

Innovation Of Yogurt Of Wuluh Belimbing Fruit Source (*Averrhoa Bilimbi L.*) And Its Effect On Reduction Of Blood Glucosa Rate Of Mencit (*Mus Musculus*)

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Abstract

This study aims to determine the juice of star fruit can be formulated in the form of yogurt and know at what concentration the yogurt of star fruit juice (*Averrhoa bilimbi L.*) can affect the blood sugar levels of mice (*Mus musculus*). Mice were induced glucose and compared its effectiveness with acarbose. Five treatment groups were used: yogurt without juice (negative control), acarbose (positive control), 0.35 ml star fruit juice yogurt, 0.50 ml, and star fruit juice yogurt, 0.65 ml star fruit juice yogurt. Blood glucose measurement for 3 days. The results showed that star fruit juice can be formulated in yogurt, meeting the standards of good physical stability, namely organoleptic, pH, and viscosity tests. In the antidiabetic test, the results showed that all concentrations can affect the blood sugar levels of mice, with a decrease of 90 mg/dl at a concentration of 0.35 ml, a decrease of 92 mg/dl at a concentration of 0.50 ml, and a decrease of 100 mg/dL at a concentration of 0.65 ml, it was concluded that the concentration that most effectively affected the blood sugar levels of mice was a concentration of 0.65 ml with a decrease of 100 mg/dl.

Key words: Antidiabetes, *Averrhoa bilimbi L.* glucose, *Mus musculus*

1. Introduction

According to the World Health Organization, non-communicable diseases were the leading cause of death worldwide in 2018, increasing to 71%. In Indonesia, the incidence of non-communicable diseases continues to increase. Data from the [1] shows that the rate of non-communicable diseases is 69.91%. One of them is diabetes mellitus, from 6.9% to 8.5%. According to the International Diabetes Federation (IDF) in 2021, Indonesia currently ranks 5th out of ten countries with the highest number of diabetics in the world. Meanwhile, the World Health Organization estimates that the number of people with diabetes in Indonesia has increased from 8.4 million in 2000 to around 21.3 million in 2030.

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to a lack of insulin secretion, insulin action, or both [2]. Diabetes mellitus is a chronic and multiethological metabolic disease or disorder characterized by high glucose levels accompanied by impaired carbohydrate metabolism [3] Diabetes is a disease or disorder that affects the body's ability to convert food into energy [4].

Plants are natural diversity that always exist around us, both wild plants and plants that are deliberately cultivated. Plants have been used as medicinal plants since ancient times, although their use has been passed down from generation to generation or by word of mouth [5]. One of the plants that can be used as traditional medicine is belimbing wuluh (*Averrhoa bilimbi L.*). The fruit of the star fruit plant is usually only used as a mixture of sour vegetables. Star fruit is usually also a lot that just falls in the yard and is just wasted. The fruit contains compounds and flavonoids

that are useful as antidiabetics [6]. Flavonoids are alpha-glucosidase inhibitors that function to delay carbohydrate absorption so that blood glucose will decrease [7].

One way to utilize star fruit is by making it into yogurt. Yogurt is already known as a popular processed product. In recent decades, yogurt sales have increased worldwide. Of course, this is due to the increasing number of discoveries that mention the benefits of this dairy product. The raw material used to make yogurt is milk, which has many nutritional contents such as protein, essential amino acids stored in the form of casein and whey protein, and yogurt as a dairy product is said to have a higher protein content than milk [8]. The novelty in fermented milk drinks is yogurt with the addition of star fruit juice (*Averrhoa bilimbi* L.).

Based on the discussion that has been described, the author is interested in innovating yogurt with star fruit juice (*Averrhoa bilimbi* L.) and its effect on reducing blood glucose levels in mice (*Mus musculus*).

2. Methodology

This type of research uses the type of research that is laboratory experimental. Conducted in July 2023 and conducted at the Pharmaceutical Technology Laboratory, Pharmacy Study Program, Faculty of Health Sciences, Muhammadiyah University of Palopo. In this study, the population used was star fruit (*Averrhoa bilimbi* L.) obtained in Botta Village, Kec. Suli and the sample used was ripe star fruit juice (*Averrhoa bilimbi* L.).

a. Tools and Materials

1) Tools

In this study the tools used were knives, basins, beaker glass, analytical scales, digital scales, stirring rods, electric stoves, blenders, filter cloths, spoons, containers for yogurt, pH meters, glucometers.

2) Materials

In this study the materials used were star fruit (*Averrhoa bilimbi* L.), plain yogurt, UHT milk, distilled water, glucose, sodium carboxymethyl cellulose, acarbose, mice, spoit, glucose strips.

b. Research procedures

1) Collection of Wuluh Starfruit (*Averrhoa bilimbi* L.) Samples

Taken wuluh star fruit (*Averrhoa bilimbi* L.) which is ripe and fresh, yellowish green in color. As much as possible wuluh star fruit that has been obtained then wet sorting is done to separate from foreign particles. Then the washing process is carried out with running water, after which the washed star fruit is chopped into small pieces.

2) Preparation of Wuluh star fruit juice (*Averrhoa bilimbi* L.)

a) Taken star fruit that has become a small piece.

b) Then blended until smooth.

c) Then the smooth star fruit is squeezed using a filter cloth to get the juice.

3) Making Yogurt of Wuluh star fruit juice (*Averrhoa bilimbi* L.)

- a) Tools and materials are prepared
- b) Then sterilize the tools
- c) Then UHT milk and star fruit juice are heated to a temperature of 75°C or until lukewarm for 3 minutes while stirring.
- d) After that the milk was cooled, put into an airtight container.
- e) Then plain yogurt (starter) was added and stirred until smooth.
- f) Then the container is closed so that the milk that has been given the starter is not damaged, then put into a larger container, cover it from the inside with a cloth to prevent the entry of light so that fermentation can run well.
- g) After that, it was fermented for 24 hours at room temperature.

4) Evaluation of Wuluh Starfruit Juice Yogurt (*Averrhoa bilimbi* L.)

a) pH test

In the pH test, the acidity of yogurt was determined. A pH meter [9] was used to determine the pH of yogurt, by calibrating the PH meter first by inserting the pH meter electrode into a buffer solution with pH 4 and pH 7.

b) Viscosity Test

In this study, testing the viscosity or viscosity of yogurt was tested using a viscometer. The sample is put into a beaker glass and then dipped the spindle into the sample and then set the height of the viscometer until the line mark is dipped and then press the ON button.

5) Preparation of 1% Na-CMC colloidal solution

1% Na-CMC colloidal solution was made by dissolving 1 g Na-CMC in distilled water heated at 70 °C, and then added to 100 ml, then stirred until dissolved. The advantage of Na-CMC is as a suspending agent that can increase viscosity/viscosity and increase the stability of the resulting suspension [10].

6) Preparation of Acarbose Suspension

The dose of acarbose in adult humans is 50 mg and the human to mice conversion dose is 0.0026, so the dose of mice per 20 g is 0.13 mg.

- a) 10 acarbose tablets were weighed one by one
- b) Then the average weight is calculated
- c) Then entered into the calculation formula
- d) Then acarbose tablets were finely crushed and then added 1% Na-CMC colloidal solution little by little and then crushed until well mixed (homogeneous).

The use of acarbose as a comparator because the mechanism of action of acarbose is the same as the mechanism of action of flavonoid compounds as antidiabetics contained in belimbing wuluh fruit, which delays the absorption of carbohydrates so that blood glucose levels will decrease [11].

7) Preparation of Glucose Solution

- a) Weighed 12 grams of glucose, put into a 100 ml volumetric flask
- b) Then added 50 ml of distilled water, stirred until dissolved
- c) Then the volume is filled with distilled water to 100 ml.

8) Measurement of Blood Sugar Levels of Mice

Measurement of blood sugar levels (pretest) of mice is by disinfecting the tail end of the mice using 70% alcohol, then injuring the tail end, then the dripping blood is touched to the glucometer test strip (Autocheck™) [12].

9) Treatment of Experimental Animals

a) Mice Adaptation (*Mus musculus*)

Mice are adapted to the cage environment for 7 days with the aim of adapting to the new environment and to prevent stress [13]. At this stage all mice are given standard AD-II feed. Then the mice are fed for 8 hours before treatment, by removing the husks and food but the mice are still given water. Then weighing the mice is done to determine the body weight of each mice.

This study used male mice (*Mus musculus*). The reason for using male mice is because male mice have a hormonal system and psychological factors that are more stable than female mice so that they can affect the results of the study [14]. The mice used with a body weight of 20-30 gr were 25 heads divided into 5 treatment groups, each group consisting of 5 mice. The calculation of the number of mice used is calculated using the Federer formula as follows:

$$(n-1)(t-1) \geq 15$$

Description: n: sample size of each group

t: number of groups

b) Glucose Induction

The induction of diabetes in test animals is done by using glucose orally. If there is an increase in blood glucose levels of mice exceeding 175 mg/dl, then the mice have developed diabetes [15].

c) Experiment Design

The treatment of the division of groups of test animals in the study was:

- Group 1 was given yogurt without juice (negative control)
- Group 2 as a positive control was given acarbose

- Group 3 was given yogurt with star fruit juice (*Averrhoa bilimbi* L.) concentration 0.35 ml
- Group 4 was given star fruit juice yogurt (*Averrhoa bilimbi* L.) concentration of 0.50 ml
- Group 5 given star fruit juice yogurt (*Averrhoa bilimbi* L.) concentration 0.65 ml

c. Data Analysis

Data analysis used for the results of blood sugar levels is One Way ANOVA (Analysis of Variance) using the procedure for One Way ANOVA blood glucose level data in the SPSS 24 program. The level of difference between the mean values between treatments was tested using Duncan's multiple interval test $P < 0.05$.

3. Result and Discussion

3.1. Result

Tabel 3.1 Organoleptic Test Results

Formula	Result			
	Odor	Taste	Shape	Color
F0	Typical	Asidic	Viscous liquid	White
F1	Typical	Asidic	Viscous liquid	White
F2	Typical	Asidic	Viscous liquid	White
F3	Typical	Asidic	Viscous liquid	White

Description:

F0: obtained results of characteristic odor, sour taste, thick liquid shape, white color (plain yogurt)

F1: obtained results of a distinctive odor, sour taste, thick liquid shape, white color (juice concentration 0.35 ml)

F2: obtained results of a distinctive odor, sour taste, thick liquid shape, white color (juice concentration 0.50 ml)

F3: obtained results of distinctive odor, sour taste, thick liquid shape, white color (juice concentration 0.65ml)

Tabel 3.2 pH Test Result

Formula	Results	Range
F0	4	The pH of yogurt has a value of 3.80-4.50 (SNI, 2009).
F1	4	
F2	3,88	
F3	3,95	

Description:

F0: obtained result 4 (yogurt without juice)

F1: obtained result 4 (juice concentration 0.35 ml)

F2: obtained a result of 3.88 (juice concentration of 0.50 ml)

F3: obtained results 3.95 (juice concentration 0.65 ml)

Tabel 3.3 Viscosity Test Result

Formula	Result	Range
F0	83	The viscosity of yogurt has a value of 3.80-4.50 (SNI, 2009).
F1	103	
F2	102	
F3	95	

Description:

F0: results obtained 83 (yogurt without juice)

F1: results obtained 103 (juice concentration 0.35 ml)

F2: obtained results 102 (juice concentration 0.50 ml)

F3: obtained results 95 (juice concentration 0.65 ml)

Tabel 3. 4 Antidiabetic Test Results

Group	Mean blood glucose levels on days ke 1,2, dan 3(mg/dl)			Blood glucose reduction after treatment (mg/dl)
	Before induction	After glucose induction	After treatment	
Kontrol (-)	84	201	131	70
Kontrol (+)	83	212	126	91
Yogurt sari 0,35 ml	79	200	112	90
Yogurt sari 0,50 ml	84	209	117	92
Yogurt sari 0,65 ml	89	218	118	100

Description:

Control (-) : Giving yogurt without juice

Control (+) : Administration of acarbose suspension

mg/dl : Milligrams Per Deciliter

Tabel 3.5 One Way ANOVA Test Results

ANOVA					
Rata-rata					
	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>Sig.</i>
<i>Betweengroups</i>	3592.000	4	898.000	3.264	0.033
<i>Within groups</i>	5502.000	20	275.100		
<i>Total</i>	9094.000	24			

Post Hoc test

Average			
Duncan			
Treatment	N	<i>Subset for alpha = 0.05</i>	
		1	2
Negatifve Control	5	70.40	
Positive Control	5		91.00
0,35	5		91.80
0,50	5		98.20
0,65	5		106.60
Sig		0.066	0.047

3.2. Discussion

This study was conducted with the aim of knowing the juice of star fruit (*Averrhoa bilimbi* L.) can be formulated in the form of yogurt and knowing at what concentration the yogurt of star fruit (*Averrhoa bilimbi* L.) can affect blood sugar levels in mice. Belimbing wuluh contains flavonoids as antidiabetics, flavonoids are antidiabetic substances that inhibit the alpha-glucosidase enzyme which delays the absorption of carbohydrates thereby lowering blood glucose levels for people with DM. Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia caused by insulin resistance and or decreased insulin secretion due to pancreatic beta cell failure [16].

The formulated star fruit juice is mixed into yogurt. Yogurt is a sour-tasting milk drink, in the form of thick or semi-solid liquid obtained from fermented milk with the help of two bacteria, namely *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [17]. In this study using mice (*Mus musculus*) because the number of children per birth is large, the life cycle is relatively short, easy to find, and has similarities in anatomical structure and physiology with humans [18]. The concentrations of star fruit juice used consisted of 0.35 ml, 0.50 ml, and 0.65 ml. Determination of the concentration of juice is determined from the conversion of doses in rats to mice. The dose taken is in the research of [19], 2 ml / 200 kgBB rats can affect the blood glucose levels of rats experiencing hyperglycemia. Before the induction of yogurt, tests were carried out on the preparation, namely organoleptical tests, pH tests and viscosity tests and then antidiabetic tests were carried out.

Organoleptical test aims to determine the physical state of yogurt preparations using the five senses. Based on table 3.1, the results obtained from all formulas have a distinctive smell of yogurt which comes from the results of fermentation, sour taste from lactic acid bacteria contained in yogurt, thick liquid shape and white color which comes from the color of milk. Then in the pH test aims to determine the degree of acidity in star fruit juice yogurt (*Averrhoa bilimbi* L.), yogurt preparations are measured using a pH meter. Based on table 3.2, the results obtained F0 obtained a value of 4, F1 obtained a value of 4, F2 obtained a value of 3.88, and F3 obtained a value of 3.95. All formulas meet the requirements in accordance with the statement [20], namely the acidity level (pH) that meets the standardization of yogurt preparations, which is 3.80-4.50.

Viscosity of yogurt is a measure of viscosity in the yogurt produced. The viscosity test in this study aims to determine the viscosity of belimbng wuluh (*Averrhoa bilimbi* L.) yogurt. The viscosity test of the preparation uses a viscometer with a spindle of 62 and a speed of 60. Based on table 3.3, the average results of F0 obtained a value of 83 cP, F1 obtained a value of 103 cP, F2 obtained a value of 102 cP, and F3 obtained a value of 95 cP. From these results, all formulas meet the requirements [20], namely thick to semi-solid viscosity with a value of 58 cP-187 cP.

Anti-diabetic testing of wuluh starfruit yogurt juice (*Averrhoa bilimbi* L.) was carried out on mice by first acclimatizing for 7 days. The purpose of this acclimatization is to adjust the mice to be given star fruit juice yogurt (*Averrhoa bilimbi* L.) with a concentration of 0.35 mg in the environment, to know visually that the mice to be used in the study are healthy [21] and to prevent stress [13]. After that, before induction, the mice were fed for 8 hours, then each mice was weighed. The number of mice used was 25 based on the Federer formula. Then the mice were grouped into 5 groups, in 1 group consisting of 5 mice. Group 1 as a negative control was given yogurt without juice, group 2 as aquadest was given Acarbose, group 3 was given star fruit juice yogurt (*Averrhoa bilimbi* L.) with a concentration of 0.35 mg, group 4 was given star fruit juice yogurt (*Averrhoa bilimbi* L.) with a concentration of 0.50 mg, and group 5 was given star fruit juice yogurt (*Averrhoa bilimbi* L.) with a concentration of 0.65 mg.

Before the mice were induced, the blood glucose levels of the mice were measured first, with the aim of knowing the initial glucose levels of the mice. After that, glucose was induced orally with the aim of increasing blood sugar levels in mice. This method is the oral glucose tolerance test (TTGO) method. TTGO is a test to measure blood glucose levels after consuming

glucose. The reason for choosing glucose as an inducer of mice is because glucose is one of the compounds that can increase blood glucose levels. This is adjusted to the condition of people who experience excess glucose in their bodies. Glucose induction in mice orally has successfully raised the blood glucose levels of mice [21]. If there is an increase in blood glucose levels of mice exceeding 175 mg/dl, then the mice have developed diabetes [15].

Measurement of mice blood sugar levels using glucose test strips, in group 1, which is a negative control given yogurt without juice with the aim of seeing the antidiabetic effect of yogurt without star fruit juice (*Averrhoa bilimbi* L.), based on table 3. 4, the results obtained were a decrease, this is in accordance with the research of [22] which states that the ability of lactic acid bacteria to inhibit the alpha glucosidase enzyme contributes to a decrease in blood glucose levels and can be a potential antidiabetic and in the research of [23] yogurt without the addition of juice can be used as an alternative snack for people with type 2 diabetes mellitus because of the effect of lowering fasting blood glucose it causes. Furthermore, group 2 is acarbose as a positive control. Based on table 3.4, the results showed a significant decrease in blood glucose levels after 1 hour and on days 1, 2 and 3 with a decrease of 91mg/dl, because acarbose is an inhibitor of the alpha glucosidase enzyme found in the small intestine wall. Inhibition of this enzyme system can effectively reduce the digestion of complex carbohydrates and their absorption, so that diabetics can reduce the increase in post-prandial glucose levels. Acarbose also inhibits the pancreatic alpha amylase enzyme which functions to hydrolyze polysaccharides in the small intestinal lumen [4]. The mechanism of action of acarbose is by inhibiting polysaccharide alpha glucosidase which has the function of breaking down disaccharides into glucose, thus inhibiting glucose absorption in the digestive tract [24].

Then in table 3.4, group 3, namely the administration of star fruit juice yogurt (*Averrhoa bilimbi* L.) with a concentration of 0.35 mg/l, obtained a decrease in blood glucose levels after 1 hour as well as on days 1, 2 and 3 with a decrease of 90 mg/dl. Then yogurt juice 0.50 ml and yogurt juice 0.65 ml also showed a significant decrease with a decrease of 92 mg/dL and 100 mg/dl. So it is known that star fruit juice yogurt can reduce blood glucose levels of mice from day 1 to day 3, this is in accordance with the research of [25] which states that star fruit wuluh contains flavonoids as antidiabetic substances. Flavonoids contained in belimbing wuluh are types of luteolin and epigenin. Flavonoids are believed to be an antidiabetic substance with its role as an alpha glycosidase enzyme inhibitor that functions to delay carbohydrate absorption so that blood glucose levels will decrease [11]. According to [26] the inhibition of alpha-glucosidase enzyme in hyperglycemia conditions can help overcome hyperglycemia conditions because the amount of monosaccharides that can be absorbed by the small intestine is reduced. In addition, flavonoids also act as antioxidant substances. Antioxidants are able to suppress beta cell radical apoptosis without altering the proliferation of pancreatic beta cells. Antioxidants are also able to bind free radicals so as to reduce Reactive Oxygen Species (ROS) so as to reduce insulin resistance [27].

Furthermore, in table 3.5 there are the results of the *One Way ANOVA* test, data analysis using the SPSS program. Data analysis in this study is using *One Way ANOVA* or one-way analysis used to test whether the means of several samples are the same or significantly different [28]. In this test using a significant value, if the p value > 0.05 is not followed by the Duncan post

hoc test and if the p value <0.05 is followed by the Duncan post hoc test to see which groups are significantly different. The results after descriptive analysis of *One Way ANOVA* can be seen in table 3.5 obtained p value <0.05 which is 0.033. This indicates a significant difference between groups and indicates or affects blood glucose levels. After the *One Way ANOVA* test continued with the Duncan post hoc test, in accordance with the research of [29], [30] which explains this test to determine which treatment has a significant difference in determining the most effective concentration in reducing blood glucose levels in mice.

The results obtained in the Duncan's post hoc test showed that the negative control (yogurt without juice) was significantly different from the positive control group (Acarbose), the yogurt juice treatment groups of 0.35 ml, 0.50 ml, and 0.65 ml, characterized by the negative control being in a different subset from the other groups. While the positive control with yogurt juice treatment groups of 0.35 ml, 0.50 ml, and 0.65 ml is not significantly different, this is characterized by the positive control group being in the same subset as the yogurt juice treatment groups of 0.35 ml, 0.50 ml, and 0.65 ml, which means that at these concentrations yogurt juice of belimbing wuluh (*Averrhoa bilimbi* L.) can reduce the blood glucose levels of mice as effectively as the control group.

So, in this study in the preparation test, namely the organoleptical test in accordance with the physical state of yogurt, namely the shape of thick liquid, distinctive odor, white color and sour taste, the pH test meets the requirements, namely pH ranging from 3.86-4, the viscosity test also meets the requirements, namely with results ranging from 60-159 cP and the antidiabetic test obtained significant reduction results, based on the results of the *One Way ANOVA* test, namely $p < 0.05$ with a value of 0.033, while in the further test, namely the Duncan post hoc test, the most effective concentration in reducing blood sugar levels is at a formula concentration of 0.65 mg.

4. Conclusion

Based on the research that has been done, it can be concluded that star fruit juice (*Averrhoa bilimbi* L.) can be formulated in the form of yogurt because it has met the standards of good physical stability which includes organoleptical test, pH or acidity test and viscosity test or viscosity in yogurt.

The antidiabetic test of star fruit juice yogurt (*Averrhoa bilimbi* L.), the results obtained are that all concentrations can affect the blood sugar levels of mice, with a decrease of 90 mg/dl at a concentration of 0.35 ml, a decrease of 92 mg/dl at a concentration of 0.50 ml, and a decrease of 100 mg/dL at a concentration of 0.65 ml, it can be concluded that the concentration that most effectively affects the blood sugar levels of mice is at a concentration of 0.65 mg with a decrease of 100 mg/dl.

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