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POTENTIAL OF KOMBUCHA LEAVES *ANNONA MURICATA* L. AND FLOWER *CLITORIA TERNATEA* AS AMYLASE ENZYME INHIBITORS AND TOTAL SUGAR LEVELS ANALYSIS

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Abstract

Preliminary: Soursop leaf kombucha and telang flower contain organic acids such as acetic acid, gluconic acid, glucuronic acid, phenolic group compounds, which have potential as antioxidants, anti-inflammatory and antimicrobial, and antihyperglycemic. The purpose of this study was to determine the potential of soursop leaf and telang flower kombucha as an inhibitor of amylase enzymes and total sugar content.

Method: Soursop leaf kombucha (*Annona muricata* Linn) and telang flower (*Clitoria ternatea*) in various comparisons of boiling time 5 minutes vs 10 minutes vs 15 minutes, then tested the activity of amylase enzyme using the method, absorbance was measured by wavelength 550 nm. The method of measuring total sugar content using the Nelson-Somogy method. The measurement results were recorded in tabular form. Data analysis used one way ANOVA test with a significance of $p < 0.05$. Analysis using SPSS 23 software.

Results: In the combination of soursop leaf kombucha and telang flower with boiling time of 5 minutes vs 10 minutes vs 15 minutes there was no significant difference for total glucose levels, which was around 2.63 ± 1.7 . Amylase activity at 5 minutes of boiling time on kombucha soursop leaves and telang flowers was better than 10 minutes and 15 minutes of boiling time, with a comparison of 34.3 vs. 10 minutes of boiling, 34.3 vs. 15 minutes of boiling.

Conclusion: The best sugar content and amylase enzyme activity was found in boiling the combination of kombucha telang flower and soursop leaf for 5 minutes.

Keyword: Kombucha, *Annona muricata* L, *Clitoria ternatea*, Amylase, Total Sugar

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Kombucha is a probiotic product that has several antibacterial properties, improves intestinal microflora, can increase body resistance and lower blood pressure. The content of active compounds in kombucha is affected by boiling time, size of simplicia, drying method, type of leaf, and the presence of inhibitory factors¹. The antioxidant activity of kombucha tea was shown to be higher than black tea, namely 93%, while black tea was 90%. This is caused by the breakdown of complex polyphenols into smaller molecules by enzymes produced by bacteria and yeast during fermentation so that they are easily absorbed and used. The sugar used in making kombucha tea is broken down into organic acids during the fermentation process so it is safe for consumption by patients with diabetes mellitus.¹⁰

Digestion of carbohydrates begins in the mouth. The food bolus obtained after chewing food is mixed with saliva which contains the enzyme amylase. Amylase hydrolyzes starch or starch into a simpler form, namely dextrin. If it stays in the mouth long

enough, some of it is converted into the disaccharide maltose² .. Obesity occurs due to the accumulation of excess fat due to an imbalance in food consumption and energy expenditure. Obesity can cause an increased risk of several diseases such as Alzheimer's, type 2 diabetes mellitus, cancer, increased circulating cholesterol³. The effect of giving probiotic drinks such as kombucha is able to lose weight by slowing down the breakdown of complex carbohydrates by inhibiting the amylase enzyme thereby reducing the availability of carbohydrates into calories⁴

Soursop leaves and telang flowers contain phenolic compounds, steroids/terpenoids, tannins, alkaloids and flavonoids which have potential as antioxidants, anti-inflammatory and antimicrobial. However, the potential for the combination of soursop leaf kombucha and telang flower to reduce blood glucose levels has not been studied by other researchers.

The purpose of this study was to analyze the effect of boiling time on the process of making kombucha soursop leaf (*Annona muricata* Linn.) and telang flower (*Clitoria ternatea*) on the ability to reduce α -amylase enzyme activity in vitro and sugar content in kombucha.

Methods

Research Design, Time and Place

Experimental research in vitro. The making of soursop leaf and telang flower kombucha was carried out at the Laboratory of the Faculty of Medicine, Islamic University of Malang (UNISMA) and the Amylase Enzyme and Sugar Level Test was carried out at the CHEMICAL Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. The research was conducted in August 2021 and was conducted at the BIOCHEMICAL LAB, University of Muhammadiyah Malang.

Sampling

Soursop leaves are selected in the order of 3 to 5 from the base. Wash and chop then let stand for 12 hours, weigh the soursop leaves then dry at a temperature of $\pm 60^{\circ}\text{C}$ for 5-6 hours. Then blend until it becomes a powder. Telang flowers are selected that are good then washed and drained. The telang flower is then dried using an oven at a temperature of $\pm 60^{\circ}\text{C}$ for 5-6 hours then blended. The two simplicia were mixed in a ratio of 1:1 and put into a tea bag.

Soursop Leaf and Telang Flower Kombucha Making

Before the process of making kombucha all tools are sterilized first. Prepared soursop leaves and telang flowers that have been put in a tea bag with a weight of 2 grams each. 1000 mL of water is heated to boiling at 100°C . Simplicia packs of soursop leaves and telang flowers are put into boiling water for 5 minutes, then filtered. The infusion is then added 150 g of sugar and stirred until dissolved and cooled in a glass jar. When the solution has reached room temperature ($25\text{-}30^{\circ}\text{C}$) added 30 ml of liquid kombucha starter (SCOBY). Cooling time should not exceed 4 hours. The jar is covered with a white cotton cloth and tied with rubber. Then the fermentation process is left for 7-14 days at room temperature and should not be exposed to direct sunlight. The same procedure was performed for the infusion for 10 minutes, and 15 minutes. 6

In vitro Alpha Amylase Enzyme Activity Inhibition Test

Each kombucha (5 minutes, 10 minutes, 15 minutes) was weighed as much as 5 ml. Add 50 ml of acetate buffer (0.1-0.5) pH 5.5 then leave for about 30 minutes while stirring occasionally. Filter with filter paper, the filtrate is a crude enzyme solution. Weigh 12 g of

filtrate in a centrifuge tube. Centrifugation 1500 rpm for 30 minutes. The extracted supernatant from each kombucha was taken as much as 1 ml and then prepared starch substrate. Dissolve 0.2 g of soluble starch in 20 ml of water. Stir and heat the solution until the appearance is clear and homogeneous making a standard solution of maltose. Dissolve 50 mg of maltose into 50 ml of HCl buffer, the solution is then diluted to obtain a standard stock with a concentration of 1000 ppm.

Enzyme Activity Alpha Amylase = $CX \frac{1}{TX} \frac{1}{U/1 \text{ mm}}$

Information:

C: Concentration of maltose/ml (micromole)

T : incubation time ((minutes)

1 unit of the enzyme alpha amylase = the amount of enzyme activity needed to liberate 1 micromole of maltose / minute / ml of the enzyme

Kombucha Sugar Level Test Using the Nelson-Somogy Method

In determining glucose levels, make glucose standards with various concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Pipette 1 ml of each standard solution into a test tube. Add 1 ml of Nelson's reagent. The mixture is heated in boiling water for 20 minutes. The mixture is then cooled at room temperature and added 1 ml of arsenomolybdate reagent. Add 7 ml of distilled water. Absorbance was measured at a wavelength of 540 nm. In the test the sample used was kombucha each with 3 samples (boiling 5 minutes, 10 minutes and 25 minutes) made variations in concentration of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Pipette each sample of kombucha with 5 variations of concentration as much as 1 ml of solution in a test tube. Add 1 ml of Nelson's reagent then the mixture is heated in boiling water for 20 minutes. The mixture is cooled at room temperature and added 1 ml of arsenomolybdate reagent then add 7 ml of distilled water to reduce the concentration. The absorbance of the solution was measured at a wavelength of 540 nm.

Calculation of % sugar content = $\frac{(FP \times \text{Concentration (X)} \times \text{filtrate volume})}{\text{Sample weight (mg)}} \times 100\%$

Statistical Data Analysis

The amylase enzyme inhibition test was carried out based on the breakdown of the substrate to produce a product, whose absorbance was measured. The results of the absorbance control and sample absorbance values were used to calculate the % inhibition or the ability of the inhibitory power.

From statistical analysis, it can be seen that the potency of soursop leaf and telang flower kombucha in various variations of boiling time compared to control. The results are said to be significantly different if the significance value is $p < 0.05$.

Findings and Discussion

The results of the amylase activity test of the combination of soursop leaf kombucha and telang flower were carried out to determine the decrease in amylase enzyme activity in breaking down starch. The principle of the test is to see the reaction of maltose and glucose with DNS to produce a color.

The highest yield was obtained in the combination of soursop leaf kombucha and telang flower with 5 min boiling time, amylase inhibitor activity with a value of 103,000 ppm and an absorbance value of 0.139. Can be seen in table 1

Table 1 Results of Amylase . Enzyme Activity Analysis

NO	SAMPLE	ABS	MAL(ppm)	Amylase Enzyme Activity
1	5 minutes	0.139	103,000	34.3
2	10 minutes	0.142	118,000	39.3
3	15 minutes	0.169	253,000	84.3

The results of the total sugar content in soursop leaf and telang flower kombucha can be seen in table 2. It can be seen in table 2 that kombucha with 5 minutes of boiling time has higher glucose levels than 10 minutes and 15 minutes of boiling time. The lowest glucose levels were in 10 minutes of boiling, with an average of $SD2.27 \pm 1.99$.

Kombucha Samples		Glucose Level (mg/dl)	SD Average
5 minutes	100 ppm	5,727	3.54 $\pm$ 1.58
	80 ppm	4,364	
	60 ppm	3,455	
	40 ppm	2,545	
	20 ppm	1,636	
10 minutes	100 ppm	4,818	2.27 $\pm$ 1.99
	80 ppm	3,909	
	60 ppm	1,636	
	40 ppm	0.727	
	20 ppm	0.273	
15 minutes	100 ppm	4,364	2.63 $\pm$ 1.68
	80 ppm	3,909	
	60 ppm	3,000	
	40 ppm	1,636	
	20 ppm	0.273	

The amylase inhibitor test was carried out by making differences in the concentration of starch substrate. These different starch substrate concentrations serve to compare and determine the best starch substrate concentration in inhibiting the amylase enzyme. Testing the activity of amylase inhibitors using a spectrophotometer so that the absorbance value of control and absorbance of the combination of soursop leaf and telang flower is known.

Best concentration on boiled kombucha

According to Yanti (2016), enzyme activity increases in proportion to the increase in substrate concentration and reaches maximum activity. This increase increases the amount of enzyme-substrate, but at a certain concentration it will be in a saturated condition when there is no more free enzyme available. Increasing the substrate concentration will increase the reaction rate until the maximum value is reached. A further increase in the substrate concentration does not increase the rate of the reaction because the enzyme is already saturated with the substrate. After the optimum concentration was obtained, the enzyme activity decreased due to the inhibition of enzyme activity. According to Soeka (2015) the rate of starch hydrolysis by -amylase depends on several conditions such as substrate concentration, pH, temperature, activator and inhibitor. The mechanism of action of the enzyme is also determined by the concentration of the available substrate. If the substrate concentration is low, the rate of action of the enzyme is also low. On the other hand, if the available substrate concentration is large, the enzyme work is also fast. In the case of excess substrate, the enzyme activity does not decrease but remains constant

Combination processing of soursop leaf kombucha and telang flower affects the glucose levels of the product, especially in boiling kombucha with 5 minutes, 10 minutes and 15 minutes of boiling time. The highest glucose levels are found in kombucha with 5 minutes of boiling and the most optimal boiling is in kombucha with 10 minutes of boiling because longer boiling will convert sugar into nutrients from SCOBY.

This is in accordance with the opinion of Palguna et al. (2013), that the ratio of water content in cooking has a positive correlation with the gelatinization value of starch. The results showed that the highest treatment is on heating for 30 minutes and a water content level of 75%, which is characterized by the highest carbohydrate content.

Conclusion and Suggestion

Telang flower and soursop leaf kombucha with 5 minutes of boiling time had better absorbance values for the amylase enzyme than kombucha with 10 minutes and 15 minutes of boiling time. Kombucha with 10 minutes of boiling has a more optimal glucose level than kombucha with 5 minutes of boiling.

There are several suggestions to improve this research in the future, namely conducting in vivo tests

Thank-You Note

The researcher would like to thank DIKTI, supervisory lecturer, Islamic University of Malang who have helped this research.

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