PHYTOCHEMICAL COMPOSITION AND BIOCHEMICAL STUDIES OF THE AQUEOUS LEAF EXTRACTS OF *Brillantaisia guianensis P.beauv* ON ALLOXAN TREATED WISTAR ALBINO RATS.

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This work was carried out in collaboration among all authors. 'All the authors' designed the study, Author A performed the statistical analysis, wrote the protocol, managed the literature searches. and wrote the first draft of the manuscript. 'Authors B & C' managed the analyses of the study and edited the final draft. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out on the phytochemical composition and biochemical studies of the leaf extract of *Brillantaisia guinensis peuv* on alloxan treated Wistar albino rats. The experimental rats were administered with 80 mg/kg body weight of alloxan, via the tail vein. After five days treatment with alloxan, the treatment with the extracts commenced. Extracts were administered orally at 100, 200 and 300 mg/kg bw (both to normal and treated rats) for twenty-one days. Metformin, which served as a standard drug was administered at 50 mg/kg. Chromatographic analysis of the phytochemical content of the leaf extract, revealed the presence of flavonoids (30.7mg/100g), saponins (50.6mg/100g), phytosterol (6.22mg/100g), tannins (7.50mg/100g) and glycosides(29.3mg/100g). Compared to test and normal control, the extracts dose-dependently and significantly lowered (P<0.05) plasma glucose and triglycerides, during the experimental period. This study revealed the presence of pharmacologically bioactive compounds in the leaf extract and showed that the leaf extract had a dose-dependent hypoglycemic and hypotriglyceridemic effect on the Wistar albino rats. The findings suggest a likely protective role of the extracts against hyperglycemia and hypertriglyceridemia thereby useful in the treatment and management of diabetes mellitus, obesity and other related cardiovascular diseases.

Key words: Brillantaisia guianensis P. beauv; hypoglycaemic; hypotriglyceridemia; phytochemicals, alloxan-induced diabetes mellitus

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1. INTRODUCTION

Diabetes mellitus is one of the commonest metabolic disorders characterized by a chronic hyperglycemic condition arising from defects in insulin secretion, insulin action or both. The American Diabetes Association [1], noted that a person is confirmed diabetic if (i) the fasting blood sugar (FBS) is greater than 6.1mmol/L or 110mg/dL (ii) and random blood sugar is greater than 7.8mmol/L or 140mg/dL. Type 1 diabetes also know n as Insulin dependent diabetes mellitus is the result of an autoimmune reaction to proteins of the islets cells of the pancreas. The pathogenesis of selective β-cell destruction within the islet in type 1 diabetes mellitus is difficult to follow due to marked heterogeneity of the pancreatic lesions. [2]. Type-2 or non-insulin dependent diabetes mellitus is a long term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. [3]. Common symptoms include unexplainable weight loss, feeling tired, sores that do not heal, polydipsia (increased thirst), frequent urination and increased hunger, that may come in slowly[4]. Other types of diabetes include Type 3c diabetes (also known as Pancreatogenic diabetes) a form of diabetes that is currently being researched on.

World Health Organization (WHO) report, estimated that 1.7 million people in Nigeria had diabetes, with the projection that the number will be tripled by 2030 [5]. Several researchers had reported the prevalence of diabetic in Nigeria. For instance, Nyenwe *et al* reported the prevalence rates of Types 2 diabetes mellitus in Nigeria to be to be 2.2% and 6.8% in 1997 and 2003 respectively [6]. A research carried out by Chijioke *et al*., [7] showed a prevalence rate of 5.12% while the one carried out in a tertiary hospital in Nigeria by Ogbera *et al*.,[8] revealed a prevalence of 15%. Another study in the city of Port Harcourt, River State of Nigeria by Nwafor and Owhoji [9] showed a prevalence of 16% among the low socio-economic group and 23.45% among the high socio-economic group.

Currently, there is an increased interest in the use of herbal products most especially in the developing countries of the world. This may be attributed to the down turn in the economy, as herbal medicine is perceived to be a cheaper means of treatment [10,11].Herbal products over the years had shown to improve glucose metabolism and maintain overall condition of individuals with diabetes, not only by exerting a hypoglycemic effects but also by improving lipid metabolism, antioxidant status, and capillary functions [12]. Despite the various precautionary measures taken in the control and treatment of diabetes mellitus, its management still remains insufficient as already existing remedies are known for numerous side effects and high cost implication as the case may be. Thus the need for therapies with low cost and lesser side effects.

Brinllantaisia guianensis p. Beauv is a perenial herbaceous shrub. Its common name is Giant Tropical Blue African Salvia, (the Acanantaeceae). The genus *Brinllantaissia,* has more than 20 species that are distributed across tropical Africa. *B guianensis* is a perennial shrub which grows up 1.5m high and found in Central and West Africa as well as East Camerouns.

B.guainensis is widely used in African traditional medicine to treat skin infections and toot-ache, [13],[14], It has been shown to possess antinoceptive effects [15] or are traditionally used for their antihypertensive activity. [14]. In Cameroon, a decoction of *B.guainensis* is used by traditional healers of Central Province for the management of cardiovascular diseases especially hypertension.

Several researches have validated the medicinal value of *B.guianensis.* The methanol leaf extract had been reported to lower arterial blood pressure of normotensive Wistar rats[16].The relaxant effects *B. guianensis* on rats vascular smooth muscle had also been reported, [17]. It is also known to have shown haematinic activity [14], In south Eastern Nigeria with high prevalence of both malaria-induced and iron-deficiency anaemia, aqueous decoctions of the leaves of *B. guianensis are* very popular among village women in combating malaria-induced anaemia in children. [18]. The root taken as soup in southern Nigeria is used to reduce pain during pregnancy. [19]. Recently, Kalu et al., had reported that the leaves of B. *guianensis* are rich in essential nutrient that can adequately meet the required daily allowance (20). In Ohafia in Abia state of Nigeria , the leaves extracts are used in the treatment and management of diabetes mellitus. This research was aimed at investigating the phytochemical compostion, hypoglycemic and hypotriglyceridemic potential of the aqueous extracts of the leaves of *B.guianensis.*

2. MATERIALS AND METHODS

2.1 Chemical Used

All reagents and drugs were obtained commercially and were of analytical grade.

2.2 Collection and Preparation of Plant Material

The leaves of *B. guianensis* used in this study were harvested fresh from Ude Plantation in Okon Aku, Ohafia Local Government Area of Abia State and were identified and given a voucher number of (EH-P-052) by a Taxonomist (Dr Edwin Wosu) in the Herbarium Unit of the Department of Plant Science and Biotechnology, University of Port Harcourt.

The samples were rid of dirt and the leaves removed, oven dried at 60^0C for 24 hours and then ground into powder. The resultant powder was soaked in hot, boiled distilled water for 12 hours, after which the resultant mixture was filtered and the filtrate, hereinafter referred to as the aqueous extract was stored in a refrigerator for subsequent use. Ten milliliters of the extract was evaporated to dryness and the weight of the residue used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract. The resultant residue was used for phytochemical analysis.

Percentage recovery of the crude aqueous extract $= \frac{\text{weight of extract (g)}}{\text{Weight of dry sample (g)}} x\ 100$

2.3 Analysis of the Phytochemical Profile

2.3.1 Calibration, identification and quantification

Standard solutions were prepared in methanol for flavonoids and tannins, chloride and ethanol for, glycosides and saponines. The linearity of the dependence of response on concentrations of the extract was ascertained by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards.

2.3.2 Determination of flavonoids

The extraction was carried out according to the method of Millogo-Kone (21)

One gram of the sample was weighed into the 250ml conical flask and 100ml of distilled water was added and boiled for 10 minutes. The flavoniods extract was obtained by pouring 100ml of boiling methanol:water (70:30) volume:volume into the conical flask. The mixture was allowed to macerate for about 6 hours and then concentrated to 5ml for gas chromatography analysis.

The gas chromatograph was an HP 6890 (Hewlett Packard, wellington, DE,USA), GC apparatus, fitted with flame ionization detector (FID), powered with HP Chemstation Rev.A 09.01(1206) software, to identify and quantify compounds. The column was a capillary DB-5MS (30mx0.25mmx0.25µm thickness). The inlet and detection temperatures were 250◦C and 320^{o}C. Split injection was adopted with a split ratio of 20:1. The Carrier gas was nitrogen gas .The compressed air and hydrogen pressures were 38 psi and 28psi. The oven programmed was; initial temperature at 60◦C for 5 mins. First ramping at 10◦C/min for 20 min was followed by a second ramping at 15◦C /min for 4 min.

2.3.3 Determination of tannin

The extraction was carried out by following the modified method of Luthar [22] The tannin fraction of the crude aqueous extract was extracted with methanol and subjected to gas chromatographic analysis. Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01(1206) software, to quantify and identify compounds. The column was HP 5 Column (30mm × 0.25 mm × 0.25µm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 40 psi. The oven was programmed as follows: initial temperature at 120 °C, followed by ramping at 10 °C/min for 20 min

2.3.4. Determinations of saponin

The extraction was carried out by following the modified method of analytical sciences according to the described by Guo [23]

The sample was pulverized and the saponin was extracted three times with redistilled methanol. The saponins were removed with 20ml of the solvent for 20 minutes with the aid of sonication. The combined extracts were concentrated to a syrup under reduced pressure, and then suspended in water. The suspension was extracted with petroleum ether, chloroform and 1- butanol saturated with water, successively, to give the respective extract after the removal of the solvent. The combined extract was filtered and concentrated to 1 ml in the vial for gas chromatography analysis. Analysis was performed using HP 6890 gas chromatograph (Hewlett Packard, wellington, DE,USA) fitted with flame ionization detector (FID), powered with HP Chemstation Rev.A 09.01(1206) software, to identify and quantify compounds. The column was a capillary Hp 5(30mx0.25mmx0.25µm film thickness). The inlet and detection temperatures were 250◦C and 320^{o}C, respectively. Split injection was adopted with a split ratio of 20:1. The Carrier gas or mobile phase was

nitrogen gas. The compressed air pressure and hydrogen pressure were 28 psi, and 40 psi. Oven Program was as follows; Initial temperature was at 60^0C for 5 minutes and first ramping at 12^0C/min for 18min and second ramping at $15\ ^0$C/min for 5mins.

2.3.4 Determination of phytosterols

The oil in the extract was extracted according to AOAC Method 999.02 [24], while the sterols were analyzed according to the modified AOAC Method 994.10 and AOAC 970.51 Official methods. This involved extraction of the lipid fraction from homogenized sample material, followed by alkaline hydrolysis (saponification), extraction of the non-saponifiables, clean-up of the extract, derivatisation of the sterols, and separation and quantification of the sterol derivatives by gas chromatography (GC) using a capillary column. Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 (1206) software, to quantify and identify compounds. The column was HP INNOWax Column (30 m × 0.25 mm × 0.25 µm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. Nitrogen was used as the carrier gas. The hydrogen and compressed air pressures were 22 psi and 35 psi. The oven was programmed as follows: initial temperature at 60 °C, first ramping at 10 °C/min for 20 min, maintained for 4 min, followed by a second ramping at 15 °C/min for 4 min, maintained for 10 min.

2.4 Experimental Design for the Experimental Diabetes Mellitus

Forty-five Wistal albino rats weighing 150-200g were sorted into nine groups of five animals each, (Table 1), so that their average weights were approximately equal. The animals were housed in plastic cages, in the Animal House of the Department of Biochemistry, University of Port Harcourt. After one week acclimatization period on guinea growers mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria), the animals were fasted overnight, and their baseline fasting blood glucose level (FBS) and triglyceride concentration were determined using multiCarein^{TM} glucose and triglyceride strips and glucometer. Blood was obtained via tail cut. The treatment and treatment control (TC) were given, three different dose concentrations of the extract; 100mg/kg, 200mg/kg and 300mg/kg body weight. Diabetes was induced by injection of a freshly prepared solution of alloxan (80 mg/kg body weight) in normal saline, while the control rats were injected with normal saline alone. The dosage of administration of alloxan was adopted from Radwan [25]. Three days after administration of alloxan, the animals were again fasted and blood collected via tail cutting [26], [27], for the determination of their fasting glucose and triglyceride levels. After three days, It was found that the rats had moderate diabetes, having hyperglycemia. Then the rats were kept for 3 days to stabilize the diabetic condition [28] before commencing the treatment, which lasted for three weeks. The Diabetmin^{TM} (metformin HCl) and extracts were administered daily by intra-gastric gavages. The fasting glucose levels and triglyceride concentrations were taken on days , 12, 19 and 26. The animals were allowed normal feed and water ad libitum.

At the end of the treatment period, the rats were fasted overnight and anesthetized by exposure to chloroform. While under anaesthesia, they were painlessly sacrificed and whole

blood was immediately used to determine glucose and triglyceride concentration using multiCarein™ glucose and triglyceride strips and glucometer.

Table 1. Experimental design for the diabetes screening

S/N	ID	Treatment
1.	Normal	Normal saline and water
2.	Test control	Alloxan (80mg/kg) and water
3.	Reference Treatment.	(Alloxan (80mg/kg) and Metformin (50mg/kg body weight)
4.	*Brinllatasia guainensis* treatment control 1(BGC1)	Normal saline and *Brinllatasia* extracts(100mg/kg)
5.	*Brinllatasia guainensis* treatment control 2 (BGC2)	Normal saline and *Brinllatasia* (200mg/kg)
6.	*Brinllatasia guainensis* treatment control 3 (BGC3)	Normal saline and *B. guainensis* (300mg/kg)
7.	*Brinllatasia guainensis* treatment 1 (BGT1)	Alloxan(80mg/kgand *B. guainensis* (100mg/kg)
8.	*Brinllatasia guainensis* treatment 2.(BGT2)	Alloxan(80mg/kg and *B. guainensis* (200mg/kg)
9.	*Brinllatasia guainensis* treatment 3.(BGT3)	Alloxan (80mg/kg and B. *guainensis* (300mg/kg)

2.5 Statistical Analysis

Data from pharmacological screening were analyzed for statistical differences between treatment groups, by means of one way analysis of variance (ANOVA) and post hoc Benferoni on SPSS17. In all, $p<0.05$

RESULTS

Composition of flavonoid in *Brillantassia guianensis P. beav*

The composition of flavonoid in *Brillantassia guianensis P Beav.* composition is shown in

Table 2. The result shows that the leaves of *Brillantassia guianensis P Beav* had twenty-eight flavonoid prominent among which include kaemferol (59.9%), leuteolin (15.87%, narigenin (7.87%), myricertin(5.4%), quercertin (8.97%), catechin(2.90%),isoquercetine (0.10%) daidzein (0.4%) and others in insignificant figure

Composition of Saponins in *Brillantassia guianensis P. beav*

The composition of Saponins in the of the leaves of *Brillantassia guianensis P Beav* is shown in Table 3. The result indicates that ten saponin were detected , notable among which are sapogenin (60.20%), saponine (31.28%), neochlorogenine (7.86%), hispigenin (0.65%) and others in minute quantities.

Composition of glycosides in *Brillantassia guianensis P. Beav*

The composition of glycosides in *Brillantassia guianensis P Beav* leaves is shown in Table 4 with artemetin (48.45%), digitoxin (36.65%), digoxin (9.64%), amygdalin (5.45%) in prominent amount and others in insignificant quantities.

Composition of Tannins *Brillantassia guianensis P Beav.*

The *Brillantassia guinensis P.Beauv* leaves contained tannins consisting of 100% tannic acid.

Composition of Phytosterols in *Brillantassia guianensis P Beav*

Seven phytosterol compound were detected in the leaves *Brillantassia guianensis P Beav* as shown in Table 5 with the most prominent being sitosterol (67.87%), savenasterol (14.60% stigmasterol (10.10%), campesterol (7.41%) and others in insignificant amount

Table 2. Composition of flavonoid in *Brillantassia guianensis P. beav*

S/N	Compound	Amts (mg/100g)x10^{-6}	% Composition
1.	Catechin	887878	2.90
2.	Resveratrol	9.033821	$2.95.10^{-5}$
3.	Genistein	8.61355	$3.05x10^{-5}$

4.	Daidzein	9.36465	0.46
5.	Apegenin	141165	$3.38x10^{-5}$
6.	Daidzein	10.3521	$4.97x10^{-5}$
7.	Butein	15.2374	$9.35x10^{-4}$
8.	Biochanin	28.6793	7.23
9.	Naringenin	2218220	15.87
9.	Luteolin	4873810	59.09
10.	Kaemferol	18121190	59.09
11.	Epicatechin	2139.46	$6.98x10^{-5}$
12.	Epigallocatechin	3151.06	0.01
13.	Gallocatechin	44.0948	$1.44x10^{-4}$
14.	Quercetin	2750290	8.97
15.	Epicatechin-3-gallate	229.737	$7.49x10^{-4}$
16.	Epigalloqcatechin-3-gallate	207.410	$6.76x10^{-4}$
17.	Isorhamnetin	2.81181	$9.17x10^{-6}$
18.	Robinetin	11.5683	$3.77x10^{-5}$
19.	Myricetin	1658740	5. 41
20.	Baicalein	7.03776	$2.29x10^{-5}$
21.	Nobiletin	3.54165	$1.15x10^{-5}$
22.	Baicalin	2.04076	$6.65x10^{-6}$
23.	Isoquercetin	3.1587.4	0.10
24.	Tageretin	2.53060	$8.25x10^{-6}$
25.	Artemetin	1.62620	$5.30x10^{-6}$
26.	Silymarin	1.90256	$6.20x10^{-6}$
27.	Hesperidin	2.92945	$9.55x10^{-6}$
	Total	30668770	

Table 3. Composition of Saponins in *Brillantassia guianensis P. beav*

S/N	Compound	Amt.(mg/100g) ($x10^{-1}$)	% composition
1	Saponine	158.238	31.28
2	Hispigenin	329177j 36	0.65
3	Solagenin	20.7812	$4.09x10^{-3}$
4	Diosgenin	2.12617	$4.18x10^{-4}$
5	Tigogenin	6.7225	$1.37x10^{-3}$
6	Neochlorogenin	39768.6	7.86
7	Hecogenin	1.38573	$2.74x10^{-4}$
8	Sapogenin	304580.3	60.20
9	Tribuloin	4.18108	$8.26x10^{-4}$
10	Yanogenin	6.15374	$1.22x10^{-3}$
11	Sconyzorgin	0.55266	$1.09x10^{-4}$
	Total	505.9	

Table 4. Composition of glycosides in *Brillantassia guianensis P. Beav*

S/N	Compound	Amt. (mg/100g) ($x10^{-5}$)	% composition
1.	Arbutin	1.2348	$4.21x10^{-5}$
2.	Linamarin	0.00456	$1.53x10^{-7}$
3.	Salicin	21.162	$7.22x10^{-4}$
4.	Artemetin	1420485	48.45
5.	Amygdalin	159844	5.45
6.	Ouabain	181.618	$6.19x10^{-3}$
7.	Dhunin	7.1487	$2.44x10^{-4}$
8.	Prunasin	6.30645	$1.24x10^{-4}$
9.	Cucurbitin	3.63030	$1.24x10^{-4}$
10.	Digitoxin	1074012	36.63
11.	Digoxin	277367	9.64
12.	Lotaustralin	65.9406	$2.25x10^{-3}$
	Total	**2931995**	

Table 5. Composition of Phytosterols in *Brillantassia guianensis P. beav*

S/N	Compound	Amount (mg/100g)	% composition
1.	Cholesterol	0.000204	$3.28x10^{-3}$
2.	Cholestenal	0.0000042	$6.74x10^{-5}$
3.	Ergosterol	0.0183	0.03
4.	Campesterol	0.461	7.41
5.	Stigmasterol	0.628	10.10
6.	Savenasterol	0.909	14.60
7.	Sitosterol	4.23	67.86
	Total	**6.227**	

3.4 Pharmacological Profile

The effects of aqueous extracts of the leaves of *Brillantassia guianensis P Beav.*on the plasma glucose concentration of alloxan-induced diabetic rats is shown in Table 6. On day 0, the plasma glucose level of the test control was significantly lower ($p<0.05$) than that of the test groups. While BGT2 was significantly higher ($p<0.05$) than the normal, reference, and the treatment control (BGC1, and BGC2). On day 5 the treatment groups (BGT1, BGT2 and BGT3) were significantly higher than the normal and (BGC1), while the reference group was significantly higher ($p<0.05$) than test control and test groups on days 5 and 12. Also on day 12, the test control was significantly ($p<0.05$) higher than normal group and B GT1. On day 19 and 26, the test control group significantly ($p<0.05$) was higher than the test groups.

On day 26, the test control was ($p<0.05$) higher than the test groups. The normal did not differ significantly at 5% level.

Table 7 shows the weekly effects of aqueous extracts of the leaves of *Brillantassia guianensis P Beav.* on the plasma triglyceride concentration of alloxan-induced diabetic rats. On day 0, the plasma triglyceride concentration of the reference group was significantly lower ($p<0.05$) than the test control. On day 5, there was a significant ($p<0.05$) increase in treatment groups (BGT1, BGT2 and BGT3) and reference compared to test control and treatment control (BGC1, BGC2 and BGC3). Days 12, 19 and 26 records shows that the extracts significantly reduced ($p<0.05$) the triglyceride concentration in the test group compared to the test control group although the reduction was not dose dependent.

Table 6. Effects of aqueous extracts of the leaves of *Brillantassia guianensis P. beav* on the plasma glucose concentration of alloxan-induced diabetic rats

Treatment	**Plasma glucose**		**Concentration (mg/dL)**		
Group	**Day 0**	**Day 5**	**Day 12**	**Day 19**	**Day 26**
Normal	73.4±2.19^{b}	94.6±5.39^{c}	97.4±2.54^{b,c}	84.8±2.70^{c}	70.8±3.44^{b}
Test control	59.0±6.26^{c}	127.4±11.60^{b,c}	132.2±11.50^{a,b}	142.8±10.52^{a}	135.4±8.46^{a}
Reference	73.0±3.88^{b}	220.3±29.10^{a}	162.2±20.63^{a}	107.6±2.62^{b}	68.4±2.62^{b}
BGC1	82.6±1.16^{b}	100.4±3.03^{c}	89.4±3.01^{c}	80.2±3.37^{c}	75.2±1.36^{b}
BGC2	78.2±3.01^{b}	111.4±3.12^{b}	108.7±2.9^{b,c}	100.2±3.14^{b}	68.2±2.01^{b}
BGC3	80.4±2.85^{b}	105.9 ± 3.6^{b}	99.11± 2.45^{b,c}	90.2 ± 3.25^{b}	71.70±1.2^{b}
BGT1	77.6±2.81^{b}	144.4±12.5^{b}	120.8±6.12^{b,c}	111.2±2.95^{b}	73.0±3.65^{b}
BGT2	97.0±2.30^{a}	146.0±10.89^{b}	118.0±1.40^{b,c}	106.0±3.69^{b}	79.8±2.69^{b}
BGT3	86.3±2.4^{b}	145.3 ± 14. 7^{b}	115.4± 2.5^{b,c}	105.2± 2.23^{b}	76.4 ±3.20^{b}

Values are Mean± S.E.M.,n=5,per group; ^{abc} Values in the same column with different superscripts are significantly different at p<0.05

Table 7. Effects of aqueous extracts of the leaves of *Brillantassia guianensis P Beav.*on the plasma triglyceride Concentration

TR EATMENT	Plasma triglyceride	Concentration (mg/dL)			
GROUP	DAY 0	DAY	DAY 12	DAY 19	DAY 26
NORMAL	103.40±10.57[a]	106.30±5.9 [b]	105.80±5.0 [b]	105.00±4.09[b]	106.00±2.8 [b]
TEST CONTROL	115.80±8.42[a]	132.60±5.5[b]	134.40±5.1 [a,b]	142.60±8.04[a]	126.00±2.0 [a]
REFERENCE	75.40±3.58[b]	178.60±49.5[a]	153.00±33.6 [a]	112.20±13.61[b]	108.00±3.1[b]
BGC1	116.20±12.57 [a]	128.00±10.6 [b]	119.80±8.1[a,b]	108.40±6.38[a,b]	101.03±3.0[b,c]
BGC2	128.60±12.87 [a]	134.80±6.1[b]	124.60±5.0 [a,b]	117.00±4.55[a,b]	100.80±2.6[b,c]
BGC3	122.4± 11.64[a]	131.4 ± 8.3 [a]	122.2 ±6.5[a]	112.7± 3.23[a]	102.3±3.5 [c]
BGT1	112.40±5.35[a]	170.00±5.6 [a]	150.60±5.8[a]	121.20±2.83[b]	95.20±1.89[c]
BGT2	120.60±8.86[a]	199.60±8.6 [a]	160.20±10.1[a]	120.20± 3.39[b]	94.60±1.67 [c]
BGT3	116.5 ±6.43[a]	184.8 ±6.3 [a]	155.2±7.43[a]	118.7 ± 4.3[b]	92.90±1.3[c]

Value s are Mean± S.E.M ,n=5,per group.

[abc] Values in the same column with different superscripts are significantly different at p<0.05

Table 7 shows the time course of the effect of aqueous extract of the leaves of *Brillantassia guianensis P Beav* on the plasma glucose of alloxan-induced diabetic rats. On days, 12, 19 and 26, the percentage reduction in the plasma glucose levels of BGT2 and BGT3 were significantly ($p<0.05$) higher than that of the test control. The extracts 200mg/kg and 300mg/kg dose dependently lowered the plasma glucose concentration on these days.

Table 10 shows the time course of the effect of aqueous extract of the leaves of *Brillantassia guianensis P Beav.* on the plasma triglycerides concentration of alloxan-induced diabetic rats. There was a significant ($p<0.05$) higher percentage reduction in the plasma triglyceride concentration in the test groups than the test controls in days 12, 19, and 26.

Table 8. Time course of the effect of aqueous extract of the leaves of *Brillantassia guianensis P Beav.* on the plasma glucose of alloxan-induced diabetic rats

Treatment	Plasma triglyceride	Concentration (mg/dL)							
Group	Day 0 (mg/dl)	Day 5		Day 12		Day 19		Day 26	
		Value (mg/dL)	% Reduction		% Reduction		% Reduction		% Reduction
Normal	73.4 ±3.0^{b}	94.6±5.39^{c}	-28.71±2.1^{a}	97.4±2.5^{b,c}	-32.86±2.0^{a}	84.8±2.7^{c}	-15.6±1.0^{b}	70.8±3.4^{b}	3.5±4.5^{a}
Test control	59.0±6.23^{c}	127.4±11.6^{b,c}	-116.59±3^{b}	132.2±11^{a,b}	-120.9±3.0^{c}	142.8±10.5^{a}	-143.8±8.7^{e}	135.4±8.5^{a}	-129.49±48.5^{b}
Reference	73.0±3.88^{b}	220.3±29.1^{a}	-199.6±24.2^{c}	162.2±29.1^{a}	-128.0±52^{c}	107.6±2.6^{b}	-47.9±4.3^{d}	68.4±2.6^{b}	6.3±6.3^{a}
BGC1	82.6±1.16^{b}	100.4±3.03^{c}	-21.37±2.0^{a}	89.4± 3.0^{c}	-8.2±2.1^{a}	80.2±3.4^{c}	2.9±3.6^{a}	75.2±1.4^{b}	8.3±1.5^{a}
BGC2	78.2±3.0^{b}	111.4±3.12^{b,c}	-42.57±1.1^{a}	108.7±2.9^{b,c}	-38.8±0.3^{a}	100.3±3.1^{b}	-28.7±8.0^{b,c}	68.2±2.0^{b}	12.82±3.2^{a}
BGC3	80.2±2.6^{b}	105.9±3.50^{b,c}	-32.04±4.3^{a}	103.4±3.5^{b,c}	-28.92±0.2^{b}	95.6±5.3^{b}	-19.20±0.34^{b,c}	75.3±2.1^{b}	6.11±0.5^{a}
BGT1	77.6±2.8^{b}	144.4±12.46^{b}	-85.41±9.4^{b}	120.8±6.1^{b,c}	-55.2±2.3^{b}	111.2±3.0^{b}	-43.6±4.8^{c,d}	73.0±3.7^{b}	5.8±4.4^{a}
BGT2	97.70±2.3^{a}	146.1±18.87^{b}	-49.82±12.2^{a}	118.4±1.4^{b,c}	- 25.1±0.3^{a}	106.0±3.7^{b}	-13.6±1.6^{b}	79.8±2.7^{b}	17.73±1.3^{a}
BGT3	100.4±1.3^{a}	135.4±12.3^{b}	-34.86±6.1^{a}	123.6±5^{b,c}	-23.11±0.^{a}	97.8±2.4^{b}	2.59 ±3.1^{b}	68.5±5.1^{b}	31.78±0.4^{a}

*Values are Mean± S.E.M. ,n=5,per group; ^{abc} Values in the same column with different superscripts are significantly different at p<0.05; *P<0.05 compared to corresponding values on day 0; percentage reduction=Percentage reduction from the corresponding values on day 0*

Table 9 Time course of the effect of aqueous extract of the leaves of *Brillantassia guianensis P Beav.* on the plasma triglyceride of alloxan-induced diabetic rats.

TREATMENT GROUP	MAGNITUDE								
	DAY 0 (mg/dl)	DAY 5		DAY 12		DAY 19		DAY 26	
		Value (mg/dL)	*% Reduction		*% Reduction		*% Reduction		*%Reduction
Normal	103.40±10.57[a]	106.0±10.27[b]	-2.90±6.3[a]	105.8±8.61[b]	-2.50±1.2[a]	105±7.09[a]	-1.55±2.1[a,b]	106.±2.8[b]	-2.5[c]
Test control	115.80±8.42[a]	132.6±9.45[b]	-14.51±5.0[a]	134.4±8.8[a,b]	-16.1±0.5[a]	142.6±8.0[a]	-23.14±1.2[c]	126±2.0[a]	-8.8[c]
Reference	75.40±0.58[b]	178.60±85.8[a]	-132.6±59.4[b]	153.0±58.2[a]	-103.±39.3[a]	112.2±23.58[b]	-50.1±11.8[d]	108±3.1[b,c]	-43.24[d]
BGC 1	116.20±12.57[a]	128.0±18.39[b]	-9.87±2.3[a]	119.8±14[a,b]	-3.1±0.6[a]	108.4±11.0[b]	6.7±0.3[a]	101±3.0[b,c]	12.22[b]
BGC2	128.0±5.87[a]	134.8±10.52[b]	-4.98±1.1[a]	124.6±8.6[a,b]	3.1±1.7[a]	117.0±8.17[b]	8.34±1.8[a]	100.8±2.6[b,c]	21.62[a]
BGC3	117.43±3.3[a]	123.6±12.4[a]	-5.25**±2.1**[a]	117.5±4.7[a]	-7**±4.2**[a]	1O9.7±3.12[b]	6.58±2.4[b]	89.4±3.2[b,c]	23.87[a]
BGT1	112.40±5.35[a]	170±9.62[a]	-51.26±0.8[a]	150.6±9.9[a]	-34.0±1.1[a]	121.2±4.90[b]	-7.83±0.4[b]	95.20±1.9[c]	15.30[a]
BGT2	120.60±8.8[a]	199.6±14.9[a]	-0.66±6.9[a]	160.2±17.5[a]	-32.8±2.7[a]	120.8±5.78[b]	-0.16±1.2[b]	94.60±1.7[c]	19.2[a]
BGT3	116.5±6.43[a]	184.8±3.6[a]	-58.6**±4.1**[a,b]	155.2±7.32[a]	-33.22[a]	118.7±4.3[b]	-1.89 ± 2.2[b]	92.90±1.3[c]	20.26[a]

Values are Mean± S.E.M n=5,per group.

[abc] Values in the same column with different superscripts are significantly different at p<0.05.

*P<0.05 compared to corresponding values on day 0.

percentage reduction=Percentage reduction from the corresponding values on day 0.

4. DISCUSSION

The phytochemical investigation revealed that the aqueous leaf extract of *Brinllatasia guainensis* contained bioactive agents such as flavonoids, saponin, β-sistosterol (phytosterol) and tannins. These compounds have been scientifically proven and validated by several researchers to possess potent pharmacological activities especially as antimicrobial, anti-cholesterolemic, antioxidant, anti-diabetic, anti-hypertensive and hepatoprotective agents. (29, 30, 31, 32,33) These justify their use for medicinal purposes in the treatment and management of diseases such as diabetic mellitus, hypertension, obesity, liver diseases etc.

Alloxan-induced diabetes mellitus is often characterized by decreased insulin level, hyperglycemia, elevated triglycerides and total cholesterol, and decreased HDL-cholesterol (31). The high percentage reduction in plasma glucose and triglyceride levels, produced by the extracts in this study give credence to the use of the leaves in the management of diabetes mellitus.

The hypoglycemic effects of the leaf extracts may have been produced by the flavonoids, saponins, tannic acid and β-sistosterol, present in the leaves and their extracts. Saponins (31) tannins (36,37, 38 ,34) and flavonoids such as apigenin, quercetin, epicatechin, kaempferol, narigerin, genistein and myricetin [36,37,38,39, 40,41,42,43] are compound with established hypoglycemic activity. The extracts may have exerted their anti-hyperglyceridemic activity by enhancing glucose uptake in the cells, (by tannin) stimulating insulin secretion from pancreatic β cells (by flavonoids and/ or β-sistosterol) and increasing insulin activity, or by converting pro-insulin to insulin, or by inhibiting hepatic gluconeogenesis.

A high plasma triglyceride level had been reported to be an independent and synergistic risk factor for cardiovascular diseases (44, 45,46); and is often associated with hypertension (47,48), abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus (49,44,50). The extracts dose dependently and significantly reduced plasma triglyceride concentration. This effect may have been mediated by the flavonoids and tannic acid which were found to be abundantly present in the leaf extracts. This also give credence to the use of this plant in the management of cardiovascular diseases (14)

Conclusion

The study revealed the presence of bioactive agents in the aqueous leaf extracts of *Brillantassia guianensis P Beav* . It also showed that the leaf extract had a dose dependent hypoglycemic and hypotriglycemic effect on the Wistar albino rats, thus suggesting a likely protective role of the extract against hyperglycemia and hypertriglyceridemia, thereby useful in the treatment and management of diabetes mellitus, obesity and other related cardiovascular diseases. This research therefore supports the inclusion of this plant in traditional anti-diabetes preparations.

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