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Md. Atiq Ashhab, Md. Monirul Islam, Md. Muhaimanul Haque, Md. Sohan Ahmed, Md. Meheub Al Raji, et al.. Assessing the Antidiabetic Properties of Methanolic extract of *Baccaurea motleyana* leaf in Alloxan-induced Diabetic Mice. Asian Journal of Biochemistry, Genetics and Molecular Biology, 2025, 17 (3), pp.30-37. <10.9734/ajbgmb/2025/v17i3447>. <hal-04949538>

HAL Id: hal-04949538

<https://hal.science/hal-04949538v1>

Submitted on 15 Feb 2025

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Assessing the Antidiabetic Properties of Methanolic extract of *Baccaurea motleyana* leaf in Alloxan-induced Diabetic Mice

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Authors' contributions

This work was carried out in collaboration among all authors. This experiment was designed by authors MAA, MMH, MSA and MMA-R. The plant material was collected, Prepared extract and performed *In vivo* and *In vitro* experiments by authors MAA, MR, MMH, MMA-R, MSA, ZSJ, MAA, MMI, MMH and MAI conducted data analysis and data interpretation. Authors MAA and MMI prepared draft copy of that manuscript. Authors MAA, MAI, MMI, MMH, MSA and MMA-R revised drafted manuscript very carefully. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/ajbgmb/2025/v17i3447>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/130494>

Original Research Article

Received: 10/12/2024

Accepted: 12/02/2025

Published: 15/02/2025

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ABSTRACT

Background: Medicines made from *Baccaurea motleyana* are used to treat a wide range of illnesses. The current study targeted to investigate the methanolic extract of *Baccaurea motleyana* leaf's antidiabetic qualities.

Results: The leaf extract of *Baccaurea motleyana* at a concentration of 100µg/ml, the α-amylase activity was found to be 37.01% inhibited and the α-glucosidase activity to be 55.05% inhibited in the α-amylase inhibition experiment. The leaf extract considerably ($P < 0.05$) reduced blood glucose levels and improved lipid profile levels such as (TG, LDL, TC, VLDL & HDL) in diabetic mice, based on the findings of antidiabetic tests conducted in *in-vivo*. When compared to diabetic animals that were not treated, treatments with the extract of methanol of *Baccaurea motleyana* leaf for 24 days significantly ($P < 0.05$) decreased the effects of the ALT and AST enzymes in the diabetic mice (alloxan induced) also.

Conclusion: According to this study, the leaf extract of *Baccaurea motleyana* has strong antidiabetic effects. Because of this, they may be able to help avoid diabetes mellitus and its complications.

Keywords: *Baccaurea motleyana*; antidiabetic effects; α-amylase; α-Glucosidase; alanine transaminase; aspartate aminotransferase lipid profile.

ABBREVIATIONS

ANOVA : Analysis of Variance
 TC : Total Cholesterol
 TG : Triglyceride
 HDL : High Density Lipoprotein
 LDL : Low Density Lipoprotein
 VLDL : Very Low-density Lipoprotein
 ALT : Alanine Transaminase
 AST : Aspartate Transaminase
 SD : Standard Deviation

1. INTRODUCTION

Feedstocks are inexpensive and renewable for that reason phytoextract is a dependable source for discovering new bioactive compounds and developing new drugs. When it comes to drug development, phytobioactive compounds are superior to synthetic compounds because of their profound ability to interact or attach with a wide range of drug targets, such as transporters, signaling molecules, hormones, nucleic acids, enzymes, receptors and ligands (Kittakoop, 2015). Because product related to phytoextract are believed to be less harmful effects and have less adverse effects than synthetic products, there has been an increase in demand for it in recent years as flavoring and agrochemicals, food additives, cosmetics, and perfumes (Thiyagarajan & Venkatachalam, 2012). Therefore, finding plants with potentially advantageous pharmacological effects is an important research objective that will help create new treatments for a range of illnesses.

Diabetes mellitus is a chronic condition that occurs when the body is unable to use insulin properly or the pancreas is not able to produce enough insulin. Consequently, diabetes mellitus is characterized by elevated blood glucose levels (Triplitt et al., 2015). In patients with diabetes mellitus, elevated blood glucose levels have an impact on lipid, protein, and carbohydrate metabolism (Saltiel & Kahn, 2001). Insulin resistance and insufficiency affect numerous metabolic pathways and enzymes involved in the metabolism of lipid. Diabetes mellitus is defined by elevated levels of triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), and decreased levels of HDL (High-density lipoprotein) (Saltiel & Kahn, 2001; Laakso et al., 1986). Every system in a patient's body is impacted by diabetes mellitus, also known as metabolic illness, including the liver. (Mohamed et al., 2016). There have been prior reports of non-alcoholic liver steatosis, liver cirrhosis, liver cancer, and elevated liver enzymes including ALT and AST in people with diabetes mellitus (Goyal & Singh, 2007; Beckman et al., 2002). Polysaccharides are broken down into monosaccharides and disaccharides by the enzymes α-glucosidase and α-amylase. In patients with type I and type II diabetes mellitus, the inhibitor of these enzymes are also utilized to reduce blood glucose levels (Tundis et al., 2010). Many medications are commercially available to treat diabetes mellitus, however long-term usage of these medications can have negative side effects. Thus, researchers are searching for phytomedicines with low levels of toxicity and little to no negative

effects (Michael, 2007; Chen et al., 2001). Finding new medications that treat diabetes mellitus more effectively and with fewer side effects is therefore crucial (Wang et al., 2013).

The Phyllanthaceae family includes *Baccaurea motleyana* (Debnath et al., 2022), which is found in Bangladesh and South-East Asia, especially in Indonesia, Malaysia, and Thailand. The plant *Baccaurea motleyana*, also known as Lotkon, is well-known for its fruits and is used as medicine traditionally to treat a variety of conditions, such as ulcers, fevers, blood disorders, and sensations of burning. Another sign of peel extract's of this fruits has possible anti-diabetic effects is its ability to increase liver glycogen levels (Prodhan & Mridu, 2021). This experiment was conducted to determine the antidiabetic effects of extract of methanol of *Baccaurea motleyana* leaf in alloxan induced diabetic rats as well as Verify scientifically the plant's medicinal preparation for diabetes management. From our searching, this is the first experimental report that works on antidiabetic potential of *Baccaurea motleyana* in alloxan induced rats.

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

Fresh leaves of *Baccaurea motleyana* were gathered from Rajshahi, Bangladesh's Puthia Nursery, located at Puthia-6260. A well known taxonomist from the Botany Department at the University of Rajshahi in Bangladesh (Voucher number: AA056) thoroughly recognized the sample. Only Fresh leaf were gathered and thoroughly cleaned. These leaves had dried properly. Following that, it was ground into a powder. 300ml of methanol were used to soak 60g of powdered *Baccaurea Motleyana* leaves in a conical flask. After that, the 500mL conical flask was properly sealed, and the flask was maintained in a rotating shaker for ten days. Following a thorough filtering process using Whatman No. 1 filter paper, the filtrate was gathered. Ultimately, a dry leaf extract was produced by evaporating the methanol.

2.2 In vitro Assays

α -Amylase inhibition activity assay: With minor modifications, the method outlined by Xiao et al. (Xiao et al., 2006) was used to screen for extracts' ability to inhibit α -amylase. A solution of 0.5 mg/ml of α -amylase (from pig pancreas, Sigma-Aldrich, USA) was added to 0.5 ml of 0.02 M Na_3PO_4 buffer (6 mM NaCl; pH = 6.9) containing varying amounts of 0.5 ml leaf

extracts. The whole mixture was then incubated for around 10 min at 37°C. After that, 0.5 ml of soluble starch (1% w/v) was mixed to the test tube, and it was incubated at 37°C for 15 min. Later, stopping the enzymatic reaction by adding with 20 μ l of 1 M HCl, 0.1ml of iodine reagent was added (5 mM Iodine and 5 mM Potassium Iodide). The color of that solution was changed and take final absorbance was at 620 nm. For conducting this experiment, acarbose was used as a Standard. Using the following formula, the results were expressed as the percentage of inhibition.:

$$\text{Inhibition activity of } \alpha - \text{amylase} = [1 - \{(A_1 - A_2)/A_0\}] * 100$$

Where A_2 is the absorbance of the product control (sample without α -amylase solution), A_1 is the absorbance of the test sample, A_0 is the absorbance of the negative control (α -amylase without extract)

α -Glucosidase inhibition assay: The method explained by Schmidt et al. (2012) was used to perform the Inhibition of α -glucosidase enzyme activity. In this method, 90 μ l of 0.1M Na_3PO_4 buffer pH containing 0.02% sodium azide and 10 μ l of the extract and standard at different concentrations were placed in a 96-well microplate. It was then treated for 10 minutes at 28°C with 80 μ l of α -glucosidase enzyme (Sigma-Aldrich, USA) solution (2.0 U/ml) in Na_3PO_4 buffer in each well. After the incubation period, 20 μ l of PNPG (4-Nitrophenyl β -D-glucopyranoside) (0.4 mM, dissolved in Na_3PO_4 buffer) was added to the solution to start the chemical reaction. The rate of 4-Nitrophenyl β -D-glucopyranoside conversion to p-nitrophenol was measured using a Multiscan FC microplate photometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) by measuring the absorbance of the color solution of p-nitrophenol at 405 nm. Acarbose was used as a standard. The following formula was used to measure the proportion of α -glucosidase enzyme inhibition:

$$\alpha - \text{glucosidase enzyme inhibition activity} = \{(\text{absorbance of blank solution} - \text{absorbance of sample solution}) / \text{absorbance of blank solution}\} * 100$$

Animal care: The Institute of the Biological Sciences (IBSc) regulations of the University of Rajshahi in Bangladesh served as the guidelines for all animal studies. (Memory number: 320/IAMEBBC/IBSc/249(35)). For the aim of acclimatization prior to the experiment, male Swiss albino rats weighing 25–30g were

appropriately maintained in a laboratory setting with a temperature of 25 ± 3 °C and relative humidity of $55^\circ\text{C} \pm 5\%$. A 12-hour cycle of light and dark was maintained throughout this experiment.

2.3 In vivo Study

Diabetes induction and assessment: Age-matched control rats were given citrate buffer, while overnight-deprived rats got a single intraperitoneal injection of Alloxan (80mg/kg) produced in 0.1M citrate buffer, pH 4.5. After six days of alloxan administration, hyperglycemia was confirmed by drawing blood from the tail vein and measuring plasma glucose levels using a Glucometer (CERA-CHEK 1070, Korea). Mice with blood glucose levels between 15 and 17 mmol/L were selected for this investigation. The following five groups (n = 5) of acclimated, healthy mice were randomly assigned: untreated mice (control), alloxan-induced diabetic control mice group, alloxan-induced diabetic mice treated with standard medicine (glibenclamide tablet, 5.0mg/kg body weight), alloxan-induced diabetic mice treated with extract of methanol of *Baccaurea motleyana* leaf with two dose (200 mg/kg), and (400 mg/kg) group.

Oral glucose tolerance test (OGTT): For this experiment, A previously reported technique was used, but with minor changes (Eidi et al., 2012). All Swiss-albino mice were starved for 16 hours before to the testing. Mice (Groups 4 and 5) were given oral doses of MEBML 200mg/kg and 400mg/kg Body weight, respectively, and then given an oral glucose solution (1g/kg body weight). Mice in Group 3 received oral glucose delivery (1 g/kg body weight) after being treated with glibenclamide, an antidiabetic medication, at dose of 5mg/kg body weight. In contrast, first group mice did not receive glucose solution.

After administration oral glucose solution, tail vein blood samples were obtained at 0 min, 30 min, 60 min, 90 min, and 120 min. Glucose levels were then measured by vein puncture from tail by using a portable glucometer (Cera-Chek 1070, Korea) (Ahangarpour et al., 2018).

2.4 Statistical Analysis

The mean $\pm$ SEM (standard error of mean) was used to express all values. Dunnett's test was performed after the information was analyzed using ANOVA (one-way analysis of variance); a p-value of less than 0.05 was deemed statistically significant.

3. RESULTS

α -Amylase and α -glucosidase enzyme inhibition activity: *Baccaurea motleyana* leaf showed the significant inhibitory activity against α -amylase and α -glucosidase enzyme and the results are presented in (Fig. 1 a, b).

Evaluation of in-vivo anti-diabetic activity: *Baccaurea motleyana* leaf significantly reduced blood glucose levels ($p < 0.005$) for the course of the 24-day treatment. In comparison to diabetic control mice, the methanolic extract of *Baccaurea motleyana* leaf reduced the glucose level by 50.92% and 60.64% at each doses (200mg/kg and 400mg/kg BW) (Fig. 2a)

Serum transaminase and lipid profile: Glibenclamide (5 mg/kg) and leaf of methanolic extract supplementation (200mg/kg and 400mg/kg) decreased TG, LDL, TC, and VLDL compared to diabetic mice after 24 days. HDL, on the other hand, jumped considerably. Simultaneously, the diabetic control group's serum transaminase levels (ALT and AST) increased dramatically before returning to normal. The information is shown in Figs. 2b and c.

Table 1. Effects of the MEBML on blood glucose levels of diabetic mice in OGTT

Group	Animal Groups	Dose (mg/kg BW)	Blood Glucose level				
			Time after administration of a single dose of Extracts (minutes)				
			0	30	60	90	120
Group 1	Normal	-	4.8 $\pm$ 1.2	6.2 $\pm$ 1.5	7.3 $\pm$ 1.2	5.6 $\pm$ 0.7	4.6 $\pm$ 1.8
Group 2	Diabetic	-	17.3 $\pm$ 1.4	22.8 $\pm$ 0.7	24.9 $\pm$ 1.4	19.4 $\pm$ 1.3	15.6 $\pm$ 2.3
Group 3	Positive control	5	7.2 $\pm$ 0.8	9.3 $\pm$ 0.9	11.4 $\pm$ 1.8	9.2 $\pm$ 1.3	6.1 $\pm$ 0.6
Group 4	MEBML	200	9.2 $\pm$ 1.2	14.9 $\pm$ 2.8	13.0 $\pm$ 1.1	10.2 $\pm$ 1.9	9.1 $\pm$ 2.6
Group 5	MEBML	400	7.9 $\pm$ 1.5	15.9 $\pm$ 2.7	15.1 $\pm$ 3.1	10.8 $\pm$ 2.1	8.1 $\pm$ 1.9

All values are expressed as mean $\pm$ SD (n; number mice = 5). Here *** indicates $P \leq 0.05$ compared to diabetic control mice

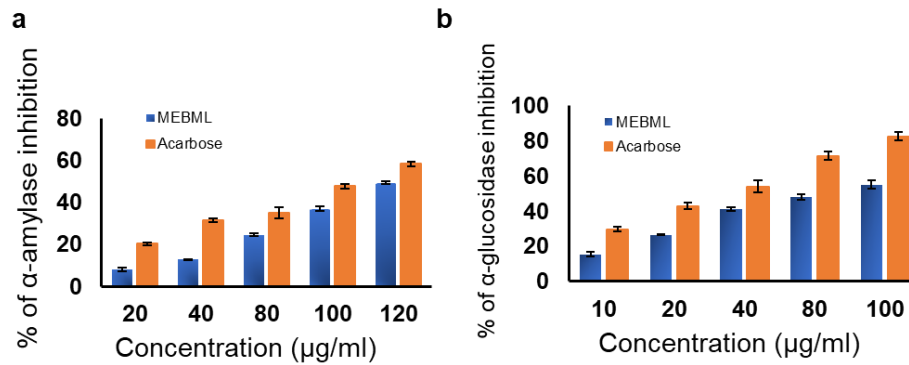


Fig. 1. α-Amylase and α-glucosidase inhibition activity: (a, b) α-amylase and α-glucosidase inhibition activity of methanolic extract of *Baccaurea motleyana* leaf (MEBML). All data are expressed as mean ± SD (n = 3)

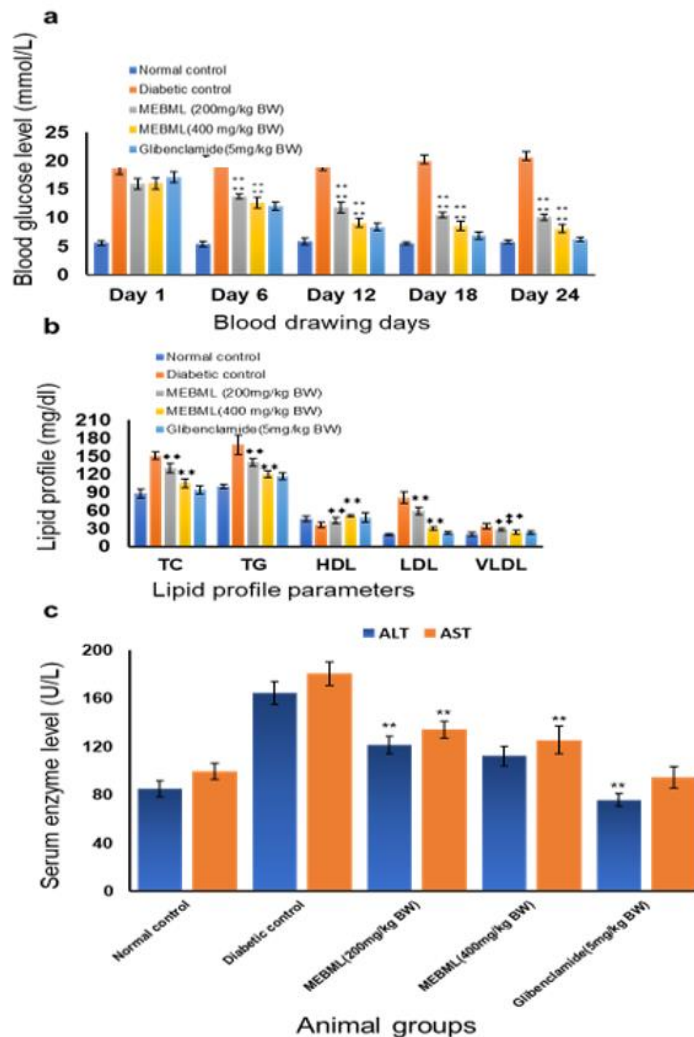


Fig. 2. Evaluation of *in-vivo* anti-diabetic activity: (a, b, & c) Effects of Methanolic extract of *Baccaurea motleyana* leaf (MEBML) treatment on blood glucose levels, lipid profile level and ALT, AST level in alloxan-induced diabetic mice. All data are expressed as mean ± SD (n = 5). Here “***” indicates $P \leq 0.05$ vs diabetic control mice

4. DISCUSSION

The hallmark of diabetes mellitus, a common metabolic disorder defined by elevated level of plasma glucose caused by peripheral insulin resistance or inadequate synthesis pancreatic insulin, is a high postprandial blood glucose level (De Sales et al., 2012). By using this technique, the important enzymes α -amylase and α -glucosidase, which are participated in the digestion of starch and glycogen (Tan et al., 2017), help to regulate postprandial glucose levels. (Wang et al., 2010). Inhibiting α -amylase and α -glucosidase, which reduces postprandial hyperglycemia by delaying glucose intake, is therefore acknowledged as a successful technique for managing type 2 diabetes and related issues (Neha et al., 2019). The results clearly showed that *Baccaurea motleyana* methanolic leaf extract has significantly inhibited α -amylase and α -glucosidase enzyme activity, suggesting that the leaf of that species may have a stronger ability to suppress elevated blood sugar levels through its expanded reducing capability for the digestion of starch substrates. Using alloxan-induced diabetic mice, the postprandial plasma sugar level suppression capacity of solvent extracts was used to further evaluate the therapeutic efficacy of *Baccaurea motleyana* leaf extract against diabetes (Fig. 2 a). Based on earlier research, we may infer that leaf extracts from *Baccaurea motleyana* may have ability to repaired impaired glucose level homeostasis through an insulin-secreting mechanism or by encouraging β -cell regeneration by lowering oxidative stress and preventing damage of DNA (Ghorbani, 2017). Because of the altered metabolism of carbohydrates, lipids, and patients with diabetes mellitus often have lower levels of HDL and higher levels of TC, TG, LDL, and VLDL in their serum (Laakso et al., 1986; Renard et al., 2004; Parhofer, 2015). These abnormalities are connected to the development of cardiovascular diseases in individuals with diabetes mellitus as well as the course of the disease (Laakso et al., 1986). When compared to diabetic control mice, diabetic mice fed a methanolic extract of leaves had considerably lower blood levels of TC, LDL, TG, and VLDL and higher levels of HDL. These findings indicate potential benefits of utilizing *Baccaurea motleyana* leaves to prevent or reduce lipid metabolism-related DM problems.

Because it absorbs and stores glucose as glycogen, changes it into glucose when needed, and creates glucose from non-carbohydrate sources such amino acid, the liver is an essential

organ for regulating blood sugar levels. (Eidi et al., 2012). Amino acid conversion to keto acids is carried out by the liver's enzymes ALT and AST, and elevated levels of keto acids may result from liver damage that allows them to seep into the blood. (Yerlikaya et al., 2017). AST and ALT levels may rise and liver damage may result from diabetes mellitus (Harris, n.d.; Radziuk & Pye, 2001; Zhu et al., 2016). The AST and ALT levels of the alloxan-induced mice were significantly higher than those of the control animals in this study. (Fig. 2 c). AST and ALT levels in the plasma were considerably lower after 24days of treatment with *Baccaurea motleyana* leaf extract (200 mg/kg and 400mg/kg) than in the group of diabetic control mice. According to the study's findings, when diabetic rats were given a methanolic extract of *Baccaurea motleyana* leaf, their plasma levels of the enzymes ALT and AST were reversed to normal levels in comparison to the mean values of the alloxan induced diabetic group. As a result, this study also shows that *Baccaurea motleyana* leaf extract may lower the risk of liver and cardiovascular damage linked to diabetes mellitus.

5. CONCLUSION

In conclusion, our study found that *Baccaurea motleyana* leaf methanolic extract has potent antidiabetic effects both *In vitro* and in animals. Furthermore, the altered levels of TG, TC, LDL, HDL, VLDL, ALT, and AST may be restored in diabetic mice treated with *Baccaurea motleyana* leaf extract. Therefore, our study suggests that *Baccaurea motleyana* leaf may be utilized to prevent and treat diabetes mellitus and its associated conditions. The specific anti-diabetic compounds included in *Baccaurea motleyana* leaves and their mode of action, however, require further investigation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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DECLARATIONS

All authors have approved this manuscript. The content of this manuscript or any portion thereof

has not been published or submitted for publication elsewhere.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This research work was approved by the Institutional Animal, Medical Ethics, Bio-Safety and Bio-Security Committee (IAMEBBC) for Experimentations on Animal, Human, Microbes, and Living Natural Sources, Memo No: 249(35)/320/IAMEBBC/IBSc. Institute of Biological Sciences, University of Rajshahi, Bangladesh.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Biochemistry and molecular biology of Rajshahi University and to the Pharmacy department of Jahangirnagar University, for supplying the Swiss albino mice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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