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RESEARCH ARTICLE

# Potential antioxidant and hypoglycaemic effect of the flower extract of *Bougainvillea spectabilis* Willd. (Nyctaginaceae)

Tata Yohanna Fave[1*], Hafsat Ali Sa'ab[2], Abdulkadir Bukar Bababe[3], Muhammad Mustapha Hassan[1], Wazis Chama Haruna[2] and Cletus Anes Ukwubile[3]

**Abstract**

The study evaluated the antioxidant and hypoglycaemic potentials of the ethanol flower extract of *Bougainvillea spectabilis*. Flower extract of *B. spectabilis* were obtained by cold maceration method. The preliminary phytochemical screening and hypoglycaemic effect were carried out using standard methods. Antioxidant screening was carried out using DPPH antioxidant assay test. Five rats were used for each group; normal control, standard control, 100, 200 and 400 mg/kg b.w. doses of the crude ethanol flower extract of B. spectabilis to test hypoglycaemic effect in alloxan induced diabetic rats. Blood glucose levels were measured at different time intervals. The preliminary phytochemical Screening revealed the presence of alkaloids, saponins, strerols, terpenoids, flavonoids and carbohydrates. The crude ethanol fraction demonstrated least antioxidant activity while the chloroform fraction showed the highest antioxidant activity followed by hexane fraction. The ethanol flower extract showed a significant dose dependent hypoglyceamic effect after 24 hours of administration. Of the doses tested, highest hypoglycaemic effect was observed by the ethanol flower extract of the dose 400 mg/kg at 24 hours. The findings revealed that non-polar fractions have more antioxidant activity than the polar fractions while the ethanol flower extract of Bougainvillea spectabilis possess a delayed hypoglycaemic effect.

**Keywords:** Alloxan; antioxidant; Bougainvillea spectabilis; DPPH; hyperglycaemia

## Introduction

Diabetes mellitus is a chronic metabolic disease characterised by hyperglycaemia which occur due to inherited and/or acquired deficiency in insulin production or utilization by the beta cells of the pancreas [1]. Hyperglycaemia often result to the classic symptoms such as excessive; urination (polyuria), thirst (polydipsia), eating (polyphagia) as well as blurred vision, weight loss, neuropathy and retinopathy. The consequences of uncontrolled diabetes often ensue to diabetic ketoacidosis, lactic acidosis, hyper-osmolar non-ketotic with subsequent progressive metabolic complications and organ damage particularly in the blood vessels and nerves [2] [3]. Decrease of antioxidant activity in diabetes is usually accompanied by increased production of free radicals or oxidative stress due hyperglycaemia [4] [5]. Hyperhylcaemia produces super-oxide anions and hydroxyl radical which results in protein glycation and peroxidation of membrane lipids which adversely damages the biomolecules. Excessive production of oxidants (Reactive oxygen or nitrogen species) in the body escalate the harmful effect of the free radicals which are often associated with the pathogenesis of many chronic diseases such as diabetes [6]. Antioxidants prevent the free radical mediated damages by scavenging them and hereby protecting from oxidative stress [4]. Many medicinal plants possess strong an-

*Correspondence:

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria

Full list of author information is available at the end of the article.

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tioxidant and free radical scavenging abilities that are essential in the prevention and treatment of some of the chronic diseases (cardiovascular diseases, diabetes mellitus, obesity and neurodegenerative diseases) caused by oxidative stress [7] .

According to the reports of International Diabetes Federation (IDF) and the World Health Organization (WHO), the estimated global prevalence of diabetes for adult between the ages of 20 and 79 in year 2015 was 415 million with about 1.5 million annual deaths directly attributed to diabetes [8] [9] and by the year 2030, 438 million people are expected to have diabetes globally [10]. According to reports of WHO, people living with diabetes in Africa increased from 4 million to 25 million between 1980 and 2014. In Nigeria, there were about 1,702,900 cases of diabetes in 2015 with prevalence rate estimated at 4.7% [8] [11]. The increased prevalence of the disease could be associated with population growth, ageing, unhealthy diets, obesity and sedentary lifestyles [12] .

The mainstay approach to treatment of diabetes mellitus is pharmacological (oral hypoglycaemic drugs and insulin) and non-pharmacological (dietary modification and physical activity). The conventional oral hypoglycaemic agents and insulin are associated with some setbacks in many developing countries due to unavailability, inaccessibility, unaffordability and low quality of these drugs. Secondary failure rates and adverse effects like hypoglycaemia of insulin, haematological disorders and rise in hepatic enzyme level are other challenges with the use of these drugs [13]. Therefore, many have resort to the use of plant species identified with hypoglycaemic activity in the form of crude extracts, decoction, infusion or tincture to treat this disease which is becoming wide and common practice in many developing countries where their primary health care needs depend largely on traditional medicines [14] [15]. However, searching for new antidiabetic drugs from natural products still continues because they contain substances which demonstrate alternative and safe effects on diabetes mellitus [16], which can be used in management of diabetes alone or in combination with orthodox/conventional antidiabetic drugs.

*B. spectabilis* Wild used in this study is commonly known as bougainvillea, great bougainvillea (family: Nyctaginaceae Genus: bougainvillea). *B. spectabilis* was reported to possess various biological activities which include; hypoglycaemic, cholesterol lowering effect, antibacterial, nematocidal, antifeedant and insecticidal, antiviral and anti-inflammatory activities were reported of *B. spectabilis* [17].

# Materials and methods

## Drugs and chemicals

Alloxan monohydrate (Kem Light Laboratories PVT. LTD), ethanol (JHD), insulin (M.J. Biopharm Private Limited)

## Collection and identification of plant materials

The flowers of B. spectabilis were freshly collected in the morning in November 2018 from Faculty of Science, University of Maiduguri, Maiduguri, Borno State. The plant material was identified by a taxonomist, Prof. S.S. Sanusi in the Department of Biological Science University of Maiduguri Borno State and was deposited with an herbarium number UM/FPH/14a/001/001 for future studies and reference.

## Preparation and extraction of the plant material

The flowers of B. spectabilis collected were shed-dried at room temperature for seven days and powdered using wooden pestle and mortar. The weight of Powdered material used for extraction was 900 g. the obtained and stored in an air tight glass container at room temperature. The Powdered plant material was extracted with 95% ethanol (JHD) using cold maceration method of extraction. The powdered plant material (0.9 kg) was soaked in five (5) litres of 95% ethanol (JHD) in a bottle and kept for 72hours with occasional agitation. After 72 hours, the mixture was filtered through a glass funnel using Whatman's filter paper. The filtrate obtained was concentrated with a reduced pressure in a rotary evaporator and allowed to dry. The percentage yield for the extract was then calculated using the formula below:

$$\text{Percentage yield } (\%) = \frac{weight\ of\ extract}{weight\ of\ powdered\ plant\ material}\ x\ 100$$

The crude ethanol extract of *B. spectabilis* was stored in an air tight container for the study.

Stock solutions of the reference drug (Biosulin) and the extract were prepared in this study by dissolving a known quantity in specified volume of distilled water for administrations.

### *Partitioning*

Extraction using solvent partitioning involves primarily the use of two immiscible solvents in a separating funnel. Twenty grams (30g) the ethanol extract was dissolved 300 mLof distilled water. It was then transferred to the separation funnel; the resulting solution is extracted with an equal volume of n-hexane usually three times, to give a fraction containing nonpolar compounds. The solution (suspension) was further partitioned with chloroform, and n-butanol successively to give fractions containing mid-polar and polar compounds respectively. The fractions from this process were then concentrated using a rotary evaporator and allowed to try.

***Phytochemical Screening***

The ethanol flower extract of Bougainvillea spectabilis was used for phytochemical screening using the standard methods of [18] [19].

### Experimental animals

Healthy albino rats of both sexes weighing (150-280g) were used in this study. The rats were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Borno State. The animals were housed in clean cages embedded with saw dust and were allowed to get acclimatized to the laboratory environment for seven (7) days under controlled environmental condition of room temperature, and had free access to food and water ad libitum before they were used for the study.

***Acute Toxicity***

The acute toxicity ($LD_{50}$) of the ethanol flower extract of B. spectabilis was determined using modified Lorke's method [20], which was adopted in this study to evaluate the acute toxicity in Wistar strain albino rats under standard conditions as the animals were allowed free access to food and water. The animals (n=2 per dose) were fasted for four (4) hours prior to the experiment oral administration of the extract. In phase I, Animals were orally administered the extract at the doses of 10, 100 and 1000 mg/Kg and observed for mortality and signs of toxicity within the first 24 hours. When death was not record in phase II after 24 hours, furthermore, another group of animals were given 1600, 2900 and 5000 mg/kg of the extract in the phase II.

***Evaluation of Antioxidant Activity***

Scavenging activity of di- phenyl-2-picrylhydrazyl (DPPH) radicals of the plant extracts were measured according to the method described by Krishna [21]. Assays was performed in 3 mL reaction mixtures containing 2.0 mL of 0.1 mM DPPH-methanol solution, 0.9 mL of 50 mM Tris-HCl buffer (pH 7.4), and 0.1 mL of deionized $H_2O$ (as control) or test plant extracts. After 30 minutes of incubation at room temperature, absorbance of the reaction mixtures at 517 nm was taken. The inhibitory effect of DPPH was calculated according to the formula below:

$$\text{Inhibition } (\%) = \frac{(Abs\ of\ control\ \breve{}\ Abs\ of\ sample)}{Abs\ of\ control}\ x\ 100$$

$IC_{50}$ represents the level where 50% of the radicals would be scavenged by test samples.

***Induction of experimental diabetes and Anti-diabetic effects of B. spectabilis***

Thirty rats weighing between (150 and 289 g) were grouped into six groups each containing five rats. The animals were fasted overnight and their baseline blood glucose level was measured prior to the induction of diabetes. Group I received normal saline which was used as normal control (NC) while groups II-VI received single dose of intraperitoneal injection of alloxan monohydrate (Kem Light Laboratories PVT. LTD) dissolved in distilled water at the dose of 150mg/kg to induce experimental diabetes in the animals. The blood glucose of the animals was measured at the interval of 24 hours. After 72 hours of alloxan administration, the glucose levels of the rats were greater than 200 mg/dl. Those with blood glucose level greater than 200mg/kg were considered diabetic and used for this study.

After induction of diabetes, the animals in each group were treated. Group I which served as the normal control group was orally administered 2 mL/kg of normal saline, while the diabetic rats in group II used as diabetic control received no treatment. Group III which served as the positive control, received 0.75 IU/kg of soluble insulin (M.J. Biopharm Private Limited) through intraperitoneal route. The diabetic rats in Groups IV, V and VI were treated with 100, 200 and 400 mg/kg of ethanol crude flower extract of Bougainvillea spectabilis respectively. The blood glucose of all the experimental animals were periodically measured after 1, 3, 6, 9, 24 and 48 hours using Accucheck active glucometer Roche Diabetes Care GmbH 68305 Mannheim, Germany. The code on the glucometer was set to correspond with that on the glucometer strips. This is to avoid errors and to ensure accuracy of the results. The tail of each rat was pricked with lancet and a drop of blood was collected on the strip then inserted into the glucometer and readings on the screen of the glucometer were recorded in mg/dl.

### Statistical Analysis

Data obtained from this study were analysed using one-way Analysis of Variance (ANOVA) to determine the relationship between the variables means using Statistical Package for Social Sciences (SPSS) version 16 and the results were expressed as mean and standard error of the mean (Mean±SEM). The p-Value<0.05 is considered significant.

# Results

### Percentage yield and phytochemical constituents of ethanol crude flower extract of *B. Spectabilis*

The percentage yield of the ethanol crude flower extract of B. Spectabilis was 8.53% obtained from 900g of powdered material with a characteristics colour of dark brown and a smooth texture. The extract showed the presence of cardiac glycosides, saponins, anthraquinones, triterpenes, flavonoids, alkaloids, and carbohydrates, however tannin was not present (Tables 1 and 2).

### Acute Toxicity Study

The oral acute toxicity study of the crude ethanol flower extract of B.spectabilis was determined using Lorke's method and there were no death of the animals recorded in both phases of the study at the different doses used. Thus, the $LD_{50}$ of the crude extract was found to be greater than 5000 mg/kg body weight (Table 3).

### Antioxidant Activities of the Various Fractions of the Flower Extract *B. Spectabilis*

The fractions of the flower extract of *B. Spectabilis* exhibited antioxidant activity in a concentration dependant manner with the highest dose at 100 $\mu$g/mL and the least at 6.25 $\mu$g/mL. The chloroform fraction exhibited the most scavenging activity with an $IC_{50}$ of 43.8 $\mu$g/mL followed by the n-hexane fraction with an $IC_{50}$ of 43.8 $\mu$g/mL. The n-butanol fraction exhibited the least scavenging activity ($IC_{50}$ of 159.3 $\mu$g/mL). These scavenging activities are however not comparable to standard drug ascorbic acid ($IC_{50}$ of 41 $\mu$g/mL) (Table 4).

### Effect of ethanol crude flower extract of *B. Spectabilis* on blood glucose level in Alloxan i nduced diabetic rats

The result of this study showed that alloxan was able to induce hyperglycaemia after 72 hours of intraperitoneal administration in the rats besides the normal control (NC) group that received normal saline. A progressive increase in the blood glucose level of the extract treated groups was observed in all the doses at 1, 3 and 6 hours after the oral administration of the crude extract except the dose of 400 mg/kg at 6 hours compared to the normal control. The rise in blood glucose level of the extract treated rats was highly significant after treatment compared to the insulin group.

A remarkable and significant reduction in the blood glucose levels were observed at all doses of the ethanol crude extract after 24 hours of oral administration. The decrease in the blood glucose level was not significant ($p>0.05$) in comparison with standard insulin (positive control). The reduction in the blood glucose levels of the extract treated rats at 24 hours of administration were dose dependent and significantly lower as compared to the diabetic control ($p<0.05$). The moderate dose of the ethanol crude extract sustained reduction in the blood glucose level up to 48 hours of administration. A progressive increase in the blood glucose level was observed in the diabetic control group (DC). The standard drug (insulin) demonstrated a highly significant hypoglyceamic effect at 1, 3 and 6 hours of administration in the alloxan induced diabetic rats compared to the crude extract treated groups ($p<0.05$). However, there was no statistically significant difference in the hypoglyceamic effect of insulin and that of the ethanol crude flower extract of Bougeinvillea spectabilis at 24 hours of administration as insulin started to lose its effect gradually with time (table 5).

**Table 1 Percentage yield and physical characteristics of the extract**

| Extraction parameters | Results |
|---|---|
| Colour | Dark Brown |
| Texture | Smooth |
| Taste | Acrid |
| Weight of dried plant (g) | 900 |
| Weight of plant extract (g) | 76.8 |
| Percentage yield (%) | 8.53 |

**Table 2 Qualitative phytochemical constituents of ethanol flower extract of *B. spectabilis*.**

| Phytochemical Constituents | Observation |
|---|---|
| Alkaloids | + |
| Anthraquinones | + |
| Carbohydrates | + |
| Cardiac glycosides | + |
| Flavonoids | + |
| Saponins | + |
| Terpenoids | + |
| Tannins | - |

+ = detected, - = not detected

**Table 3 Oral acute toxicity of ethanol flower extract of *B. spectabilis.***

| Experimental phases | Dose (mg/kg) | Observation of death within 24 hours |
|---|---|---|
| Phase I | 10 | 0/2 |
| | 100 | 0/2 |
| | 1000 | 0/2 |
| Phase II | 1600 | 0/2 |
| | 2900 | 0/2 |
| | 5000 | 0/2 |

$LD_{50}$>5000 mg/kg b.w

Value were expressed in Mean±SEM, n=5, Key: A= Blood glucose level after 24 hours of fasting, B= Blood glucose 72 hours after alloxan administration, CT = Control, DC= diabetic control, LD= low dose, MD= moderate dose, HD = high dose, BS=Bougainvillea spectabilis, One way ANOVA, *= $p<0.05$ (significant compared with DC), $\beta$ = $p<0.05$ (significant compared with insulin

## Discussion

The findings from this study revealed the presence of some phytochemicals that are associated with antioxidant and antidiabetic effects. Studies have shown that polyphenolic compounds such

**Table 4 Scavenging activity of the various fraction of ethanol flower extract of *B. Spectabilis***

| Samples | DPPH-radical scavenging Activity (%) 6.25 $\mu$g/mL | 12.5 $\mu$g/mL | 25 $\mu$g/mL | 50 $\mu$g/mL | 100 $\mu$g/mL | $IC_{50}$ ($\mu$g/mL) |
|---|---|---|---|---|---|---|
| Hexane fraction | 0.0 | 0.0 | 0.0 | 22.6 | 98.3 | 60.8 |
| Chloroform fraction | 0.0 | 0.0 | 66.5 | 90.1 | 90.5 | 43.8 |
| n-Butanol fraction | 0.0 | 0.0 | 0.0 | 0.0 | 41.8 | 159.3 |
| Crude ethanol fraction | 0.0 | 0.0 | 0.0 | 0.0 | 20.2 | 329.8 |
| Ascorbic Acid | 57.5 | 82.6 | 83.8 | 86.2 | 84.5 | 41.0 |

**Table 5 Hypoglycaemic Effect of the ethanol flower extract of *B. spectabilis* on glucose levels in alloxan induced diabetic rats**

| Groups | Doses | A (mg/dl) | B (mg/dl) | Glucose level after treatment Mean±SEM 1 hr | 3 hr | 6 hr | 24 hr | 48 hr |
|---|---|---|---|---|---|---|---|---|
| NC | 2 mL/kg | 109.2±3.8 | 112.6±2.1 | 112.8±2.1 | 108.2±3.2 | 111.0±2.8 | 107.0±2.9 | 111.4±2.1 |
| DC | 2 mL/kg | 108.6±5.6 | 485.4±22.6 | 520.0±20.5 | 538.4±14.6 | 531.0±13.9 | 557.2±12.8 | 580.0±6.1 |
| Insulin | 0.75 IU/kg) | 111.6±3.0 | 395.8±74.6 | 165.4±71.5 | 116.0±40.0 | 179.6±32.3 | 262.8±22.2 | 384.6±35.2 |
| BSLD | 100m g/kg | 121.2±8.2 | 382.6±51.2 | 469.8±78.8$^{\beta}$ | 586.6±11.5$^{\beta}$ | 420.8±32.9$^{\beta}$ | 293.4±44.6* | 519.8±31.9$^{\beta}$ |
| BSMD | 200m g/kg | 117.6±8.8 | 408.2±59.0 | 462.0±63.1 | 544.2±48.0$^{\beta}$ | 498.6±16.9$^{\beta}$ | 204.6±20.4* | 295.2±48.2$^{\beta}$ |
| BSHD | 400 mg/kg | 99.4±22.2 | 407.8±57.1 | 502.2±43.8 | 457.8±23.3 | 369.0±34.2 | 202.0±16.5* | 425.6±46.0 |

flavonoid, tannins are associated with antioxidant and antidiabetic activities in mice and rats [5] [22]. The preliminary phytochemical screening of ethanol flower extract of Bougainvillea spectabilis shown the presence of alkaloids, anthraquinones, flavonoids, saponin, cardiac glycosides, terpenoids and carbohydrates while tannins was found to be absent. Similarly, the study of Zahidul [23] reported the presence of alkaloid, reducing sugars, flavonoid, saponin, phenolic compounds, tannins, in the methanol flower extract of B. spectabilis but differ with the absence of tannins in this study. Furthermore, the report of Ghogar [24] indicated presence of alkaloids, flavonoids, quinones, saponins, steroids, tannins and terpenoids from the stem, flower and leaf extracts of B. spectabilis. Inconsistency in presence of phytochemical of extract may result from difference in geographical area of the plant, solvent used in extraction, period and time of collection [25]. These phytochemical constituents may be responsible for the glucose lowering effect of the extract [26] [23].

Many phytochemicals exhibit more than one biological activity such as antioxidant, antidiabetic, anticancer effects with many other health benefits. The DPPH scavenging activity is based on the ability of sample to donate hydrogen atom or transfer electron to DPPH, thus neutralize the free radical character and then gives rise to the reduced form of DPPH (non-radical) with the loss of violet colour. In this study, the four fractions of the extract (hexane, chloroform, butanol and crude), chloroform exhibited the highest antioxidant activity with $IC_{50}$ 43.8 as compared with $IC_{50}$ of standard ascorbic acid (41.0 mg/mL). similarly, the result of Omar [27] shown that the chloroform fraction exhibited a high antioxidative and DPPH-radical inhibitory activity. The hexane, butanol and the crude fractions had $IC_{50}$ of 60.8, 159.3 and 329.8 mg/mL respectively. This is contrary to the study of Dhankar [28] which reported that the aqueous extract has potential scavenging activity followed by the chloroform. The DPPH radical scavenging activity increases in a dose concentration-dependent manner from the concentrations 6.25 - 100 mg/mL.

The antioxidant and antidiabetic activities of the polyphenolic constituents demonstrated could occur by blocking proinflammatory cytokines or endotoxin-mediated kinases and transcription factors, inhibition of $\alpha$-glucosidase, lipase or the formation of nitric oxide protecting pancreatic $\beta$-cells against cytokine-induced toxicity [29] [30] [31].

The oral acute toxicity (LD50) of the ethanol flower extract of Bougainvillea spectabilis was found to be greater than 5000 mg/kg body weight which means that is relatively non-toxic on acute exposure. This study is similar with the work of [17] which

reported that stepwise doses of the ethanol stem bark extract was administered from 300 mg/kg to 5000 mg/kg orally and no considerable signs of toxicity were observed.

The study also revealed that the ethanol flower extract of Bougainvillea spectabilis showed a significant hypoglycaemic activity at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg after 24 hours of administration (Table 5). Compared with insulin (standard drug), there is a significant decrease in blood glucose level than the extract at 1, 3 and 6 hours of administration. The antidiabetic effect of ethanol flower extract is dose-dependent considering the order of significant decrease in blood glucose level. This is similar to the study of kumar [32] on the methanol stem bark extract of B. spectabilis which reported that the hypoglycemic effect is dose- dependent with 500 mg/kg exhibited the highest activity. This study is in contradiction [17] which reported that the 100 mg/kg body weight exhibited the highest activity.

## Conclusion

Diabetes mellitus as a chronic disease remain a global health challenge. This study revealed that the ethanol flower extract of Bougainvillea spectabilis contained many phytochemical constituents such as flavonoids, cardiac glycosides, tannins and terpenoids known to exhibit antioxidant and hypoglycaemic effects. Chloroform fraction of the flower extract has significant antioxidant activity with an $IC_{50}$ of 43.8 %. The hypoglycaemic activity was dose dependent as it exhibited significant activity at the highest dose. Furthermore, the extract is fairly non-toxic on acute exposure with the lethal dose greater than 5000 mg/kg body weight. Bougainvillea spectabilis possess huge potentials which can be explored further through identification and isolation of the bioactive components as well as their mechanism of actions

### Conflict of Interest

We have none to declare.

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### Author details

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria. [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria. [3]Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, FY, Maiduguri, Nigeria Tata.

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