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ANTIOXIDANTS IN THE EXTRACT OF BALINESE SALAK RIND (*Salacca amboinensis (Becc) Mogea*) AS AN ALTERNATIVE SUBSTANCE TO DECREASE BLOOD SUGAR LEVELS AMONG *Mus musculus L.* WITH HYPERGLYCEMIA

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Abstract: This study aims to determine the antioxidant potential of Balinese salak rind extract (*Salacca amboinensis (Becc) Mogea*) *(BSRE)* as an antihyperglycemic agent in male white mice with hyperglycemia. The research was conducted in three stages extraction of Balinese salak rind using 96% ethanol, phytochemical screening, and antihyperglycemic activity testing. Mice were hyperglycemia induced by alloxan. Mice were divided into five groups consisting of negative control (Aquadest), positive control (Glibenclamide 13 mg/Kg BW), dose 1 (200 mg BSRE/kg BW), dose 2 (400 mg BSRE/kg BW), dose 3 (600 mg BSRE/kg BW). Phytochemical screening showed that the BSRE was positive for containing alkaloids, flavonoids, tannins, saponins, and steroids. The analysis results on the BSRE showed an IC^{50} value of 81,76 ppm, including strong antioxidant activity. The activity of blood glucose levels showed that BSRE could decrease blood glucose levels of *Mus musculus L.* with hyperglycemia.

Keywords: *Balinese Salak Rind, Antioxidants, Hyperglycemia*

INTRODUCTION

Hyperglycemia in uncontrolled diabetes mellitus (DM) patients can cause severe damage to body tissues, such as nerves and blood vessels. DM sufferers require lifelong treatment to reduce symptoms, prevent disease progression and prevent the development of complications. DM is a metabolic disease characterized by increased blood glucose levels beyond normal (hyperglycemia) and disturbances in carbohydrate, fat, and protein metabolism associated with a lack of insulin sensitivity and secretion, as well as progressive changes in the structure of the pancreas beta cell. Hyperglycemia or high blood sugar levels play a role in cell damage by increasing reactive oxygen species (ROS), which can cause tissue oxidative stress and cause cell injury through the mechanism of lipid peroxidation and protein oxidative damage. In addition, high levels of ROS will produce oxidative stress, and antioxidants cannot reduce oxidant levels, causing damage to cells, tissues, and organs [1].

Antioxidants can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. Free radicals can damage cell-forming macromolecules that can cause degenerative diseases. Humans have endogenous antioxidants in their bodies that can reduce free radicals. However, if the amount is smaller than free radicals, exogenous antioxidants are needed to minimize the negative effects of free radicals [2]. One factor affecting the decrease in blood glucose levels is compliance with undergoing diabetes mellitus treatment therapy. Therefore, adherence is very closely related to blood glucose levels. The higher the level of patient compliance, the blood sugar level will decrease; conversely, the lower the patient's adherence to treatment, the blood glucose level cannot be controlled, which means that the blood glucose level will remain high [3]. People with diabetes taking antidiabetic drugs in the long term or for life can cause side effects, which are only used to relieve symptoms and prevent complications. Therefore, alternative treatments of medicinal plants, such as extracts and herbal medicines, are urgently needed to reduce drug side effects. People affected by diabetes mellitus often consume synthetic drugs and are very dependent. Synthetic drugs consumed by the public tend to have fewer therapeutic effects than their side effects, and most synthetic drugs cause side effects that are unacceptable to the body [4].

Salak is one of the plants that can be used in the community as an antidiabetic. Salak is a fruit native to Indonesia. It is considered an exotic fruit that can be consumed not only for the flesh but also for the skin in extract form because it has medicinal properties. Salak rind extract can be processed into a healthy drink that contains phenolic compounds known as natural antioxidants. Salak rind has a flat scaly texture that contains pterostilbene compounds as

antidiabetic substances [5]. Balinese salak rind has strong antioxidant activity due to the presence of very high content of polyphenolic compounds, namely flavonoids. Flavonoids which are antioxidants are believed to be able to prevent several diseases, one of which is being able to protect cells and DNA damage by cleaning cells from ROS, which causes complications from diabetes mellitus. Community knowledge of the use of medicinal plants is very diverse, from the method of processing, how to use it, the parts used, and the efficacy of each type of plant in curing disease. The community knows that the flesh of the salak fruit can be used as a processed food ingredient [6]. Public knowledge about the benefits of Balinese salak rind waste is minimal, and it is still rare to know how to process it as medicine.

Another potential that can also be developed from Balinese salak rind extract products is as an antidiabetic. Several active compounds that are usually believed to be drugs for the prevention and treatment of diabetes include containing active compounds such as alkaloids, flavonoids, saponins, steroids and triterpenoids, phenolic hydroquinones, and tannins [7]. Balinese salak rind contains active elements that work simultaneously on the patient's body to cure the disease. The active elements contained in the rind of salak, which is efficacious for curing diabetes, are ferulic acid and proline, cinnamic acid derivatives, arginine, and pterostilbene [8]. DM management is effective in the early stages before symptoms or prediabetes appear. One way to control blood glucose levels can be done traditionally using natural ingredients.

This study aims to determine the antioxidant potential of Balinese salak rind extract (*Salacca amboinensis (Becc) Mogea*) as an antihyperglycemic agent in male white mice with hyperglycemia. This research is also useful for informing the public about the potential of a processed salak product for health.

RESEARCH METHOD

Extract of Balinese Salak Rind

The dried simplicia of Balinese salak rind *Salacca amboinensis (Becc) Mogea* was cleaned and then crushed. The powder is then put in a container, macerated with ethanol solvent, and allowed to stand for a certain period. The macerate is separated by filtration and repeated at least twice with the same amount and type of solvent until the solvent is clear. All the macerates were collected, and the solvent was evaporated using a rotary evaporator to obtain a thick extract. Balinese salak rinds dried and reduced in particle size are then extracted using 96% ethanol [9]. The extractant was filtered and then thickened using rotary vapor to extract Balinese salak rind [10].

Phytochemical Screening

Pegagan leaves were then screened for their phytochemical content in alkaloids, flavonoids, tannins, and saponins and analyzed for antioxidant activity.

Antioxidant Activity (DPPH Method)

Preparation of DPPH solution by weighing 5 mg of DPPH and then adding methanol p.a up to 50 mL. Determination of the maximum absorption wavelength of DPPH by determining the optimum wavelength, measuring the absorbance at a wavelength of 510-525 nm. The mother liquor was made in series with 4, 8, 12, 16, 20, and 100 ppm concentrations determination of % Inhibition and IC_{50}.

$$\% \text{ Inhibition} = \frac{\text{Abs. blank} - \text{Abs. sample} \times 100\%}{\text{Abs. Blank}}$$

Treatment of Experimental Animals

Male white mice that had been induced by alloxan were divided into five groups and given intake of aquadest (control), glibenclamine 13 mg/Kg BW (positive control), Balinese salak rind extract 200 mg/Kg BW (BSRE1), 400 mg/Kg BW (BSRE2) and 600 mg/Kg BW (BSRE3). Mice were measured for blood glucose content on days 7:7-14 and 21:7-21.

RESULT AND DISCUSSION

Phytochemical Screening and Antioxidant Activity

Before analyzing the effect of the ethanolic extract of Balinese salak rind extract on blood glucose levels, the extract of Balinese salak rind was screened for phytochemicals on the extract. The results of the phytochemical screening analysis are shown in Table 1.

Table 1. Phytochemical Screening Analysis

No.	Identification of compounds	Result
1.	Flavonoids	+
2.	Tannins	+
3.	Alkaloids	+
4.	Saponins	-
5.	Steroids	+

The results of the phytochemical screening of the extract Balinese salak rind showed that the extract contained alkaloids, flavonoids, tannins, saponins, and steroids. It can be seen that the ethanolic extract of Balinese salak rind contains several phytochemical compounds, such as alkaloids, steroids, flavonoids, and tannins. This study's results align with the results of research conducted by others containing phenols, flavonoids, tannins, and monoterpenoids. Another

study also reported that snake fruit skin extract contains flavonoids, saponins, phenols, tannins, steroids/triterpenoids, and alkaloids [11]. The phytochemical content of the ethanolic extract of Salak skin provides various benefits, such as antioxidants or skin lightening. In addition to the quality of the extract, the antihyperglycemic effect of the Balinese salak rind extract was also related to the phytochemical content of the extract. Saponins and flavonoids have antioxidant and inhibitory effects on glucosidase enzymes. The glucosidase enzyme is inhibitory effect can absorb glucose in the digestive tract, thereby reducing postprandial blood glucose levels [12]. In addition, the antioxidant effect of the Balinese salak rind extract ethanol also contributes to the antidiabetic effect. It is related to the mechanism of pancreatic damage caused by alloxan. Alloxan will be reduced by GSH, which forms unstable dialuric acid and can undergo autoxidation to form alloxan radicals.

Table 2. IC_{50}

Material	IC_{50} (ppm)
Balinese Salak Rind extract	81.76

The amount of antioxidant activity is indicated by the IC_{50} value, which is the concentration of sample solution required to inhibit 50% of DPPH free radicals. Table 2 shows the IC_{50} value in Balinese Salak Rind extract of 81,76 ppm, which is included in the strong antioxidant activity.

Table 3. Absorbance and % Inhibition

Material	Concentration (ppm)	Abs. Blank	Abs. Sample	% Inhibition
Balinese Salak Rind extract	4	0.77	0.629	12.84
	8		0.638	11.39
	12		0.624	12.76
	16		0.617	13.29
	20		0.619	14.35
	100		0.257	62.82

Table 3 shows that the greater the concentration of the sample solution, the smaller the absorbance value obtained. The smaller the absorbance value, the greater the % inhibition value. It is because the higher the concentration of the solution, the higher the antioxidant activity.

Blood Glucose Levels

The test results showed that the extract had antihyperglycemic activity. Male diabetic white mice experienced a decrease in blood sugar content on the 14th and 21st days after administration of Balinese Salak rind extract in treatments P1, P2, and P3 by 24.3% and 41.6%, respectively; 25.4% and 42.3%; 37.8% and 55.3%. The results of the decreased measurement are shown in Table 4.

Table 4. Blood Glucose Levels

Group	Day-14 (%)	Day-21 (%)
Positive Control	36.8	57.2
BSRE1	26.3	47.3
BSRE2	27.6	44.5
BSRE3	38.6	57.3

Table 4 shows the group's results given the Balinese salak rind extract, which decreased blood sugar levels. The decrease in blood sugar levels in mice is thought to be due to the presence of phytochemical compounds in Balinese salak rind extract. Flavonoids, alkaloids, tannins, and steroids have shown their antihyperglycemic properties. Phytochemical compounds found in Balinese salak rind extract can modulate metabolic pathways in which glucose can act as a substrate or a product. These phytochemical compounds affect gluconeogenesis, glycogenolysis, the pentose phosphate pathway, and glycogenesis. These phytochemical compounds also work by interfering with glucose absorption and inhibiting α-glucosidase and α-amylase activities. The next mechanism of phytochemical compounds is thought to be able to reduce triglyceride levels and cholesterol content, which will impact hyperlipidemia, one of the pathophysiological features of DM [13].

Flavonoids and alkaloids can protect against β-cell damage, increase proliferation and stimulate insulin secretion. Flavonoids, alkaloids, tannins, and steroids can protect themselves from oxidative stress associated with diabetes complications. Meanwhile, alkaloids can stimulate cellular glucose uptake and reduce insulin resistance. Tannins also have an antidiabetic effect with a working mechanism of increasing the propagation of cell recovery and reducing the absorption of carbohydrates by inhibiting the activity of a-amylase and a-glucosidase [14]. Meanwhile, saponins display their antidiabetic activity through possible mechanisms of improving insulin resistance, stimulating insulin secretion, and protecting pancreatic cells. The antioxidant activity in the skin of the Balinese salak rind will

complement the body's defense system to ward off free radicals to limit the damage. The antioxidant defense system works by interacting directly with free radicals to prevent the formation of reactive oxygen compounds or convert reactive compounds to become less reactive [15]. The ethanolic extract of salak fruit contains several phytochemical compounds, such as alkaloids, steroids, triterpenoids, flavonoids, and tannins. This study's results align with the results of research conducted by other studies, which contain phenols, flavonoids, tannins, and monoterpenoids. Another study also reported that salak skin extract contains flavonoids, saponins, phenols, tannins, steroids, triterpenoids, and alkaloids [16]. The phytochemical content of salak skin ethanolic extract provides various benefits such as antioxidants, the antihyperglycemic effect of salak fruit extract is also related to the phytochemical content of the extract. Saponins and flavonoids have antioxidant and inhibitory effects on glucosidase enzymes. Based on the results of salak fruit extract with concentrations of 2%, 4%, and 8% with the observed parameters of lipid profile and blood glucose profile showed a significant effect on the lipid profile of rats for all parameters on profile lipid and blood glucose levels ($p < 0.05$) [17]. There was a difference in fasting blood glucose levels in rats before and after treatment ($p = 0.015$) by testing the antioxidant content of salak seed extract [18]. The administration of salak fruit extract with concentrations of 10%, 20%, and 40% with observation parameters on body weight and blood glucose profile showed a significant effect on blood glucose profile and body weight ($p < 0.05$). There were significant differences between each treatment group on glucose profile parameters and body weight of rats [19].

The effect of glucosidase enzyme inhibition can absorb glucose in the digestive tract, thereby reducing postprandial blood glucose levels; the antioxidant effect of salak fruit extract ethanol also contributes to the antidiabetic effect. It is related to the mechanism of pancreatic damage caused by alloxan. Alloxan will be reduced by glutathione which forms unstable dialuric acid and can undergo autoxidation to form alloxan radicals. These alloxan radicals can damage pancreatic beta cells through damage to the nucleotide acid structure of beta cells and inhibition of the thiol group of the glucokinase enzyme. Damage to the nucleotide acid structure of pancreatic beta cells causes beta cell death. At the same time, inhibition of the glucokinase enzyme's thiol group disrupts Adenosine Triphosphate's formation in pancreatic beta cells, causing a decrease in insulin secretion. Based on the mechanism of action of alloxan, salak fruit extract containing saponins and flavonoids is able to provide antioxidant effects by donating electrons to the alloxan radicals formed so as to form more stable alloxan compounds and reduce the danger of alloxan in rat pancreas tissue [20-22]. It can be seen from the results of other studies, which showed the antioxidant content in doses of pare fruit extract [23], dragon fruit extract [24], and salak fruit extract combined with sappan wood extract [20]. There is an improvement in the structure of pancreatic tissue which can potentially prevent and treat degenerative diseases.

CONCLUSION

The antioxidant activity of Balinese salak rind extract (*Salacca zalacca Var. Amboinensis (Becc) Mogea*) is 81,76 ppm which is included in the strong antioxidant activity. The effect of glucosidase enzyme inhibition can absorb glucose in the digestive tract, thereby reducing postprandial blood glucose levels; the antioxidant effect of salak fruit extract ethanol also contributes to the antidiabetic effect. Balinese salak rind extract can decrease blood sugar levels among *Mus musculus L.* with hyperglycemia.

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