Comparison of Total Phenolic Content in Green Tea Kombucha (Camellia sinensis) and Lavender Flower Kombucha (Lavandula angustifolia) Using UV-VIS Spectrophotometry

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Abstract: Kombucha is a fermented beverage produced through the fermentation of tea and sugar by a Symbiotic Culture of Bacteria and Yeast (SCOBY). The substrates used in kombucha production can include ingredients high in phenolic compounds, such as green tea leaves and lavender flowers. This study aims to compare the total phenolic content between green tea kombucha and lavender flower kombucha. The analysis method utilized UV-Visible spectrophotometry with the Folin-Ciocalteu method and gallic acid standard with results expressed in mg gallic acid equivalents (GAE) per gram of simplicia. The analysis results indicated that kombucha made from green tea leaves had a total phenolic content of 162.35 mg GAE/g, whereas kombucha made from lavender flowers had a total phenolic content of 136.43 mg GAE/g. These findings show that the total phenolic content in green tea kombucha is higher compared to lavender flower kombucha, with a ratio of 5:4.

1 INTRODUCTION

Tea is a beverage product that has benefits for the human body. Tea is made from tea shoots (Camellia sinensis) which contain chemical compounds, namely catechins, vitamin E, vitamin C, tannins, flavonoids, theophylline, polyphenols and minerals such as Mg, Ge, Mo, Se and Zn (Arisudin et al., 2021) . Tea can be fermented using microorganisms which provides a variety of ways to consume the tea such as kombucha. Kombucha tea is a traditional tea that is known to be interesting because it is the result of fermentation carried out by a symbiotic culture (De Filippis et al., 2018). Kombucha is a fermented drink produced through the fermentation process of tea and sugar by SCOBY (Symbiotic Culture of Bacteria and Yeast). Kombucha is known to have quite high levels of antioxidants (Pebiningrum et al., 2023).

The substrate used in making kombucha can come from materials that contain high phenolic compounds, such as green tea leaves and lavender flowers (Sulistiawaty and Solihat, 2022). Green tea is a type of non-fermented or unfermented tea that contains a lot of catechins, one of the components of flavonoids (Kusnan, 2022). In the research results of Kusmiyati et al. (2015) reported that green tea has antioxidant activity and high total phenol compounds of 334.68 + 0.89 mg/L GAE. Lavender flowers are native to Switzerland which have anti-aging antioxidants (Sayuti, 2017). The results of research by Dobros et et al. (2022) reported that the value of total phenolic content in Lavandula angustifolia extract varied from 14.88 to 32.82 mg/L GAE.

This study aims to determine the comparison of total phenolic levels in green tea kombucha (Camellia sinensis) and lavender kombucha (Lavandula angustifolia) using the spectrophotometric method. Phenolic compounds are compounds that contain hydroxyl groups and are found in many plants. Phenolic compounds are aromatic compounds with benzene-derived structures that have aromatic rings and one or more hydroxyl groups (OH) (Kurang and

Malaipada, 2021). The method used to determine the phenolic content of kombucha in green tea and lavender flowers is UV-Vis spectrophotometry.

UV-Vis spectrophotometry is a compound analysis method using UV and Visible wavelengths as absorption areas (Zurweni and Sanova, 2023). UV-Vis spectrophotometry is an instrument to identify compounds that have chromophore groups and auxochrome groups (Sahumena et al., 2020). Auxochrome groups are functional groups that have free electrolyte pairs. Chromophore groups are organic compounds with conjugated double bonds that are responsible for electrical absorption (Krisdiyanto and Sa'ad, 2023).

2 METHODS

Time and Location of Research

This research was conducted at the Instrumentation Laboratory Campus II Sunan Ampel State Islamic University Surabaya in May 2024.

Tools and Materials

The tools used in the research are measuring flask, measuring pipette, bulb, vortex, centrifuge, cuvette, test tube, beaker glass, and UV-vis spectrophotometer. While the materials used are green tea kombucha, lavender flowers, water, kombucha culture starter, distilled water, granulated sugar, gallic acid, methanol p.a, Folin-ciocalteu, Na2CO3.

Kombucha Starter Preparation

100 ml of water was brought to a boil, 100 grams of sugar (10% b/v) was added and 5 grams of 0.5% tea (b/v) was added. Filtered and covered with aluminum foil then allowed to stand at room temperature. Next, 100 ml of kombucha culture starter (10% b/v) was added to the tea and then sealed. Left for 14 days to propagate the kombucha culture starter.

Green Tea Kombucha Preparation

Brewed 5 grams of green tea with 200 ml of boiling water, then added 50 grams of sugar and stirred. The brewed green tea was covered tightly with a cloth and allowed to stand at room temperature. Added 20 ml of liquid kombucha starter and fermented for 12 days in a tightly closed container.

Lavender Flower Kombucha Preparation

Brewed 5 grams of lavender flowers with 200 ml of boiling water, then added 50 grams of sugar and stirred. The lavender flower brew was covered tightly with a cloth and allowed to stand at room temperature. Added 20 ml of liquid kombucha starter and fermented for 12 days in a tightly closed container.

pH Level Test

The pH measurement was measured using a pH meter. Green tea kombucha and chamomile flower kombucha were put in a beaker glass and then dipped in a pH meter.

Phenolic Content Test

Determination of Maximum Wavelength

Determination of the maximum wavelength of gallic acid was done by measuring gallic acid solution with a concentration of 10 ppm at a wavelength of 400-800 nm using a uv-vis spectrophotometer instrument. The maximum wavelength can be known based on the highest absorbance of the measurement results using a uv-vis spectrophotometer instrument (Tetha and Sugiarso, 2016).

Standard Curve of Gallic Acid Standard Solution

The standard solution in this study uses gallic acid standard. Gallic acid standard solution was made with concentration variations of 10, 20, 30, 40, 50 ppm. Taken each standard concentration of gallic acid as much as 1 ml and put into a test tube. The solution was reacted with Folin-ciocalteu as much as 0.5 ml then shaken and left for 8 minutes. Added 7% Na2CO3 solution as much as 4 ml then homogenized using a vortex for 1 minute. The solution was put in a 500 rpm centrifuge for 3 minutes. Measurements were made using a uv-vis spectrophotometer instrument at a wavelength of 760 nm. The absorbance measurement results of gallic acid standard solution were used to make a calibration curve where gallic acid concentration as the X-axis and absorbance as the Y-axis. The regression equation of the curve was used to determine the concentration of the sample

Determination of Phenolic Content of Samples

Samples of green tea kombucha and lavender flower kombucha were each taken as much as 1 ml and reacted with 0.5 ml of Folin-ciocalteu then shaken and left for 8 minutes. The sample solution was added with 4 ml of 7% Na2CO3 solution and then homogenized for 1 minute. Then the absorbance of the sample was measured using a uv-vis spectrophotometer instrument at a wavelength of 760 nm. The blank solution used was methanol p.a.. Phenolic content in green tea kombucha and lavender flower kombucha samples was calculated using the formula:

$$TPC = c. v. fp$$

Description:

TPC : total phenolic content (mg/L GAE)

c : concentration (x value) (ppm)

v : volume of extract (ml)

fp : dilution factor

g : sample weight (gram)

3 RESULTS

Kombucha from lavender flowers is a fermented beverage processed from a decoction of lavender flowers. green tea, and sugar which is then fermented with SCOBY. This kombucha drink has a distinctive flavor and aroma. Its sour and sweet taste creates a fresh sensation. The new flavor is created by acetic acid bacteria collaborating with yeast. The result of lavender flower kombucha fermentation is shown in Figure 1.

Figure 1: Apperarance of Lavender Flower Kombucha

The results of lavender flower kombucha fermentation will be analyzed for total phenolics using a UV-Vis speltrophotometer. Before that, it is necessary to measure gallic acid standard solution first. Variations of ppm concentrations used are 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. The wave used in the measurement of gallic acid standard is 760 nm. Here are the absorbance results of gallic acid standard solution.

Table 1: Concentration Data of Gallic Acid Standardizers

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

The concentration variation solutions used were 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. Absorbance was obtained using UV-VIS instrument. Variations of concentration and absorbance are made curves to get regression results. The results of the gallic acid standard curve can be seen below.

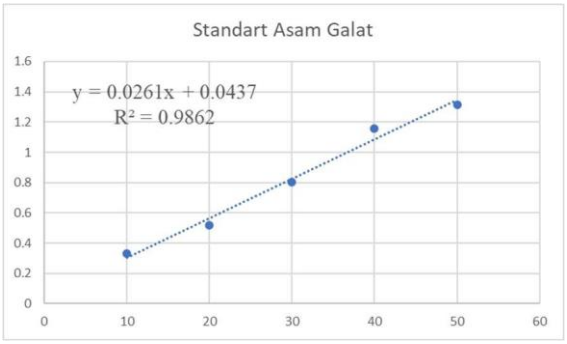

Figure 2: Gallic Acid Standard Calibration Curve

Based on the results of the calibration curve, the regression equation obtained is y (Absorbance) = 0.261x (concentration) + 0.0437. The coefficient of determination R2 = 0.9862. Determination (R2) has a value of 0.9862 can be said to be linear because it is close to the number one. Phenolic concentration will be known if substituting absorbance in equation Y. The amount of phenolic concentration in the sample is indicated by X.

The known concentration results are continued with the calculation of TPC. TPC determination is one of the data analysis to determine the relationship between phenolic and antioxidant in the sample. Phenolic compounds have a role to prevent oxidation. Total phenol activity testing is carried out with the aim of knowing antioxidant activity. The higher the

total phenol content, the higher the antioxidant because antioxidants are mostly composed by phenol compounds (Djapiala, et al. 2013). Gallic acid equivalents are determined from the total phenolic content of the sample. Based on the concentration and absorbance of phenolic, the TPC value is obtained as follows.

Table 2: TPC value of green tea and lavender flower kombucha

Sampel	Absorbance	Phenolic Concentration	TPC (mg/L GAE)
Green Tea Kombucha	4.281	162.348659	162.35
Lavender Kombucha	3.561	134.762452	134.76

Phenolic concentration can be known by substituting the sample absorbance into Y in the equation. The amount of X shows the amount of phenolic concentration in the sample. The calculation stage of the TPC (Total Phenolic Content) value is carried out after the phenolic concentration is known. TPC determination is part of the analysis that has to do with phenolic content and antioxidant activity. Samples with high phenolic secondary metabolite compounds usually have high antioxidant activity (Handayani et al., 2022). The total phenolic content in each sample was expressed as Gallic Acid Equivalent (GAE). GAE is a general reference used in measuring phenolic compounds contained in a material (Wibisino et al., 2020).

Based on the concentration of phenolics present, the TPC value of green tea and chamomile kombucha was obtained. The TPC value of each sample can be seen in table 3.

Table 3: TPC Value of Kombucha Green Tea, Telang, Rosella, Lavender and Chamomile

Sampel	Absorbance	Phenolic Concentration	TPC (mg/L GAE)
Kombucha Green Tea	4.281	162.348659	162.35
Kombucha Chamomile	0.968	35.4137931	35.41

The TPC value obtained by green tea kombucha has a value of 162.35 mg/L GAE, while the lavender flower kombucha has a value of 134.76. Based on these two values, it can be seen that green tea kombucha has higher phenolic content compared to lavender flower kombucha. The next test continued with pH testing on green tea kombucha and lavender flower kombucha. The pH test results are shown in Figures 3 and 4.

Figure 3: Green tea kombucha pH test results

Figure 4: Lavender flower kombucha pH test results

The results of the pH value are shown in the table below.

Table 1: TPC value of green tea and chamomile kombucha

Sample	pH Value
Green Tea Kombucha	3.32
Lavender Kombucha	2.9

The pH test on green tea and lavender flower kombucha was carried out using a pH meter. The pH level in green tea kombucha is 3.32, while in lavender flower kombucha is 2.9. Based on the above results, it can be seen that green tea kombucha has higher wetness than lavender flower kombucha.

4 DISCUSSIONS

Kombucha is a fermented beverage from tea and sugar fermented by Acetobacter xylinum bacteria and several other yeasts that convert sugar content into essential amino acids that can benefit the body (Saputra et al., 2017). The main ingredients of kombucha that are often used are green tea, black tea, can also be made with several ingredients such as fruits or flowers. Kombucha has properties that are good for the body. According to Khaerah and Akbar (2019), these properties are due to the presence of phenolic content which has high antioxidant activity. The higher the content of phenolic compounds, the

higher the antioxidant activity. The fermentation process of tea will increase the amount of phenolics in tea thus increasing antioxidant activity as well.

Lavender flowers contain Phenolic compounds of lavender flowers include hydroxybenzoic acid (p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, gentisic acid, gallic acid), hydroxycinnamic acid (rosmarinic acid, caffeic acid, p- cumaric acid, ferulic acid, chlorogenic acid, sinapic acid, cinnamic acid, 4-O- caffeoylquinic, 5-O-caffeoylquinic) and flavonoids (apigenin and luteolin glycosides, catechins, naringenin, vanillin) (Dobros, et al. 2022). Antioxidant and polyphenol contents are also found in green tea.

Antioxidant activity and polyphenols have a role in counteracting free radicals. Free radicals come from outside the body that can have a harmful impact on the body. Free radicals have molecules that contain more than one unpaired electron. Electrons that do not have a pair can cause free radicals because they have reactive properties and are easily attracted to magnets (Yuslianti, 2018). The content of chemical compounds in green tea contains catechins and polyphenols. Catechins are composed of epicatechin, epigalotekin, epitecin, epitecin gallate, catechin gallate, and epigalatocatechin gallate. Flavonols are composed of kaemfenol, quecetin, minicertin. Catechins are the main compounds with flavan-3-ol skeleton that determine the quality of tea leaves (Hasanah et al., 2012). About 42% of dried tea leaves contain catechin polyphenol compounds (Rabbani et al., 2019). The following is a picture of the molecular compounds of catechin derivatives.

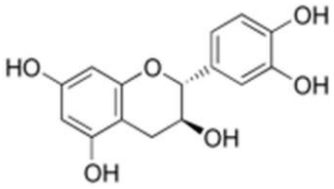

Figure 5: Molecular Structure of Catechins

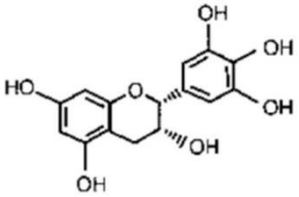

Figure 6. Molecular Structure of Epigalocatechin

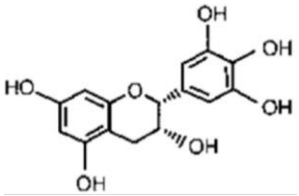

Figure 7. Molecular Structure of epicatechin

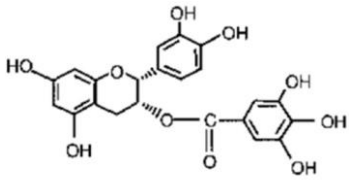

Figure 8. Molecular Structure of Epigalocatechin Galate

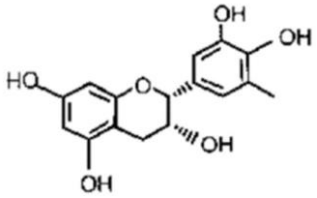

Figure 9. Molecular Structure of Epicatechin (Anesini et al., 2008)

Suoeroxide anion radicals such as reactive oxygen species (ROS) that are formed can cause oxidative stress that triggers disease to death in cells. The formation of antioxidants can inhibit oxidative damage caused by free radicals (Anand et al., 2017) Antioxidants become compounds that can inhibit free radicals because they have electron donor properties. Epigalotekin is one of the compounds that has high antioxidant activity (Du et al., 2012). The following is the mechanism of free radical capture carried out by epigalocatechin.

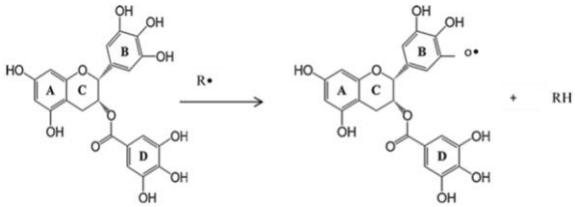

Figure 9. Mechanism of Free Radical Capture by Epigalocatechin (Sajilata *et al*., 2008)

The compound epicalocatechin gallate is a compound that belongs to the hydroxyl group of rings B and D. This compound has antioxidant properties that work

by transferring single electrons to free radicals. Free radical compounds that have a pair because they have received an electron donor from epigalocatechin gallate turn into non-free radicals. The antioxidant activity of epigalocatechin gallate also has metal chelating properties and can inhibit the formation of ROS. This compound can increase superoxide dismutase activity and gluation levels so that oxidative damage can be minimized (Fadhila et al., 2021).

Absorbance testing on gallic acid is used as a standard solution because it is one of the natural, stable and cheap phenols. Gallic acid is a phenolic compound derived from hydroxybenzoic acid which is classified as a simple phenolic acid (Ahmad et al., 2015). Gallic acid has O-H groups and conjugated double bonds on each benzene ring, it can form complex compounds with Folin-ciocalteu reagent (Supriningrum et al., 2020). Folin-ciocalteu oxidizes phenolic- hydroxy or phenolic groups to reduce heteropoly acid (phosphomolybdate- phosphotungstate) to a bluish-green molybedenum-tungsten complex (Martono et al., 2020). The sample solution is added with Na2CO3 solution which aims to create an alkaline atmosphere so that phenolic compounds undergo proton dissociation into phenolic ions (Safitri and Herdyastuti, 2021). The phenolic ion will react with Folin- ciocalteu reagent to produce a blue color.pH testing is done to determine the level of acidity or wetness in the sample. pH measurement uses the concept of loharithm with the aim of getting the right ion concentration. pH is a quantity measured from a scale of 0 to 14 (Astria, 2014). pH is said to have acidic properties if it is less than 7 and is said to have alkaline properties if it is more than 7, while a pH with a value of 7 is said to be neutral (Ihsanto, 2009). pH can decrease due to the fermentation process which produces organic acetic acid, lactic acid, and so on. Fermentation itself can increase antioxidant compounds. The more organic acids, the higher the phenolic content and the higher the antioxidant activity. The higher the phenolic content and antioxidant activity, the lower the pH value (Novita and Dyah, 2011).

5 CONCLUSIONS

The results of this study showed that the phenolic content in green tea kombucha was 162.35 mg/L GAE, while in lavender flower kombucha it was 134.76 mg/L GAE. The total phenolic content of green tea kombucha is higher than lavender flower kombucha. This is because the basic ingredients of green tea already contain high phenolic compounds. The polyphenol group that dominates in green tea is catechin which has several derivatives. Derivatives of these catechins can have the ability to ward off free radicals because of their reactive nature. The pH value of green tea kombucha is 3.32, while the pH value of lavender flowers is 2.9. Both samples are said to be acidic because they have a low pH of less than 7.

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