

Evaluation of the Hypoglycemic Effects of Methanolic *Bougainvillea spectabilis* Leaf Extract in Alloxan-Induced Male Albino Mice

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Abstract

Diabetes Mellitus (DM) is a critical global health concern, necessitating research into affordable and accessible treatment options like herbal medicine. This study investigated the hypoglycemic potential of a methanolic extract from *Bougainvillea spectabilis* leaves, a widely cultivated plant rich in bioactive phytochemicals, using an alloxan-induced diabetic male albino mouse model. The research addressed a gap in local Philippine studies by employing an effective, internationally validated methanolic extraction method. The study utilized a quantitative, pre-test/post-test experimental design with thirty diabetic mice divided into six treatment groups (four extract dosages: 500, 1000, 1500, and 2000 mg/kg; a positive control: Insulin Glargine; and a negative control: Normal Saline Solution). Phytochemical analysis confirmed the presence of sterols, flavonoids, saponins, glycosides, and tannins, compounds previously linked to blood sugar control. Experimental results demonstrated that the *B. spectabilis* extract exhibited a dose-dependent hypoglycemic effect. All extract dosages showed a consistent decline in blood glucose levels from the third to the fifth hour of monitoring. Notably, the 2000 mg/kg dose displayed a significantly greater decrease in blood sugar compared to other extract treatments during the 3rd to 5th hours. Statistical analysis (One-Way ANOVA and Tukey's HSD) confirmed significant differences in efficacy among the treatment groups during the first three hours. While effective, the extract treatments were not as potent as the positive control, Insulin Glargine. The findings support the use of the methanolic *B. spectabilis* leaf extract as an effective hypoglycemic agent and provide a foundational basis for further investigation into its development as an affordable anti-diabetic herbal medicine.

Keywords: *Alloxan-Induced Mice, Bougainvillea spectabilis, Diabetes Mellitus, Hypoglycemic, Methanolic Extract.*

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Introduction

Diabetes Mellitus (DM) is a pervasive global health crisis, defined by the World Health Organization (WHO, 2024) as a chronic condition where the body either fails to produce sufficient insulin or cannot effectively utilize the insulin it produces. The Center for Disease Control and Prevention (CDC, 2020) identify three main types: type 1 (autoimmune-related insulin deficiency), type 2 (poor insulin utilization and blood sugar control), and gestational diabetes. The scale of this epidemic is staggering; the International Diabetes Federation (IDF) reported approximately 537 million adults (20–79 years old) living with diabetes worldwide in 2021, a number projected to surge to 783 million by 2045. In the Philippines specifically, the IDF recorded over 4.3 million adult cases in 2021. Untreated, DM can lead to severe and life-threatening complications, including kidney failure, blindness, limb amputation, and cardiovascular disease (International Diabetes Federation, 2025).

The urgent need for accessible and affordable treatment options has driven significant interest in herbal medicine. The Philippine Council for Health Research and Development notes the wide availability of herbal remedies for blood sugar control in the market. Scientific studies support this traditional practice, with research indicating that many plants are rich in natural antioxidants such as tannins, flavonoids, and vitamins C and E, which can protect cells and help lower blood glucose levels (Kooti, W. et al., 2016). Globally, over 400 plant species are recognized for their potential in treating diabetes, yet only a fraction have undergone rigorous scientific validation (Kumar, S. et al., 2021).

This study focuses on *Bougainvillea spectabilis*, a tropical perennial shrubby vine belonging to the Nyctaginaceae family, which is native to South America and widely cultivated globally (Ornelas Garcia et al., 2023). Beyond its aesthetic value, the plant is a rich source of phytochemicals—biologically active compounds known to have therapeutic effects—including alkaloids, flavonoids, cardiac glycosides, saponins, and cyanins (Adebayo GI, 2009; Piatteli M., 1970).

Crucially, recent research has highlighted the antidiabetic potential of *Bougainvillea*. The plant contains D-Pinitol, which has demonstrated significant reductions in hyperglycemia in diabetic rat models (Elghiet et al., 2023). Local Philippine studies have also shown the promising hypoglycemic properties of *Bougainvillea* leaf and bract extract on Dexamethasone-induced male albino rats (Batiquin, M.C. et al., 2019). Furthermore, a number of in vivo experiments conducted elsewhere have shown a significant hypoglycemic effect for various *Bougainvillea* extracts, including those prepared with methanol and aqueous solvents, on streptozotocin- or alloxan-induced diabetic rodents (Abo-Elghiet et al., 2023; Devi and Ramesh, 2018; Chauhan et al., 2015; Adebayo et al., 2009; Bhat et al., 2008).

Despite the compelling existing evidence, there is a recognized gap in the local application of specific extraction methods. Prior studies in the Philippines have not yet explored the use of a methanolic solvent for *Bougainvillea spectabilis* leaf extraction, a method that has proven effective in international in vivo models. Moreover, the use of the well-established and cost-effective alloxan-induced diabetes model in male albino mice for this specific extract preparation has not been widely documented locally. Hence, this study is a humble attempt to evaluate the potential hypoglycemic effects of *Bougainvillea spectabilis* leaf extract using a methanolic solvent on alloxan-induced male albino mice. The research aims to contribute to the body of knowledge by utilizing



an accessible and widespread local resource with a validated, yet locally unapplied, extraction technique. The primary objective is to determine the effect of various dosages of the extract on blood glucose levels and identify the most effective treatment concentration. The findings are intended to serve as a foundational basis for further investigation, including subsequent human trials, to offer a potentially affordable and accessible herbal medicine for individuals living with high blood sugar levels.

Materials and Methods

The research was conducted utilizing a quantitative approach, specifically experimental research design. The study applied a pre-test, post-test, and random block design with six treatments. A pre-test was done on all Albino mice to obtain baseline data on fasting blood sugar levels. On the other hand, a post-test was used to obtain changes in the blood sugar level of the animals after receiving interventions. Additionally, the researchers employed a random block design to seventy (70) albino mice (8-12 weeks) and were then grouped into six treatment groups (5 mice per group).

Plant Extraction

The leaves of *Bougainvillea spectabilis* were sourced from a farm in Quezon Province, Philippines and were authenticated by Jose Vera Santos Memorial Herbarium in University of the Philippines Diliman. After obtaining the authentication, the leaves were brought to Department of Science and Technology's (DOST) Pharmaceutical Section of Chemicals and Energy Division (CED) for the extraction process. Leaves were separated from the stalk, air-dried, and were finely ground and sieved to create a powder. It was then placed in a flask with methanol as a solvent. The flask was immersed in a water bath at a predetermined temperature for a specific time duration, allowing it to produce the concentrated crude plant extract (Pineda et al., 2025). After the extraction process, the mixture was filtered to eliminate any remaining plant material or impurities, resulting in the isolation of the extracted compounds in the solvent. Subsequently, the solution was gently heated to evaporate the solvent and concentrate the extracted compounds. The *Bougainvillea spectabilis* leaf extract was also submitted for phytochemical analysis that was also done by the DOST-ITDI-CED.

Ethical Selection of Experimental Animals

The albino mice were selected based on their age in weeks (8-12 weeks) and gender (male). The animals were acclimatized to Laboratory Animal Facility (LAF) of DOST-ITDI. The albino mice were kept in cages with free access to mouse feed and water all throughout the experimental period. The experimental protocols and procedures used in this study were approved by the DOST IACUC (Institutional Animal Care and Use Committee) and Bureau of Animal Industry (BAI).

Induction of Alloxan and Administration of Methanolic *Bougainvillea* Leaf Extract

Hyperglycemia was induced in the male albino mice using Alloxan (Sigma-Aldrich, Inc., USA) dissolved in normal saline with a dosage of 100-120 mg/kg body weight, then was administered via a single intraperitoneal injection. Prior to administration, the crude extract was dissolved using TWEEN® 80 (Polyethylene Sorbitol Ester) due to the residual sap of the *Bougainvillea spectabilis* extract. The blood glucose levels of the mice were checked forty-eight (48) hours after the administration of alloxan. Blood glucose determination was done by disinfecting the tail with 70% alcohol, cutting it, and gently milking it from the body toward the tip. The blood samples of the mice were collected on a reagent strip to determine the blood glucose level using a portable glucometer (Accu-Chek®, Roche, USA). A blood sample for blood sugar determination was obtained during: (a) pre-induction of Alloxan after 12 hours of fasting; (b) forty-eight (48) hours



after injection of Alloxan; (c) first hour after administration of treatment; (d) second hour after administration of treatment; (e) third hour after administration of treatment; (f) fourth hour after administration of treatment and, (g) fifth hour after administration of treatment. Overall, the sugar level of each group of mice was checked every hour within the five (5) hour duration. Mice with fasting blood glucose levels between 150- 250 mg/dL were considered diabetic.

Albino Mice for In Vivo Experiment

The experimentation process lasted for fifteen days (15) days, during which, seventy (70) albino mice were used in this study. From this, thirty (30) mice which became diabetic were grouped to be used for treatments. These animals were divided into six groups, each comprising five male albino mice. The animal groupings were as follows: (a) animals induced with alloxan that have developed diabetes was given oral gavage of normal saline solution; (b) animals induced with alloxan that have developed diabetes was given Insulin Glargine subcutaneously (c) animals induced with alloxan that have developed diabetes was given oral gavage of 2000 mg/kg of *Bougainvillea spectabilis* leaf extract (d) Animals induced with alloxan that have developed diabetes was given oral gavage of 1500 mg/kg of *Bougainvillea spectabilis* leaf extract (e) Animals induced with alloxan that have developed diabetes was given oral gavage of 1000 mg/kg of *Bougainvillea spectabilis* leaf extract (f) Animals induced with alloxan that have developed diabetes will be given oral gavage of 500 mg/kg *Bougainvillea spectabilis* leaf extract. After the twenty-four (24) hour course of treatment, the animals used in this study were euthanized.

Data Analysis

Quantitative data analysis, defined as the methodical examination of numerical data (Eteng, 2022), was employed to interpret the experimental results. The study utilized the following statistical treatments: A One-Way Analysis of Variance (ANOVA) was initially performed to assess the overall relationship between the independent variable (different *Bougainvillea spectabilis* extract dosages and control treatments) and the dependent variable (blood glucose levels) across all three or more experimental groups (Kenton, W., 2023). This test determined if a statistically significant difference existed among the mean blood glucose levels of the treatment groups. Following a significant result from the ANOVA, Tukey's Honest Significant Difference (HSD) post-hoc test was applied. The Tukey HSD test, which utilizes a studentized range distribution to account for multiple comparisons (Tukey, 1949), was used to precisely identify which specific pairs of treatment means exhibited statistically significant differences. This allowed for a detailed comparison of the efficacy between the different extract dosages and control groups.

Results

Phytochemical Analysis for Plant Constituents

Phytochemical analysis involves identifying and quantifying bioactive compounds present in plants, offering potential medicinal, nutritional, and therapeutic advantages. The phytochemical analysis conducted on the extract from *Bougainvillea Spectabilis* Leaves, submitted to DOST-ITDI Standards and testing Division (STD), yielded results as depicted in Table 1.

Table 1. Phytochemical Analysis of *Bougainvillea spectabilis* Extract

Plant Constituent	Result	Test Method
Sterols	+	Lieberman-Burchard Test
Triterpenes	-	Lieberman-Burchard Test
Flavonoids	+	Shinoda Test



Alkaloids	-	Mayer's Test
Saponins	+	Froth Test
Glycosides	+	Fehling's Test
Tannins	+	Ferric Chloride Test

**Note: The symbol "+" indicates the presence of plant constituents in the sample analyzed.*

Blood Sugar Dose Response

During the initial hour, male albino mice exhibited a decrease in blood sugar levels at a dose of 500 mg/kg. However, by the second (2nd) hour, mice in this group showed an increase in blood sugar levels, while those in groups receiving doses of 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg experienced a decrease. Additionally, from the third (3rd) hour until the fifth (5th) hour, male albino mice across all treatment groups, spanning from 500 mg/kg to 2000 mg/kg, demonstrated a consistent decline in blood sugar levels. Nevertheless, in the third (3rd) to fifth (5th) hour, the dose of 2000 mg/kg showed a significant decrease compared to other treatments. Numerous studies have documented the hypoglycemic effects of *Bougainvillea spectabilis* on mice and rats (Devi and Ramesh, 2018; Bhat, M. 2008). These effects have been consistently linked to the bioactive compounds present within the plant. (Chauhan, P. et al., 2015; Abarca-Vargas, R.A and Petricevich, V. 2018). (See Figure 1)

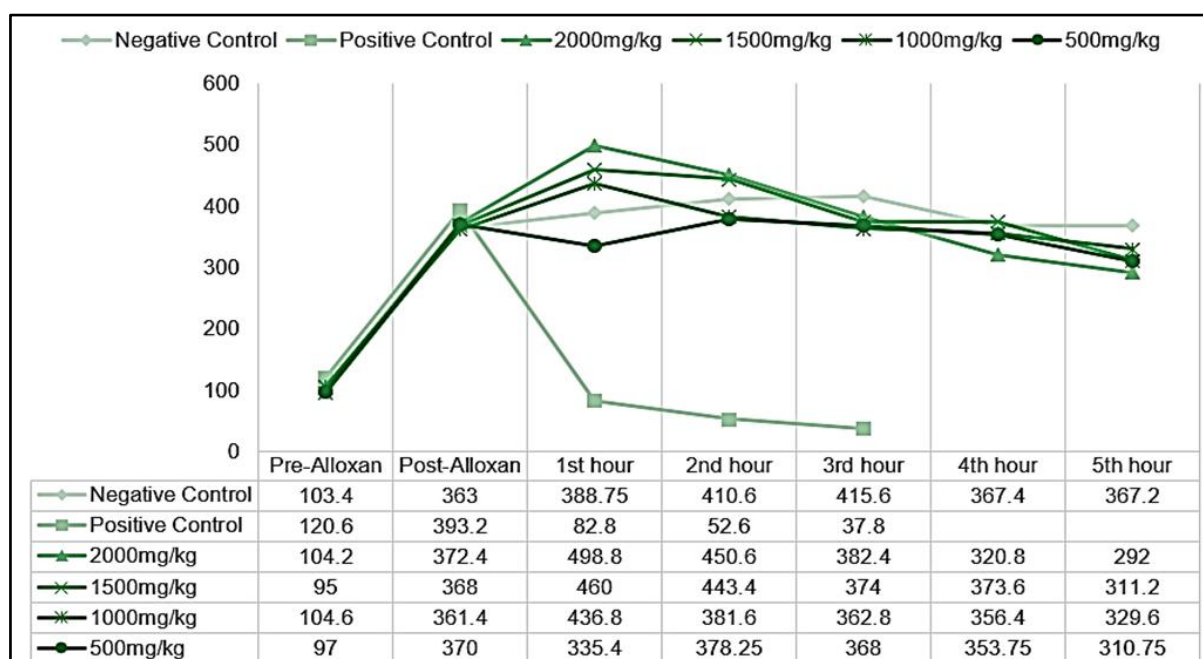


Figure 1. Blood Sugar Dose Response of Albino Mice through a 5-Hour Period

The results of the one-way ANOVA analysis yielded the following p-values for each hour: (1st hour) 0.00033, (2nd hour) 0.00103, (3rd hour) 0.0014, (4th hour) 0.99642 and, (5th hour) 0.98170. It showed that there were significant differences between the treatment groups in the first (1st), second (2nd), and third (3rd) hours, as shown by p-values that were less than the standard 0.05 level of significance (Mishra et al., 2019). These findings suggest that at least one pair of treatment groups demonstrated statistically significant variations in effectiveness during these initial time intervals. In contrast, during the 4th and 5th hours, the p-values surpassed the 0.05 threshold,



indicating a lack of statistically significant differences between treatment groups at these later time points (Kao & Green, 2008).

The of Tukey's Honest Significant Difference (HSD) test scrutinized the differences in the effects of each treatment group compared to others, focusing on data collected from hours one (1) through three (3). In the first (1st) to third (3rd) hour, a significant difference exists between the treatments at various periods. However, the treatment 5 vs 6 at the second (2nd) hour does not show a significant difference.

Discussion

The results showed that the *Bougainvillea spectabilis* leaf extract contains sterols, flavonoids, saponins, glycosides, and tannins, however, presence of triterpenes or alkaloids were not found (Singh et al., 2022). The plant components that are present in *Bougainvillea* Leaf extract have the potential to help manage diabetes. Sterols have been linked to improved lipid metabolism and insulin sensitivity (Prasad et al., 2022), which may potentially help to manage diabetes. Another important plant component found was flavonoids, which have antioxidants and anti-inflammatory characteristics that may help reduce oxidative stress and inflammation, both of which are commonly related with diabetes problems. Flavonoids also regulate hyperglycemia by inhibiting carbohydrate-digesting enzymes and glucose transporters, which aids in achieving normal sugar level within the bloodstream (Yen et al., 2021; Singh, 2018). Saponins have hypoglycemic effects by improving insulin secretion and glucose uptake, thereby assisting in blood glucose level regulation (Kumar et al., 2023). Glycosides have been associated with blood glucose lowering actions and may contribute to improved insulin sensitivity (Xu et al., 2014). Lastly, tannins have anti-diabetic and anti-obesity bioactivities. It can lower blood glucose levels by delaying intestinal glucose absorption and inducing an insulin-like effect on insulin-sensitive tissues. Tannins also delay the onset of insulin-dependent diabetes mellitus by regulating the antioxidant environment of pancreatic β -cells (Mendoza and Silva, 2018; Sieniawska & Baj, 2017). The varied range of plant components found in *Bougainvillea spectabilis* leaf extract highlights its potential as a natural diabetes treatment, with each component providing health advantages.

Results demonstrated the successful induction of diabetes in male albino mice, as evidenced by the analysis of pre- and post-alloxan induction data. Alloxan is widely acknowledged as an effective diabetogenic agent, commonly employed by researchers to induce diabetes in vivo experiments (Ighodaro et al., 2017). Furthermore, the data showed that among the different treatments tested, insulin glargine has shown a drastic change in lowering the blood sugar levels of male albino mice. It's worth noting that insulin glargine is FDA-approved for the treatment of diabetes in humans (Cunningham, A., Freeman, A., 2022), and its efficacy has also been demonstrated in the treatment of diabetes-induced rats and mice. Moreover, the Plain Normal Saline Solution, serving as the negative control, displayed an initial increase in blood sugar levels during the first(1st) to third (3rd) hour, followed by a decline in levels during the fourth (4th) and fifth (5th) hour of monitoring. In addition to this, for the other treatments administered with varying doses and time intervals from the first (1st) hour up to the fifth (5th) hour, notable observations were made.

This result implies that using a different treatment and dose of *Bougainvillea spectabilis* extract yields a different effect on their blood glucose level. Similar results were observed by Jawla et al., 2012; Kumar et al., 2012) after an oral administration of *K. pinnata* in streptozotocin-induced diabetic rats at doses of 250 and 500 mg/kg body weight for 21 days. Moreover, Price and Panel (2023) state that a drug's bioavailability—its ability to enter the bloodstream and remain effective—is largely dependent on its dosage and mode of administration. As a result, a drug with a lower bioavailability will require a higher dosage to achieve the minimum effective concentration. On the



other hand, in a study conducted by Njogo et. al. (2018) where they tested the in vivo hypoglycemic effect of *Kigelia africana* leaf extracts, the results showed that the extracts were found to have antidiabetic or blood sugar lowering properties regardless of the exact amount or dose given, possibly due to their constituent plant compounds and minerals.

Conclusion

Contingent on the analysis and interpretation of the data, it revealed that while *Bougainvillea spectabilis* leaves extract shows lowering blood sugar levels in male albino mice, its effectiveness varies across different doses of treatments. Specifically, the extract exhibited a consistent decrease in blood sugar levels from the 3rd to 5th hour across all treatments. However, the 500 mg/kg dose displayed a unique pattern, with an initial decrease in blood sugar levels in the first hour followed by an increase in the second hour. Surprisingly, the treatment with 2000 mg/kg showed a significant decrease during the third (3rd) to fifth (5th) hour compared to other treatments. This indicates that since *Bougainvillea spectabilis* leaves contains phytochemicals such as Sterols, Flavonoids, Saponins, Glycosides, and Tannins that are associated with potential blood sugar control, the *Bougainvillea spectabilis* leaves extract is an effective hypoglycemic agent to the male albino mice. With this, the hypothesis is therefore rejected. However, it's noteworthy that none of the treatments: 500 mg/kg, 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg were as effective as the Insulin Glargine in regulating blood sugar levels.

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Conflict of Interests

The researchers declare that there is no conflict of interest for publication of this paper.

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