Analysis Total Phenolic Content of Chamomile Flower (Matricaria chamomilla) and Green Tea (Camellia sinensis) Kombucha with Spectrophotometry UV-Vis Method

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Abstract: Kombucha is a probiotic drink made from fermented tea. The basic ingredients for making kombucha can come from organic ingredients, such as chamomile flowers & green tea. Chamomile flowers (Matricaria chamomilla) and green tea (Camellia sinensis) are one type of plant that has phenolic content and is beneficial for the health of the body. Phenolic compounds are compounds that are found in many plants and play a role in producing natural plant antioxidants. This study aims to determine the phenolic content contained in chamomile kombucha and green tea. The method used is a quantitative test with UV-Visible spectrophotometry to determine phenolic levels. The results showed that the total phenolic content of chamomile flower kombucha was 35.41 mg/L GAE and green tea kombucha was 162.35 mg/L GAE. It can be concluded that the phenolic content of green tea kombucha is higher than chamomile flower kombucha.

1 INTRODUCTION

Chamomile is one of the most well-known plants in the West and has been used medicinally since ancient times. Chamomile is often used in herbal teas due to its calming, carminative, and spasmolytic properties. Chamomile has daisy-like flowers with a yellow center and white petals. Chamomile's fresh and dried flower parts can be used to make infusions, liquid extracts, and essential oils. Chamomile has two main species from the *Asteraceae/Compositae* family that are often used in traditional herbal medicine. These species are *Matricaria chamomilla* (German chamomile) and *Chamaemelum nobile* (Roman chamomile) (Zadeh *et al*., 2022).

Camellia sinensis is a plant that originated in Southeast China and spread to India, Sri Lanka, and other tropical and subtropical countries. The plant grows with sufficient rainfall, good drainage, and acidic soil. *Camellia sinensis* is known as the basic ingredient for making tea. The main types of tea plants often used are *Camellia sinensis* var. sinensis (Chinese tea), which is widely grown in China, Japan, and Taiwan, and *C. assamica* var. assamica (Sour tea), which is dominant in South and Southeast Asia, including Malaysia and Australia. *Camellia sinensis* is one of the plants that has phenolic and antioxidant content that is beneficial to the health of the body (Felicia et al., 2016).

Chamomile flowers and green tea are types of plants that can be used as basic ingredients for making kombucha. Kombucha is a microbial fermented beverage that utilizes bioprocess technology, with bacteria and yeast as starter cultures (Oktavia *et al*., 2021). Kombucha is produced from the fermentation process by *Acetobacter xylinum* bacteria and several other types of yeast that convert sugar into essential amino acids that are beneficial to the body. In addition, there are several other microbes that play a role in the kombucha fermentation process, including *Bacterium* sp, *Gluconobacter gluconicum*, *Acetobacter aceti*, *Acetobacter ketogenum*,

Saccharomyces cerevisiae, and *Phicia fermentan* (Jayabalan, 2007).

Microorganisms in the kombucha fermentation process will produce several acidic compounds, such as acetic acid, glucoronic acid, lactic acid, carbonic acid, folic acid, gluconic acid, chondroitin sulfate, hyaluronic acid and usnat acid through the process of converting tea and sugar. In addition, it also produces several kinds of vitamins, such as B1, B2, B3, B6, B12, B15, and C) and polyphenolics that play an important role in the antioxidant process (Naland, 2008; Setyaningrum *et al*., 2023). Kombucha has various properties, one of which is to increase endurance. In addition, kombucha is also able to prevent cell aging, and also prevent cancer which is usually found in the gastrointestinal tract and can be used as anti-aging because it is rich in antioxidant content (Oktavia *et al*., 2021).

The use of chamomile flowers and green tea as basic ingredients for making kombucha because they contain high phenolic compounds. Phenolic is a secondary metabolite that is spread in plants (Lerebulan *et al*., 2018). Phenolic compounds are known to have antioxidant activity (Onkar *et al*., 2012). The structure of this compound includes phenolic quinones, flavonoids, phenylpropanoids, simple monocyclic phenols, and polyphenols such as tannins, lignin, melanin, and phenolic quinones (Marjoni & Afrinaldi, 2015; Pamungkas *et al*., 2016). Phenolic compounds contain conjugated double bonds and chromophore groups.

Fadhilah *et al* (2022) said that chamomile contains phenolic compounds such as flavonoids and tannins that have strong antioxidant activity and function to fight free radicals and protect the body from various diseases. Putri & Astuti (2017) explains that chamomile flowers contain total phenolic content of 67.4 mg/GAE per gram of dry extract. One of the compounds contained in chamomile flowers is apigenin. Apigenin can act as an antioxidant, anti-inflammatory, and anxiolytic and supports the therapeutic effects of chamomile in improving sleep quality and reducing anxiety.

Leslie and Gunawan (2019) stated that phenolic compounds were found as the main secondary metabolites in *Camellia sinensis* with the results of phytochemical tests showing very high strength (+4). In addition, Hunandra (2017) revealed that green tea-based kombucha contains phenolic compounds with total phenol levels in green tea reaching 1003.8 ppm. Kombucha made through fermentation of green tea using kombu mushrooms and granulated sugar will produce phenolic compounds that have benefits as antioxidants.

The levels of chemical compounds that have conjugated double bonds and chromophore groups can be measured by UV-Visible spectrophotometric methods (Sari & Ayuchecaria, 2017). UV-Vis spectrophotometry is an analytical method that utilizes ultraviolet (UV) and visible light wavelengths to detect compounds. Compounds that can generally be identified by this method are compounds that have chromophore and auxochrome groups. Testing using a UV-Vis Spectrophotometer is fast and simple when compared to other methods (Sahumena *et al*., 2020).

This study aims to determine the phenolic content contained in chamomile kombucha and green tea. The method used is a quantitative test with UV-Visible spectrophotometry to determine phenolic level.

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 this research are measuring cup, beaker glass, measuring flask, erlenmeyer, glass jar, stirrer, drop pipette, volume pipette, spoon, analytical balance, stove, oven, vortex, aluminum foil, pH meter, UV-VIS spectrophotometer. The materials used in this study are chamomile flowers, green tea, water, tea, kombucha culture starter, distilled water, sugar, methanol p.a, Na2CO3, gallic acid, *Folin-ciocalteu*.

Kombucha Starter Preparation

1000 ml of water is brought to a boil and 100 grams of sugar (10% b/v) of the amount of water used and 5 grams of 0.5% (b/v) tea is added. Then filtered and the filtrate is covered with aluminum foil and let stand until the tea has room temperature. After that, 100 ml of kombucha culture starter (10% b/v) was added to the steeped tea and the container was tightly closed. The kombucha culture starter propagation was left for 14 days.

Kombucha Starter Preparation

Boil 1000 ml of water until it boils and add 100 grams of sugar (10% w/v) from the amount of water used and add 5 grams of 0.5% (w/v) tea. 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.

Preparation of Green Tea Kombucha

5 grams of green tea is brewed using 200 ml of boiling water. Then added sugar with a concentration of 50 grams stirred. Green tea brew is covered tightly with a cloth and allowed to stand until room temperature. Liquid kombucha starter as much as 20 ml is added and fermented for approximately 12 days in a closed container.

Preparation of Chamomile Kombucha

5 grams of chamomile flowers are brewed using 200 ml of boiling water. Then added sugar with a concentration of 50 grams stirred. Chamomile tea was covered tightly with a cloth and allowed to stand until room temperature. Liquid kombucha starter of 20 ml was added and fermented for approximately 12 days in a 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

Standard Curve of Gallic Acid Standard Solution

Gallic acid standard solution was made concentration variations of 10, 20, 30, 40, 50 ppm. Gallic acid standard solution of each concentration was taken 1 ml put into a test tube and added 0.5 ml of *Folin-ciocalteu* then allowed to stand 8 minutes while shaking. Into the solution was added 4 ml of 7% Na2CO3 solution and homogenized using a vortex for 1 minute. Measurements were taken at a wavelength of 760 nm.

Sample Absorbency Measurement

Kombucha each sample was taken as much as 1 ml and added 0.5 ml of Folin-ciocalteu, allowed to stand for 8 minutes while shaking. 4 ml of 7% Na2CO3 solution was added and homogenized using a vortex for 1 minute. Absorbance was calculated with a wavelength of 760 nm. Measurements were made at a wavelength of 760 nm. Total phenol content can be calculated using the following formula:

$$TPC = c.\ v.\ Fp \qquad (1)$$

Information:

TPC : total phenolic content (mg/L GAE)

c : concentration (x value) (ppm)

v : sample volume (ml)

fp : dilution factor

3 RESULTS

Kombucha of chamomile flowers and green tea is a fermented beverage made from a decoction of chamomile flowers, green tea, and sugar with a sweet taste and distinctive sour aroma. Kombucha fermentation is carried out through symbiotic culture between acetic acid bacteria and yeast. Chamomile flower kombucha fermentation produces a cream color, while green tea kombucha produces a brownish red color. The results of chamomile flower kombucha fermentation are presented in Figure 1.

Figure 1: Apperarance of Chamomile Flower Kombucha & Green Tea Kombucha

The pH value is one of the parameters used to determine the quality of kombucha through the fermentation process. The pH measurement aims to identify the acidity level of kombucha and the level of decomposition of raw materials required during the fermentation process. The pH value of each sample can be seen in table 1.

Table 1: pH value Data

No	Sample	pH Value
1	Kombucha Green Tea	3.32
2	Kombucha Chamomile Flower	2.78

The phenolic compound levels of chamomile flower kombucha and green tea were analyzed a uv-vis spectrophotometric instrument. The first step in determining phenolic content is the preparation of a standard standard curve of gallic acid with absorbance values of each concentration. The absorbance value data for determining the standard curve of gallic acid can be seen in table 2.

Table 2: Absorbance Value Data of Gallic Acid Standard Curve Determination

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

Measurement of total phenolic content determination was carried out at a wavelength of 760 nm. The standard curve of gallic acid obtained for the measurement of total phenolic content is y=0.0261x+0.0437 with a correlation coefficient (R2) of 0.9862. The measurement results of gallic acid standard solution can be seen in Figure 2.

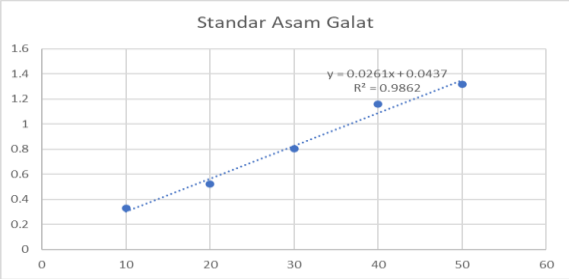

Figure 2: Gallic Acid Standard Calibration Curve

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 of green tea kombucha is 162.35 mg/L GAE, while chamomile kombucha is 35.41 mg/L GAE. Based on the data obtained, it is known that the highest phenolic content is found in green tea kombucha. Green tea contains more phenolic compounds or compounds with OH groups. This can be seen in table 4. Data on compound content in green tea samples is comparable to the TPC value obtained. The highest TPC value was the kombucha tea sample from green tea, namely 162.35.

4 DISCUSSIONS

Kombucha is a fermented beverage made from a solution of tea and sucrose sugar (as nutrients) fermented by *Acetobacter xylinum* bacteria as well as several other types of yeast. This fermentation process converts the sugar into essential amino acids (Jayabalan, 2007). These essential amino acids have various benefits for the body, such as improving the immune system, kombucha can then prevent cell aging and cancer, especially in the digestive tract (Oktaviani *et al*., 2021). This efficacy is due to the content of phenolic compounds that have antioxidant activity (Khaerah & Akbar, 2019).

Phenolic compounds are compounds that have antioxidant activity. Antioxidants are indispensable for the healing and treatment of degenerative diseases such as diabetes, liver damage, inflammation, cancer, cardiovascular, neurological disorders and the aging process. Antioxidants are very useful because they can inhibit free radicals (Sari & Ayuchecaria, 2017). Phenolic compounds have varied structures such as phenolic quinones, flavonoids, phenylpropanoids,

simple monocyclic phenols, polyphenols (including tannins, lignin, and melanin), and phenolic quinones (Pamungkas *et al*., 2016). Phenolic compound levels can also affect antioxidant activity. The higher the total phenolic content in a sample, the higher the antioxidant activity (Faidah *et al*., 2024).

Fermentation in kombucha tea drinks can increase phenolic compounds and antioxidants (Martinez Leal, et al. 2018). The fermentation process of kombucha tea is known to produce four isomers of epicatechin including epigalocatechin gallate, epicatechin gallate, epigalocatein, and epicatechin which have an effect on increasing phenolic compounds (Karsidin *et al*., 2022). These isomers will cause an increase in polyphenols during the fermentation process due to the biotransformation process by enzymes produced from the metabolism of microorganisms such as epigalocatechin gallate into epigalocatein, while epicatein gallate into epicatein (Suhardini & Zubaidah, 2016).

Quantitative analysis in this study was carried out by measuring the amount of total phenolics in kombucha using the *Folin-ciocalteu* method, which is a common technique for determining phenolic content in plants. *Folin-ciocalteu* reagent is used because phenolic compounds can react with Folin to form a solution whose absorbance can be measured (Chun *et al*., 2003). The reaction oxidizes phenolics and phenolic-hydroxy groups that will reduce the heteropoly acid in the *Folin-ciocalteu* reagent into a blue molybdenum-tungsten complex compound that can be measured absorbance on a spectrophotometer with a maximum wavelength of 765 nm (Alfian & Susanti, 2012).

Measurement of absorbance of phenolic content requires a standard solution, namely gallic acid as a comparison solution. Gallic acid is used as a comparator because the compound has a phenol group with good stability and is relatively affordable compared to other standard solutions (Syarif *et al*., 2016). In addition, gallic acid has high purity so that it can increase accuracy in the process of analyzing a compound (Waterhouse, 1999). Na_2CO_3 compound is needed in the process of making gallic acid standard solution. Na_2CO_3 or what is called sodium carbonate is an organic compound that is included in the salt group. The use of Na_2CO_3 is because sodium carbonate is composed of strong base compounds (NaOH) and weak acids (H_2CO_3). The basic salt in which if this salt is dissolved it will react and produce OH- ions. The weak acid H_2CO_3 will increase the yield of hydrophobic properties of alginate (Tambunan & Rudiyansyah, 2023).

Food ingredients commonly used for herbal products through the fermentation process have the aim of increasing the content of compounds present in the sample. The samples used in this study were chamomile flower kombucha (*Matricaria chamomilla*) and green tea (*Camellia sinensis*). According to research by Prasetyadi *et al* (2024) stated that the crown of chamomile flowers (*Matricaria chamomile* L.) contains several active compounds including essential oils, phenolics, flavonoids *(Matricaria chamomile* L.) such as apigenin, quercetin, patuletin, and luteolin), and glucosides. Phenolic compounds found in chamomile flowers are known to play a role in antibacterial and anti-inflammatory activities.

Alim & Hayuningtyas (2023) also mentioned that chamomile (*Matricaria chamomile* L.) contains various phytochemical compounds including flavonoids, alkaloids, saponins, terpenoids, tannins/phenols, steroids, and chamazulene. Meanwhile, green tea (*Camellia sinensis*) contains flavonoid compounds, phenolic acids, and carotenoids that have an effect on increasing antioxidant activity (Vu & Alvarez, 2021). Increased antioxidant activity can also be influenced by antioxidant activity in the basic ingredients processed into kombucha (Khaerah & Akbar 2019).

Chamomile flowers are known to contain several kinds of compounds, including organic acid compounds, flavonoids, coumarins, essential oils, and so on. Organic acid compounds found in chamomile flowers are isobutyric acid ($C_4H_8O_2$), tiglic acid ($C_5H_8O_2$), 4-hydroxybenzoic acid ($C_7H_6O_3$), galacturonic acid ($C_6H_{10}O_7$), chlorogenic acid ($C_{16}H_{18}O_9$), and so on. These organic acid compounds are known to have the potential to handle the treatment of immune system diseases, cancer, and cardiovascular diseases (Tvrzicka *et al*., 2011).

Flavonoid class compounds in chamomile flowers include apigenin ($C_{15}H_{10}O_5$), quercetin ($C_{15}H_{10}O_7$), luteolin ($C_{15}H_{10}O_6$), rutin ($C_{27}H_{30}O_{16}$), and so on. These compounds act as anti-inflammatory, antibacterial, antioxidant, anticancer, and so on (Dai *et al*., 2023). Coumarin ($C_9H_6O_2$), esculatin ($C_9H_6O_4$), skimmin ($C_{15}H_{16}O_8$), daphnin ($C_{15}H_{16}O_9$), 7-methoxycumarin ($C_{10}H_8O_3$) re coumarin class compounds found in chamomile flowers. According to Pratiwi *et al* (2021) coumarin class compounds act as anti-inflammatory, antioxidant, antiallergic, antiviral, antioxidant, antithrombotic, and anticancer.

Essential oils are oxygenated hydrocarbon compounds that contain a lot of oxygen. Essential oils

are mostly used as aromatherapy products because they have a distinctive aroma. In addition, essential oils also have potential as antibacterial, antifungal, antiviral, antioxidant, anti-parasitic, anticancer, and anti-inflammatory (Eiska, 2021). The compounds of the essential oil group contained in chamomile flowers include benzaldehyde (C_7H_6O), isobutyl phenylacetate ($C_{12}H_{16}O_2$), benzyl acetate ($C_9H_{10}O_2$), and so on (Dai *et al*., 2023).

Green tea (*Camellia sinensis*) contains several active compounds from the polyphenol group, organic acids, methylcanthine, minerals, and so on. Active compounds that mostly dominate come from the polyphenol group, namely catechins. According to research by Purwanto *et al* (2022) the catechin content in green tea brew is known to reach 30-42%. Catechins are polyphenol group compounds that have high antioxidant activity. The main catechin compounds found in green tea are EC (*Epicatechin*), ECG (*Epicatechin-3-gallate*), EGC (*Epigallocatechin*), and EGCG (*Epigallocatechin-3-gallate*) (Trivana et al., 2023). The chemical structure of catechin compounds can be seen in Figure 3.

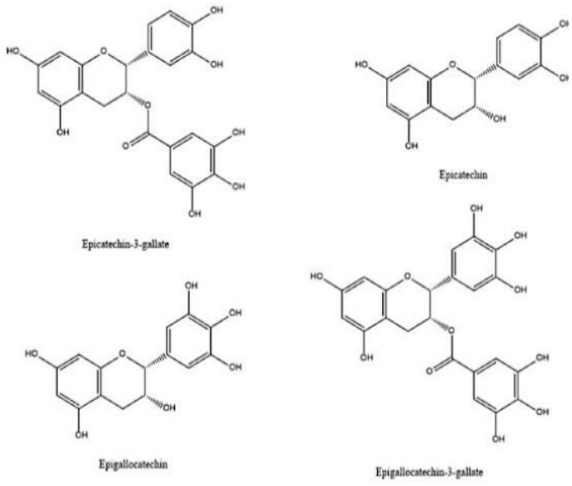

Figure 3. Chemical Structure of Catechin Compounds (Purwanto *et al*., 2022)

EC (*Epicatechin*) catechin compounds have potential as anti-cancer substances with good toxicity levels that are anti-hepatotoxic, anti-carcinogenic, anti-mutagenic, and have a safe LD50 (Sururi *et al*., 2023). Meanwhile, the catechin compound ECG (*Epicatechin-3-gallate*) acts as a mediator of inhibitory effects on cancer cells. In addition to regulating intracellular signals, ECG is also able to inhibit the enzymatic activity of RNase A and matrix metalloproteinase through metal chelators (copper and zinc) in cancer cells (Li *et al*., 2022). The catechin compound EGC (*Epigallocatechin*) functions to reduce oxidative stress that triggers neurodegenerative diseases, and has antioxidant, anticancer, and antiallergic effects (Leslie & Gunawan, 2019).

The catechin compound EGCG (*Epigallocatechin-3-gallate*) is a polyphenol found in green tea and has been shown to have anti-inflammatory, anti-apoptotic, anti-edema effects, as well as properties that stimulate regeneration or reduce the presence of inhibitory molecules in the environment (Errachid *et al*., 2021). EGCG is a catechin compound used as a quality indicator because it is the most active with a large amount (Ananingsih *et al*., 2013; Purwanto *et al*., 2022). According to Albuquerque *et al* (2016), these catechin compounds act as antioxidants, antiobesity, anti-inflammatory, anticancer, antitumor, and so on. In addition, there are also other compounds contained in green tea such as kaempferol, quercetin, gallic acid, glycosides, and mirisetin.

The pH value plays an important role in determining total phenolic content. Changes in pH value in the fermentation process can increase antioxidant activity by changing the content and structure of phenolic compounds (Martínez Leal *et al*., 2018). The pH value affects the kombucha fermentation process because several acidic compounds, such as acetate and gluconate are formed. Kombucha drinks that are suitable for consumption have a pH value ranging from 2.5-4.6 (Hapsari *et al*., 2021). Based on the pH value data, it is known that green tea kombucha has a pH of 3.32, while chamomile flower kombucha has a pH of 2.78. The acid value in Kombucha Green Tea brew increases and the pH of the Kombucha Green Tea solution decreases, this is because during the fermentation process the yeast and *Acetobacter xylinum* bacteria contained in the starter or kombucha fungus metabolize the sucrose or sugar contained in the green tea brew solution and produce a number of organic acids, the high sugar content in the tea solution causes an increase in microorganism activity and organic acids. The higher the organic acids contained in kombucha, the higher the total acid produced, thus lowering the pH of the tea solution. The decrease in pH value in fermentation will support the life of *Acetobacter xylinum* bacteria in kombucha culture to carry out its metabolic activities. The dissolved acid will dissociate to release free protons which reduce the pH of the solution in Kombucha Green Tea (Hassmy *et al*., 2017).

5 CONCLUSIONS

The results showed that the total phenolic content of green tea kombucha was 162.35 mg/L GAE, while chamomile kombucha was 35.41 mg/L GAE. The total phenolic content of green tea kombucha is higher than chamomile flowers. This is due to the presence of phenolic compounds found in green tea. Polyphenol class compounds in the form of catechins are one of the phenolic compounds in green tea with high antioxidant activity.

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