

Analysis of Phenolic Content of Green Tea Kombucha and Butterfly Pea (*Clitoria Ternatea L.*) Flower Kombucha Using Spectrophotometer Uv-Vis

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Abstract: Kombucha is a health drink that comes from fermented green tea and microorganisms such as *Acetobacter xylinum*. Kombucha is believed to have high antioxidant content. Butterfly pea flowers (*Clitoria ternatea L.*) can be used as a basic ingredient for making kombucha because they are believed to have high phenolic content. Phenolics are compounds that have hydroxyl groups (-OH) and contribute most to antioxidant activity. The aim of this research was to determine the levels of phenolic compounds in green tea kombucha and butterfly pea flower kombucha. This research used experimental research by testing phenolic levels using a spectrophotometer uv-vis instrument. The results showed that the phenolic content of green tea kombucha was 162.35 mg/L GAE and butterfly pea flower kombucha was 124.46 mg/L GAE. Thus, the highest phenolic content is found in green tea kombucha.

1 INTRODUCTION

Green tea is a drink that is popular among people and is very popular in Asian countries, especially in Japan (Tamon *et al.*, 2021). Green tea has many health benefits, such as preventing and lowering high blood pressure, reducing bad cholesterol levels, reducing the risk of stroke, and smoothing the skin. All types of tea contain catechin compounds, but green tea is currently more popular because its catechin content is higher than black tea. Therefore, green tea is known to help prevent cancer growth (Lelita *et al.*, 2019). Research conducted by Wang *et al.* (2022) showed that green tea has a very strong antioxidant activity value, with an IC50 value (50% inhibitory concentration) of $8.45 \pm 0.41 \mu\text{g/mL}$ in the DPPH (2,2-diphenyl-1-picrylhydrazyl) test.

Green tea can be used as a fermented drink, namely kombucha. Kombucha is a fermented drink between tea and sugar made by a kombucha starter culture called SCOBY (Symbiotic Culture of Bacteria and Yeast). Kombucha has the form of a gelatinous mass or resembles a white gelatinous biofilm which is similar to nata de coco but the medium is different. The activity of microorganisms during fermentation produces various compounds that are beneficial for health such as vitamins, minerals, enzymes and

phenolic compounds (Khamidah and Antarlina, 2020). Apart from green tea, the raw materials for making kombucha can be obtained from plants high in antioxidants such as butterfly pea flowers.

Butterfly pea flower (*Clitoria ternatea*) is a compound flower that is identical to the purple color on its petals. Butterfly pea flower is a plant that can be found in home gardens, plantations and the edges of rice fields. This plant functions as an ornamental plant which is used as a traditional eye medicine and food coloring. Apart from that, this plant produces green beans which are legumes (Angriani, 2019). Based on several studies collected by Jeyaraj *et al.* In 2021, butterfly pea flowers are known to contain quite a variety of bioactive compounds. The bioactive compounds contained in it include kaempferol, quercetin, and myrisetin. Apart from that, telang flowers are also known to contain several other compounds such as fatty acids, phytosterols and tocopherols. Several compounds contained in telang flowers are known to have good antioxidant properties. In research conducted by Saha *et al.* (2021), butterfly pea flower extract showed a total phenol value of $271.4 \pm 12.7 \text{ mg GAE/g}$ (gallic acid equivalent per gram) and a DPPH radical scavenging

activity value of $89.2 \pm 3.4\%$. Based on these data, it can be seen that butterfly pea flowers can be used as kombucha because they have good phenolic compound value.

Phenolic compounds are the result of secondary metabolites from plants with a combination of mono and polysaccharides linked to one or more phenolic groups, or as ester or methyl ester derivatives. This compound is an aromatic compound whose structure is derived from benzene so that it has an aromatic ring and the presence of one or more hydroxyl groups (-OH). Phenolic compounds tend to dissolve in water, generally bind to sugar as glycosides and are located in cell vacuoles (Mahardani and Yuanita, 2021). There are around eight thousand types of plants that contain compounds belonging to the group of phenolic compounds, where the structures of these compounds are known. These phenolic compounds include flavonoids, phenyl propanoids, phenolic quinones, polyphenols (such as lignin, melanin and tannin), as well as simple monocyclic phenols (Sundu *et al.*, 2022). One of the instruments used to determine the levels of phenolic compounds is a spectrophotometer uv-vis. This study aims to determine the phenolic content of green tea kombucha and butterfly pea flower kombucha using a spectrophotometer uv-vis.

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, spectrophotometer uv-vis.

Materials

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

Kombucha Tea Preparation

Kombucha Strater 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 Telang Kombucha

5 grams of butterfly pea flowers are brewed using 200 ml of boiling water. Then add sugar with a concentration of 50 grams and stir. Cover the steeping telang 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 butterfly pea 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. The pH value of each sample can be seen in table 1.

Table 1: pH Value

No	Sample	pH Value
1	Kombucha Teh Hijau	3.32
2	Kombucha Telang	2.86

Kombucha is a fermented drink that is high in secondary metabolite compounds so it is considered to have good antioxidant activity. Kombucha has a sour taste due to the increase in organic acid compounds during the fermentation process. This results in a decrease in the pH of the kombucha. The longer the fermentation time, the greater the total acid (Wistiana and Zubaidah, 2015). In general, the degree of acidity of a material is indicated by the pH value. The pH value of green tea kombucha and butterfly pea 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 organic acids such as acetic acid, gluconic acid, and glucuronic acid (Wistiana and Zubaidah, 2015). The decrease in the pH of kombucha tea also occurs because during the

fermentation process, yeast will synthesize sugar into ethanol. Then it will be broken down into organic acids by acetic bacteria into acetic acid and gluconic acid and several concentrations of organic acids (Puspitasari *et al.*, 2017).

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). The group is attached to an aromatic ring called phenol. 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 butterfly pea flower kombucha were analyzed using a spectrophotometer uv-vis instrument. Spectrophotometer uv-vis is an instrument used to quantitatively determine the compound content in a sample which is measured in the ultraviolet-visible light region with a wavelength of 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. This data can be seen in table 2.

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

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

Solutions of varying 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 standard curve for gallic acid can be seen in Figure 1.

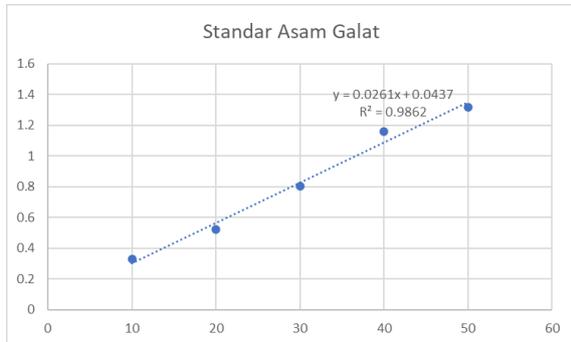


Figure 1: This caption has one line so it is centered

The measurement results from this instrument are in the form of absorbance based on the Lambert-Beer law from several concentrations of standard solutions or samples. The absorbance was analyzed to obtain a standard curve. The standard curve provides an illustration of the correlation coefficient (r) and the linear regression equation, $y = ax + b$. This equation is used to determine the compound content of a sample being analyzed (Wahyuni and Marpaung, 2020). The standard standard curve for gallic acid has the regression equation y (absorbance) = $0.0261x$ (concentration) + 0.0437 with a coefficient of determination $R^2 = 0.9862$. The curve can be said to be linear because the determination coefficient (R^2) 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 ($C_6H_2(OH)_3CO_2H$). Gallic acid is used as a standard solution because gallic acid has a hydroxyl group and a conjugated

double bond on each benzene ring so that this compound easily reacts to form a complex with the Folin-Ciocalteu reagent which is a constituent unit of phenolic compounds (Adawiah *et al.*, 2015). In addition, gallic acid is the choice as a standard due to the availability of a stable and pure substance (Niwele *et al.*, 2020). The reagents used are Folin Ciocalteu and Na_2CO_3 . Folin Ciocalteu is a polymeric ion complex solution made from phosphomolybdic acid and heteropoly phosphotungstic acid consisting of water, sodium tungstate, sodium molybdate, phosphoric acid, hydrochloric acid, lithium sulfate and bromine. The principle of the Folin Ciocalteu method is a colorimetric oxidation and reduction reaction to measure all phenolic compounds in the sample. Phenolic compounds react with gallic acid to produce phenolic compounds and a blue molybdenum-tungsten complex. The high intensity of the blue color contained means that there are a lot of phenolic compounds in the sample. Another reagent is Na_2CO_3 , which is a chemical compound that functions as a chemical aid in the neutralization process. Gallic acid is reacted with Folin-Ciocalteu reagent to produce a yellow color, after which it is added with Na_2CO_3 solution to produce a blue color.

After knowing the phenolic concentration, then calculate the total phenolic content (TPC). Determination of TPC is part of the analysis which is related to phenolic content and antioxidant activity. Samples that have a relatively high content of phenolic secondary metabolite compounds usually 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 Telang Flower Kombucha

Sample	Absorbance	Phenolic Concentration	TPC (mg/L GAE)
Green Tea Kombucha	4.281	162.348659	162.35
Telang Kombucha	3.292	124.4559387	124.46

The TPC value of green tea kombucha is 162.35 mg/L GAE, while the TPC value of rosella flower kombucha is 124.46 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 butterfly pea flower kombucha. In this research, the

sample used was kombucha made from green tea leaves and butterfly pea flowers. This sample selection was based on previous studies which showed that both samples contained secondary abolute, proven in table 4.

Table 4: Compound Content of Green Tea and Butterfly Pea Flower

No	Aspect	Green Tea	Butterfly Pea Flower
1	Flavonoids	Catechin, epicatechin, epigallocatechin, and epigallocatechin gallate.	Kaempferol glycosides, quercetin glycosides, and mirisetin (Andiarni & Murtisiwi, 2018).
2	Anthocyanin	-	Delphinidin-3,5-glucoside (Putri & Baharza, 2023) cyanidin-3-O-glucoside, Cn-3-(6"-p-coumaroylgluc-oside) (Saputri et al., 2023).
3	Polyphenols	epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC)	phenolic acids and flavonoids
4	Phenolic	catechins, epicatechin, epigallocatechin, and epigallocatechin gallate.	Phenolic acids (gallic acid, chlorogenic acid) (Marpaung, 2020).

Based on the results table 4, aspects of the compounds in green tea and butterfly pea flowers were obtained. The flavonoids in green tea contain the compounds catechin, epicatechin, epigallocatechin and epigallocatechin gallate. Meanwhile, in butterfly pea flowers the compounds are kaempferol, quercetin and myricetin. Green tea does not have anthocyanins, while telang contains anthocyanins whose compounds are delphinidin, cyanidin and peonin. The compounds contained in polyphenols in hiau tea are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). Meanwhile, the polyphenol compound contained in telang is phenolic acid (gallic acid, chlorogenic acid). Furthermore, phenolic compounds in green tea contain catechin, epicatechin, epigallocatechin and epigallocatechin gallate. Meanwhile, in telang the phenolic compounds are phenolic acid (gallic acid, chlorogenic acid) and

flavonoids (kaempferol, quercetin, myricetin). There are no steroid compounds in green tea and telang.

Butterfly pea flower (*Clitoria ternatea* L.) is a herb that has many benefits for human health. Butterfly flower petals contain high amounts of flavonoids (Putri and Baharza, 2023). This plant also contains various active compounds or phytochemicals which have pharmacological potential, such as antioxidants, anti-inflammatory, antidiabetic, anticancer, antihistamine and immunomodulatory. Apart from that, the compounds in Telang Flower can also affect the central nervous system. Based on these contents, butterfly pea flowers have the potential to be an innovative ingredient in making kombucha tea (Siregar *et al.*, 2023). Butterfly pea flowers can be used as a drink, either by using fresh flowers that have just been picked or by using flowers that have been dried first, then blended with warm water (Ikhwan *et al.*, 2022).

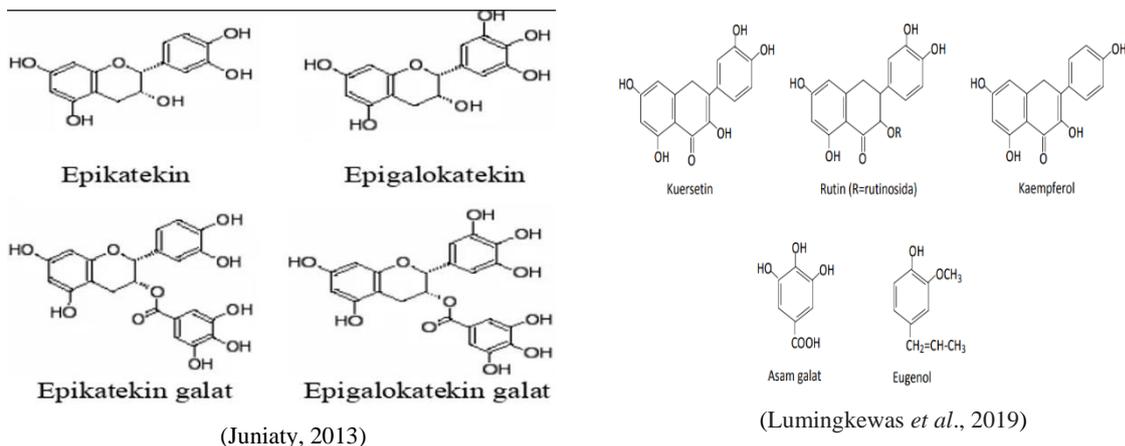


Figure 2: Green tea phenolic compounds (left) and Telang phenolic compounds (right)

Green tea and telang flowers contain compounds that provide many health benefits. Based on Table 4, it can be seen that green tea and telang flowers have differences in compound content. The content of

phenol compounds is more found in green tea than bayang flowers. However, green tea does not contain anthocyanin compounds. This can be proven by the results of the study, where the TPC value of green tea

kombucha is higher when compared to telang flower kombucha.

The total phenolic content is related to the metabolic activity of microbes during fermentation. These microbes can modify bioactive components such as polyphenols, tannins, and flavonoids. The activity of microorganisms during fermentation produces compounds that are beneficial to health, including various vitamins, minerals, enzymes, and phenolic compounds. The higher the level of phenolic compounds produced, the higher their antioxidant activity (Khamidah & Antarlina, 2020). Lactic acid bacteria in kombucha can produce phenolic compounds because they metabolize ferulic acid and cinnamic acid into 4-vinyl phenol and 4-vinyl guaiacol, thus increasing the amount of phenolic compounds (Winarsi et al., 2019).

The fermentation process plays a crucial role in altering the components and bioactivity of active compounds. Biochemical changes occurring during fermentation also alter the ratio of nutrient and antinutrient components, which in turn affect product characteristics, such as increasing the content of biologically active phenolic components as well as their antioxidant activity. Fermentation also enhances antioxidant properties by increasing the content of free flavonoids. Furthermore, fermentation stimulates cell wall degradation, which ultimately releases or even induces the synthesis of some bioactive components. The fermentation process also triggers the release of microbial enzymes that liberate free forms of plant chemical components such as flavonoids, tannins, and alkaloids (Rahmi et al., 2016).

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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