

Phytochemical Screening and Blood Glucose Level Effects of *Bhee* Fruit Extract (*Melastoma sp*) on Diabetic Mice

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Submitted: 20/11/2022 **Revised:** 3/12/ **Accepted:** 22/02/2023 **Published online:** 20/04/2023

doi: <https://doi.org/10.35308/j-kesmas.v7i2.7366>. **How to cite this article:** Rinawati., Muhsin, S.W., Sari, W., Hayuningtyas, A., Safrida., Putri, S.E., Nursia, L.E., & Siregar, S.M.F. (2023). Phytochemical Screening and Blood Glucose Level Effects of Bhee Fruit Extract (*Melastoma sp*) on Diabetic Mice. *J-Kesmas: Jurnal Fakultas Kesehatan Masyarakat (The Indonesian Journal of Public Health)*. 10(1): 15-19

Abstract

Diabetes is a non-communicable disease among the highest causes of death worldwide, including in Indonesia. Factors that cause diabetes include pancreatic β -cell damage, genetic factors, and an unhealthy lifestyle. Diabetes is characterized by high blood glucose levels (hyperglycemia) beyond normal limits. Hyperglycaemia can be treated using synthetic chemical drugs or insulin therapy, which has adverse long-term effects. The community believes herbal medicine is an alternative diabetes treatment. *Melastoma sp* is a plant that has the potential as an antidiabetic agent. This study aimed to determine the effectiveness of ethanol extract of *Bhee* fruit (*Melastoma sp*) on blood glucose levels of diabetic mice. This study is a laboratory experiment using laboratory animals. The mice were divided into seven treatment groups with three replicates: KN (normal control), KP (treatment control), KD (diabetes control), KO (drug control), P1 (treatment dose of 100 mg/kgBB extract), P2 (treatment dose of 200 mg/kgBB extract), and P3 (treatment dose of 400 mg/kgBB extract). The study's results, the administration of doses of ethanol extract of *the* fruit 100, 200, and 300 mg /kgBB for 14 days reduced glucose levels in diabetic mice. However, they could not exceed the ability metformin at a dose of 500 mg/kgBB.

Keywords: Diabetes; *Melastoma sp*; hyperglycemia; Herbal Medicine

Introduction

Diabetes mellitus is one of the diseases that has become a global concern because of its increasing prevalence (Saputra et al., 2023). Indonesia is in the seventh position in Asia for a person with diabetes. The number of people with diabetes is increasing by about 9.3% of the world population and is expected to continue to increase in 2035 from 8.5 million to 14.1 million (IDF, 2019). Aceh ranks seventh out of 35 provinces because its prevalence in 2016 reached 80,178 people, or 20% (Zulkarnaini et al., 2022). The prevalence of diabetes in 2020 in the West Aceh Regency is also high because it is up to 3,533 (West Aceh Health Office, 2021).

Diabetes occurs due to metabolic disorders in the endocrine system, which results in low insulin secretion (Fauza, 2023). Insulin functions to break down glucose so that it can be utilized by the body (Thongsom, 2013).

Insulin resistance causes an increase in blood glucose levels. Measurement of blood glucose levels when reaching >200 mg/dl and >126 mg/dl when fasting indicates that a person has hyperglycemia (Maulana et al., 2022). Diabetes treatment can be done by taking chemical drugs or regular insulin injections. Chemical drugs and insulin injections can cause specific side effects in the long term. Side effects of chemical drugs such as glucose level control can include hypoglycemia, liver toxicity, weight gain, and enlargement of the abdomen (Indarto & Supriyanto, 2021). The use of injection insulin can cause side effects such as hypoglycemia, allergies, edema, and potential infection due to injection (Putri & Halimah, 2022). The community chooses herbal plants as an alternative because they have fewer side effects. Plants can be utilized as antidiabetic agents because they contain various secondary metabolites (Margono & Sumiati, 2019).

Indonesia has various types of plants that have the potential as antidiabetic herbal medicines. One of them is *Melastoma sp* which has regional names such as *Senggani* (Java), *senduduk* (Sumatra) *kemanden* (Madura) (Laia et al., 2019). In Aceh, *Melastoma sp* is known as "*Bhee*." This plant is efficacious as a fever-reducing medicine and pain reliever, cures vaginal discharge, and stops bleeding. Despite its abundance, the *Bhee* plant in western Aceh has yet to be widely utilized. Parts of the plant used as medicine are leaves, roots, fruit, and seeds (Suwita & Meldawati, 2022). *Bhee* fruit has not been widely used as an antidiabetic agent. *Bhee* fruit contains several secondary metabolites, such as alkaloids, triterpenoids/steroids, flavonoids, and phenols. The flavonoid groups found in this fruit are anthocyanins and flavonoids (Wardatun, 2020). *Bhee* fruit, when ripe, will open its shell, dark purple, has a bitter, slightly sweet taste, and, if consumed, will leave a black color on the tongue (Wong, 2008).

The potential of *Bhee* plant parts antidiabetic agents has been proven through the following studies: ethanol extract from leaves can overcome hyperglycemia and reduce lipid levels significantly in diabetic mice (Kumar et al., 2013) with a dose of 300 mg/KgBB able to reduce 11, 51 ± 1.8 mg/dL and methanol extract at a dose of 500 mg/KgBB able to reduce 86.2 ± 1, 28 mg/dL. *Bhee* leaf ethanol extract has low toxicity, so it is safe to use as an antidiabetic herbal preparation (Prayoga et al., 2020).

This plant's secondary metabolites and antioxidant activity can inhibit glucose absorption in the intestinal tract through carbohydrate digestion (Aryal et al., 2021). *Bhee* fruit contains high levels of vitamin C (Putri et al., 2020) and organic acids (Zheng, 2021). Not many references have been obtained regarding the effect of *Bhee* fruit on diabetes. A reference states that SNEDDS preparation of *Bhee* fruit ethanol extract) in alloxan-induced zebrafish can reduce blood glucose levels (Novitalia & Miladiyah, 2022).

Methods

Place of Research

This research was conducted in several places: 1). Biology Laboratory MIPA Syiah Kuala University for phytochemical screening on *Bhee* fruit (*Melastoma sp*) 2). Animal Structure and Development Laboratory of FMIPA Syiah Kuala University. The laboratories were used for testing the extract of *Bhee* fruit (*Melastoma sp*) on test animals.

Tools and Materials

The tools used in this study were as follows: Sonde

needles, syringes, cages, and weight scales to measure the development of body weight of experimental animals before, during, and after treatment Blood glucose level checker brand "Easy touch" and blood glucose level measuring strips Easy touch brand. The materials used in this study were *Bhee* fruit (*Melastoma sp*) distilled water, male mice, mice food, and Alloxan to condition the mice to become diabetic.

Research Stages

Extraction of simplistic

Bhee fruit (*Melastoma sp*) was taken from the West Aceh region. The fruit is separated from the stalk and petals and then washed thoroughly so that no dirt is attached. All samples were drained and dried at 40-50°C using the oven for 4-5 days until the moisture content remained. The dried samples were then pulverized with a blender and sieved with a 40-mesh sieve. The obtained sample was wrapped in plastic and stored for further testing.

Phytochemical Test of *Bhee* Fruit (*Melastoma sp*).

The extraction process used the maceration method with ethanol solvent at room temperature for 3x24 hours. Every 24 hours, filtered, and then the solvent was replaced with a new one. The extract was concentrated using a rotary evaporator (Ripaldo, 2020). Phytochemical screening was carried out using the Harborne method (1997). The maceration method was a simple extraction method and could penetrate the cell wall and enter the cell cavity containing the active substance. The quality and equipment were easier to do. The choice of solvent for the maceration process would provide high effectiveness by showing the solubility of natural material compounds to the solvent (Pinasthika, 2014).

Preparation of diabetes test animals

Mice were acclimatized first for seven days in a quiet room with food, drink, and sufficient air circulation. The purpose of acclimatization is to prevent mice from experiencing stress. After grouping, mice that would be conditioned diabetes must be induced alloxan intraperitoneally at a dose of 175 mg/kgBB; after 48 hours, blood glucose levels were checked. Mice were already in diabetic condition if glucose levels reached > 200 mg / dL (Susilawati, 2016). The number of mice used was obtained through the calculation of the Federer formula $[(t-1)(n1) \geq 15]$, where t was the number of treatments to be given, and n was the number of samples group to be sought.

Extract testing on experimental animals.

Bhee fruit extract (*Melastoma sp*) was given to test

animals in vivo using a sonde needle at a dose of mg/KgBB, 200 mg/KgBB and 300 mg/KgBB, and 400 mg/KgBB for 14 days. Measurement of blood glucose levels and body weight were measured on days 7 and 14.

Research Design

Mice were grouped using a completely randomized design. 21 mice were divided into seven groups with three replicates as follows:

1. Normal Control (KN) = Group of mice (only given food and aquadest)
2. Drug Control (KO) = a group of mice given 500 mg Metformin hcl as a comparator
3. Treatment Control (KP) = Group of normal mice given a dose of *Bhee* fruit extract 400 Mg/KgBB
4. Diabetes Control (KD) = Group of mice that remain conditioned to diabetes without administering extracts or fruit extracts *Melastoma sp.*
5. Treatment 1 (P1) = Group of diabetic mice given a dose of 100 Mg/KgBB of *Bhee* fruit extract extract
6. Treatment 2 (P2) = Group of diabetic mice given a dose of *Bhee* fruit extract 200 Mg/KgBB
7. Treatment 3 (P3) = Group of diabetic mice given a dose of *Bhee* fruit extract at a dose of 400 Mg/KgBB

The Normal Group served as a comparison in case of changes in blood glucose levels of mice conditioned with diabetes and extract treatment. Drug control (KO) compared the effectiveness of the extract or the drug Metformin in reducing blood glucose levels. The treatment group (KP) aimed to determine the effect of *Bhee* fruit extract on blood glucose levels in normal mice. Diabetes control (KD) is a group of diabetic mice that act as a parameter in lowering blood glucose levels treated with *Melastoma sp.* Groups P1, P2, and P3 were diabetic mice treated with different doses of extracts to determine the most appropriate dose. Examination of mice glucose levels used easily touched by taking blood from the peripheral blood vessels of the tail of mice as much as 0.05 ml using a small and sterile needle (Rusmini, 2019).

Data Analysis

Data analysis was carried out descriptively in table data processing and computer aids. ANOVA analysis was carried out to measure blood glucose levels in each group. If there were differences between treatment

groups, they were analyzed by the DMRT test (Duncan multiple range test).

Results

Phytochemical Screening of *Bhee* Fruit Extract (*Melastoma sp*)

Phytochemical screening was conducted to detect what secondary metabolites were found in *Bhee* fruit extract using ethanol solvent. The results can be seen in the following table:

Table 1. Phytochemical screening results of ethanol extract of *Bhee* fruit (*Melastoma sp*) by maceration method

Metabolite Content	Reagen	Test Results
Alkaloid	Mayer	+
	Wagner	-
	Dragendorff	+
Steroid	Liebermann-Burchard analysis	-
Terpenoid	Liebermann-Burchard analysis	+
Saponin	Aquadest	+
Flavonoid	Hcl & Logam Mg	+
Fenol/Tanin	FeCl3	+

The table above shows that the ethanol extract of *Bhee* fruit (*Melastoma sp*) was positive for alkaloids, terpenoids, saponins, flavonoids, phenols, and tannins. However, steroids were not detected in the extract. The results of this study were the same as those conducted by (Purwaningsih et al., 2023). Namely, steroids were not detected in the methanol extract of the *senggani* fruit.

Blood Glucose Levels

This study used mice as test animals which were divided into seven treatment groups, namely Normal Control (KN), Treatment Control (KP), Diabetes Control (KD), Drug Control (KO), P1, P2, and P3. Blood glucose levels were measured four times before alloxan induction, after alloxan induction, and H7 and H14 after extract administration. The results of blood glucose level measurements were presented in the following table:

Table 2. Mean blood glucose levels of mice before and after administration of *Bhee* fruit extracts

Group	Average Blood Glucose Level (mg/dL)			
	Before Alloxan Induction	After Alloxan Induction	H7	H14
KN	133.67±5,77 ^a	127.00±3,00 ^a	123.67±2,30 ^a	122.67±4,61 ^a
KP	127.67±2,08 ^b	132.33±4,04 ^a	132.67±4,61 ^b	133.00±6,92 ^b



KD	105.33±2,08 ^a	245.00±7,81 ^b	258.33±2,08 ^e	279.33±3,05 ^f
KO	108.33±7,09 ^a	268.67±6,02 ^c	176.33±5,50 ^c	159.00±4,35 ^c
P1	134.33±4,07 ^c	267.00±4,58 ^c	264.00±5,56 ^e	251.00±3,60 ^e
P2	135.33±4,72 ^c	271.67±5,77 ^c	261.67±2,88 ^e	246.00±3,60 ^e
P3	121.67±4,93 ^b	266.33±3,21 ^c	244.00±2,64 ^d	191.00±3,60 ^d

Description: KN= Normal Control (without alloxan induction and extract administration). KP = Treatment Control (normal mice + extract). KD = Diabetes Control (without extract treatment). KO = Drug Control (diabetic mice + Metformin hcl 500 mg), P1 = Diabetic mice + extract dose of 100 mg / kgBB. P2 = Diabetic mice + extract dose of 200 mg / kgBB, P3 = Diabetic mice + extract dose of 400 mg / kgBB.

The results of measuring blood glucose levels during observation changed in each group. Blood glucose levels of mice before alloxan induction was still expected. Although there were differences in each group, they were not significant. Different physiological factors of each mouse caused this condition. Induction of alloxan in the mice group conditioned to diabetes caused an increase in blood glucose levels in the KD, KO, P1, P2, and P3 groups. Alloxan had toxic and selective properties against pancreatic β-cells (Tapehe, 2022). Alloxan could selectively increase the number of calcium ions in pancreatic β cells. The increase in calcium ions in the cell results in impaired insulin secretion and inhibited blood glucose absorption into the cell (Irawan, 2022).

Bhee fruit extract was administered after the group of mice conditioned with diabetes had experienced hyperglycemia. Measurement of blood glucose levels on day seven and day 14 showed mixed results. The blood glucose levels of the KN and KP groups remained normal. *Bhee* fruit extract did not affect changes in blood glucose levels in the KP group. Blood glucose levels in the KD group increased. Glucose levels in the P1, P2, and P3 groups decreased. This proves that the treatment of *Bhee* fruit extract could affect blood glucose levels. The KO group given Metformin hcl 500 mg also showed a decrease in glucose levels, and was more significant than in groups P1, P2, and P3. To see how much influence the administration of *Bhee* fruit extract can be seen in the percentage table of blood glucose levels below:

Table 3. Percentage change in blood glucose level after alloxan induction and after administration of *Bhee* fruit extract

Group	Blood Glucose Level (%)	
	After Induction Alloxan	After Extract Administration
KD	Increase 132.59%	Increase 14.01%
KO	Increase 148.59%	decrease 40.82%
P1	Increase 98.76%	decrease 5.99%
P2	Increase 101.97%	decrease 10%
P3	Increase 118.90%	decrease 28.29%

Based on the table above, alloxan induction in

diabetic-conditioned mice has increased significantly. The percentage increase in glucose levels due to alloxan induction differs in each group. Different physiological factors in mice could cause this condition. Blood glucose levels in the KD group increased to 132.59% until day 14. The KD group was not given any extract or drug treatment. The administration of extracts for 14 days in groups P1, P2, and P3 affected blood glucose levels. Group P1, treated with extract at a dose of 100 mg/KgBB, experienced a decrease in blood glucose levels by 5.99%. The P2 group treated with extract at a dose of 200 mg/KgBB experienced a decrease in blood glucose levels by 10%. Group P3 treated with 400 mg/KgBB extract showed the highest percentage, which reduced glucose levels by 28.29% mg/KgBB. Metformin hcl given to the KO group reduced glucose levels by 40.82% higher than in groups P1, P2, and P3.

Discussion

The results of the above studies prove that alloxan injection could affect the blood glucose levels of mice. Glucose levels of mice before alloxan induction were still at normal levels. Measurement of glucose levels after alloxan induction showed a significant increase and exceeded expected levels. Alloxan works specifically to damage pancreatic β cells (Lina et al., 2022). The damage was characterized by reduced cell density, changes in cell nuclei, paling cytoplasm, and vacuolization. Damage to pancreatic β-cells would stimulate the formation of reactive oxygen species (ROS), which causes disturbances in insulin secretion (Kurniatanty & Wadhihah, 2022). These triggers could increase blood glucose levels in alloxan-induced mice. Normal mice in the KP group treated with the extract showed no change in blood glucose levels. The average glucose level before treatment in the KP group was 132,33 mg/dL and became 133.33 mg/dL after being given the extract for 14 days. This proves that giving extracts to normal mice is safe and does not cause hyperglycemia.

The normal blood glucose levels at the time of measurement ranged from 62.8-175 mg/dL (Noena et al., 2020) while fasting ranges from 50-109 mg/dL (Ismail & Naki, 2023). The maximum limit of normal blood glucose levels was 199 mg/dL (Irwan, 2018).



Blood glucose levels were categorized as hyperglycemia if the number reached >200 mg/dL (Kartikasari et al., 2018).

The treatment of extract doses of 100, 200, and 400 mg/KgBB could affect blood glucose levels in mice groups P1, P2, and P3. The group of mice that experienced the highest decrease in blood glucose levels occurred in P3 with the administration of a dose of 400 mg/KgBB extract by 28.29%. Before being treated with extracts, average glucose levels in the P3 group reached 266.33 mg/dL. After giving the extract for 14 days, the average blood glucose level of the P3 group reached 191.00 mg/dL. These levels were still classified as high, almost reaching the maximum limit of normal glucose levels.

The treatment of Metformin hcl 500 mg, which was converted according to the body weight of mice as a comparison drug, showed greater effectiveness than the administration of the extract. The percentage decrease in blood glucose levels in the mice group could be due to the components of Metformin hcl that were formulated as an appropriate drug. However, it could also be due to the drug dose being more significant than the extract dose. Metformin reduced blood glucose levels in the KO group by 40.82% for 14 days of treatment. When viewed from the percentage of the effect of giving extracts and giving drugs, then giving extracts were considered safer because it could reduce blood glucose levels slowly. In addition, the content of secondary metabolites in the extract requires a more extended treatment duration to regenerate damaged pancreatic β -cells effectively. A significant decrease in blood glucose levels in the KO group was feared to cause hypoglycemia in the long term.

The activity of secondary metabolites in plant extracts that have potential as antidiabetic agents was sensitive to insulin receptors. The way secondary metabolites work mimics insulin when affecting blood glucose levels (Ishak et al., 2021). Antioxidant activity in secondary metabolites could increase the absorption of cell glucose levels. When insulin secretion increases, the body uses glucose in the body's metabolic processes, such as glycolysis and lipoproteins (Datu et al., 2023).

Bhee plants contain flavonoids, tannins, phenylpropanoids, organic acids, terpenoids, and steroids with hypoglycemic activity (Zheng et al., 2021). The purple-colored part of the *Bhee* fruit indicates a high antioxidant content. Phytochemical screening results show that the ethanol extract of *Bhee* fruit contains metabolites in the form of alkaloids, terpenoids, flavonoids, phenolics, saponins, and tannins. These metabolites acted as antidiabetic agents by inhibiting the work of alpha-amylase enzymes (Muregesan et al., 2015).

Secondary metabolites in the extract acted as antidiabetic agents in various ways. Tannins acted as chelators that could wrinkle the membrane of epithelial cells in the small intestine. This causes the absorption of glucose levels after eating to be slower, and blood glucose levels could be well controlled. In addition, tannins could help damage pancreatic β -cell cells through increased muscle *glycogenolysis* (Fajarizki, 2022).

Terpenoids help increase insulin secretion by inhibiting carbohydrate metabolic signaling pathways and the α -glucosidase process (Shehadeh et al., 2021). Saponins increased the insulin signal transduction of the IRS-1/P13K/AKT pathway, which could minimize liver damage (Feng et al., 2021). Flavonoids and alkaloids acted as antidiabetic agents through intra-pancreatic and extrapancreatic mechanisms. The intrapancreatic mechanism was a secondary metabolite mechanism that worked to repair parts of the pancreas by protecting pancreatic β -cells, increasing insulin secretion, and helping to repair pancreatic β -cell damage. The extrapancreatic mechanism was the ability of secondary metabolites to control blood glucose levels outside the pancreas by suppressing the absorption ability of blood glucose in the intestine and reducing gluconeogenesis activity due to inhibiting the enzyme glucose 6-phosphatase fructose 1,6-biphosphate (Oswari, 2021). Secondary metabolites contained in *Bhee* fruit extract work together to regulate the rate of blood glucose levels so that it can overcome hyperglycemia.

Conclusion

Bhee fruit ethanol extract contains alkaloids, terpenoids, flavonoids, phenolics, saponins, and tannins. Giving ethanol extract of *Bhee* fruit for 14 days can reduce blood glucose levels in mice induced by alloxan.

Acknowledgments

Thanks to LPPM Teuku Umar University for providing research funds through internal grants 2022 with Decree Number: 099/UN59.7/SPK-PKK/2022. In addition, thank you also to fellow researchers and related parties who helped carry out this research well.

Author Contribution and Competing Interest

RN provided the concept for writing and overseeing the writing process. Others provided input and suggestions for improving the article. All authors made contributions to the writing of the article.

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