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Research Article

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In vitro antidiabetic evaluation of *Bombax buonopozense* methanol leaf extract

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ABSTRACT

Diabetes mellitus (DM) is a major worldwide health burden that requires research into cost-effective treatment alternatives. It has been claimed that herbal medicines, have improved glucose metabolism in diabetics. It has been showed that several plant extracts are useful in preserving glucose homeostasis. Therefore, the present study aimed to evaluate the phytochemical and antidiabetic activities of *Bombax buonopozense* methanol leaf extracts. Standard methods were used for the identification of alkaloids, tannins, flavonoids, saponins, phenolics, cardiacglycosides and steroids. The enzyme inhibitory activities of the extracts of *B. buonopozense* was evaluated on α-amylase and α-glucosidase. The crude leaf extract of *B. buonopozense* exhibited a more effective inhibition on α-glucosidase with IC_{50} (Half maximal inhibitory concentration) values 888.20±35.06 µg/mL when compared to control (ascorbic acid) with values 1076±2.77 µg/mL. Fractions of ethyl acetate showed lower inhibitory property of alpha glucosidase with IC_{50} value 18.44±2.63 µg/mL compared to the control (ascorbic acid with IC_{50} value 16325±1318 µg/mL). This study showed the scientific basis for the traditional therapeutic usage of *B. buonopozense* by proven its antidiabetic activity in vitro. This is the first time that *B. buonopozense*'s antidiabetic efficacy and possible mechanisms have been documented.

KEYWORDS: Diabetes, *Bombax buonopozense*, α-amylase,α-glucosidase

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by abnormally high blood glucose levels. The two primary categories of endocrine cells found in the pancreatic islets of Langerhans are alpha cells, which secrete glucagon, and beta cells, which produce insulin. The external supply of glucose constantly influences the amount of hormones secreted by beta and alpha cells. When glucagon and insulin are out of balance, glucose levels become unnecessarily unbalanced. Insulin is either absent or acts slowly (insulin resistance) in people with diabetes mellitus, which results in hyperglycemia (Sapra & Bhandari, 2023). Two are the most common types of diabetes out of all of them. insulin-dependent diabetes mellitus, or type I diabetes, is an autoimmune disease; on the other hand, type II diabetes is defined by a disruption of insulin action that results from disruptions to cell membrane transporters or receptors (Malik *et al.*, 2019). Herbal treatments have long been used to treat diabetes. Compared to synthetic medications, plant medicines containing a range of chemicals may lower blood glucose levels through multiple pathways, perhaps yielding more significant therapeutic results and fewer side effects. α-glucosidase, an enzyme that breaks down carbohydrates into glucose in the small intestine, is the main enzyme in charge of regulating blood glucose levels after meals. α-glucosidase inhibitors delay glucose production and absorption, which can reduce postprandial glucose increase in diabetics (Wang *et al.*, 2022). Reducing postprandial hyperglycemia through delayed digestion and absorption of carbohydrates, such as those facilitated by the digestive tract's alpha-glucosidase and alpha-amylase enzymes, is one of the treatment options for managing diabetes mellitus. Alpha-glucosidase and alpha-amylase enzyme inhibitors slow down and extend the pace of carbohydrate digestion, which lowers the rate at which glucose is absorbed and, as a result, lowers postprandial hyperglycemia (Okechukwu *et al.*, 2020). Many diseases have been successfully cured and prevented by natural therapies, with fewer side effects. Even though there are many synthetic medications on the market for treating various conditions, most consumers are not happy with these medications because of the detrimental consequences they have on their health. Because of this, natural herbal remedies have gained popularity in recent years, despite their slow start to action and ability to effectively treat illnesses at their source (Das *et al.*, 2023). Many undesirable side effects of conventional DM drugs include weight gain, hypersensitivity, diarrhea, hypoglycemia, heart and liver failure, nausea, and

hypersensitivity. Herbal remedies with high flavonoids, phenolic compounds, terpenoids, alkaloids, glycoside, and coumarin content help regulate the metabolism of carbohydrates. Due to insufficient standards, traditional medicine has tremendous hurdles even though it is more successful than the present medication system (Malik *et al.*, 2023).

The *Bombax buonopozense* is a significant tropical medicinal tree that is found throughout Africa. It has a height of 40 m, tall buttresses (6 m), massive conical spines, compound leaves with 5-9 leaflets, and whorled branches. *B. buonopozense's* flowering part is used as a vegetable in Nigeria. The plant has been known to have a great deal of biological activity due to its medicinal qualities. Its antibacterial, antidiarrheic, and antipyretic properties have all been reported in earlier research (Tilaoui *et al.*, 2021). In southwest Nigeria, the leaves of *B. buonopozense* (Bombacaceae) have long been used as a remedy for arthritis (Fadogba *et al.*, 2024). Therefore, the goals of this research were to look into the phytochemical profiles and anti-diabetic properties of leaf extract from *B. buonopozense.* It is anticipated that the findings of this study will offer useful knowledge about *B. buonopozense* pharmacological potential, thereby validating and enhancing the plant's usage.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The *Bombax buonopozense* leaf was collected in Karmo, Abuja at the National Institute for Pharmaceutical Research and Development (NIPRD) in Herberium, Abuja, Mr. Hakeem identified the plant. The number of the voucher specimen is NIPRD/H/7347l.

Preparation of Plant Extract

The leaves and stem bark of *Bombax bounopozense* were air dried for 28 days at room temperature in a cool dry room. The dried parts of *B. bounopozense* were further processed, by pulverizing the plant parts. The dry leaves using a wooden mortar and pestle were ground into fine powder. The powdered samples were stored in a polyethylene bag for further work. 1.4 kg of *B. buonopozense* leaf was macerated separately in 12 L of methanol (75%) for 72 h, 4 times consecutively. The extract was filtered using muslin cloth and concentrated at 78 °C using a rotary evaporator and the percentage yield was calculated using the method described by Benchaachoua *et al.* (2018). The stock extracts were preserved by storing them in an airtight glass container and kept inside the refrigerator at 4 °C (Adebayo *et al.*, 2010).

Fractionation of the Crude Methanol Leaf Extract of *B. buonopozense*

The crude methanol extract of the *B. buonopozense* was then portioned using a solvent of varying polarity starting with n hexane, dichloromethane and ethylactate.

Qualitative Phytochemical Analyses of the Crude and Fractions of the Methanol Leaf Extract of *B. buonopozense*

Standard phytochemical screening tests using standard protocols to classify the phytochemicals present were carried out on the crude extracts and solvent fractions (n-hexane, ethyl-acetate and dicholomethane) of *B. buonopozense* plant extracts. The following phytochemical tests were carried out using already established methods (Chen *et al.*, 2019).

Identification of Tannins

Extracts of *B. buonopozense* (0.5 g each) were dispersed in 10 mL of distilled water and then strained. A small amount (3 drops) of 0.1% ferric chloride ($FeCl_3$) was introduced into the filtrate and thereafter monitored for a change in colour from a brown-like green or a blue-black colour, which confirmed that tannins were present.

Identification of Saponins

Test for the presence of saponins in the extracts was determined based on the capacity of saponins to create an emulsion with oil. A weighted amount (20 mg) of the extracts of *B. buonopozense* was boiled for 5 min each in 20 mL of distilled water in a water bath and filtered. A filtrate volume (10 mL) was diluted with 5 mL of distilled water, and briskly vortexed to allow the froth to develop. A few (3) drops of olive oil were then added to the froth, briskly vortexed again and observed for the development of an emulsion afterward.

Identification of Steroids

A portion of *B. buonopozense* (0.5 g) extracts was added with acetic anhydride (2 mL) and 2 mL of sulphuric acid. There was an observation for color changes; colors from violet to blue or green were observed and indicative that steroids were present (Shaikh & Patil, 2020).

Identification of Terpenoids

In this step there was a combination of 0.5 g samples with chloroform (2 mL), and careful addition of concentrated sulphuric acid (3 mL) to form a layer. The presence of a reddish-brown coloration confirmed the presence of terpenes (Liu *et al.*, 2020).

Identification of Cardiac Glycosides

Glacial acetic acid (2 mL) containing a drop of ferric chloride solution was treated with the extracts (0.5 g). This was overlaid with 1 mL of concentrated sulphuric acid. A brown ring at the interface showed that cardiac glycosides have a deoxysugar characteristic (Ugoeze *et al.*, 2021).

Identification of Alkaloids

There was an immersion of 0.5 g of grounded plant samples into a 10% ethanol acetic acid. This was left for 4 h and then eventually washed. There was an addition of 2 mL of distilled hydrochloric acid to filtrate, and then few drops of Meyer's reagent additionally added. The presence of alkaloids was indicated by a green color appearance (Ugoeze *et al.*, 2021).

Identification of Flavonoids

Heating was done with 20 mL of water using a beaker and extracts of *Bombax bouneponzense* (0.5 g) were filtered. A part of the filtrate was added 5mL of dilute ammonia solution and then subsequent addition of concentrated H_2SO_4. The presence of Flavonoids was indicated by a yellowish coloration (Shaikh & Patil, 2020).

Identification of Phenolics:

Ethanol (10 mL) was added to the extract and the resulting solution was transferred to a test tube and warmed in a water bath for 15 minutes. Three drops of freshly prepared ferric cyanide solution were added to the extract solution. The formation of a blue green colour indicated the presence of phenols (Velavan *et al.*, 2015).

In vitro Antidiabetic Activity Assay

The *in vitro* alpha amylase activity was determined according to the method described by Iheagwam *et al.* (2020).

Determination of Alpha-amylase (α – amylase) Inhibitory Activity

To 250 µL of each extract concentration in a test tube (0-360 µg/mL), the following were added sequentially: buffered α-amylase (250 µL, 0.05 mg/mL), starch (250 µL, 1%), the reaction mixture was incubated for 10 min at 25 °C. DNSA (500 µL) was added subsequently and then boiled for 5 mins. It was then cooled and diluted with 5 mL of water. The control was prepared in the same manner as the test samples with distilled water replacing the extract. The absorbance of each test tube content was taken at 540 nm and the percentage inhibition was calculated as follows;

% Inhibition= Ac-AtAcx 100 where A_c and A_t are the absorbance of the control and tests, respectively.

The concentration of the extracts resulting in 50% inhibition of the enzyme activity (IC_{50}) was determined graphically.

Determination of Alpha-Glucosidase (α – Glucosidase) Inhibitory Activity

The *in vitro* alpha glucosidase activity was determined according to the method described by Kim *et al.* (2005)

α-glucosidase: To 50 µL of each extract concentration in a test tube (0-40 µg/mL) the following were added sequentially: buffered α-glucosidase (100 µL, 1.0 U/mL) and incubated at 37 °C for 10minutes, then pNPG (50 µL, 3.0 mM) and incubated at 37 °C, for 20 min, then Na_2CO_3 (5% w/v), cooled to 25 °C and lastly 5 mL water was added and vortexed. The absorbance of the yellow p-nitrophenol from the different test tubes will be taken at 405 nm and the percentage inhibition was calculated as follows;

% Inhibition= Ac-AtAcx 100 where Ac and At are the absorbance of control and tests respectively

The concentration of the extracts resulting in 50% inhibition of the enzyme activity (IC_{50}) will be determined graphically.

Statistical analysis

The statistical analysis was carried out with Graph Pad Prism version 8.0. The experimental data were presented as mean ± standard deviation (SD) of three repeated experiments. The significant differences between groups were analyzed by One-way analysis of variance (ANOVA). The differences between groups are considered statistically different at p value <0.05.

Result of the qualitative phytochemical assessment of *B. buonopozense* leaf extracts is shown in Table 1. The results of *B. buonopozense* showed that crude extract gave a positive reaction to phenols, flavonoids, Saponins, cardiac glycoside, steroids and tannins while hexane and dichloromethane fraction gave a negative reaction to the phenols and saponins. Ethylacetate fraction gave a positive result for flavonoids.

The result of the inhibitory effect of the crude extracts of *B. buonopozense* on α-amylase and α-glucosidase showed that the extract is a more effective inhibition on α-glucosidae with IC_{50} values 888.20±35.06 µg/mL when compared to control (ascorbic acid) with values 1076±2.77 µg/mL (Table 2).

The result of the inhibitory activities of the extracts of *B. buonopozense* on α-glucosidase showed ethylacetate fraction of *B. buonopozense* leaf extract exhibited more effective inhibition with IC_{50} values 1.31±0.05 µg/mL when compared to control (ascorbic acid) with values 1552±463.21 µg/mL (Table 3).

The result of the inhibitory activities of the extracts of *B. buonopozense* was evaluated on α-amylase. The ethylacetate fraction of *B. buonopozense* leaf extract exhibited a more effective inhibition on α-amylase with IC_{50} values 18.44±35.06 µg/mL when compared to control (ascorbic acid) with values 16390±1318 µg/mL (Table 4).

DISCUSSION

The Ethnopharmacological use of plants was the basis for phytochemical, antioxidant and pharmacological investigation (Builder *et al.*, 2020). *B. buonopozense* is utilized extensively in diverse cultures across nearly every continent to address various health conditions, such as diabetes, antimicrobial, antioxidant, anti-diarrhoeic and antipyretic activities (Tilaoui *et al.*,

Table 1: Phytochemical Constituents of the Extracts and Solvent Fractions of *B. buonopozense*

Phytochemicals	Phenols	Flavonoids	Saponin	Cardiac glycosides	Steroids	Terpens	Alkaloids
Crude methanol extract	+++	–	++	++	+	++	–
N-hexane extract	–	–	–	+	+	+	–
Dichloromethane	–	–	–	+	+	+	–
Ethylacetate	+	+	–	+	+	+	–

+++ represent highly present, ++ represent moderately present, + represent slightly present, – represents absence of phytochemical.

Table 2: IC_{50} of alpha amylase and α-glucosidase inhibitory activities of methanol leaf extract of *Bombax buonopozense* and Acarbose

Samples	α-glucosidase IC_{50} (μg/mL)	*α*-amylase inhibitory activity IC_{50} (μg/mL)
Methanol extract	888.20±35.06	739.80±22.74
Acarbose	1076±23.53	136.90±3.64

Data are represented as Mean±SD (standard deviation) *p<0.05 significantly different from each other.

Table 3: IC_{50} of alpha amylase inhibitory activities of fractions of *Bombax buonopozense*

Samples	*α*-amylase Inhibitory ActivityIC_{50} (μg/mL)
Ethylcetate fraction	1.31±0.05*
Dichloromethane fraction	6.26±0.12*
N hexane fraction	2.94±2.99*
Aqueous fraction	5.41±0.1447*
Acarbose	1552±463.21

Data are represented as Mean±SD. *p<0.05 significantly different from Acarbose.

Table 4: IC_{50} of alpha glucosidase inhibitory activities of fractions of *Bombax buonopozense*

Samples	α-Glucosidase Inhibitory Activity IC_{50} (μg/mL)
Ethylcetate fraction	18.44±2.63*
Dichloromethane fraction	11000.33±354.84
n hexane fraction	15568.67±865.92
Aqueous fraction	12696±118.29
Acarbose	16390±1318

Data are represented as Mean±SD. *p<0.05 significantly different from Acarbose.

2022). The extraction yield determined for dichloromethane, ethyl acetate, hexane and water fractions from leaf parts of *B. buonopozense* indicated that the aqueous crude extract recorded a higher yield percentage for both plants. This could be due to the high polarity of the water, which can extract a greater variety of plant components compared to the other solvents. The phytochemical assessment of *B. buonopozense* revealed the presence of several phytochemicals including phenols, tannins, flavonoids, cardiac glycosides and steroids. The phytochemicals present could be responsible for the different biological activities of the plant. Phenols, flavonoids and tannins are the most common phytoconstituents of fruits, medicinal plants, and aromatic plants and they are available in both plants. Tannins are known to interact with proteins to create a protective layer on mucous membranes (Airaodion *et al.*, 2019). Flavonoids are identified to have properties that stabilize membranes, influence certain metabolic processes, and inhibit lipid peroxidation in various systems. The action mechanisms of flavonoids involve their scavenging or chelating processes. Phenols possess antioxidant properties, which protect cells by either preventing the creation of free radicals or neutralizing the free radicals already produced in the body. Phenolic compounds belong to a category of antioxidant substances that function as terminators of free radicals. Flavonoids derived from plants have attracted a wide range of attention due to their various biological activities, including their anti-oxidant, anti-inflammatory, anti-tumor, anti-diabetic, anti-obesity, anti-hypertensive, and anti-viral actions (Niisato & Marunaka, 2023).

Reducing the activity of α-glucosidase is a crucial strategy for controlling blood sugar levels after meals (Wang *et al.*, 2022). The plant realm, renowned for its abundant phenolic and flavonoid compounds, has demonstrated significant antioxidant effects, providing protection against various oxidative stress-related ailments, including diabetes mellitus (Akter *et al.*, 2024).

The result of the qualitative phytochemical assessment of *B. buonopozense* leaf extracts is shown in Table 1. The results of *B. buonopozense* showed that crude extract gave a positive reaction to phenols, flavonoids, Saponins, cardiac glycoside, steroids and tannins while hexane and dichloromethane fraction gave a negative reaction to the phenols and saponins. Ethylacetate fraction gave a positive result for flavonoids.The enzyme inhibitory activities of the extracts of *B. buonopozense* were evaluated on α-amylase and α-glucosidase. The crude leaf extract of *B. buonopozense* exhibited a more effective inhibition on α- glucosidase with IC_{50} values 888.20±35.06 μg/mL when compared to control (ascorbic acid) with values 1076±2.77 μg/mL. A fractions of ethyl acetate showed lower inhibitory properties of alpha glucosidase with IC_{50} value 18.44±35.06 μg/mL compared to the control (ascorbic acid with IC_{50} value 16390±1318 μg/mL Diabetes is defined by elevated levels of glucose in the blood, leading to potentially severe consequences, including organ damage or failure, particularly affecting kidneys and eyes, as well as numerous cardiovascular diseases. Consequently, treatment strategies predominantly aim to minimize variations in blood sugar levels and associated complications. One such therapeutic strategy involves curbing post-meal high blood sugar levels by slowing down glucose absorption through the suppression of carbohydrate-breaking enzymes like α-amylase and α-glucosidase (Rocha *et al.*, 2019). α-Glucosidase are enzymes that facilitate the absorption of processed glucose from dietary polysaccharides within the small intestine. Using *B. buonopozense* to inhibit α-glucosidase and α-amylase represents an effective tactic in diabetes management by reducing glucose absorption. This

inhibitory action of ethylacetate fraction of *B. buonopozense* on α-glucosidase may be due to their capacity to bind proteins. The polyphenolic components of *B. buonopozense* might disrupt the functioning of digestive enzymes at the brush border of the small intestine. This can slow down the release of d-glucose from oligosaccharides and disaccharides, causing a delay in glucose absorption and thereby lowering post-meal glucose levels. Multiple studies suggest that phenolic compounds extracted from medicinal plants hold promise for the treatment of diabetes mellitus. A new therapeutic strategy for managing diabetes mellitus involves inactivating enzymes that hydrolyze carbohydrates, such as α-amylase and α-glucosidase, to prevent glucose from being absorbed from the gastrointestinal tract and, as a result, lessen postprandial hyperglycemia and its complications.Because they impede the breakdown of carbohydrates, α-amylase and α-glucosidase inhibitors lower postprandial plasma glucose levels, which helps manage hyperglycemia (Abomughaid *et al.*, 2024). Additionally, it has been shown that flavonoids, tannins, and saponins can help manage diabetes conditions.

CONCLUSION

The current investigation focuses on biological and phytochemical examinations of *B. bouneponzense* plants grown in Africa. The herb's hypoglycemic qualities are well-known in traditional medicine across the globe. The current study shows that the plant acts as a natural inhibitor of these enzymes and supports the herbalist's claim that it has antidiabetic qualities.

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