HYPOGLYCAEMIC STUDIES OF THE LEAF EXTRACTS OF *Brillantaisia guianensis P.beauv* ON BLOOD GLUCOSE LEVELS OF ALLOXAN-INDUCED DIABETIC WISTAR ALBINO RATS.

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Abstracts

Preliminary phytochemical screening of aqueous leaf extract of *Brillantaisia guianensis P.beav* revealed the presence of flavonoids, saponins, tannins and phytosterol. The hypoglycemic effects of aqueous leaf extract of *Brillantaisia guianensis P.beav was also* investigated in alloxan-induced diabetic wistar rats. Diabetes was induced by administration of freshly prepared alloxan at 80 mg/kg body weight, to the Diabetic Control (Group 1), Reference drug control (Group 3), and test Treatment groups (Groups 7-9) The treatment and treatment control (TC) were given, three different dose concentrations of the extract; 100mg/kg, 200mg/kg and 300mg/kg. The drug reference control was given metfomin 50mg/kg while the normal control rats (group 1) and Diabetic (Control group 2) were given appropriate volumes of water for 26 days. Compared to the Diabetic control (126±2.0 mg/dL), after treatment, the fasting glucose levels of Groups 1,3,4,5,6,7,8,9 (106.00± 2.8, 108.00±3.1, 101.03±3.0, 100.8±2.6, 102.3± 3.5, 95.20± 1.67± 94.60± 1.67, 92.90±1.3 respectively) were significantly lower ($p<0.05$). This corresponded to 8.3%, 12.82%,6.11% reduction of glucose level in these groups, 5.5%,17.73% and 31.78% in the groups administered with the extracts , hence, suggesting that the extracts dose dependently lowered the plasma glucose with 300mg/kg being most efficient. These results suggest that the aqueous extract of the leaf of *Brillantaisia guianensis P.beauv* possess hypoglycaemic properties that could be beneficial for the management of diabetics mellitus.

key words: *Brillantaisia guianensis P.beauv*, hypoglycaemic , alloxan, diabetes, phytochemical.

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1.0 INTRODUCTION

Diabetes mellitus can be seen as a group of 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 the (i) the fasting blood sugar (FBS) is greater than 6.1mmol/L or 110mgdL (ii) and random blood sugar is greater than 7.8mmol/L or 140mg/dL. Type 1 diabetes also known 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 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). Back here in Nigeria, 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 (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 function (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.

1.2 The resource plant *(Brillantaisia guianensis P.beauv.)*

Figure 1 *Brinllantaisia guianensis p. Beauv*

(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 tropical Africa. B guianensis is a perenial shrub which grows up 1.5m high and found in central and west Africa as well as east Camerouns.

Table 1: Botanical classification of *Brillantaisia guianensis P.beauv*

Kingdom	Plantae
Subkindom	Viridiplantae
Infrakingdom	Streptophyta
Superphylum	Embryophyta
Phylum	Tracheophyta/Magnoliophyta
Subphylum	*Euphyllophytina*
Infraphylum	*Radiotopses*
Subclass	*Magnolldae*
Superorder	*Asteranae*
Order	*Lamiales*
Famiy	*Acanthaceae*
Genus	*Brillantaisia*
Specific epiphet	*Guianensis*
Species	*Brillantaisia guianensis P.beauv*
Common Name	*Giant Tropical Blue African Salvia.*
Synonym(s) ** old name(s)	*Brillantaisia nitens, Leucorhaphia Lamium*

Different ethnic groups across Africa have varieties of names for *B.guainensis P.beav.* Burkil et al (13) had reported several names in Africa, for instance it is known as a-guare-(a)nsra amomg the AKAN-ASANTE people of ghana. It is known as kissi, manding-maninka and loma all in Guinea know it to be pediindo, bolobolo and bolobolоye. In sierra leone, the mendes call it

daninye. In the same vein in Nigeria it is called ebohohede in Edo state, polo-polo in Awka , Anambra state. In Okon- Ohafia L.G.A of Abia state it is known as nri atu.

B.guainensis is widely used in African traditional medicine to treat skin infections and pain-like took ache, Burkil (13), Adjanohoun et al.,(14), some have been shown to possess antinociptive effects (15) or are traditionally used for their antihypertensive activity. (14). In Cameroon, the decoction of *B.guainensis* is used by traditional healers of centre province for the management of cardiovascular diseases especially hypertension

Several researches have validated the medicinal value of *B.guianensis.* The m ethylene/chloride/me thanol leaf extract had been reported to lower arterial blood pressure an d heart rate 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 had 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 is very popular among village women in combating malaria induced anaemia in children. (18).The root taken in soup in southern Nigeria is used to reduce pain during pregnancy. (19). In Ohafia in Abia state of Nigeria , the leaves are used in the treatment and management of diabetes mellitus. This research was aimed at evaluating the hypoglycemic potential of the aqueous extracts of this plant as a leaves used in the treatment and management of diabetes mellitus.

2.0 MATERIALS AND METHODS

Chemical used.

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

2.1 Collection of Plant Material

The leaves of *Brillantaisia guianensis P.beuv* used for this studies were harvested fresh from ‘ude’ plantation in Okon Aku, Ohafoa Local Government of Abia State and was identified and given a herbarium no. of E. Hebarium id. No. EH-P-052) by a taxonomist in the Herbarium of the department of plant science, University of Port Harcourt Dr Edwin Nwosu. The leaves were

destalked, washed with clean cold tap water. In order to determine determine the dosage of administration of the extracts to the animals, the plants leaves were oven dried at 55◦C and ground into powder. A known weight (500g) of *Brillantaisia guianensis P.beuv* of the powder was soaked in 2 litres of boiled, hot distilled water for 12 hr after which the resulting mixture was filtered and the filtrate (aqueous extract) evaporated to dryness. The residue was weighed and stored in a refrigerator (4ºC). This residue was analyzed. The percentage recovery of the crude extract was calculated as follows.

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

2.2Analysis of the phytochemical profile

2.3 Calibration, Identification and Quantification

Standard solutions were prepared in methanol for flavonoids , chloride and; ethanol for, glycosides and saponines. The linearity of the dependence of response on concentrations 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.

2.3.1 DETERMINATION OF FLAVONIDS

The extraction was carried out according to the method of Millogo-Kone.(20)

The dried extract of ethanolic and aqueous extraction, was sampled and made to be free of water by ensuring constant weight for a period of time in the laboratory. 1.00g of the sample was weighed into the 250ml conical flack capacity with addition of 100ml of distilled water and boiled for 10minutes. The flavoniods extract was obtained by pouring 100ml of the boiling methanol;water (70:30) v. v into the materials. The mixture was allowed to macerate for about 6 hours and then concentrated to 5ml for gas chromatography analysis.

Chromatographic conditions

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.

2.3.2 DETERMINATION OF TANNIN

The extraction was carried out by following the modified method of Luthar (21)

Five grammes of the powered sample were extracted with 125mL of redistilled methanol for 20 min at room temperature (29 ±1◦C). The resultant extract was concentrated for gas chromatography analysis. The standard mixture was prepared from 1000mg/L stock solution of tannic acid.

Chromatographic conditions

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 220^{o}C, respectively. The adopted Split injection was with a split ratio of 20:1. The Carrier gas or mobile phase was nitrogen gas. The compressed air pressure and hydrogen pressure were 40 psi, and 28 psi. The oven was programmed as follows; initial temperature at 120◦C followed by ramping at 10◦C/min for 20min.

2.3.4. DETERMINATIONS OF SAPONIN

Saponin Extraction

The extraction was carried out by the following the modified of analytical sciences according to the method of Guo (22)

Procedure

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 the sonication. The combined extracts were concentrated to 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 and 1ml was injected into the injection pot of GC.

Chromatographic conditions

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^{0}C for 5 minutes and first ramping at 12^{0}C/min for 18min and second ramping @15 C/min for 5min

2.3.5 DETERMINATION OF PHYTOSTEROLS

The oil were extracted according to AOAC method 999.02 (23), while the sterols were analyzed according to the modified AOAC method 994.10 and AOAC 970.51 official methods.

Procedure

Extraction of Oil

After preparation and installation of the extraction cell and collection vessel in the SFE instrument, 3.0g of the pulverized sample was placed in the extraction cell an extracted for 60 mins with CO_2 and 15% ethanol, under the following conditions; 51.7 kpa, 100^0C, nominal fluid flow rate, 2.1g/min; and restrictor temperature such that CO_2 evolve at temperature between 80-100^0C

Analysis of Sterols

The aliquot of the extracted oil was added to the screw-capped test tubes. The sample was saponified at 95^0C for 30 minutes, by using of 3ml of 10% KOH in ethanol, to which 0.20ml of benzene had been added to ensue miscibility. Deionised water (3mL) and 2ml of hexane was used in extracting the non-saponifiable materials (sterol, etc) .Three extractions each with 2ml of hexane, were carried out for 1hour, 30 minute and 30 minutes respectively, to achieve complete extraction of the sterols. The hexane was concentrated to 1ml in the vial for gas chromatography analysis and 1µL was injected into the pot of gas chromatogram..

The gas chromatography conditions for the analysis.

Chromatographic conditions

The gas chromatograph used was an HP 6890 (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 AC-5column (30mx0.32mmx0.25µm thickness).The Injection temperature was 250^0 and detector temperature 320^0C. Split injection was adopted with a split ratio of 20:1.The Carrier gas was nitrogen gas. Hydrogen and Compressed air Pressures were 22psi, and 35 Psi respectively. Oven program was programmed as follows: Initial Temperature is at 60^0C. First ramping was at 10^0C /min for 20min and maintained for 4min while Second ramping was at 15^0C/Min for 4min and maintained for 4 mins.

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, 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.

Table 2 Experimental Design for the anti-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 contol 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 *Brinllatasia* (300mg/kg)
7	*Brinllatasia guainensis* treatment 1 (BGT1)	Alloxan (80mg/kg and *Brinllatasia* (100mg/kg)
8	*Brinllatasia guainensis* treatment 2.(BGT2)	Alloxan (80mg/kg and *Brinllatasia* (200mg/kg)

9*Brinllatasia guainensis* treatment 3.(BGT3) Alloxan (80mg/kg and *Brinllatasia* (300mg/kg)

The treatment and treatment control (TC) were given, three different dose concentrations of the extract; 100mg/kg, 200mg/kg and 300mg/kg. 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 (24). Three days after administration of alloxan, the animals were again fasted and blood collected via tail cutting (25), (26), for the determination of their fasting glucose and triglyceride levels. It was found that the rats had moderate diabetes, having hyperglycemia. Then the rats were kept for 3 days to stabilize the diabetic condition (27) 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 blood was collected from each rat into heparin and EDTA sample bottles. Whole blood was immediately used to determine the triglyceride level. The heparin anti-coagulated blood samples were centrifuged at 1000g for 10 minutes, after which their plasma was collected and stored in the refrigerator (4^0C) for subsequent analysis, while the EDTA anti-coagulated blood samples were used for the haematological analysis.

3.0 Statistical analysis

Data from pharmacological screening were analyzed for statistical differences between treatment groups, by means of one way analysis (ANOVA) and post hoc Bonferoni, on SPSS 17. In all, $p<0.05$

RESULT

The Flavonoid composition of *Brillantassia guianensis P Beav.*

The Flavonoid composition of *Brillantassia guianensis P Beav.* composition is shown in table 3 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.

Table 3 Flavonoid composition of *Brillantassia guianensis P Beav.*

S/N	Compound	Retention time(mins)	Amounts (mg/100gx10^{-6})	% Composition
1.	Catechin	13.740	887878	2.90
2.	Resveratrol	15.035	9.033821	2.95.10^{-5}
3.	Genistein	15.499	8.61355	3.05x10^{-5}
4.	Daidzein	15.618	9.36465	0.46
5.	Apegenin	15.812	141165	3.38x10^{-5}
6.	Daidzein	16.246	10.3521	4.97x10^{-5}
7.	Butein	16.668	15.2374	9.35x10^{-4}
8.	Biochanin	17.090	28.6793	7.23
9	Naringenin	17.359	2218220	15.87
9.	Luteolin	17.767	4873810	59.09
10.	Kaemferol	18.049	18121190	59.09
11.	Epicatechin	19.515	2139.46	6.98x10^{-5}
12.	Epigallocatechin	20.462	3151.06	0.01
13.	Gallocatechin	21.817	44.0948	1.44x10^{-4}
14.	Quercetin	22.596	2750290	8.97
15.	Epicatechin-3-gallate	22.851	229.737	7.49x10^{-4}
16.	Epigalloqcatechin-3-gallate	23.462	207.410	6.76x10^{-4}
17.	Isorhamnetin	23.963	2.81181	9.17x10^{-6}
18.	Robinetin	24.982	11.5683	3.77x10^{-5}
19.	Myricetin	24.787	1658740	5. 41
20.	Baicalein	25.701	7.03776	2.29x10^{-5}
21.	Nobiletin	25.479	3.54165	1.15x10^{-5}
22.	Baicalin	25.861	2.04076	6.65x10^{-6}
23.	Isoquercetin	25.952	3.1587.4	0.10
24.	Tageretin	26.501	2.53060	8.25x10^{-6}
25.	A 84rtemetin	26.833	1.62620	5.30x10^{-6}
26.	Silymarin	26.948	1.90256	6.20x10^{-6}
27.	Hesperidin	28.524	2.92945	9.55x10^{-6}
	Total		30668770	

The Saponins composition of the of the leaves *Brillantassia guianensis P Beav* is shown in table 4. The result indicates that among the ten saponin were detected , notable among which are sapogenin (60.20%), saponine (31.28%), neochlorogenine (7.86%), hispigenin (o.65%) and others in minute quantities.

Table 4 Saponins composition of *Brillantassia guianensis P Beav.*

S/N	Compound	Retention time(mins)	Amount. (mg/100g($x10^{-1}$)	% composition
1	Saponine	17.607	158238	31.28
2	Hispigenin	18.704	3291.36	0.65
3	Solagenin	19.539	20.7812	$4.09x10^{-3}$
4	Diosgenin	19.938	2.12617	$4.18x10^{-4}$
5	Tigogenin	20.518	6.7225	$1.37x10^{-3}$
6	Neochlorogenin	21.749	39768.6	7.86
7	Hecogenin	22.637	1.38573	$2.74x10^{-4}$
8	Sapogenin	23.177	304580.3	60.20
9	Tribuloin	23.985	4.18108	$8.26x10^{-4}$
10	Yanogenin	24.741	6.15374	$1.22x10^{-3}$
. 11	Sconyzorgin	26.329	0.55266	$1.09x10^{-4}$
Total			50.59204	

The glycosides composition of *Brillantassia guianensis P Beav* leaves as shown in table 5 contain twelve glycosides, with artemetin(48.45%), digitoxin (36.65%), digoxin (9.64%), amygdalin (5.45%) and others in insignificant quantities.

Table 5 Glycosides composition of *Brillantassia guianensis P Beav.*

S/N	Compound	Retention time (mins)	Amount. (mg/100g($x10^{-5}$)	% composition
1.	Arbutin	17.768	1.2348	$4.21x10^{-5}$
2.	Linamarin	18.050	0.00456	$1.53x10^{-7}$
3.	Salicin	18.838	21.162	$7.22x10^{-4}$
4.	Artemetin	19.099	1420485	48.45
5.	Amygdalin	19.516	159844	5.45
6.	Ouabain	20.466	181.618	$6.19x10^{-3}$
7.	Dhunin	21.099	7.1487	$2.44x10^{-4}$
8.	Prunasin	21.435	6.30645	$1.24x10^{-4}$
9.	Cucurbitin	21.905	3.63030	$1.24x10^{-4}$
10.	Digitoxin	22.065	1074012	36.63
11.	Digoxin	22.600	277367	9.64
12.	Lotaustralin	23.965	65.9406	$2.25x10^{-3}$
	Total		2931995	

Tannins composition of *Brillantassia guianensis P Beav.*

The tannins composition of *Brillantassia guianensis P Beav.* is shown in table 6. The result shows that the leaves contained tannins consisting of 100% tannic acid

Table 6 Tannins. composition of *Brillantassia guianensis P Beav.*

Compound	Retention time (mins)	Amount mg/100g(10^{-1})	% composition
Tannic acid	19.521	7.46400	100
Total		7.46400	

Phytosterols composition of *Brillantassia guianensis P Beav*

Seven phytosteroid were detected in the leaves *Brillantassia guianensis P Beav* is shown in table 6 with the most prominent being sitosterol (67.87%), savenasterol (14.60%), stigmasterol (10.10%), campesterol (7.41%) and others in insignificant amount.

Table 7 Phytosterols composition of *Brillantassia guianensis P Beav.*

S/N	Compound	retention time (mins)	Amount (mg/100g(10^{-1})	% composition
1.	Cholesterol	19.488	0.00204	$3.28x10^{-3}$
2.	Cholestenal	20.460	0.00004196	$6.74x10^{-5}$
3.	Ergosterol	21.394	0.0183	0.03
4.	Campesterol	22.311	4.61259	7.41
5.	Stigmasterol	23.062	6.28789	10.10
6.	Savenasterol	23.726	9.0925	14.60
7.	Sitosterol	25.261	42.2552	67.86
	Total		6.22686	

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 than ($p<0.05$) the normal, reference, and the treatment control (BC1, and BC2). On day 5 the treatment groups (BGT1, BGT2 and BGT3) were significantly higher than the normal and (BCG1), while the reference group was significantly higher ($p<0.05$) than test control and test groups on days 5 and 12. Also Day 12 saw the test control significantly ($p<0.05$) higher than normal group and BGT1. 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.

Table 8 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 8 Effects of aqueous extracts of the leaves of *Brillantassia guianensis P Beav.* on the plasma glucose concentration of alloxan-induced diabetic rats..

TREATMENT GROUP	Plasma glucose Concentration (mg/dL) 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 9 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.

NT	Plasma triglyceride	Concentration (mg/dL)							
	DAY 0 (mg/dl)	DAY 5		DAY 12		DAY 19		DAY 26	
		Value (mg/dL)	% Reduction		% Reduction		% Reduction		% Re
	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
ol	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.
	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
	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
	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
	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±
	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
	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
	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

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.

4.2 Discussion

There are many local herbs that are currently being used by the population as alternative therapy for the treatment of diabetes. Most of these herbs have not been subjected to scientific scrutiny to determine their phytochemistry and potency. This study has brought to the fore the need to thoroughly examine herbs and also provide reasons for their efficacy.

The results obtained from the phase 1 (phytochemical profile) of this study revealed that the aqueous root extract of *Brinllatasia guainensis* contains bioactive agents such as , flavonoids,

saponin, β-sistosterol and tannins. These compounds have potent pharmacological activities especially as antimicrobial, anti-cholesteromic, antioxidant, anti-diabetic, anti-hypertensive and hepatoprotective agents. These justify their uses for medicinal purposes in the treatment of diseases such as diabetic mellitus, hypertension, obesity, malaria, liver diseases etc.

Alloxan-induced diabetes mellitus is often characterized by decreased insulin level, hyperglycemia, elevated triglycerides and total cholesterol, and decreased HDL-cholesterol (28).The high percentage reduction in plasma glucose levels, produced by the extracts in this study give credence to the use of this leaves in the management of diabetics mellitus

The results obtained from the phase 2 (biochemical effect) of this study show that; there was no significant difference ($p<0.05$) in glucose level between all the groups before induction of diabetes. But after induction, the glucose level of group 2, 3,, 7 8 and 9 increased significantly ($p<0.0$ 5) when compared to groups 1 and 4.5, 6 respectively). After treatment, there was a significant decrease ($p<0.05$) in the fasting blood glucose levels of groups 1, 4,5,6,7, and 9 when compared to the normal, diabetic control and drug reference control.. Further statistical analysis show that this corresponded to This corresponded to 8.3%, 12.82%,6.11% reduction of glucose level in group, 5.5%,17.73% and 31.78% in the groups administered with the extracts , hence, suggesting that the extracts dose dependently lowered the plasma glucose with 300mg/kg being most efficient.. The hypoglycemic effects of the extracts may have been produced by the saponins (Table 4), tannic acid (Table 6)and β-sistosterol (Table 7) and flavonoids (Table 3) presents in the leaves and their extracts. Saponins (29), tannins (30), (31), (32) (33) (34) and flavonoids such as apigenin, quercetin, epicatechin, kaempferol, narigerin ,genistein and myricetin (35) (36)(37)(38) (39)(40)(41)(42) are compound with established hypoglycemic activity. The extracts may have exerted their anti-hyperglycemic activity by enhancing glucose uptake (tannins), stimulating insulin secretion from pancreatic β cells (by flavonoids and/ or β-sistosterol) and insulin activity, or by converting pro-insulin to insulin, or alternatively, by inhibiting hepatic gluconeogenesis. Thus anyone or a combination of some or all of the above

mentioned component could be responsible for the hypoglycemic effects of the extracts, observed in this study.

Conclusion

These results 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 effect of the wistar albino rats, thus suggesting a likely protective role of the extract against hyperglycemia and the treatment of diabetes mellitus.

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