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Determination of Total Phenolic with Different Tea Varieties by Spectrophotometry UV Visible

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Abstract

Tea has a high content of total phenolic with potent antioxidant activity and beneficial for health. This study aimed to determine the total phenolic content in tea with three varieties such as green tea, black tea and Roselle tea. The phenolic compounds in the dried tea were extracted by maceration method with aquadest as solvent. Total phenolic content was determined by spectrophotometry UV visible method with Folin Ciocalteau reagent. The complex of phosphotungstate- phosphomolybenols were reduced by phenolic compounds in an alkaline condition that could be measured by spectrophotometry UV visible. Gallic acid was used as a standard for total phenolic. Total phenolic levels of green, black, and rossella tea were 0.18%, 0.19% and 0.12%, respectively. It can be concluded that total phenolic of black tea higher than total phenolic of green and Roselle tea.

Keywords: green tea, black tea, Roselle tea, total phenolic, Folin Ciocalteau method

Introduction

Tea, an aqueous extract derived from *Camellia sinensis* plant, is one of the most widely consumed beverage in the world. Based on the manufacturing process, teas are classified into several groups such as non-fermented (green tea and Roselle tea), semi-fermented (oolong tea) and fully fermented (black tea) (Chan et al., 2011). Black tea is consumed mostly in North America, Europe and South Asia, where as green tea and Roselle tea are consumed mainly in East Asian countries (Hossain et al., 2014). Numerous studies have reported that drinking tea give various physiological and pharmacological benefits which include, antidiabetic, anti-inflammatory, antioxidant, anticholesterolemic, antimutagenic, anticarcinogenic and antibacterial activities (Zhao et al., 2014).

The active components playing key roles in most of the biological activities of tea are known as catechins (also known as total phenolic). Moreover, total phenolic are well-known for possessing antioxidant properties. The presence of different

forms of catechins and their derivatives in both green and black teas made them capable of working as potential antioxidants (Taheri and Sariri, 2011). Numerous studies have indicated that tea catechins and total phenolic are effective scavengers of free radicals or reactive oxygen species generated due to various oxidative stress. We have recently reported that tea extract prevents damage of oxidative stress (Akter et al., 2015). Given the importance of the function from total phenolic compounds as antioxidants, the total phenolic content levels contained in green tea, black tea and Roselle tea should be performed. Thus the utilization of tea can be more leverage to serve as an alternative treatment of herbs in the healing of various diseases. By looking at the total phenolic content contained in the tea extract it can be estimated large antioxidant activity. Therefore, this study aims to compare the total phenolic contents of three varieties such as green tea, black tea and Roselle tea.

Material and Method

Sample Preparation: Dried green tea crushed into a coarse powder then weighed 3 g then extracted using 25 mL of distilled water at 90 °C for 45 minutes. Furthermore, separated by filtration. Followed by the same treatment for black tea and Roselle tea samples.

Preparation of 100 ppm induct solution of gallic acid: 0.025 grams of gallic acid in a beaker was dissolved with a small amount of distilled water and transferred in a 250 mL measuring flask. The solution was diluted with distilled water to a precise mark of border and shaken until homogeneous.

Preparation of the concentration of gallic acid 20; 30; 40; 50; 60; 70; 80; 90; 100 ppm: 100 ppm induct solution of gallic acid was taken as much as 5; 7.5; 10; 12.5; 15; 17.5; 20; 22.5; 25 mL and put in a 25 mL measuring flask. The solution was diluted with distilled water to a precise mark of border and cornered to homogeneous.

Make curve calibration of gallic acid standard: Solution was made from a blank solution of different concentrations proportionally that is 0; 2; 4; 6; 8; 10 ppm. The amount of volume taken from the 100 ppm solution were 0; 0.5; 1.0;

1.5; 2; 2.5 mL put in a 25 mL measuring flask. The solution was diluted with aquadest to the limit mark and cornered to homogeneous.

Preparation solution of Na_2CO_3 7,5%: 3.75 g of Na_2CO_3 crystals was weighed in a beaker and added 5 mL of distilled water. The solution was removed in a 50 mL measuring flask and diluted with aquadest to the limit and then cornered to homogeneous.

Determination of Total Phenolic on Sample Solution: 1 mL sample of green tea extract put into a 25 mL measuring flask and added with 2.5 mL of 10% Folin-Ciocalteau reagent, shaken out and allowed to stand for 4-8 minutes. Added 2.5 mL of Na_2CO_3 7.5% then shaken out and silenced for 1 hour dark room then measured its absorbance using UV-Visible spectrophotometer at maximum specified wavelength. Repeated for samples of black tea and Roselle tea.

Result and Discussion

The total phenolic test was performed using Folin-Ciocalteau reagents containing a mixture of sodium tungstate, sodium molybdate, lithium sulfate, concentrated hydrochloric acid, 85% phosphoric acid, bromine, and distilled water. The Folin-Ciocalteau reagent was used because the phenolic compound can react with Folin to form a color solution which can be measured for its absorbance at certain wavelengths. This reactant oxidized phenolics (alkali salts) or phenolic-hydroxy groups reducing heteropoly acids (phosphomolibdat-phosphotungstat) contained in the Folin Ciocalteau reagent into a molybdenum-tungsten complex. The phenolic compound reacted with the Folin Ciocalteau reagent only in an alkaline condition to allow proton dissociation in the phenolic compound into phenolic ions. To make base condition used Na_2CO_3 7.5%. The hydroxyl group of phenolic compounds reacted with the Folin Ciocalteau reagent to form a blue-tungsten molybdenum complex that could be detected by a UV visible spectrophotometer. The greater concentration of phenolic compounds, the more phenolic ions that would reduce the heteropoly acid (phosphomolibdat-phosphotungstat) into molybdenum-tungsten complex so that the resulting blue color became more concentrated. In the determination of total phenolic content with a defined wavelength of 765 nm from gallic acid 6 ppm as standard solution. The standard

solution used was gallic acid with concentration variations of 0, 4, 6, 8 and 10 ppm (Table 1).

Table 1. Measurement of Absorbance Standard Gallic Acid Solution

Concentration (ppm)	Absorbance
0	0.000
4	0.405
6	0.589
8	0.723
10	0.933

Based on Figure 1, the standard curve of the gallic acid regression equation obtained Y = 0.0917x + 0.0165 where Y was absorbance (A) and X was concentration. Subsequently the sample that has been treated the same as the standard solution is measured at λmax, then calculated the total phenolic content by using the basis of the obtained regression line equation. The qualitative results of the total phenolic determination on green tea, black tea and Roselle tea gave positive results, which were marked with blue or green color when added Na_2CO_3 7.5% reagent and Folin Ciocalteau reagent which showed that this reactant oxidized phenolic (alkali salt) or a phenolic-hydroxy group reduces the heteropolyac acid (phosphomolibdat-phosphotungstat) contained in the Folin Ciocalteau reagent into a blue or green as a molybdenum-tungsten complex showed in Figure 2.

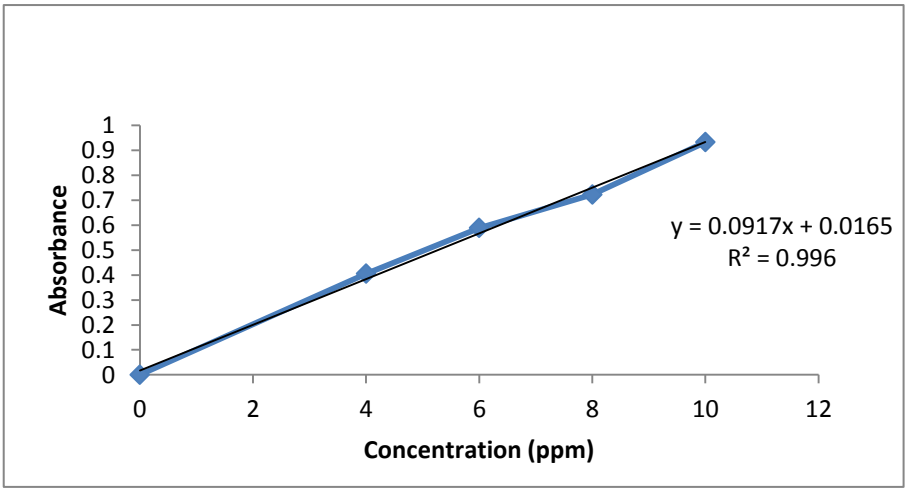

Fig. 1 The Relationship of Concentration and Absorbance of Gallic Acid as Standard

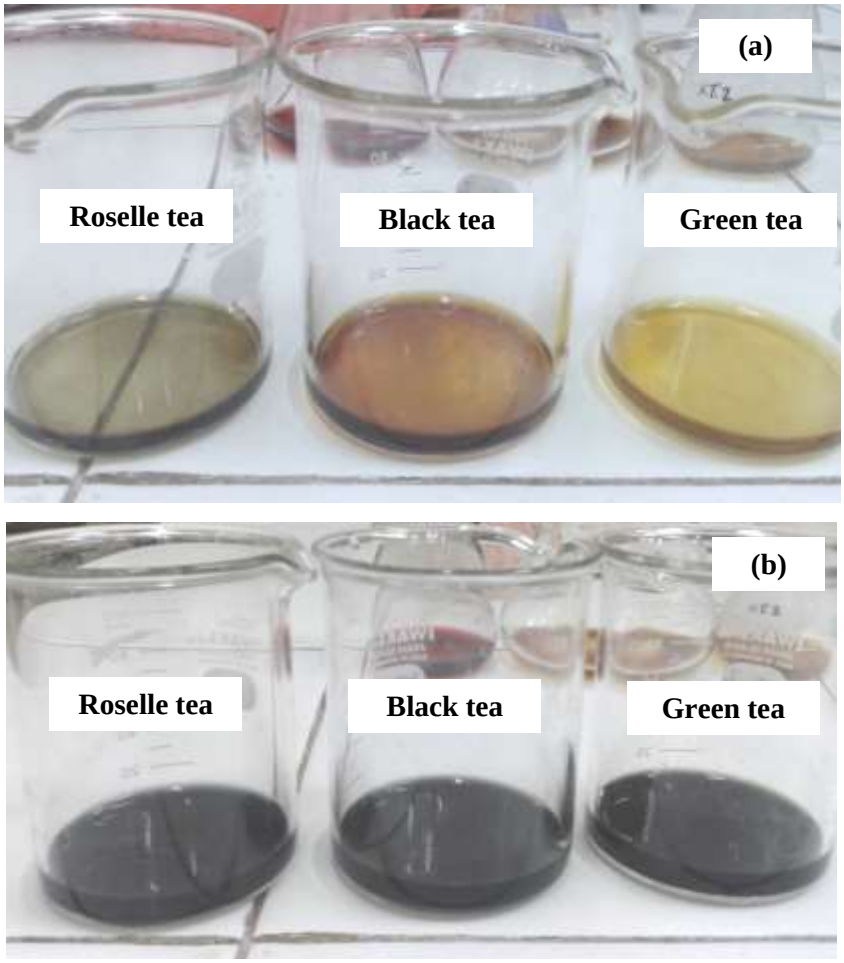

Fig. 2. Green tea, Black tea and Roselle tea before (a) and after (b) the addition of reagents

The results of the calculation of total phenol content in green tea, black tea and Roselle tea samples could be seen on Table 2. The lowest phenolic tea was Roselle tea. Green tea and black tea had similar number of total phenolic about 0.18-0.19%. The precision of phenolic extraction showed in Table 3. Based on Table 3, it could be concluded that maceration using aquadest was the stable method for phenolic extraction of tea because it had %RSD below than 2%.

Table 2. The Total Phenolic Content of Green, Black and Roselle tea

Samples	Absorbance	Content of total phenolic (%)
Green tea	0.8143	0.18
Black tea	0.8887	0.19
Roselle tea	0.5513	0.12

Table 3. Precision levels of black tea, green tea and Roselle tea

No	Black Tea		Green Tea		Roselle Tea	
	Absorbance	**Content of total phenolic (%)**	**Absorbance**	**Content of total phenolic (%)**	**Absorbance**	**Content of total phenolic (%)**
1	0.8807	0.1963	0.8154	0.1814	0.5513	0.1214
2	0.8983	0.2003	0.8183	0.1821	0.5500	0.1212
3	0.8800	0.1961	0.8181	0.1821	0.5510	0.1214
4	0.8960	0.1998	0.8153	0.1814	0.5523	0.1217
5	0.8887	0.1981	0.8183	0.1821	0.5520	0.1216
Average of content		0.19 %		0.18 %		0.12 %
% RSD		0.97 %		0.19 %		0.17 %

As published in the Japanese Journal of Cancer Research, when Japanese scientists combine conventional phenolic cancer treatment, the results are 20 times more effective than conventional treatments. This study, published in the Archive of Internal Medicine, says phenolics may play a role in preventing bad cholesterol (LDL, Low Density Lipoprotein), which triggers the buildup of plaques that can clog arteries. In addition, these antioxidants in tea also facilitate the arteries to send blood full of nutrients to the heart and to the entire body.

Based on the explanation, it can be understood that the presence of phenolic compounds as one of the antioxidants contained in tea, especially in green tea, black tea and Roselle tea can be a deterrent to the presence of free radicals that enter into our body. The presence of phenolic compounds in green tea, black tea and Roselle tea allows the tea to be a beverage capable of counteracting free radicals derived from foods containing oxidizing easy fatty acids, especially fried foods involving cooking oil. Because cooking oil is easily damaged and oxidized to hot temperatures used for cooking, whereas today many foods are sold in the form of fried foods, be it bananas, tempe, fried tofu, tempura and fried dishes such as catfish, fish, chicken, and others. Thus, drinking green tea and black tea will be more effective to prevent free radicals because it contains total phenolic content as higher antioxidant than Roselle tea.

Conclusion

The total phenolic content of green tea was 0.18%, black tea was 0.19% and Roselle tea was 0.12%. It can be concluded that drinking green tea and black tea will be more effective to prevent free radicals because it contained total phenolic content as higher antioxidant than Roselle tea.

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