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ANALYSIS OF ANTIOXIDANT CONTENT IN TELANG FLOWERS *(Clitoria ternatea)* KOMBUCHA TEA BY DPPH (1,1-diphenyl-2-picrylhydrazin) METHODS

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ABSTRACT

Kombucha is a drink made from fermented tea liquid and sugar. Some of the benefits of kombucha include antioxidants, antibacterials, improving gut microflora, increasing body resistance and lowering blood pressure. These properties are due to the content of phenolic compounds that have antioxidant activity. One of the plants that can capture free radicals is telang flower. This study utilizes telang flower plants as a kombucha drink that contains antioxidants. This study aims to prove the antioxidant activity of telang flower kombucha using the DPPH (2,2-diphenyl-1- picrylhydrazil) method with vitamin C as a positive control. DPPH is a free radical compound. Tests were carried out on kombucha with 5 different concentrations of 50, 100, 150, 200, and 250 ppm. Samples of each concentration will be reacted with DPPH compounds, then measured the absorbance value using UV-Vis Spectrophotometry at a wavelength of 517 nm. In making kombucha, 20 g of dried telang flowers were used. The results obtained in this study are the IC50 value of telang flower kombucha of 0.668 ppm which is categorized as very strong with the IC50 value of Vitamin C of 0.583 ppm. From these results it can be concluded that telang flower kombucha has very strong antioxidant activity to ward off free radical compounds.

Keywords: : *Kombucha, telang flowers, vitamin C, Antioxidant, DPPH*

INTRODUCTION

Kombucha drinks are made when tea and sugar are fermented with a kombucha culture known as SCOBY (Symbiotic Cultures of Bacteria and Yeasts). In countries like China and Russia, where kombucha is popular, kombucha is consumed as a health drink. Kombucha tea tastes like apple cider and is tangy and refreshing. The fermentation process of kombucha tea is done with the help of bacteria and yeast microbes. The symbiotic culture consists of various forms of yeast (Torolupsis sp.), lactic acid bacteria (Lactobacillus, Lactococcus), acetic acid bacteria (Acetobacter xylinum, Acetobacter aceti), and Saccharomyces ludwigii, Saccharomyces bisporus, and Zygosaccharomyces sp. These yeasts and bacteria are capable of preventing microorganism infections.

Kombucha has health benefits including antibacterial, antioxidant, digestive system stabilization, inflammation prevention, immune enhancement, and blood pressure reduction. The presence of phenolic chemicals that have antioxidant action is what causes these effects. Antioxidants are molecules that have the ability to prevent oxidation of other molecules and provide endogenous protection and exogenous oxidative stress by capturing free radicals. Many plants that have antioxidant properties are plants that contain carotenoids and polyphenols, especially flavonoids such as bay flowers. By counteracting free radicals, antioxidants are compounds that provide exogenous oxidative stress and endogenous protection. Molecules known as antioxidants have the ability to prevent other molecules from being oxidized (Lai-Cheong, 2017; Puspitasari, et all, 2017)

Telang flower or its Latin name (*Clitoria ternatea*) contains bioactive compounds including kaempferol, quercetin, and mirisetin. Telang flowers also contain several compounds such as fatty acids, phytosterols, and tocopherols. The human body can overcome free radicals with certain antioxidant compounds (Jearaj, 2021).

The DPPH (1,1-diphenyl-2-picrylhydrazyl) method is simple, easy to use and does not require much time to test the antioxidant activity in pineapple, turmeric and bay flower kombucha. The ability to capture DPPH radicals is a way to measure antioxidant activity. In the presence of antioxidants, DPPH radicals will dissolve by donating electrons to DPPH which changes the color of the sample from purple to yellow. This change in color is proportional to the number of electrons taken up by DPPH, so it can be measured by spectrophotometry (Jaya, et all, 2012).

In Rahadi Santoso's research, before fermentation, DPPH antioxidant inhibition in green tea was 73.67% and black tea was 66.83%. After the fifth day of fermentation, DPPH antioxidant inhibition increased to 86.95% for green tea and 77.77% for black tea. On the seventh day of fermentation, the highest antioxidant DPPH inhibition occurred at 92.57% for green tea and

84.75% for black tea. However, on the 9th day of fermentation, the DPPH antioxidant inhibition of both types of tea was reduced (Nadialista, 2021).

Based on this background, a study was conducted to analyze the antioxidant content in telang flowers using the DPPH method.

METHODS

1. Preparation of telang flower juice

Telang flowers are sorted to select good quality flowers and discard damaged or unfit ones. The sorted flowers are then cut into smaller pieces. The pieces were washed thoroughly to remove any dirt or residue that may be present. The flowers were weighed as much as 20 grams. The weighed flowers were boiled in 500 ml of water for 10 minutes. After boiling, the mixture was filtered using a sieve to separate the pulp from the boiled liquid (Parindra, et all, 2016).

2. Kombucha Preparation

Add 50 grams of palm sugar to the pineapple juice and stir until the sugar is completely dissolved. Cover the mixture and leave it until the temperature drops to room temperature. When the juice has cooled, put it in a jar and add the starter as much as 10% of the volume of pineapple juice. Slowly add the SCOBY (Symbiotic Culture Of Bacteria and Yeast) to the jar. Close the jar and store in a dark place away from direct sunlight. This process was then repeated for turmeric juice and bay flower juice samples with the same steps (Parindra, et all, 2016).

3. Preparation of Stock Solutions of kombucha and vitamin C Samples

Preparation of test solutions was carried out by making test solutions in various concentrations using 96% ethanol solvent. A total of 25 mg of kombucha was weighed and dissolved in 25 mL of ethanol solvent to obtain an initial solution with a concentration of 1000 ppm which would be used for dilution of the test solution. The stock solution of each Kombucha sample was diluted again to make 5 series of solution concentrations (50, 100, 150, 200, and 250 ppm). Similarly, vitamin C was diluted to produce concentration variations of 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm (Musman,et all, 2017).

4. Preparation of 40 ppm DPPH Working Standard Solution

Preparation of DPPH solution is done by dissolving 0.001 g of DPPH in 25 mL of 96% ethanol, so that a solution with a concentration of 40 ppm is obtained. This solution is stored at low temperature and protected from light for immediate use.

5. Measurement of Absorbance of Kombucha and Vitamin C Samples

Measurement of % inhibition of kombucha drinks and vitamin C was carried out by adding 1 mL of DPPH solution to each test solution (3 mL) of kombucha drinks and vitamin C with various concentrations. This mixture was then homogenized and incubated for 30 minutes in the dark. After that, the absorbance was measured using a UV-Vis Spectrophotometer at a predetermined maximum wavelength. This process was repeated three times to ensure the accuracy of the results (Hasibuan, 2017; Suharti, 2017).

RESULTS AND DISCUSSION

The linear regression value on vitamin C is Y = 85.688 + 0.016x and the correlation coefficient value is 0.99223 while the linear regression value of telang flower is Y = 74.84 + 0.044x with the correlation coefficient value is 0.98438. The results of the calculation of DPPH absorption inhibition, telang flower and IC50 values are shown in table 1.

Table 1. Inhibition and IC50 values of vitamin C and telang flower

Sample Uji	concentration	Absorbance	Inhibition	IC_{50} (ppm)
Vitamin C	50	0,036	86,48	0,583
	100	0,034	87,23	
	150	0,032	87,98	
	200	0,029	89,11	
	250	0,028	89,49	
Telang flower kombucha tea	50	0,062	76,72	0,668
	100	0,06	77,47	
	150	0,056	78,97	
	200	0,046	82,73	
	250	0,036	86,48	

The main metabolites of kombucha fermentation are organic acids, active ingredients that have health effects such as antioxidants. When compared to the unfermented substrate, the starter culture secreted during the fermentation process enhances the antioxidant properties of kombucha. Regardless of the underlying structure of the substrate, the starter culture provides a major microbial inoculum to the solution. Sucrose is converted into glucose and fructose by the yeast produced by the organisms as they ferment the substrate. In this way, the organisms can convert the monomers into carbon dioxide and ethanol. then bacterial enzymes oxidize the ethanol, which forms acetic acid with a low pH. then these microorganisms form cellulose, which aids in biofilm formation.

Furthermore, the kombucha beverage made of bay flower was tested for antioxidants through the DPPH method with vitamin C as a comparator. The DPPH method, which is 2,2-diphenyl-1-picrylhydrazyl, was used to measure the absorbance value of the kombucha drink using a UV-Vis spectrophotometer at a wavelength of 517 nm. The IC50 value or 50%

inhibitory concentration, is a measure of the antioxidant concentration sufficient to stop the activity of 50% of free radicals. The ability to capture DPPH radicals is one way to measure antioxidant activity. If there are antioxidants in the kombucha drink, by donating their electrons to DPPH, the antioxidants will neutralize the DPPH radicals. This causes the initially purple sample to turn yellow. This happens because the unpaired electrons become paired with hydrogen donors, which form stable DPPH radicals. This color change will continue until the sample is no longer purple at all.

The percentage of inhibition shows the percentage of material that has the ability to resist free radical compounds. then calculated by comparing the absorbance of the blank and sample with the absorbance of the blank to get the percentage value of inhibition.

Based on table 1 shows the IC50 value of kombucha telang flower 0.668 ppm, this indicates very strong antioxidant activity in reducing free radicals. Vitamin C in its pure form has the ability to reduce free radical substances (DPPH). This is evidenced by the IC50 value of less than 50 ppm which is 0.583 ppm. From these results, it shows that the value of vitamin C is higher than kombucha of telang flower.

From these results that kombucha has very strong antioxidant activity to counteract free radical compounds. In theory, it is known that IC50 values below 50 ppm indicate that a compound has very strong antioxidant activity. IC50 values between 50-100 ppm indicate strong antioxidant activity. IC50 values between 101-150 ppm indicate moderate activity, IC50 values between 150-200 ppm indicate weak activity, and IC50 values above 200 ppm indicate very weak antioxidant activity.

According to research by Andriani and Mustiwi (2020) found that ethanol extract of telang flowers has an IC50 of 41.36 ppm, which is included in the very strong category. So that when compared, kombucha tea of telang flowers has 60 times the strength compared to fresh.

CONCLUSION

Based on the results obtained in this study, it can be concluded that the IC50 value of telang flower kombucha is 0.668 ppm and has very strong antioxidant activity to ward off free radical compounds.

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