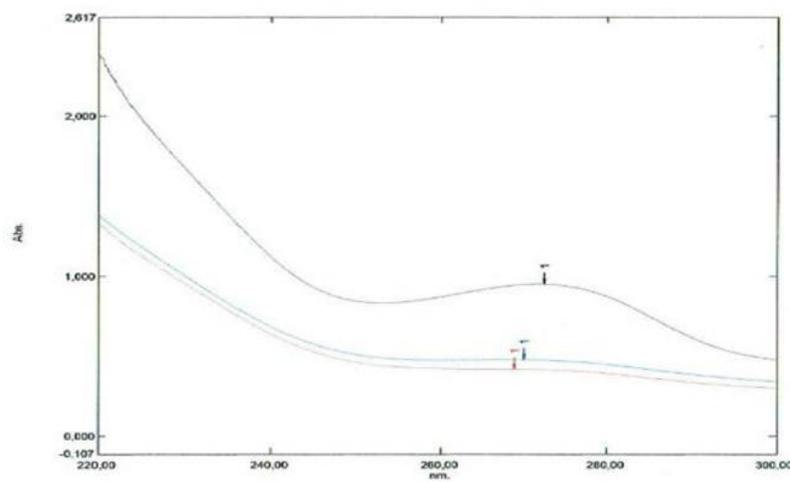
The next stage is the identification process of catechin compounds in the steeping water of green tea, oolong tea, and black tea. Catechin identification was carried out using FeCl_3 and steasny reagents (Formaldehyde 20 (%v/v) and Concentrated HCl 2: 1) as well as the UV spectrophotometer profile of each sample. Catechins are polyphenolic compounds that can form complexes between phenol (-OH) groups from the sample in the presence of Fe^{3+} (iron), characterized by the formation of a complex color change to green, blue, or red to black [13]. Table 1 shows the color changes that occurred in each sample using different extraction methods.

The results in Table 1 show that the sample gave a positive reaction to form a black-green and red-orange complex with the addition of 1 (%w/v) FeCl_3 reagent. This reaction indicates the presence of galliccatechin [13, 14]. These results are also reinforced by using a steasny reagent (formaldehyde 20%: HCl p (2: 1)) which showed a red-black colour change. The colour change of the sample shows that it contains catechin tannins [14, 15]. Based on the identification results using chemical reagents, it shows that in green tea, oolong tea and black tea from Indonesia, China and Taiwan contain positive catechins.

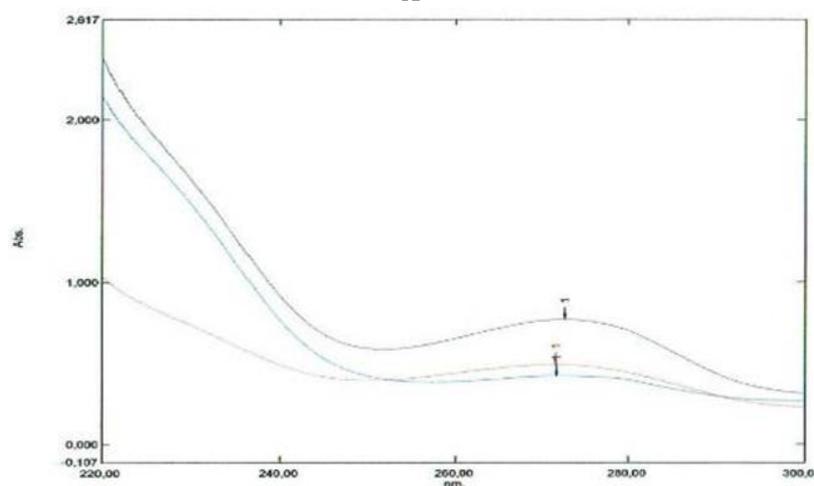
Table 1 Identification of catechins in black tea, oolong tea and green tea using chemical reagents and spectrophotometer UV.

<i>Sample</i>		Discoloration by Chemical Reagent			UV spectra	
		FeCl_3	Steasny	Result *	λ_{max} (nm)	Result **
<i>Black Tea</i>	1A	Blackish Green	Blackish Red Precipitate	+	277	EGCG
	1B	Blackish Green	Blackish Red Precipitate	+	274	EGCG
	1C	Blackish Green	Blackish Red Precipitate	+	268	ECG
<i>Green Tea</i>	2A	Blackish Green	Blackish Red Precipitate	+	275	EGCG
	2B	Blackish Green	Blackish Red Precipitate	+	274	EGCG
	2C	Blackish Green	Blackish Red Precipitate	+	273	EGCG
<i>Oolong Tea</i>	3A	Blackish Green	Blackish Red Precipitate	+	274	EGCG
	3B	Blackish Green	Blackish Red Precipitate	+	275	EGCG
	3C	Blackish Green	Blackish Red Precipitate	+	273	EGCG
<i>Standard</i>		Blackish Green	Red Orange Precipitate	+	280	Catechins

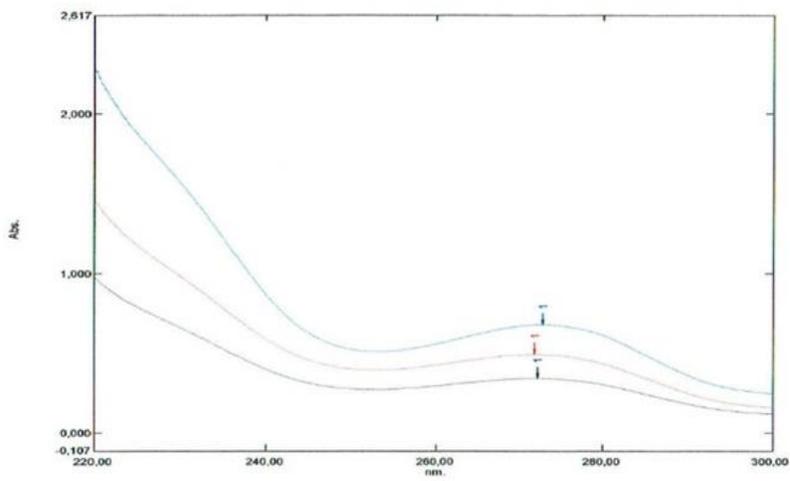
Sample 1A (Indonesian Black Tea), 1B (Chinese Black Tea), 1C (Taiwan Black Tea), 2A (Greentea Indonesia), 2B (Greentea China), 2C (Taiwan Green Tea), 3A (Indonesian Oolong Tea), 3B (Chinese Oolong Tea), 3C (Taiwanese Oolong Tea). * (+) Positive samples contain catechins (galliccatechin), ** (EGCG) epigallocatechin gallate and (ECG) epicatechin gallate



A



B



C

Figure 1 UV-Visible spectra of some tea samples. (A) black tea, (B) oolong tea, (C) black tea

The identification of the type of catechins in each tea sample was also carried out using a UV spectrophotometer. The absorption spectrum of various green tea, oolong tea and black tea products was measured using a spectrophotometer in the 200-400 nm wavelength range with the concentration of each sample 0.5 (% v/v). UV spectra were recorded in

the spectral bandwidth ranges of 2 nm and reading speed of 240 nm per minute. The analysis showed that there was a peak over the long range almost the same wave from each sample (Figure 1 and Table 1).

Figure 1A shows that the UV-spectra profile of some black teas with aqueous (steeping) solvent shows that the black tea in the aqueous solvent provides maximum absorption at λ_{\max} 271, 267, and 268 in black tea samples from Indonesia, Taiwan, and China. Figure 1B shows the UV-spectra profile of some green tea with aqueous solvent (steeping) and provides maximum absorption at λ_{\max} 272, 271, and 271 from Indonesia, Taiwan, and China respectively. Likewise, in Figure 1C, the UV-spectra profile of oolong tea with water solvent (steeping) provides maximum absorption at λ_{\max} 271, 272, and 273 in samples from Indonesia, Taiwan, and China respectively.

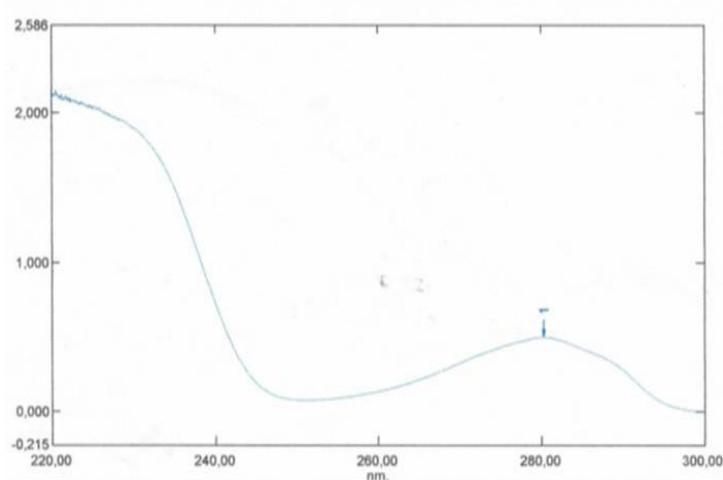


Figure 2 UV-Spectra Standard Catechin Curve

These results were similar to those of the catechins research by [16, 17] which is in the λ_{\max} range 270-274 nm in aqueous or ethanol solvent. Range these wavelengths indicate the presence of the compound EGCG class catechins. While the results are different found at pure standard λ_{\max} which gives maximum absorption at λ_{\max} 280 nm (Figure 2). The shift in wavelength can be caused by differences in the class of compounds where the standard used is pure catechins. The comparison process of catechin levels was carried out based on temperature and time treatment on catechin levels from the steeping of green tea, oolong tea, and black tea products from within and outside the country.

Comparison of catechin levels in tea samples used the linear regression equation for the catechin standard curve. The standard curve can be used to determine the catechin levels of of green tea, oolong tea, and black tea product. Standard solutions are made of concentration series, namely 0; 10; 20; 30; 40 and 50 $\mu\text{g/mL}$.

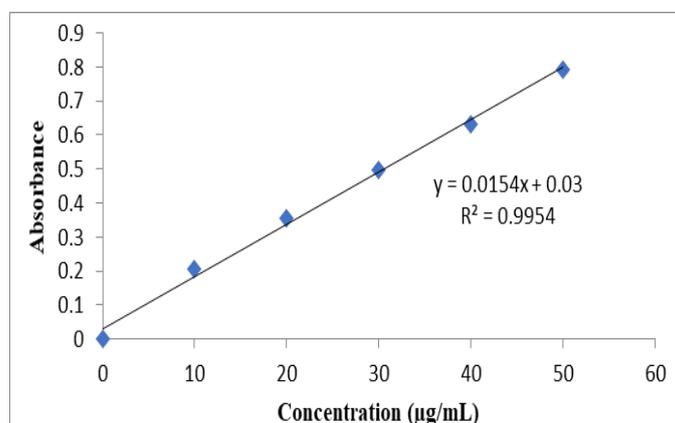


Figure 3 Standard curve of pure catechin

Based on the results of standard catechin measurements obtained the standard curve equation $y=0.0154x + 0.03$ with coefficient correlations (R^2) is 0.995. This equation was used to determine the levels of catechins from the tea sample. The data on catechin levels in green tea, oolong tea, and black tea product, which are equivalent to catechins (% g/100g).

Table 2 Levels of tea catechins from Indonesia with various treatments.

Product	Type of Tea	Temperature	Time	Content (g / 100 g)
Indonesia ^{bc}	Green tea*	75 **	5	32.05 ± 0.76
			10	24.08± 0.17
			15	26.94 ± 0.28
		85 **	5	27.08 ± 5.01
			10	28.46± 2.24
			15	19.90 ± 0.82
		95 **	5	32.05 ± 0.76
			10	24.08 ± 0.17
			15	32.25 ± 3.05
Indonesia ^{bc}	Oolong tea *	75 **	5	4.55 ± 0.28
			10	10.98 ± 0.18
			15	10.49 ± 0.14
		85 **	5	4.28 ± 0.14
			10	7.51 ± 1.01
			15	15.68 ± 0.82
		95 **	5	7.80 ± 0.81
			10	9.57 ± 0.02
			15	13.10 ± 0.77
Indonesia ^{bc}	Black tea *	75 **	5	4.77 ± 0.59
			10	7.61 ± 0.15
			15	5.24 ± 0.07
		85 **	5	7.14 ± 0.17
			10	10.97 ± 0.32
			15	7.84 ± 0.41
		95 **	5	18.10 ± 0.25
			10	17.53± 0.11
			15	16.11 ± 0.09

abc Alphabetical differences indicated a significant difference in catechin levels ($P < 0.05$) by tea product. * shows the significant difference in catechin levels ($p < 0.05$) by type of tea. ** shows the significant difference in catechin levels ($p < 0.05$) by temperature treatment

Table 3 Levels of tea catechins from China with various treatments.

Product	Type of Tea	Temperature	Time	Content (g / 100 g)
China ^{ac}	Black tea*	75 ^{**}	5	13.08 ± 0.70
			10	10.15 ± 0.51
			15	15.72 ± 0.02
		85 ^{**}	5	12.84 ± 0.49
			10	7.26 ± 0.46
			15	14.85 ± 2.62
		95 ^{**}	5	7.86 ± 0.48
			10	13.52 ± 0.43
			15	15.75 ± 0.008
China ^{ac}	Green tea*	75 ^{**}	5	12.07 ± 0.98
			10	24.08 ± 0.17
			15	11.71 ± 3.70
		85 ^{**}	5	10.03 ± 0.35
			10	28.46 ± 2.24
			15	10.31 ± 0.22
		95 ^{**}	5	27.61 ± 2.13
			10	24.08 ± 0.17
			15	17.98 ± 3.67
China ^{ac}	Oolong tea*	75 ^{**}	5	10.08 ± 1.85
			10	9.07 ± 1.26
			15	11.99 ± 1.70
		85 ^{**}	5	17.85 ± 2.94
			10	23.77 ± 0.72
			15	23.06 ± 1.50
		95 ^{**}	5	24.70 ± 1.55
			10	25.48 ± 1.15
			15	24.07 ± 1.03

abc Alphabetical differences indicated a significant difference in catechin levels ($P < 0.05$) by tea product. * shows the significant difference in catechin levels ($p < 0.05$) by type of tea. ** shows the significant difference in catechin levels ($p < 0.05$) by temperature treatment.

Table 4 Tea catechin levels from Taiwan with various treatments.

The place	Type of Tea	Temperature	Time	Content (g / 100 g)
Taiwan ^{ab}	Black tea*	75**	5	5.85 ± 0.80
			10	6.16 ± 0.42
			15	7.75 ± 0.25
		85**	5	7.42 ± 0.15
			10	7.33 ± 0.90
			15	7.44 ± 1.06
		95**	5	36.21 ± 0.51
			10	35.07 ± 0.23
			15	32.22 ± 0.19
Taiwan ^{ab}	Green tea*	75**	5	7.22 ± 0.86
			10	8.47 ± 0.94
			15	8.47 ± 0.94
		85**	5	10.31 ± 2.77
			10	19.90 ± 0.82
			15	19.90 ± 0.82
		95**	5	20.16 ± 0.86
			10	26.43 ± 2.08
			15	26.43 ± 2.08
Taiwan ^{ab}	Oolong tea*	75**	5	9.54 ± 1.19
			10	15.23 ± 0.30
			15	10.49 ± 0.14
		85**	5	14.28 ± 0.34
			10	21.95 ± 0.65
			15	15.68 ± 0.82
		95**	5	36.21 ± 0.51
			10	35.07 ± 0.23
			15	32.22 ± 0.19

abc Alphabetical differences indicated a significant difference in catechin levels ($P < 0.05$) by tea product. * shows the significant difference in catechin levels ($p < 0.05$) by type of tea. ** shows the significant difference in catechin levels ($p < 0.05$) by temperature treatment.

Based on the one way ANOVA statistical analysis showed that the treatment at temperature of 75 ± 2 , 85 ± 2 and 95 ± 20 C, there were significant differences in catechin levels in each treatment (Tukey, $p < 0.05$, $n = 3$). In contrast to the case of brewing time (5 10 and 15 minutes) it shows that statistically it does not have a significant effect on the catechin levels among in green tea, oolong tea and black tea samples from Indonesia, China and Taiwan (Tukey, $p > 0.05$, $n = 3$). When compared specifically the extraction results by steeping, it can be concluded that the green tea samples gave the highest catechin levels followed by oolong and black tea samples. In the green tea sample, which had the highest levels, was obtained from Indonesian products and the lowest levels of green tea were obtained from Taiwanese products. Whereas in the sample of oolong and black tea, the highest levels were obtained from Taiwanese products and the lowest levels were obtained from Indonesian products. The difference in catechin levels in tea is likely influenced by

several factors the type of tea variety, the height of the place where the shoots are produced, the effect of picking, and the processing in factory.

4. Conclusion

Tea products from Indonesia, Taiwan and China are predicted to contain a catechin class compound, namely EGCG. Green tea products have the largest levels of catechins compared to oolong and black tea products. The tea catechins from each product (Indonesia, Taiwan and China) provide high levels with a brewing temperature of 95° C. The higher the brewing temperature, the greater the catechin content of each tea. However, this research is limited to one tea product in each place so that it does not necessarily represent the catechin content in the product in each country.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors disclose that they have no conflict of interest.

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