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The Effect of Extraction Method on Catechin Levels in Green Tea (*Camellia sinensis* L.) Extract by TLC-Densitometric

Aktsar Roskiana Ahmad[1]*, Bahria Bahria[2], Harti Widiastuti[2]
[1]Phytochemistry-Pharmacognosy, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia
[2.3]Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia

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***Email:**
aktsar.roskiana@umi.ac.id

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Abstract

Green tea (Camellia sinensis L.*) is one of the most widely used plants in Indonesia. Green tea (Camellia sinensis* L.*) is rich in catechins which have many benefits including antioxidants, anticancer, can reduce stress, and reduce cholesterol levels in the blood. This study aims to determine the levels of catechins in Green Tea (Camellia sinensis* L.*) extract with various extraction methods. Each sample was extracted by maceration, microwave and infusion using aquadest solvent and the extract obtained in Freeze Drying. Qualitative analysis using 1% $FeCl_3$ reagent, positive catechins are indicated by a color change to blackish blue and quantitative analysis using TLC-Densitometry. The results showed the catechin content of the extract by maceration method was 331 mg/g.*

I. Introduction

Many native Indonesian plant ingredients are efficacious as drugs and their clinical properties can be justified. One of them is green tea leaves, green tea has long been useful in traditional medicine that has spread throughout the country.

Tea can be grouped into three types, namely green tea, oolong tea, and black tea (Khan & Mukhtar, 2013; T. R. et al., 2013). Green tea is made without fermentation by inactivating the enzyme polyphenol oxidase (Setiawan et al., 2021). Green tea is one of the most widely consumed plants in the world such as in the form of beverage preparations because the content of phytochemical compounds in tea leaves is known to function the cardiovascular system, reduction of body mass, decrease the risk of cancer, and neurodegenerative diseases (Prasanth et al., 2019). These phytochemical compounds belong to the polyphenolic group, which is a multi-phenol chain, green tea has a greater polyphenol content than black tea and oolong tea, especially in green tea shoots there are the largest catechin compounds around 50-80% (Fajriani et al., 2021; Musial et al., 2020).

Catechins are a class of polyphenolic compounds because of their large number of hydroxyl functional groups. Catechins and their derivatives such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), besides caffeine, tannins, theobromine, theophylline, and phenolic acids such as gallic acid are found in almost all types of tea (Khan & Mukhtar, 2013; Prasanth et al., 2019). In particular, the catechin compounds in tea leaves have properties such as antioxidants, anticancer, antitumor, reduce the risk of cardiovascular disease, reduce stress, and lower cholesterol in the blood (Musial et al., 2020; Prasanth et al., 2019).

Catechin in green tea have antioxidant properties that play a role in fighting free radicals that can cause various diseases (Nain et al., 2022) and in the research of Mukhtar and Ahmad (1999) polyphenol compounds in green tea, especially catechins have been shown to be used as preventive compounds. Cancer (Mukhtar & Ahmad, 1999; Prasanth et al., 2019). In measuring the levels of catechins in green tea, several methods can be used, including HPLC (High-Performance Liquid Chromatography), Spectrophotometry, and TLC-Densitometry.

An assay using a combination of TLC and Densitometry has high sensitivity, accuracy, specificity, and precision, and is quite economical because it uses a small mobile phase, a relatively short time, and can be assayed several samples simultaneously (Parys & Pyka-Pająk, 2022). Therefore, based on the description above, a study was conducted on the effect of extraction variations on catechin levels in green tea extract (*Camellia sinensis* L.) by TLC-Densitometry.

II. Research Method

II.1 Research Location

The research took place at the Pharmacognosy-Phytochemical Laboratory of the

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Faculty of Pharmacy, Universitas Muslim Indonesia and the Pharmacy Laboratory of Hasanuddin University.

II.2 Materials

Aluminum foil, acetic acid pa, aquadest, *n*-butanol pa, catechins, filter paper, TLC plate (E-Merck®) F_{254}, methanol pa, green tea simplicial (*Camellia sinensis* L.).

II.3 Extraction Process

II.3.1 Maceration

As much as 30 grams of green tea leaf powder was put into a maceration vessel, then 500 ml of distilled water was added and then allowed to stand for 2.5 hours at room temperature protected from light, while occasionally assisted by shaking with the help of a sonicator. Then filtered into a container vessel, then the dregs were re-extracted with 500 ml of distilled water as a solvent. Furthermore, the liquid extract obtained was concentrated by freeze-drying.

II.3.2 Microwave

One gram of extract was added to 15 ml of hot water. The solution is heated to boiling for 5 minutes and filtered. Five milliliters of filtrate are added with a few drops of $FeCl_3$ 1%, resulting in a violet-green color. The filtrate of 5 mL is added with gelatin 10% to form a white precipitate. Five milliliters of filtrate are added with NaCl-gelatin (1% gelatin solution in 10% NaCl solution) to form a white precipitate.

II.3.3 Infusion

Take as much as 30 grams of green tea simplicia powder, put in a beaker then add 500 mL of distilled water, then put it in the oven to carry out the extraction process for 15 minutes then filtered and re-extracted again with 500 mL of distilled water as a solvent. Furthermore, the liquid extract obtained was concentrated by freeze-drying.

II.4 Identification of Catechins by TLC

Identification of catechins in the extract was carried out using a TLC plate. The catechin standard and extract were spotted on a TLC plate, then eluted with *n*-butanol-acetic acid-water (5:1:4). After that, it was sprayed using 1% $FeCl_3$ reagent. Positive for catechins if it shows blue-black or dark blue spots (Sugihartini et al., 2012).

II.4.1 Assay by TLC-Densitometry

Eluent Manufacture

The eluent of *n*-butanol-acetic acid-water (5:1:4) was made as much as 30 mL, 15 mL of *n*-butanol was pipetted, 3 mL of acetic acid and 12 mL of water was pipetted into the chamber and then homogenized (Sugihartini et al., 2012).

Preparation of The Standard Solution of Catechins 1000 ppm

The comparison of catechins was pipetted 10 mg carefully and then dissolved with methanol pa in a 10 mL volumetric flask to the limit mark until a concentration of 1000 ppm was obtained.

Preparation of Sample Solution

For each sample of green tea leaf extract FD1, FD2, FD3 as much as 10 mg was dissolved in 10 mL of methanol pa.

Determination of Maximum Wavelength (λmax)

The maximum wavelength of catechins was carried out by running a standard solution of 200 ppm catechins in the wavelength range of 200-400 nm. The maximum absorbance obtained at a certain wavelength is the maximum wavelength of catechins (Sugihartini et al., 2012).

Preparation of a Standard Solution of Catechin Working Series

Stock solution 1000 ppm made a concentration of 200, 300, 400, 500, and 600 ppm. For a concentration of 200 ppm, a standard solution of 1000 ppm was pipetted as much as 1 mL, 300 ppm was pipetted with 1.5 mL, 400 ppm was pipetted with 2 mL, 500 ppm was pipetted with 2.5 mL, and 600 ppm is pipetted with 3 mL. then each concentration was filled with methanol pa in a 5 mL volumetric flask up to the mark.

Determination of Catechin Levels in The Sample

Preparing a TLC plate with a size of 10x10 cm, with the top edge marked 0.5 cm and the bottom edge marked 1.5 cm (Ahmad et al., 2020). From the standard solution that has been prepared, 2 L of each concentration was applied using a micropipette, then 2 L of extract from green tea leaves (*Camellia sinensis* L.) which had been dissolved in methanol pa was spotted using a micropipette as much as 2 L on a TLC plate. and replicated three times. The plates were eluted in the chamber with eluent *n*-butanol-acetic acid-water (5:1:4). Separate spots were observed with a UV lamp and measured by TLC-Densitometry and then analyzed for scan results (Malik et al., 2014).

III. Results and Discussion

In this study, an extraction method was used to separate the chemical components contained in green tea (*Camellia sinensis* L.). The extraction methods used are maceration, microwave and infusion methods using aquadest as a solvent.

Green tea plants are known to contain catechin compounds. Catechins in green tea are polyphenolic compounds belonging to the class of phenolic compounds, which have many hydroxyl functional groups. Catechins are also often classified as a flavonoid group because of their antioxidant properties (Fajriani et al., 2021; Nain et al., 2022; Prasanth et al., 2019).

Green tea leaf simplicia (*Camellia sinensis* L.) is first separated from the stem and then chopped and then blended, the aim is to expand the surface so as to facilitate the extraction process so that the maximum withdrawal of chemical components in

the sample can be obtained. Furthermore, the extraction process is carried out by varying the extraction method to see whether certain extraction methods have an influence on the amount of content obtained.

Extraction processes include extraction by maceration, extraction by microwave and extraction by infusion using the same solvent, namely aquadest. In the maceration process, the sample is soaked for 2.5 hours and occasionally shaken with the help of a sonicator, this process is shorter than other maceration processes, this is because the solvent used is aquadest, where if the sample is soaked longer it can cause mold growth so that it can damage extract, therefore this maceration process is assisted by shaking using a sonicator to speed up the process of withdrawing the chemical components in the sample. then filtered and remaceration using the same solvent (Abubakar & Haque, 2020). The advantage of this maceration method is that the operation is simple and the tools used are easy to obtain. Furthermore, extraction by microwave, the sample and aquadest were put into a beaker and then covered with aluminum foil, then put into a microwave oven, heated for 15 minutes, filtered and re-extracted with the same solvent. The advantage of the microwave method is that the process is also shorter, temperature control is better and can obtain a higher amount of extract content and does not damage the components of a substance (Delazar et al., 2012). Furthermore, extraction using an infusion, the sample, and aquadest that have been mixed in a beaker are put into the infusion vessel and then heated starting at 90^{o}C for 15 minutes. filtered and separated the residue from the dregs, The pulp is then re-extracted with the same solvent. The advantage of this infusion method is that the work is shorter and the tools are simple.

All extraction processes use distilled water because aquadest is very good for extracting polyphenolic compounds, where the sample to be extracted contains a lot of polyphenolic compounds. Polyphenol compounds are compounds that contain 2 aromatic rings with more than one hydroxyl group. stated that the more hydroxyl groups of a phenolic compound, the higher the solubility in water or the polar nature, so polar solvents and aquadest were chosen to be used in daily life when brewing tea (Fernández-Agulló et al., 2013; Giovanna et al., 2021).

Table 1. Extraction yield and % yield of green tea

Sample Green tea	Simplicia Weight (g)	Amount of Solvent (mL/L)	Extraction Results (g)	Extract (%)
Maceration Extract	30	1000 mL	5,320	17.73
Microwave extract	30	1000 mL	5.559	18.53
Infusion extract	30	1000 mL	5,694	18.98

All liquid extracts obtained were then freeze-dried to obtain a thick extract. Each extract with the amount of extract, the extraction by maceration was 17.73%, extraction by microwave was 18.53, and extraction by infusion was 18.98%.

The freeze-drying thick extract was then identified by qualitative testing to determine the presence or absence of catechin compounds in green tea by spotting standard catechins and extracts on a TLC plate and then eluted with eluent *n*-butanol-acetic acid-water (5:1:4). After that the plate was sprayed using $FeCl_3$ and the results obtained were in accordance with Figure 1, the sample was positive for catechins because it showed blackish-blue spots (Giovanna et al., 2021; Wang et al., 2009).

The stationary phase used is silica gel F_{254}, F_{254} which means it fluoresces in lamp 254 nm where it does not contain a binder but contains a fluorescence indicator. As for the mobile phase, the mobile phase used *n*-butanol-acetic acid-water (5:1:4) where the mixture of the three eluents could elute catechins well (Wang et al., 2009). Methanol was used as a standard solvent for catechins and extracts because it was known that methanol is a solvent that can dissolve both polar and non-polar compounds.

Determination of the maximum wavelength of catechins was carried out by running a standard solution of 200 ppm catechins at a wavelength range of 200-400 nm and obtained a maximum wavelength of 262 nm. So it is said that catechins can absorb electromagnetic radiation in the UV region so that they appear in UV lamps, this is due to the chromophore structure such as the C=C functional group and autochrome such as the OH functional group found in fluorescent catechins when exposed to UV light.

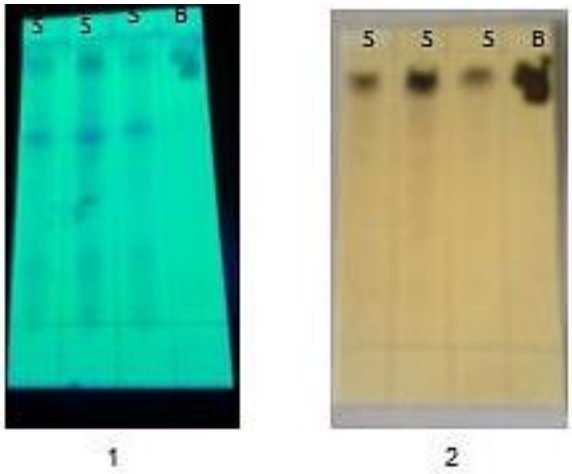

Figure 2. Qualitative identification of green tea extract (S) and catechin standard (B) densitogram profile in UV_{254} (1) and $FeCl_3$ spray (2)

Table 2. Result of the area of catechin standard solution

Amount of Catechin Standard Solution that is Spotted (µg)	Area
0.4	10532.8
0.6	17164.6
0.8	2344.5
1	23418.5
1.2	26334.4

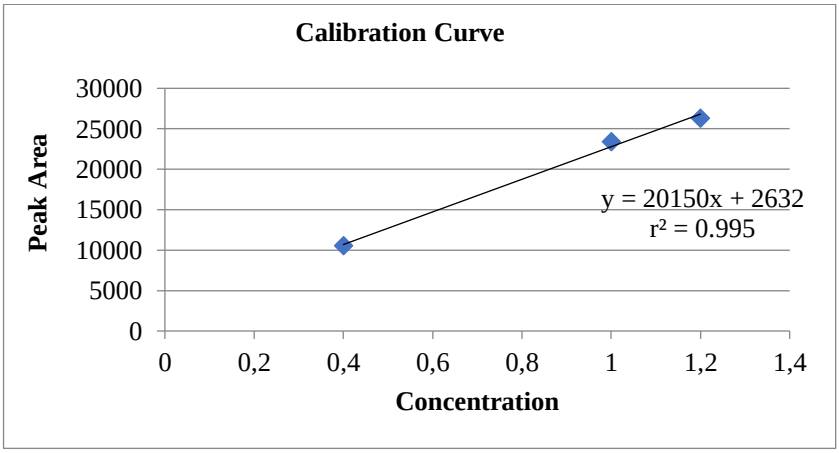

Figure 1. The linearity standard curve with r = 0.997, the regression equation y = 20150 x + 2632 and the calculation of the coefficient of variation of the function Vx0 = 4.6% is obtained.

Table 3. Results calculation of sample content of maceration extract, microwave extract, infusion extract

Sample 10mg/10mL	AUC	Catechin levels (mg/g)	Average levels of catechins (mg/g)
Maceration Extract	13312.2	265 mg/g	331 mg/g
	15912.9	329 mg/g	
	18665.8	397 mg/g	
Microwave extract	18417.9	392 mg/g	393 mg/g
	19707.4	423 mg/g	
	17328.8	364 mg/g	
Infusion extract	12090.5	234 mg/g	203 mg/g
	9557.1	171 mg/g	

Quantitative analysis of catechin levels in samples was carried out by spotting standard catechins and samples made on TLC plates with a size of 10 x 10 cm with an upper edge of 0.5 cm and a lower edge of 1.5 cm using a micropipette as much as 2 g then the plate was eluted in the chamber. with eluent *n*-butanol-acetic acid-water (5:1:4), then observed with a UV lamp and then measured with a densitometry tool and obtained a linear relationship between area and concentration as indicated by the value of the regression equation y = 20150 x + 2632 with the value of r = 0.995 (Figure 2).

IV. Conclusions

The results of the research concluded that the extraction method had an influence on the amount of catechin content in green tea extract (*Camellia sinensis* L.) by using the TLC-Densitometry method. The catechin content highest is the maceration method by 331 mg/g to another method.

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