# ANTIOXIDANT ACTIVITY TEST OF VANILLA LEAF KOMBUCHA TEA WITH VARIATIONS IN FERMENTATION TIME

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**ABSTRACT**

Kombucha tea is a fermented tea leaf drink with added sugar and *Symbiotic Culture of Bacteria and Yeast (*SCOBY). Vanilla leaves (*Vanilla planifolia*) have the potential to be the basic ingredient for making kombucha tea. Variations in fermentation time in tea making can affect the content of active compounds. The purpose of this study was to determine the levels of phenolic compounds and antioxidant activity of kombucha tea with variations in fermentation time. The fermentation time for making vanilla leaf kombucha tea is 0, 4, 8, and 12 days. The phenolic compounds were known by *the Folin–Ciocalteu* method using the gallic acid standard, while the antioxidant activity was known by the free radical inhibition *method 2,2-diphenyl-1-pycrylhydrazyl* (DPPH). The results showed that the levels of phenolic compounds at fermentation times of 0, 4, 8, and 12 days were 37.99, 42.188, 57.58, respectively, and 50.53 mg/L GAE. The highest phenolic levels are found at the 8th day of fermentation. The IC50 values for fermentation times of 0, 4, 8, and 12 consecutive days were 18%, 7.9%, 4%, and 7.7% v/v. An IC50 value < 50 indicates strong antioxidant activity. Vanilla leaf kombucha tea has the potential to be a beverage product that is beneficial for health due to its content of phenolic compounds and antioxidant activity.

***Keyword:*** antioxidants, fermentation, kombucha, phenolics, vanilla

## Introduction

Health constitutes things that are very important to humans to stay productive in every way. Maintaining health can be done in various ways, including regular exercise, adequate rest and consuming healthy food or drinks. In the present era many diseases are caused by Consumption of unhealthy food Among them are fried processed ingredients, drinks containing alcohol and so on. Unhealthy food can trigger free radical exposure, when it enters the body continuously resulting in the body being susceptible to various diseases such as cancer, arteriosclerosis and premature aging (Rahim *et al*., 2023). The thing that can be controlled to reduce exposure to free radicals is to avoid consuming food or drinks that trigger the formation of free radicals in the body. Therefore, it is very important to choose healthy foods or drinks. One of healthy foods or drinks is food or beverages that contain active compounds such as polyphenols, flavonoids and tannins. The active compound is able to Countering Free Radicals by donating one of its free electrons. The ability of a compound to ward off free radicals is called an antioxidant (Simanjuntak, 2012).

Natural compounds, antioxidants produced by Plants from the process of secondary metabolism. Secondary metabolite compounds are produced by plants. Among them are flavonoids, polyphenols, alkaloids, and steroids. The active compound donates one electron to stabilize free radicals in the body (Augustine, 2017). Innovations in the processing of natural products that contain high antioxidant

compounds have been developed, including food and beverage products, so that humans consume them more often. One of the innovations in beverage products that are rich in antioxidants and popular with the public is tea. Processed tea products continue to be developed to increase the active compounds they contain, one of which is the manufacture of kombucha tea.

Kombucha tea is a fermented beverage of tea leaves with added sugar and tea mushrooms known as SCOBY (*Symbiotic culture of bacteria and yeast*). SCOBY is a tea mushroom that results from the symbiosis between yeast and bacterial microbes. The microbes that play a role in making kombucha drinks from the acetic acid bacterial group are *Acetobacter aceti, Acetobacter pausterianus, Acetobacter xylinum, and Bacterium gluconicum* and *Schizosaccharomyces pombe, Candida* sp., *Kloeckera* sp., *S. ludwigii, S. cerevisiae, Torulospora* sp*., Zygosaccharomyces bailii,* and the species *Pichia,* which is included in yeast (Goh *et al*., 2012).

During the fermentation process of bacteria and yeast convert sugars into major compounds such as acetic acid, ethanol, and glucuronic acid, as well as minor compounds such as lactic acid, phenolic acid, B vitamins, and enzymes. The content of active compounds contained in kombucha tea will increase due to the fermentation process. The organic acids produced can act as antioxidants (Muhialdin *et al*., 2019; Purnami *et al*., 2018; Suhardini & Zubaidah, 2016). This is the reason why kombucha tea is more beneficial for health compared to regular tea. Kombucha tea has a sour taste like vinegar, which is because organic acid compounds have increased during the fermentation process, and affect the decline of pH in the Kombucha Tea (Wistiana & Zubaidah, 2015).

Tea Kombucha can be made from plants that have a high content of polyphenols, such as flavonoids and tannins. Research conducted by Suhardini and Elok in 2026 states that the antioxidant activity of kombucha tea uses coffee leaves, guava leaves, soursop leaves, bay leaves, tea leaves, and betel leaves. It was obtained that the phenol compounds contained in kombucha tea will increase due to the fermentation process. An increase in phenol levels will also affect antioxidant levels. The tannin content in the leaves affects the growth medium of bacteria and yeast during the fermentation process, resulting in kombucha tea with good quality (Suhardini & Zubaidah, 2016).

One of the factors that can affect the resulting product is the fermentation time during the tea making process. The fermentation time will affect the alcohol content and the content of the active compounds in kombucha tea. It is therefore very important to control the fermentation time of kombucha tea making. Research conducted by Goh et al in 2012 explained that Kombucha tea that uses black tea as a base is known to be the best product at fermentation time for 8 days because it produces the highest cellulose deposits of 66.9% (Goh *et al*., 2012). In 2020, Nurhayati et al. conducted a study on kombucha cascara tea (coffee skin), which showed that the fermentation time has a real effect on physical, chemical, and sensory characteristics. The best results are 8 days of fermentation time because the total levels of total phenolics are high, pH, and total acids are still within the limits of safe levels for consumption (Nurhayati *et al*., 2020). Research by Yanti et al in the year 2020 explains that making Kombucha Soursop Leaves for 12 Days has the highest antibacterial activity with resistance to *Escherichia coli* by 16.28 mm and *Staphylococcus aureus* by 17.08 mm.

The selection of fermentation time on days 0, 4, 8, and 12 is based on the dynamics of biochemical and microbiological changes during the kombucha fermentation process. Day 0 represents the initial condition of the raw materials before the occurrence of fermentation activity, while day 4 reflects the initial phase of fermentation when microorganisms begin to adapt and metabolize the substrate. Day 8 was chosen because, based on various studies, this phase often shows optimal conditions of fermentation, characterized by an increase in the content of phenolic compounds and antioxidant activity due to the release and biotransformation of polyphenols by the enzymatic activity of microorganisms. Meanwhile, day 12 represents an advanced fermentation phase, in which the accumulation of organic acids, a decrease in pH, as well as an increase in the metabolic activity of microorganisms, can lead to the degradation, oxidation, or polymerization of phenolic compounds, so that the measured phenolic content tends to decrease. Therefore, the variation in fermentation time was chosen to comprehensively describe the change in kombucha quality from the initial phase to the advanced phase of fermentation (Phung *et al*., 2023; Villarreal-Soto *et al*., 2018).

One of the plant leaves that has the potential to be an ingredient for making kombucha tea is vanilla leaves (Yanti *et al*., 2020). Vanilla is a type of plantation crop with high economic value, with relatively stable price fluctuations compared to other plantation crops (Artika *et al*., 2021). Vanilla leaves also have natural antioxidant activity that needs to be explored further. According to research conducted by Pratomo *et al*. (2018) explained that Vanilla leaves have higher antioxidant activity than nutmeg, ginger, or butylated hydroxy toluene (BHT) (Pratomo et al., 2018). Vanilla at a concentration of 1mM can capture DPPH free radicals (*1,1-diphenyl-2-picrylhydrazyl*) well. Vanilla leaf extract has antioxidant activity of 20.52-37.86 mg of caffeic acid/g sample. Strong antioxidant activity in vanilla leaves due to the presence of vanilla acid compounds (p-hydroxybenzoic acid and hydroxybenzaldehyde) (Rojas-López *et al*., 2013). However, in processed tea products, vanilla leaves have not been widely researched, especially in making kombucha tea. Therefore, in this study, kombucha tea will be made from vanilla leaves with a variety of fermentation times, aiming to find out the levels of phenolic compounds and their antioxidant activity.

## Methods

### Equipments and Materials

The equipments used in this study are measuring cups, beaker cups, measuring flasks, glass containers, stirrers, drip pipettes, volume pipettes, spoons, analytical scales, stoves, micropipettes, ovens, vortexes, autoclaves, aluminium foil, UV-VIS spectrophotometers.

The ingredients used in this study are vanilla leaves, tea, kombucha culture starters, aquades, granulated sugar, Merck methanol, 96% Pro Analysis ethanol, Merck $Na_2CO_3$, Merck gallic acid, Merck *Folin-ciocalteu* solution, DPPH Sigma Aldrich, PP Indicator from Merck, and NaOH from.

### Working Procedure

#### *Vanilla Leaf Tea Preparation*

The vanilla leaves are washed with running water until clean and cut into small pieces. Then it is dried in the sun until the leaves wither for 30-60 minutes. The wilted vanilla leaves are dried using an oven at 55°C until they are completely dry. The dried leaves are used as vanilla leaf tea.

#### *Kombucha Starter Preparation*

2000 ml of water is boiled and added by 200 g of sugar (10% w/v), and 10 g of 0.5% (w/v) tea is added. The solution was then filtered, and the filtrate is covered with aluminium foil and let stand until the tea has reached room temperature. After that, 200 ml (10% b/v) of kombucha culture starter is added to the tea brew, and the container is tightly closed. The propagation of the kombucha culture starter is left for 14 days.

#### *Vanilla Leaf Kombucha Tea Preparation*

Vanilla leaf tea as much as 36 g (0.5% b/v) is brewed using 7200 ml of boiling water. Then 20 g of sugar (10% b/v) is added under stirring. The vanilla leaf tea brew is tightly covered with aluminium foil and let sit until room temperature. A liquid kombucha starter of 20 ml is added and fermented for 0, 4, 8 and 12 days in a closed container and filtered after the fermentation time is complete.

#### *Chemical Characterisation of Vanilla Leaf Kombucha Tea*

1. Total acid level test.

   The test of the total level of titrated acid was carried out on the principle of acid-base titration. The test is carried out by means of 10 ml of samples being put into a 100 ml measuring flask and then adding water to the limit mark. After that, 10 ml of filtrate is taken and put into the Erlenmeyer and a 3-drop pp indicator. The solution is titrated with a 0.1 N NaOH solution until the sample solution is coloured from clear to pink. Total acids are calculated using the formula:

$$\text{Total Acid (\%) (1)} = \frac{V_{NaOH} \text{ X } N_{NaOH} \times BM \times 100\%}{V_{sampel} \times 1000} \quad (1)$$

Description:
$V_{NaOH}$ : The volume of NaOH used for titration
$N_{NaOH}$ : standard concentration of NaOH
$V_{sample}$: Sample volume used for titrates
BM : Molecular weight of acetic acid

2. pH level test.
   pH measurements are made using a pH meter. Vanilla leaf kombucha tea is put in a beaker, and dipped in a pH meter.
3. Phenolic rate test.
   a. Alloy acid solution standard curve.
      Standard gallic acid solutions are made in concentration variations of 10, 20, 30, 40, 50 ppm. The standard solution of gallic acid is taken at a concentration of 1 ml in a test tube, and 0.5 ml of Folin-Ciocalteu is added and left for 8 minutes while shaking. Into the solution is added 4 ml of 7% $Na_2CO_3$ solution and in the vortex for 1 minute. Measurements were made at a wavelength of 760 nm.
   b. Sample absorption measurement.
      Sample vanilla leaf kombucha tea is taken as much as 1 ml and 0.5 ml of Folin-ciocalteu is added, let stand for 8 minutes while shaking. A 7% $Na_2CO_3$ solution of 4 ml is added and in a vortex for 1 minute. Absorbance is calculated with a wavelength of 760 nm. Measurements were made at a wavelength of 760 nm. The total phenol content can be calculated using the following formula:

$$\text{TPC} = \frac{c.V.fp}{V} \quad (2)$$

      Description:
      TPC : total phenolic content (mg/L GAE)
      c : concentration (x-value) (ppm)
      fp : Dilution factor
      V : Sample volume (L)
4. Antioxidant activity test.
   Testing of antioxidant activity in vanilla leaf kombucha tea was carried out using the DPPH (*α-diphenyl--picrylhydrazyl methodβ*). The antioxidant activity test was carried out by mixing 2 ml of 50 ppm DPPH solution and 2 ml of the kombucha sample solution. The variations in the concentration of kombucha tea are 10%, 15%, 20%, 25% and 30% v/v with aquades solvent up to 10 ml. The solution was incubated for 30 minutes at room temperature, and its absorption was measured at a wavelength of 524 nm. The formula for the percentage of DPPH absorption inhibition uses the following formula:

$$\text{\% Inhibition} = \frac{(A_0 - A_s)}{A_0} \times 100 \quad (3)$$

   Description:
   $A_0$ : Absorbance control (DPPH + Aquades)
   $A_s$ : Sample absorbance and DPPH

   Control absorbance was made by adding 2 ml of 50 ppm DPPH and 2 ml of water, measured at a wavelength of 524 nm. After that, the value of IC50 from the linear regression equation is determined.

## Results and Discussion

Kombucha constitutes a processed beverage product derived from the fermentation of green tea or black tea by SCOBY biofilm (*Symbiotic Culture of Bacteria and Yeast*) (Cardoso et al., 2020). These microorganisms affect the texture, aroma, colour, and quality of fermented products. The basic

ingredient in making kombucha tea is sugar because sugar is a source of nutrients for the kombucha culture microorganisms. In general, the type of sugar that is often used in making kombucha tea is granulated sugar. The bacteria in kombucha will convert glucose into various types of acids, vitamins, and alcohols during the fermentation process of kombucha tea. Garlic produces glucose and fructose derived from sucrose inversion. Glucose will be converted by yeast and cellulose by *Acetobacter xylinum.* It becomes gluconic acid through the pentose phosphate pathway by acetic acid bacteria, while most fructose is metabolized into acetic acid and gluconic acid in small amounts. Thus, glucose serves as a substrate for cell growth and the formation of products such as acetic acid (Puspitasari *et al*., 2017).

In the manufacture of kombucha tea, one of the factors that can affect the product produced is the length of fermentation time. In this study, a variety of fermentation times were carried out, namely 0, 4, 8, and 12 days. The results of vanilla leaf kombucha tea can be seen in Figure 1 and Table 1.


**Figure 1.** Fermented Kombucha Tea 0, 4, 8 and 12 Days

**Table 1**. Aroma, Taste, and Color of Vanilla Leaf Kombucha Tea

| Fermentation Time (days) | Aroma | Taste | Colour |
|---|---|---|---|
| 0 | Acid | Slightly sour | Brownish yellow |
| 4 | Medium acid | Medium sour | Brownish yellow |
| 8 | Strong acids | Strong sour | Brownish yellow |
| 12 | Very strong acids | Sour is very strong | Brownish yellow |

The aroma and sour taste produced from the fermentation process of vanilla leaf kombucha tea are due to the change of compounds such as glucose into acetic acid. The longer the fermentation time, the taste and the sour aroma the stronger which is in line with the total value of the titrated acid and the pH shown in Table 2. The fermentation process that involves the conversion of sugars into acetic acid and gluconic acid by SCOBY microorganisms plays a role in the formation of the distinctive flavour and aroma of kombucha tea. The results of this study show that with increased fermentation time, the content of organic acids such as acetic acid increases, which is reflected in a decrease in pH and leads to an increase in sour taste.

**Table 2**. Effect of Fermentation Time on Total Titrated Acid and pH

| Vanilla Leaf Kombucha Fermentation Day | % Total Titrated Acid (TAT) | pH |
|---|---|---|
| 0 | 0.003 | 5.56 |
| 4 | 0.012 | 3.67 |
| 8 | 0.024 | 3.06 |
| 12 | 0.048 | 2.37 |

In Table 2 is known that the longer the fermentation time of vanilla leaf kombucha tea, the percentage of total titrated acid (TAT) increases and the pH value decreases. The increase in the percentage of TAT and the decrease in pH during the fermentation process indicate that the acid concentration in kombucha tea is increasing. According to Hassmy (2017) The increase in the concentration of kombucha acid is due to the fermentation process of yeast and bacteria metabolizing the sugar in the solution, so as to produce a number of organic acids such as acetic acid and gluconic acid. The higher the level of organic acids contained in vanilla kombucha leaf tea, the higher the total acid produced, so that the pH value will decrease with the increase in fermentation time. The pH value of kombucha that is acceptable and safe to consume is between 3-5.5. When kombucha tea has a pH below this value, it is necessary to dilute it because it will affect the taste of the kombucha tea produced. In this study, it is known that the fermentation time of 12 days produces a pH value below the tolerance limit of consumption; therefore, if it is to be consumed, it must be diluted first so that the taste is not too sour. While kombucha on days 0, 4, and 8 is still within normal limits for consumption (Simanjuntak, 2012).

Measurement of the phenolic content of vanilla leaf kombucha tea using the Ciocalteu-Folin method (molybdotungstofosfotungstat heteropolianion 3H2O-P2O5-13WO3-5MoO3-10H2O). This mixture of compounds will form a blue complex so that its absorbance can be measured with a uv-vis spectrophotometer instrument at the maximum wavelength. The reaction mechanism is that *the ciocalteu folin reagent* will react with the phenolate compounds in the sample, resulting in a blue colour [$(PMoW_{11}O_4)^{4-}$]. The blue colour in the solution is due to the metal molybdenum (Mo(VI)) in the reactant complex compound, reduced to Mo(V) due to electron donation by the phenol compound. The standard solution commonly used to determine the phenolic content in a sample is gallic acid, so the unit of phenolic content in the sample is mg of gallic acid equivalent per L of sample.

In determining the phenolic levels in the sample, gallic acid is used to make standard solutions with various concentration series, namely 10, 20, 30, 40, and 50 ppm. According to Rahayu and Inanda (2015) Gallic acid is used as a standard because gallic acid is one of the phenolic compounds, stable and relatively inexpensive compared to others, and has strong antioxidant activity. A standard solution of gallic acid with a concentration series is measured for absorbance with a UV-vis spectrophotometer. Measurements were made at a maximum wavelength of 763 nm. The concentration (sb x) and absorbance (sb y) values are used to create the calibration curve (Figure 2).

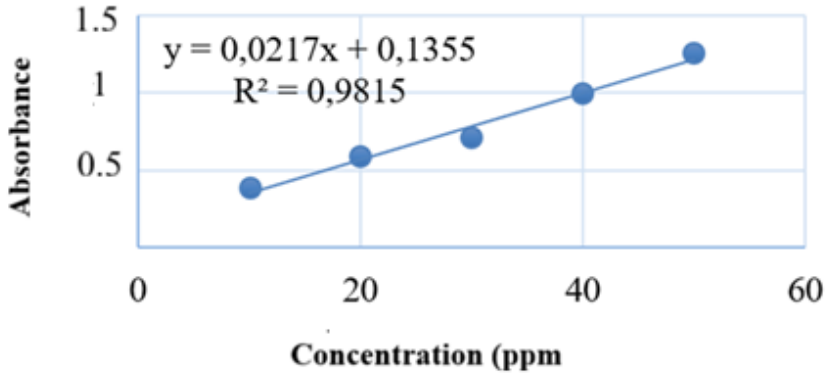


**Figure 2**. Gallic Acid Calibration Curve

Based on Figure 2, it is known that the calibration curve has a linear regression equation, namely y = 0.0217x + 0.1355, with R2 0.9815. A value of R2 close to 1 indicates that the curve is linear, so the data can be used for the determination of phenolic concentrations in the sample. The concentration of phenolic compounds in vanilla leaf kombucha tea can be determined through this equation by entering the sample absorbance value on variable y, so that an x value is obtained, which is the total concentration of phenolic compounds in vanilla leaf kombucha tea. It is then calculated with a formula

to find out the phenolic level in the sample. The levels of Phenolic Gallic Acid in the Sample (ppm) can be seen in Table 3.

**Table 3**. Effect of Fermentation Time on Phenolic Levels

| Vanilla Leaf Kombucha Fermentation Day (day) | Absorbance | Phenolic Levels of Gallic Acid in Samples (ppm) |
|---|---|---|
| 0 | 0.96 | 37.99539 |
| 4 | 1.051 | 42.18894 |
| 8 | 1.385 | 57.58065 |
| 12 | 1.232 | 50.52995 |

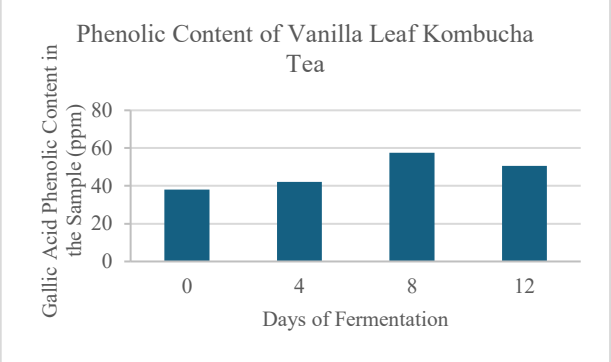


**Figure 3.** Phenolic Levels Graph of Vanilla Leaf Kombucha Tea

Based on Table 3 and Figure 3, it is known that the phenol levels of vanilla leaf kombucha tea are increasing from days 0, 4, and 8, and decreasing on day 12. Increased phenolic levels with increased fermentation time on days 0, 4, and 8 are due to microbial enzyme activity during fermentation. The fermentation process of kombucha by the enzymatic activity of microorganisms contributes to the release of phenolic compounds that were originally bound to the plant cell wall matrix and promotes the biotransformation of complex polyphenols into simpler and more readily available phenolic compounds, so that the total phenolic content in kombucha has the potential to increase compared to the original raw materials, such as the Battle of the (Phung *et al*., 2023). Enzymes such as β-glucosidase and tanase produced by microorganisms in SCOBY can hydrolyse glycoside or tannin bonds into free phenolic forms, increasing the bioactivity and antioxidant potential of the final product (Onsun *et al*., 2025). The decrease in phenolic levels on the 12th day of fermentation compared to the 8th day is suspected to occur because fermentation has entered an advanced phase, where the metabolic activity of microorganisms is increasingly intensified so that some phenolic compounds undergo degradation, oxidation, or are utilized in secondary metabolism. In addition, increased alcohol and acidity levels during further fermentation can affect the stability of phenolic compounds as well as cause structural changes or binding of phenolic compounds with other components, resulting in lower measured levels of free phenolics (Villarreal-Soto *et al*., 2018). This is in accordance with data on the increase in alcohol content on day 12.

The fermentation time also affects the antioxidant activity of vanilla leaf kombucha tea. A compound that can ward off free radicals by donating its electrons to stabilize them is called an antioxidant compound. In addition to stabilizing electron radicals, antioxidant compounds are also able to break free radical chain reactions (Fadhilah Azhar *et al.*, 2021). The fermentation process will increase antioxidant activity because bacteria and yeast will increase the number of phenols. The higher the level of phenolic compounds in the sample, the higher the antioxidant activity (Khaerah & Akbar, 2019).

In general, the method used to test non-enzymatic antioxidant activity is to use *the reagent* 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH method is one of the methods that is generally used because it is accurate, effective, and practical. The mechanism of the radical deterrence reaction is shown in Figure 4.

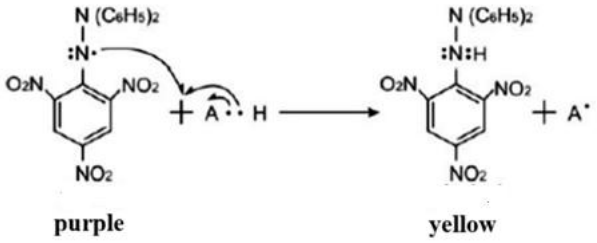


**Figure 4.** Reaction Mechanism for Identifying Color Change from DPPH

Maximum absorbance is due to the structure of DPPH containing chromophore and auxochrome clusters. The antioxidant reaction of capturing DPPH radicals can be observed by the presence of a discoloration that occurs in the sample. At first the DPPH is purple, then the longer it will turn yellow. The change in purple to yellow due to reduced DPPH produces the compound 1,1-diphenyl-2-pykrilhydrazine (Husniati *et al*., 2020). The colour intensity of the DPPH solution indicates how much DPPH is reduced. If the colour of the solution fades yellow, the more DPPH is reduced, this indicates that the radical antidote is greater (Wijaya *et al.*, 2014). The testing process with the DPPH method must be carried out in a dark room because DPPH Sensitive to light. A dark room will minimize the radicals that form in addition to the DPPH free radicals that are added deliberately (Meigaria *et al*., 2016).

The antioxidant activity of vanilla leaf kombucha tea for each treatment used the $IC_{50}$ value parameter. $IC_{50}$ or *Inhibition Concentration* 50% is the concentration value required for the sample solution to reduce DPPH free radicals by 50%. The inhibition value is the value of free radical inhibition (Handayani *et al*., 2016). The $IC_{50}$ value is widely used as an indicator of the strength of antioxidant activity, where the lower the $IC_{50}$ value, the higher the antioxidant activity. Because smaller concentrations are needed to neutralize free radicals. In kombucha fermentation, changes in $IC_{50}$ values during fermentation time reflect the dynamics of the content and effectiveness of antioxidant compounds, specifically phenolic compounds. The inhibition value of each concentration in each treatment can be seen in the Table 4.

**Table 4.** Kombucha Tea Inhibition Value Day 0

| Concentration (%) | ln Concentration | Absorbance | % Inhibition |
|---|---|---|---|
| 10 | 2.3025 | 0.3127 | 32.4598 |
| 15 | 2.7080 | 0.2607 | 43.6898 |
| 20 | 2.9957 | 0.2139 | 53.8031 |
| 25 | 3.2188 | 0.2060 | 55.5098 |
| 30 | 3.4011 | 0.1617 | 65.0649 |

**Table 5**. Kombucha Tea Inhibition Value Day 4

| Concentration (%) | ln Concentration | Absorbance | % Inhibition |
|---|---|---|---|
| 10 | 2.3025 | 0.166 | 64.1525 |
| 15 | 2.7080 | 0.157 | 66.0961 |
| 20 | 2.9957 | 0.137 | 70.4150 |
| 25 | 3.2188 | 0.126 | 72.7905 |
| 30 | 3.4011 | 0.096 | 79.2689 |

**Table 6.** Kombucha Tea Inhibition Value Day 8

| Concentration (%) | ln Concentration | Absorbance | % Inhibition |
|---|---|---|---|
| 10 | 2.3025 | 0.207 | 55.2987 |
| 15 | 2.7080 | 0.147 | 68.2556 |
| 20 | 2.9957 | 0.12 | 74.0862 |
| 25 | 3.2188 | 0.096 | 79.2689 |
| 30 | 3.4011 | 0.076 | 83.5879 |

**Table 7**. Kombucha Tea Inhibition Value Day 12

| Concentration (%) | ln Concentration | Absorbance | % Inhibition |
|---|---|---|---|
| 10 | 2.3025 | 0.196 | 57.6741 |
| 15 | 2.7080 | 0.186 | 59.8336 |
| 20 | 2.9957 | 0.122 | 73.6543 |
| 25 | 3.2188 | 0.11 | 76.2456 |
| 30 | 3.4011 | 0.087 | 81.2125 |

After knowing the inhibition value of each concentration in each treatment, a linear regression equation is sought by plotting the x-axis as the concentration and the y-axis as the %inhibition. The regression equation for fermentation times of 0, 4, 8, and 12 consecutive days was y = 17.323x ($R^2$ = 0.9919); y = 12.954x + 32.651 ($R^2$ = 0.8905); y = 25.258x - 1.7879 ($R^2$ = 0.9913); y = 23.002x + 2.4372 ($R^2$ = 0.9196). The IC50 value was obtained by calculating the concentration of the sample using the linear regression equation formula obtained from the linear regression graph of the relationship between concentration and % inhibition (Hani & Milanda, 2021). IC50 value data. Each treatment can be seen in Table 8. From the calculation data, an IC50 value was obtained that exhibits strong antioxidant activity due to IC50<50. The smaller the IC50 value, the greater its antioxidant activity (Firdayani *et al*., 2015).

**Table 1.** IC50 Tea Value of Vanilla Kombucha Tea

| Vanilla Leaf Kombucha Fermentation Time (days) | IC50 (% v/v) |
|---|---|
| 0 | 18 |
| 4 | Sec. 7.9 |
| 8 | 4 |
| 12 | Sec. 7.7 |

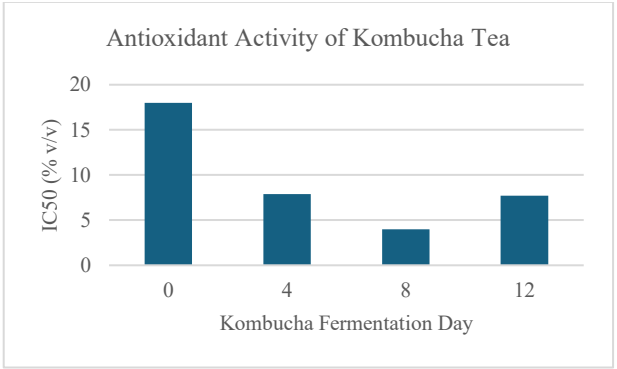


**Figure 5**. Antioxidant Activity of Kombucha Tea

Based on Table 8 and Figure 5, it is known that the antioxidant activity of Vanilla leaf kombucha tea increased from day 0 until the next day 8. The increased antioxidant activity during the fermentation process is due to the increased content of phenolic compounds, which play an important role in kombucha's ability to ward off free radicals. The higher the level of phenolic compounds, the greater their antioxidant activity, which is indicated by the lower $IC_{50}$ value. This indicates that the concentration of the sample needed to inhibit 50% of free radicals becomes smaller. The mechanism of free radical inhibition by phenolic compounds occurs through the ability of hydroxyl groups (-OH) in phenolic structures to donate hydrogen atoms or electrons to free radicals so that they become more stable and non-reactive, as shown in Figure 4. The increased content of phenolic compounds during kombucha fermentation is due to the biotransformation process that is generated by the enzymatic activity of microorganisms in SCOBY. Enzymes produced by bacteria and yeast, such as β-glucosidase and tanase, play a role in changing the chemical structure of complex polyphenols through the breakdown of glycoside or ester bonds, resulting in simpler phenolic compounds. These phenolic compounds are naturally found in the leaves of plants that are used as the base material for kombucha, and one of the phenolic compounds that acts as a natural antioxidant in plants is cinnamic acid (Suhardini & Zubaidah, 2016). On the 12th day, antioxidant activity decreases because during the fermentation process, phenolic compounds change into simpler organic acids, causing a weakening of the ability to ward off free radicals. This causes antioxidant activity to decrease or IC50 to increase on day 12 (Puspitasari *et al*., 2017).

## Conclusion

The conclusion in this study is that the phenolic compound levels of vanilla leaf kombucha tea at fermentation time of 0, 4, 8, and 12 days are 37.99; 42.188; 57.58; and 50.53 mg/L GAE, respectively. The highest phenolic levels are found at the 8th day of fermentation. The IC50 value for fermentation time of 0, 4, 8, and 12 consecutive days was 18%; 7,9% ; 4% ; and 7.7% v/v. An IC50 value < 50 indicates strong antioxidant activity. Vanilla leaf kombucha tea has the potential to be a beneficial drink for healthbecause it contains high phenolic compounds and strong antioxidant activity.

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