Analysis of Phenolic Content in Green Tea Kombucha and Rosella (*Hibiscus sabdariffa*) Kombucha Using Spectrophotometer Uv-Vis

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Abstract: Phenolic compounds are compounds that have hydroxyl groups and are most abundant in plants. Green tea and rosella flowers are types of plants that contain phenolic compounds and can be used as ingredients to make kombucha tea drinks. Kombucha is a fermented drink made from tea and sugar which is fermented by a colony of bacteria and yeast. The aim of this research was to determine the levels of phenolic compounds in green tea kombucha and rosella flowers through experimental tests using spectrophotometry uv-vis. The results showed that the phenolic content of green tea kombucha was 162.35 mg/L GAE, while rosella kombucha had a level of 101.30 mg/L GAE. Thus, the phenolic content of green tea kombucha is higher compared to rosella kombucha.

1 INTRODUCTION

Green tea (*Camelia sinensis*. L) is a type of herbal plant originating from China. This plant is widely cultivated as a raw material for making traditional medicines (Anindita *et al*., 2012). Tea is a drink that is popular with the general public because it is believed to have many health benefits. The content of bioactive compounds in tea can function as anticancer, antimicrobial, lowering blood cholesterol, reducing blood sugar, antibacterial and what is popular is antioxidant (Sasmito and Dearta, 2020). Based on research conducted by Priani *et al*., (2024) green tea has very strong antioxidant activity with an IC50 value of 6,435 ppm. Green tea extract was also developed into a nanoemulsion preparation using Tween80 surfactant and PEG400 cosurfactant. The research results showed that green tea ethanol extract had very strong antioxidant activity with an IC50 value of 2.14 ± 0.01 ppm.

One of the fermented foods made from green tea is kombucha. Kombucha is a drink made from fermented tea with a *SCOBY* (*Symbiotic Consortium of Bacteria and Yeast*) which is rich in beneficial compounds such as organic acids, minerals, vitamins, amino acids and active polyphenols (Kapp and Sumner, 2019). The advantage of kombucha tea compared to green tea usually lies in its higher content of organic acids, vitamins and amino acids. The antioxidant properties of kombucha tea have been proven to increase during the fermentation process due to the free phenolics produced, where the higher the phenolic content, the higher the antioxidant activity (Bishop *et al*., 2022). Apart from green tea, the basic ingredients for making kombucha can be obtained from plants that are rich in antioxidants such as rosella flowers.

The roselle plant (*Hibiscus sabdariff*a) also known as marsh acid, beetle acid, or susur acid, originates from the African continent. Apart from being used as an ornamental plant, roselle also has medicinal properties as a tea, and the leaves can be used as a vegetable. This plant is known for its many beneficial properties for humans, such as lowering cholesterol levels, treating hypertension, preventing osteoporosis and premature aging, reducing throat phlegm, and treating canker sores (Astuti and Fadilla, 2020). The use of rosella flowers (*Hibiscus sabdariffa* L.) is believed to have antioxidant activity related to the phenolic content in them (Pangaribuan, 2016). This is proven by research conducted by Sitanggang *et al*., (2023) which shows that rosella flowers have high

antioxidant activity, with an IC50 of 10.74 ± 0.14 mg/g and an IC50 of 202.47 µL/mL. The flower petals are rich in antioxidants which play a role in binding free radicals and are often used as natural dyes.

Green tea and rosella both contain secondary metabolite compounds which are included in the type of antioxidant, namely phenolic compounds. Phenolics have one or more aromatic benzene rings and are considered one of the most abundant types of antioxidants (Wardani *et al*., 2020). Phenolic compounds are bioactive secondary metabolites that are widely distributed in plants, mainly produced through the cicamic acid, pentose phosphate and phenylpropanoid pathways. Structurally, phenolic compounds include a variety of compounds that have aromatic rings with one or more hydroxyl groups, and can vary from simple molecules to complex polymers (Haminiuk *et al*., 2012). Phenolic compounds are chemical compounds that have conjugated double bonds and chromophore groups. Chemical compounds that have conjugated double bonds and chromophore groups can be determined using the spectrophotometric uv-vis method (Sari and Ayuchecaria, 2017). This research was conducted to determine the phenolic content of green tea kombucha and rosella kombucha using a UV-vis spectrophotometer.

2 METHODS

Tools and Materials

Tools

The tools used in this research were glass jars, spoons, stoves, measuring glasses, beakers, measuring flasks, Erlenmeyer flasks, dropper pipettes, volume pipettes, bulbs, analytical scales, vortexes, pH meters, UV-VIS spectrophotometers.

Materials

The ingredients used in this research were green tea, rosella flowers, water, kombucha starter culture, distilled water, granulated sugar, methanol p.a, Na2CO3, gallic acid, Folin-ciocalteu.

Kombucha Tea Preparation

Kombucha Starter Preparation

1000 ml of water is boiled until it boils and 100 grams of sugar (10% w/v) is added to the amount of water used and 5 grams of 0.5% (w/v) tea is added. Then filter and cover the filtrate with aluminum foil and let sit until the tea has room temperature. After that, add 100 ml of kombucha starter culture (10% w/v) to the brewed tea and then close the container tightly. Propagation of the kombucha starter culture was left for 14 days.

Making Green Tea Kombucha

5 grams of green tea is brewed using 200 ml of boiling water. Then add sugar with a concentration of 50 grams and stir. Cover the brewed green tea tightly with a cloth and let it sit until room temperature. 20 ml of liquid kombucha starter is added and fermented for approximately 12 days in a closed container.

Making Rosella Kombucha

5 grams of rosella flowers are brewed using 200 ml of boiling water. Then add sugar with a concentration of 50 grams and stir. Cover the steep rosella tea with a cloth and let it sit until room temperature. 20 ml of liquid kombucha starter is added and fermented for approximately 12 days in a closed container.

pH Level Test

pH measurements are measured using a pH meter. Green tea kombucha and rosella flower kombucha were put in a glass beaker and then dipped into a pH meter.

Phenolic Content Test

Standard Curve of Gallic Acid Standard Solution

The standard gallic acid solution was made in varying concentrations of 10, 20, 30, 40, 50 ppm. Take 1 ml of the standard solution of gallic acid of each concentration and put it into a test tube and add 0.5 ml of Folin-ciocalteu then leave it for 8 minutes while shaking. To the solution was added 4 ml of 7% Na2CO3 solution and vortexed for 1 minute. Measurements were carried out at a wavelength of 760 nm.

Sample Absorption Measurement

1 ml of kombucha from each sample and add 0.5 ml of Folin-ciocalteu, leave it for 8 minutes while shaking. 4 ml of 7% Na2CO3 solution was added and vortexed for 1 minute. Absorbance was calculated with a wavelength of 760 nm. Measurements were carried out at a wavelength of 760 nm. The total phenol content can be calculated using the following formula:

$$TPC = c \cdot v \cdot fp$$

Explanation:

TPC : total phenolic content (mg/L GAE)

c : concentration (x value) (ppm)

v : sample volume (ml)

fp : dilution factor

3 RESULTS and DISCUSSIONS

Kombucha is a health drink that has many benefits for the body. Kombucha is believed to contain phenolics and antioxidants. Kombucha has a sour taste caused by the increase in organic acid compounds during the fermentation process, and resulting in a decrease in the pH of the kombucha. The longer the fermentation time, then the total acid will increase (Wistiana & Zubaidah, 2015). Generally, the degree of acidity of a material is indicated by the pH value. The pH value of green tea kombucha and rosella kombucha can be seen in table 1. The low pH value in the two kombucha samples is caused by the metabolic process of yeast and bacteria towards sucrose which produces a number of organic acids such as acetic acid, gluconic acid and glucuronic acid (Wistiana & Zubaidah, 2015).

Table 1: pH value

No	Sample	pH Value
1	Kombucha Teh Hijau	3.32
2	Kombucha Rosella	2.62

The decrease in the pH of kombucha tea also occurs because during the fermentation process, yeast will synthesize sugar into ethanol and by bacteria the acetate is broken down into organic acids, such as acetic acid and gluconic acid and several concentrations of organic acids. This results in a decrease in the pH of the fermentation medium (Puspitasari et al., 2017). As fermentation progresses, the pH decreases, becoming more acidic. *Lactobacillus bulgaricus* is a lactic acid bacterium with the ability to convert carbohydrates into organic acids, primarily lactic acid. The accumulation of lactic acid in the fermentation medium causes a gradual decrease in pH. This influence is associated with an increase in medium acidity due to the release of organic acids (Ansory et al., 2023).

Kombucha is believed to have good antioxidant content. This antioxidant potential is obtained from the phenolic compounds contained in kombucha (Nafisah et al., 2023). Phenolic compounds are a type of organic compound that has one or more hydroxyl groups (-OH) attached to an aromatic ring called phenol. The basic structure of phenolic compounds is an aromatic ring which has hydroxyl groups scattered around the ring. This hydroxyl group gives reactive properties and unique characteristics to phenolic compounds. This compound can be found naturally in various types of plants. In the food and beverage industry, phenolic compounds play a role in providing a distinctive aroma, as natural colorants, and as antioxidant agents in food and beverage products (Christalina et al., 2018). The way phenolic compounds work involves denaturing proteins in bacteria. These compounds can be absorbed into bacterial cells because they have hydrogen bonds. If phenolic levels are low, phenolic compounds will form protein complexes with weak bonds, then decompose, followed by penetration of phenolic compounds into bacterial cells, which causes protein deposition and denaturation (Novita, 2016).

In this study, the levels of phenolic compounds in green tea kombucha and rosella flower kombucha were analyzed using a spectrophotometer uv-vis. A spectrophotometer uv-vis is a tool used quantitatively to identify the compound content in a sample by measuring absorbance in the wavelength range 200-700 nm. The first step in calculating phenolic content is to create a standard curve for gallic acid. In making a standard curve, the absorbance of each concentration is needed. The measurement results are in the form of absorbance, which is analyzed according to the Lambert-Beer law from the concentration of the standard solution or sample. Absorbance data are used to create a standard curve, which provides information about the correlation coefficient (r) and the linear regression equation ($y = ax + b$). This regression equation is then used to determine the compound content in the analyzed samples (Wahyuni and Marpaung, 2020). This data can be seen in table 1.

Table 2: Data for Determining the Standard Curve for Gallic Acid

Consentration (ppm)	Absorbance
10	0.331
20	0.52
30	0.804
40	1.159
50	1.315

Solutions of variation concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm) were searched for absorbance using a spectrophotometer uv-vis instrument. The absorbance of each concentration can be seen in table 2. After obtaining the absorbance of each concentration, the next step is to create a standard standard curve for gallic acid which is used to find equations and linear regression. The standard curve for gallic acid can be seen in Figure 1.

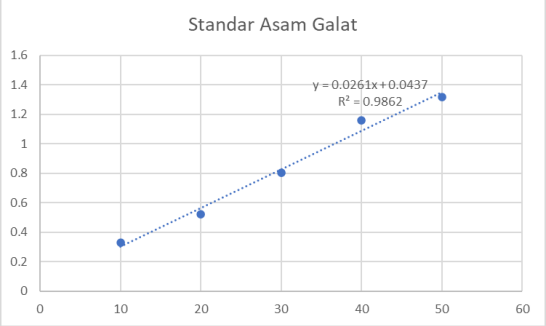


Figure 1: Standart Curve for Gallic Acid

The standard curve for gallic acid has the regression equation y (absorbance) = 0.0261x (concentration) + 0.0437 with a coefficient of determination R2 = 0.9862. The curve can be said to be linear because the determination coefficient (R2) is 0.9862 or close to one. The phenolic concentration can be determined by substituting the absorbance of the sample into Y in the equation. The size of the X indicates the concentration of phenolics in the sample.

The standard phenolic compound used is gallic acid or 3,4,5 - trihydroxybenzoic acid (C6H2(OH)3CO2H). Gallic acid is a derivative of hydroxybenzoic acid which is included in the category of simple phenolic acids (Ansory et al., 2023). In the process of testing phenolic content, kombucha was added with Folin Ciocalteu reagent and 7% Na2CO3. The oxidation reaction of Folin with phenolic compounds or phenolic hydroxyl groups will reduce the phosphomolybdate-phostungstate present in the Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent will react with the hydroxyl group and form a molybdenum-tungsten blue complex which can be detected using a spectrophotometer. The addition of Na2CO3 aims to achieve alkaline conditions in the Folin reaction, which causes the dissociation of phenolic compound protons into phenolic ions. Na2CO3 can form an alkaline environment, so that the higher the phenolic content in the extract, the stronger the intensity of the blue color produced (Gemati et al., 2013).

After knowing the phenolic concentration, then calculate the TPC. Determination of TPC is part of the analysis which is related to phenolic content and antioxidant activity. Samples with a relatively high content of phenolic secondary metabolite compounds generally have high antioxidant activity (Handayani et al., 2022). The total phenolic content in each extract is expressed as gallic acid equivalent (GAE). GAE is a general reference for measuring the number of phenolic compounds contained in a material. Based on the existing phenolic concentration, the TPC value of kombucha for each sample was obtained. The TPC value of each sample can be seen in table 3.

Table 3: TPC Value of Green Tea Kombucha and Rosella Flower Kombucha

Sample	Absorbance	Phenolic Concentration	TPC (mg/L GAE)
Green Tea Kombucha	4.281	162.348659	162.35
Rosella Kombucha	2.688	101.3141762	101.31

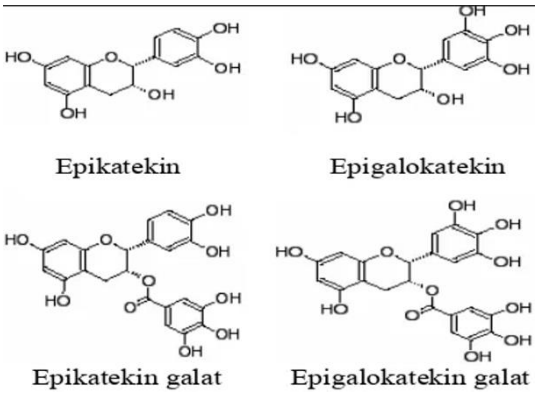
The TPC value of green tea kombucha is 162.35 mg/L GAE, while the TPC value of rosella flower kombucha is 101.31 mg/L GAE. Based on the data obtained, it can be seen that the TPC value for green tea kombucha is higher than the TPC value for rosella flower kombucha. The samples used in this research were green tea kombucha and rosella flower kombucha. The use of samples in this study was based on previous literature which showed the presence of compounds contained in green tea and rosella flowers. The compound content of each sample can be seen in table 4..

Table 4: Compound Content of Green Tea, Telang, Rosella, Lavender and Chamomile

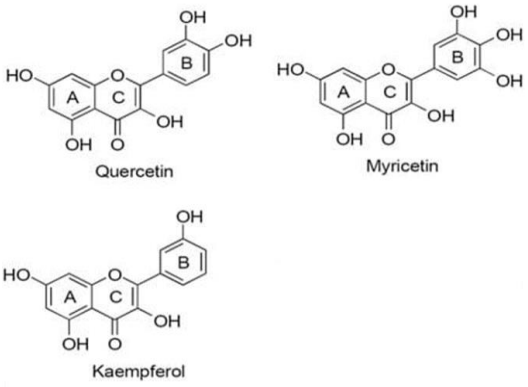
No	Aspect	Green Tea	Rosella
1	Flavonoid	Catechin (-) epicatechin (EC) (-) epigallocationchin (EGC) (-) epicatechin gallate (ECG) (-) epigallocationchin gallate (EGCG). (Fadhlurrohman & Susanto, 2024)	Kaempferol Quercetin Myricetin (Ramahani et al., 2024)
2	Anthocyanin	-	Cyanidin Peonidin Delfinidine Malvidin Petunidin (Gamgulu, 2023)
3	Polyphenols	Epigallocatechin galat Epigallocatechin (Fadhlurrohman & Susanto, 2024)	Citric acid, malic acid, ascorbic acid (Nugroho et al., 2018)
4	Phenolic	simple hydroxybenzoic acids such as gallic acid and propyl gallate (Galati, 2006)	Citric acid Hydroxycitric acid Hibiscus acid Protocatechuic acid (Boras-Linares, 2014)
5	Steroid	Beta-sitosterol (Helilusiatiningsih, 2021)	Beta-sitosterol Stigmasterol (Khan et al., 2020)

Green tea and rosella are both rich in various compounds that provide health benefits. Based on table 4, it can be seen that green tea and rosella have differences in terms of their compound content. Green tea contains flavonoid compounds such as (-) epicatechin (EC), (-) epigallocatechin (EGC), (-) epicatechin gallate (ECG), (-) epigallocatechin gallate (EGCG). Rosella contains kaempferol, quercetin, and myrisetin, which have been shown to have antioxidant and anti-inflammatory properties that are important for body health. On the other hand, rosella contains anthocyanins such as cyanidin, peonidin, delphinidin, malvidin, and petunidin, which are also known for their antioxidant and anti-inflammatory properties. Additionally, polyphenols such as epigallocatechin gallate (EGCG) and epigallocatechin (EGC) are found in green tea. Rosella flowers contain citric acid, malic acid and ascorbic acid. Phenolic compounds such as simple hydroxybenzoic acids namely gallic acid and propyl gallate are present in green tea, while rosella is rich in citric acid, hydroxycitric acid, hibiscus acid and protocatechuic acid. Lastly, steroids such as beta-sitosterol and stigmasterol can be found in green tea, providing additional health benefits. With the combination of these compounds, green tea and rosella offer various health benefits for the body.

Based on table 4, it can be seen that green tea contains several catechin compounds including epicatechin (EC), epigallochantiochin (EGC), epicatechin gallate (ECG), epigallochantiochin gallate (EGCG), polyphenolic compounds in the form of epigallocatechin gallate and epigalocatechin. and contains steroids in the form of beta sitosterol. Based on the results of the catechin compound, the function of the epicatecin compound in green tea is as a strong antioxidant, which can reduce lipid peroxidation and also inhibit platelet aggregation (Praptiwi, et al, 2015). Furthermore, the compound epigallochantiochin (EGC) is a catechin class compound that can prevent the production of IL-8 in airway epithelial cells, thereby limiting the degree of inflammation of the respiratory tract. Furthermore, the compound epicatechin gallate (ECG) is a natural flavonoid compound found in green tea



(Juniaty, 2013)



(Jia dan Lui, 2013)

Figure 2: Phenolic Compounds of Green Tea (left) and Rosella (right)

Based on the results of phenolic content analysis research which can be seen in table 3, that the phenolic content in green tea kombucha is greater than in rosella kombucha. This is proven by table 4, that compounds containing the -OH group are more commonly found in green tea so that the TPC value in green tea kombucha is higher than rosella flower kombucha.

The microorganisms in the fermentation process provides a strong rationale for the increase in phenolic content. The activities of these microorganisms during fermentation can induce damage to cell wall structures, allowing various bioactive compounds contained within to be released (Mahardani & Yuanita, 2021). Throughout fermentation, microorganisms involved in the process can produce specific enzymes capable of converting non-phenolic compounds into phenolic compounds. These enzymes can transform the structure of complex organic compounds into simpler and more measurable phenolic compounds. Microorganism metabolism activities also result in various secondary metabolites. Some secondary metabolites produced during fermentation may possess phenolic properties or can be converted into phenolic compounds by these microorganisms. Consequently, the total phenolic content in kombucha will increase. Changes in phenolic content during fermentation may depend on the type of microorganism used, fermentation conditions, as well as the composition and chemical properties of the fermented substrate (Ansory et al., 2023).

4 CONCLUSIONS

The results showed that the TPC value of green tea kombucha was 162.35 mg/L GAE and rosella flower kombucha was 101.30 mg/L GAE. Thus, green tea kombucha has a higher phenolic content compared to rosella flower kombucha. This shows that green tea has a greater opportunity as the main ingredient in making kombucha which can provide better health benefits.

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