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ORIGINAL ARTICLE

Breadfruit leaf extract: A potential hypolipidemic and antioxidant agent in diabetes

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ABSTRACT

Elevated total cholesterol levels are medically termed as hypercholesterolemia. Breadfruit leaves are recognized for their health benefits owing to the presence of secondary metabolites. This study aimed to investigate the effect of breadfruit leaf extract (Artocarpus altilis) on total cholesterol levels in white rats (Rattus norvegicus) and assess its antioxidant potential. Two control groups were established: negative control (1% Na-CMC) and positive control (simvastatin). The three treatment groups were administered different doses of the breadfruit leaf extract (100, 200, and 400 mg/kg body weight). Each group consisted of five rats. The results indicated that 400 mg/kg body weight of breadfruit leaf extract significantly reduced total cholesterol levels in rats by 29.27% ($p<0.05$). The antioxidant activity of breadfruit leaf extract was evaluated using the DPPH method at concentrations of 100, 200, 300, 400, and 500 µg/ml. The maximum wavelength used was 516 nm, and the absorbance was measured using a UV-Vis spectrophotometer. The linear regression equation obtained for breadfruit leaf extract was Y = 0.1171x + 35.76, with an IC50 value of 121.605 µg/ml, indicating moderate antioxidant activity. In contrast, the linear regression equation for vitamin C was Y = 0.6378x + 26.083, with an IC50 value of 37.499 µg/ml, indicating a very strong antioxidant activity.

Keywords: antioxidants, artocarpus altilis, diabetes, hypercholesterolemia, total cholesterol

Introduction

Cardiovascular diseases (CVDs) represent a significant global health burden, accounting for 74% of all deaths annually and equivalent to approximately 41 million individuals. Cardiovascular diseases, particularly coronary heart disease, contribute to the highest mortality rate (17.9 million deaths per year).[1] In Indonesia, 1.5% of the total population (1.02 million individuals) was diagnosed with heart disease in 2018.[2]

Impaired cardiac and vascular function can lead to coronary heart disease, heart failure, hypertension, and stroke. Several preventable risk factors contribute to the development of heart disease, including smoking, high-fat diet, excessive alcohol consumption, and physical inactivity. These habits can elevate blood pressure, total cholesterol, LDL cholesterol, and triglycerides, ultimately increasing the risk of coronary heart disease.[3]

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Elevated levels of total and LDL cholesterol, a condition known as hypercholesterolemia, can be attributed to various factors such as diets high in saturated and trans fats as well as genetic predispositions. Additionally, elevated blood glucose levels in individuals with type 2 diabetes can contribute to hypercholesterolemia. This is because glucose cannot be efficiently converted to glucose-6-phosphate, forcing the body to rely on the breakdown of fats and proteins for energy. This process increases the production of acetyl-CoA, leading to elevated levels of total and LDL cholesterol.[4]

The Indonesian population has turned to traditional medicine, particularly plant-based remedies, for the treatment of various ailments. With minimal side effects compared with synthetic drugs, approximately 15,000 plant species in Indonesia possess medicinal properties. While only approximately 7,000 of these species have been utilized in pharmaceutical preparations, the secondary metabolites found in the leaves of the breadfruit tree (Artocarpus altilis) have shown potential as therapeutic agents. These metabolites exhibit antioxidant, anti-inflammatory, antidiabetic, and antihypercholesterolemic properties.[5] Based on the aforementioned information, this study aimed to investigate the effects of an ethanolic extract of breadfruit leaves on total cholesterol levels in hypercholesterolemic diabetic male rats. The antioxidant activity of the ethanolic extracts was determined using the DPPH method.

Method

This experimental study employed a pretest-posttest controlled-group design. This study was conducted at the Research Laboratory of Universitas Prima Indonesia and the Cendikia Laboratory. The research period spanned from June 2023 to November 2023. The study population consisted of male Wistar rats (Rattus norvegicus). A sample of 25 male Wistar rats weighing–100-200 grams and aged 2-3 months, obtained from the Cendikia Laboratory, were used. Data were collected based on measurements of total cholesterol levels in rats. To test the antioxidant activity of the ethanolic extract of the breadfruit leaves, data were obtained from the absorbance values of the samples and the standard (vitamin C) at each concentration.

The equipment used in this study included an analytical balance, drying cabinet, maceration container, scissors, blender, filter paper, funnel, rotary evaporator, water bath, test tubes, dropping pipette, Bunsen burner, stirring rod, beaker, graduated cylinder, Erlenmeyer flask, rat cage, marker, autocheck, cholesterol strip, syringe, oral sonde, and UV-Vis spectrophotometer. Materials used included: breadfruit leaves, Mayer and Dragendorff reagents, 2% HCl, Mg ribbon, 10% FeCl3, 98% chloroform, 98% anhydrous acetic acid, 98% H2SO4, distilled water, 96% ethanol, Na-CMC, alloxan, standard feed, 10 kg high-fat feed (corn, beef fat 10%, egg yolk 5%), used cooking oil, Simvastatin tablets, white rats, DPPH solution, vitamin C, and methanol.

Fresh breadfruit leaves were collected from Marubun Jaya Village, North Sumatra, Indonesia. Subsequently, they were cleaned, dried, and pulverized into crude drug powder.[6] A cold maceration method using 96% ethanol as the solvent was employed for extraction over a total period of seven days. Five hundred grams of the crude drug powder was placed in a maceration vessel and mixed with 3 liters of 96% ethanol. The mixture was stirred daily for five days and then filtered to separate the filtrate from the residue. The residue was subjected to a second maceration for two days using 96% ethanol (1.5 L), followed by another filtration. The combined filtrates were concentrated using a rotary evaporator and evaporated in a water bath to obtain a viscous extract.

Phytochemical analysis was conducted on the ethanolic extract of breadfruit leaves to identify the presence of bioactive compounds. The analysis involved several tests: Mayer's and Dragendorff's reagents were used to detect alkaloids (nitrogenous bases); concentrated HCl and magnesium ribbon were employed to identify flavonoids, which are known for their antioxidant properties; a hot water and HCl test was performed to detect saponins, which produce foam; ferric chloride solution was used to detect tannins, which have antibacterial properties; and a mixture of chloroform, acetic anhydride, and sulfuric acid was used to identify triterpenoids and steroids, which are compounds with various biological activities. Positive results from these tests indicated the presence of these compounds in the breadfruit leaf extract.[7]

To quantify the free radical scavenging capacity of the breadfruit leaf extracts, researchers used the DPPH method. In this assay, leaf extract was mixed with an antioxidant-sensitive DPPH radical. The extent of antioxidant activity in the extract was determined by measuring the decrease in DPPH absorbance, which is indicative of the DPPH radical scavenging activity. A UV-Vis spectrophotometer was used to monitor the reaction. Ascorbic acid, a well-known antioxidant, was used as a positive control. By comparing the results obtained from the breadfruit leaf extract with those of ascorbic acid, researchers were able to assess the

antioxidant efficacy of the extract and establish a dose-response relationship between the extract concentration and its free radical scavenging ability.[7,8]

Fifty mice were randomly divided into five groups of ten mice each: a negative control group (administered 1% Na-CMC suspension), a positive control group (administered simvastatin), and three treatment groups receiving different doses of the extract (100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively). Following a one-week acclimatization period with standard rodent chow and water, baseline total cholesterol levels were measured.[9,10]

Results

From 5 kg of fresh breadfruit leaves, 500 g of dried crude drug was obtained after the drying process. The dried crude drug was extracted to obtain active compounds. The extraction results showed that 35 g of breadfruit leaf extract was obtained from 500 g of the crude drug. This indicates that the extraction efficiency or yield was 7%. Thus, only 7& of every 100 g of dried crude drug was successfully converted into the extract.

Table 1. Breadfruit leaf extract

Wet weight	Simplisia weight	Extract weight	Yield
5000 g	500 g	35 g	7%

Phytochemical screening revealed the presence of several bioactive compounds in the analyzed samples. Alkaloids, one of the tested compounds, yielded negative results. However, flavonoids, saponins, and tannins were positively detected, as indicated by distinct color changes when reacted with specific reagents. Flavonoids produced a red color, saponins formed a persistent foam, and tannins produced a greenish-black color. Additionally, the test for triterpenoids showed a positive result, marked by the appearance of an orange color. The presence of these compounds suggests the potential of the sample as a source of bioactive compounds with various benefits such as antioxidant, anti-inflammatory, and antimicrobial properties.

Table 2. Antioxidant activity test results

Sample	Cons. (µg/mL)	Abs.	% Inhibition	Linear Regression	IC_{50} (µg/mL)	Description
Ethanol extract of breadfruit leaves	100	0,583	46,267	Y = 0,1171x+35,76	121,605	Medium
	200	0,480	55,760	R^2 = 0,9444		
	300	0,254	76,590			
	400	0,150	86,175			
	500	0,113	89,585			
Vitamin C	20	0,660	39,170	Y = 0,6378x + 26,083	37,499	Very strong
	25	0,635	41,475	R^2 = 0,9484		
	30	0,603	44,424			
	35	0,539	50,323			
	40	0,535	50,691			

Notes: Absorbance of blank = 1.085

The antioxidant activity of an ethanol extract of breadfruit leaves was investigated and compared to that of a vitamin C standard. A 1:3 volume ratio was used, with 1.5 mL of sample and 4.5 mL of DPPH. The results indicated that breadfruit leaf extract exhibited moderate antioxidant properties, with an IC50 value of 121.605 µg/mL. This suggests that the extract has the potential to scavenge free radicals, although its activity is lower than that of vitamin C, which displays a strong antioxidant effect with an IC50 of 37.499 µg/mL. As the concentration of breadfruit leaf extract increased, its antioxidant capacity also improved, as indicated by the increasing percentage inhibition values.

Table 3. Average total cholesterol level of rats (after treatment)

Group	Average total cholesterol level of rats (mg/dl)					Mean
	Day 1	Day 4	Day 7	Day 10	Day 13	
Group I (Negative control)	122	121	121	118	115	119,4 ± 2,9
Group II (Positive control)	81	79	76	75	73	76,8 ± 3,2
Group III (100 mg/kgBB dose)	105	104	100	98	99	101,2 ± 3,1
Group IV (200 mg/kgBB dose)	100	96	97	93	92	95,6 ± 3,2
Group V (Dose 400 mg/kgBB)	89	85	81	83	80	83,6 ± 3,6

Table 3 indicates a progressive decrease in the average total cholesterol levels among the treatment groups over the 13-day period. The negative control group exhibited consistently high cholesterol levels, whereas the positive control group exhibited a marked reduction. Notably, groups administered the experimental substance demonstrated varying degrees of cholesterol reduction, with the 400 mg/kg BB dose group exhibiting the most significant decrease compared to the negative control, suggesting a potential dose-dependent cholesterol-lowering effect.

Table 4. Average body weight of rats

Group	Average body weight of rats (g)		
	Before induction	After induction	After treatment
Group I (Negative control)	143,6 ± 13,5	167,2 ± 5,1	170,6 ± 6,2
Group II (Positive control)	173,2 ± 11,3	193 ± 8,7	191,4 ± 10,4
Group III (100 mg/kgBB dose)	175,8 ± 6	189,4 ± 14,3	189,8 ± 14,2
Group IV (200 mg/kgBB dose)	172,6 ± 13,9	179,6 ± 15,8	181 ± 14,4
Group V (Dose 400 mg/kgBB)	164,2 ± 15,2	179,8 ± 17	179,8 ± 15,6
Average	165,88 ± 16,5	181,8 ± 15	182,52 ± 13,9

Observations of changes in rat body weight revealed a general trend in weight gain following induction and treatment, irrespective of group assignment. However, this weight increase was not significantly pronounced in the 400 mg/kg BW group. The positive control group exhibited slightly higher weight gain than the other groups, suggesting that the treatment administered to the positive control group may have exerted a more potent effect on rat weight gain than the other treatments.

Table 5. Average total cholesterol level of rats

Group	Average total cholesterol level of rats (mg/dl)		
	Before induction	After induction	After treatment
Group I (Negative control)	97,2 ± 5	118 ± 5,5	119,4 ± 2,9
Group II (Positive control)	86,2 ± 7	111 ± 7,3	76,8 ± 3,2
Group III (100 mg/kgBB dose)	94 ± 7	115,6 ± 5	101,2 ± 3,1
Group IV (200 mg/kgBB dose)	85,2 ± 6,4	117,6 ± 4,6	95,6 ± 3,2
Group V (Dose 400 mg/kgBB)	82,2 ± 6,4	118,2 ± 6,8	83,6 ± 3,6
Average	88,96 ± 8,2	116,08 ± 6	95,32 ± 15,4

The results of this study demonstrated that, following induction, total cholesterol levels increased in all rat groups. However, after treatment, only the positive control group exhibited a significant decrease in total cholesterol levels. The negative control and other treatment groups did not show a significant reduction. These findings suggest that the treatment administered to the positive control group was more effective in reducing total cholesterol levels than other treatments.

Discussion

The yield can be influenced by several factors, including the type of solvent, particle size of the simplisia, method used, and length of the maceration process.[11] The results of the organoleptic test were in accordance with the requirements stated in FHI Edition II Year 2017.[12] The results showed that the greater the concentration of the ethanol extract of breadfruit leaves and ascorbic acid, the lower the absorbance value. However, the percentage inhibition for each sample was greater. The antioxidant power of the ethanol extract of breadfruit leaves is in the moderate category because it has an IC50 value of 121.605 µg/mL (IC50 100-150 µg/mL), whereas ascorbic acid, which acts as a comparison, is classified as a very strong antioxidant activity category with an IC50 value of 37.499 µg/mL (IC50 < 50 µg/mL).[13]

The antioxidant activity of the ethanol extract of breadfruit leaves was lower than that of ascorbic acid. This is because ascorbic acid is the largest flavonoid group compound that can counteract free radicals with great effects, whereas breadfruit leaf ethanol extract contains a mixture of various compounds.[14] The total cholesterol levels of rats in the negative control group continued to increase after alloxan induction and high-fat feeding. This can occur because, in the negative control, the administration of 1% Na-CMC is only a suspending agent and not an active substance of the drug, so it cannot reduce the total cholesterol levels in rats.[15] The ethanol extract of breadfruit leaves at doses of 100, 200, and 400 mg/kg proved to was shown to reduce the total cholesterol levels of rats by 12.46%, 18.70%, and 29.27%, respectively.

Insulin resistance in diabetes is closely related to increased cholesterol levels, which is why alloxan is induced in rats. The beta cells in the pancreas are damaged by alloxan, leading to disruption of insulin, which plays an important role in regulating lipase enzymes. As a result, when insulin resistance occurs, enzyme

levels increase. Therefore, under diabetic conditions, fat metabolism disorders (characterized by increased cholesterol levels) often occur.[16]

Conclusion

The results of this study showed that the extract of breadfruit leaves possessed moderate antioxidant potential, as indicated by the IC50 value of 121.605 µg/ml. Linear regression analysis further corroborated the relationship between the extract concentration and antioxidant activity. Moreover, the administration of breadfruit leaf extract to experimental rats significantly reduced the total cholesterol levels, particularly at the highest dose (400 mg/kg BW). This decrease in cholesterol levels indicates a hypolipidemic effect of breadfruit leaf extract. Further research is recommended to analyze the LDL, HDL, and triglyceride levels.

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