Journal of Pharmaceutical Research International

22(1): 1-11, 2018; Article no.JPRI.39870
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Phytochemical Screening and Hypoglycemic Property of *Globimetula braunii* (*Loranthaceae*) Leaf Extracts

Gideon O. Okpanachi[1,2*], Avosuahi R. Oyi[1], Hassan Musa[1], A. Abdulsamad[1], Muhammad B. Sani[3] and Jamilu Ya'u[3]

[1]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe State, Nigeria.
[3]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors GOO, ARO, MBS and JY designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HM and AA managed the analyses of the study. Author GOO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2018/39870
Editor(s):
(1) Rafik Karaman, Professor, Bioorganic Chemistry, College of Pharmacy, Al-Quds University, USA.
Reviewers:
(1) Julie S. Berame, De La Salle University & Caraga State University, Philippines.
(2) Walter Antônio Roman Junior, University of the Region of Chapeco, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/24145

Original Research Article

Received 5th December 2017
Accepted 13th February 2018
Published 14th April 2018

ABSTRACT

Aims: To carry out phytochemical screening, acute toxicity test and to investigate the hypoglycemic potential of the ethanol extract and fractions of *Globimetula braunii* leaves.
Place and Duration of Study: Department of Pharmacognosy and Drug Development and Animal House, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. August 2016 to April 2017.
Methodology: The leaf powder (2.1 kg) was extracted with 7.0 L of ethanol using the infusion method. The extract obtained was further fractionated each with 2.7 L petroleum ether 60/80, ethyl

**Corresponding author: E-mail: okpanachigideon@gmail.com;*

acetate and n-butanol to obtain PEF, EAF and NBF fractions respectively. Phytochemical screening of the ethanol extract and TLC profiling of the different fractions were carried out. Acute toxicity testing via the oral route of administration was conducted on Laboratory mice. The hypoglycemic property of the extract and fractions on Alloxan induced hyperglycemic adult Wistar rats was investigated.

Results: The percentage yield of the ethanol extract was 10.22 % w/w. The PEF, EAF, NBF and AQF fractions yielded 3.8%, 6.59%, 14.21% and 48.36% w/w respectively. The phytochemical screening of the ethanol extract showed the presence of alkaloids, anthraquinones, flavonoids, saponins, steroids, phenols/tannins and triterpenoids. These were redistributed among the fractions of the extract. The extract was practically non-toxic when administered orally (LD_{50}>5000). Treatment with 250 and 500 mg/kg of ethanol extract produced a significant effect (*P*<.001) at 1 h, 2 h and 4 h post administration via oral route when compared with 500 mg/kg metformin. The ethanol extract, petroleum ether and n-butanol fractions (500 mg/kg) showed significant blood glucose reduction effect (*P*<.001) after 1 h, 2 h and 4 h treatment.

Conclusion: A Pharmacological study of the extract and fractions of *G. braunii* leaves have significantly proven the safety and significant blood glucose reduction effect. The hypoglycemic property may be linked to their phytoconstituents especially the flavonoids, phenols and triterpenoids.

Keywords: Globimetula braunii; flavonoids; phenol; tannins; triterpenoids; hypoglycemic property.

1. INTRODUCTION

Mistletoe is a general term for woody parasitic plant especially in the *Loranthaceae* and Viscaceae plant families [1]. In West Africa, seven genera of the *Loranthaceae-Helixanthera, Bethania, Englerina, Globimetula, Agelanthus, Tapinanthus and Phragmanthera* – and about five dozen or more species are recognized [2]. Mistletoes are hosted by many tree crops of economic importance [1-6]. A typical farmer view mistletoe either growing in wild forest, gardens or orchards as a devastating parasite that poses serious loss to economically viable fruit trees [3,4,7,8,9]. The spread of mistletoe species is by seed dispersal usually mediated by birds that thrive on mistletoe fruit or host through faecal excretions or regurgitations [2]. Mistletoes are known to cause the host plants many biological effects, chief among which is a salt imbalance. A verified formulated hypothesis states that these hemi parasites (*Loranthaceae*) contribute to a decrease in the salt content in the parasitized host boughs particularly those bearing fruits [10]. Mistletoes of the *Loranthaceae* and *Viscaceae* family are widely used in various cultures in almost every continent to treat various ailments including hypertension, cancer and diabetes or used as a diuretic agent [2,11,12].

Globimetula braunii belongs to the family of *Loranthaceae*. It is a parasitic shrub that grows on dicotyledonous trees and attaches itself to the host by modified roots [2]. The members of the *Loranthaceae* family consist of about seventy-four genera commonly known as mistletoes and are widely distributed in tropical countries like Malaysia, India and Africa. Although regarded as a threat to agricultural yield because of its parasitic characteristics [13]; reports reveal that it is an important medicinal plant which explains the use of the leaves to hasten delivery in traditional medicine practice and effective for treating many diseases ranging from a headache, leg pain to pulmonary troubles. The leaves, fruits and flowers of the subject plant have been implicated in the management of high blood pressure, while the roots attaching it to the host plant are used for other therapeutic applications like ulcer and cancer treatment [14,15, 2]. The fresh leaf-extract of the plant exhibited laxative properties [16], antioxidative properties [17], antilipemic and hypocholesteremic activities indicative of possible cardio-protective potential in normal and hypercholesteremia situations [18]. An investigation of *G. braunii* leaf extract in rats revealed it has no significant hepatic and haematological toxic effects [19]. The folkloric study showed that the Nupe tribe of Niger State and Tangale tribe in Gombe state, Nigeria, use the leaf extract in the management of Type 2 diabetes. The Yoruba tribe (South Western Nigeria) calls it *Afomo onishano*. It is called *Kauchi Dogonyaro* in Hausa (North-West Nigeria). In North Eastern Nigeria it is called *kyaushe* among the Tangale tribe in Gombe state. The leaf extract (*Cauchi dogonyaro*) is produced by infusion and it is administered three times a day. Studies also revealed that an oral

administration of hexane and petroleum ether fractions of *G. braunii* leaf collected in Niger State Nigeria has hypoglycaemic effect in rats [20].

Recent findings indicate that all herbal medicines may not be safe and about 80 percent of the world population relies on botanical preparations as medicines to meet their health needs and the number of patients is growing exponentially [21-23]. Despite the growing popularity of herbal medicines worldwide, there is a dearth of scientific evidence of efficacy and standardization for most herbal medicines [24].

This study is aimed at conducting phytochemical screening and investigating the hypoglycemic potential of the ethanol extract, PEF, EAF, NBF and AQF fractions of *G. braunii* leaves collected from Gombe State, North Eastern Nigeria.

2. METHODS

2.1 Collection and identification of Plant Material

The plant which was hemi-parasitic on *Azadirachta indica* (*Meliaceae*) tree was collected. The plant was taken to the Herbarium Unit of Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria for identification and authentication. It was identified as *Globimetula braunii* with voucher specimen number 289 and was deposited for further reference. The Fig. 1 below is a photograph of *G. braunii* plant. The leaves were air dried [25] after removal of foreign materials and powdered with pestle and mortar in the research laboratory of Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. The powdered material was stored in an air tight container at room temperature until required for use.

2.2 Extract Preparation and Physical Examination

The powder was weighed (2.1 Kg) and extraction was carried out by infusion with 7.0 L of Ethanol for 15 min and allowed to cool. The extract collected was filtered and concentrated under reduced pressure using a rotary evaporator to collect the ethanol extract. The dried extract was collected and preserved in desiccators. The percentage yield of the crude extract was determined using the formula given below:

$$\text{Percentage yield of extract} = \frac{\text{Weight of total extract}}{\text{Weight of powdered material}} \times 100\ (\%\ \text{w/w})$$

The organoleptic property of the extract was carried out.

Fig. 1. Photograph of *Globimetula braunii* plant

2.3 Fractionation of the Ethanol Extract of *G. braunii* Leaves [26]

Fractionation of the crude extract was carried out using petroleum ether, ethyl acetate, n-butanol in order of their increasing polarity to obtain Petroleum ether, ethyl acetate and n-butanol fractions respectively. The dried ethanol extract (214.6 g) was dissolved in 300 mL of distilled water and poured into a separating funnel. Starting with the least polar, 900 ml of petroleum ether was added to the extract in the separation funnel (water to solvent ratio is 1:3). The mixture in the funnel divided into two layers; the lower aqueous portion layer and the upper petroleum ether fraction layer. Both the petroleum ether and aqueous layers were collected into different beakers. The aqueous portion was reloaded into the separating funnel and fresh petroleum ether solvent (900 ml) was added to it. This process was repeated three times to ensure maximum fractionation. All the petroleum ether fractions collected were pooled and labelled PEF. This same procedure was carried out using ethyl acetate to obtain ethyl acetate fraction (EAF) and n-butanol to obtain an n-butanol fraction (NBF) respectively. The leftover after collecting the NBF was named aqueous fraction (AQF). All the fractions collected were concentrated using a water bath and the percentage yield of each fraction was calculated using the formula below:

$$\text{Percentage yields of fraction} = \frac{\text{Weight of fraction}}{\text{Weight of the extract}} \times 100\ (\%\ \text{w/w})$$

2.4 Phytochemical Screening of GBE

Preliminary phytochemical screening of GBE was carried out using standard procedure [27] to test for the presence of alkaloid, anthraquinones, flavonoid, saponins, triterpenoids and phenol/tannin.

2.5 TLC profiling of the Fractions of Ethanol Extract of *G. braunii* Leaf

All the four fractions obtained were each subjected to Thin Layer Chromatography and monitored in TLC tank in different solvent systems at a different ratio (v/v) as shown in Table 1. After the development of the chromatogram, the plates were dried and sprayed with *p-anisaldehyde*, Bontrager's, Dragendoff's, ferric chloride, aluminium chloride and Liberman Burchard as visualization detecting reagent, followed by heating at 110^{o}C. Chromatograms were viewed immediately to confirm the presence of phytoconstituents.

Table 1. Solvent systems for determination of the presence of plant constituents of fractions of ethanol leaf extract of *G. braunii* using thin layer chromatography

Fraction	Solvent system	Ratio (v/v)
Petroleum ether	Hexane: Ethyl acetate	8:2
Ethyl acetate	Hexane: Ethyl acetate: Formic Acid	9:9:2
N-butanol	Butanol: Acetic Acid: Water	10:1:1
Aqueous	Butanol: Acetic Acid: Water	10:1:1

2.6 Pharmacological Studies

2.6.1 Acute toxicity study (Median lethal dose LD_{50}) [28]

The first phase of nine rats randomly divided into three groups of adult Wistar rats of both sexes per group were given 10, 100 and 1000 mg/kg body weight orally (through a cannula) of ethanol leaf extract of *G. braunii*, respectively. The rats were observed for signs of adverse effects and death for 24 h. The second phase consisted of three adult Wistar rats were each given 1600, 2900 and 5000 mg/kg body weight of ethanol leaf extract of *G. braunii*. The rats were also observed for signs of toxicity and mortality.

2.6.2 Diabetes induction

Diabetes was induced in male Albino rats (200-250 g) by intraperitoneal administration of Alloxan injection at a dose of 150 mg/kg body weight. The rats were monitored after 72 h for diabetes induction by collecting blood samples through the caudal vein. Their blood glucose was determined with a glucometer- Accu-Chek Active (GC Roche Mannheim Germany). Blood glucose level ≥200 mg/dL were considered diabetic and used as diabetic animals in subsequent studies [29].

2.6.3 Effect of ethanol leaf extract of *G. braunii* on Alloxan-induced hyperglycaemia in adult wistar rats [30]

A group of six diabetic rats each were treated with 250, 500 and 1000 mg/kg single administration of the ethanol leaf extract of *G. braunii* (GBE) through the oral route using a

gastro enteral cannula compared with control groups of rats treated with 1 mL/kg of normal saline and 500 mg/kg metformin. Their blood was collected through the caudal vein and glucose levels were determined with a glucometer- Accu-Chek Active (GC Roche Mannheim Germany) after 1 h, 2 h, 4 h and 24 h of treatments. Rats were denied access to food during 1 h, 2 h and 4 h post treatment but were allowed freely to drink water and eat pellet diet after 4 h.

2.6.4 Effect of ethanol leaf extract, Petroleum ether, ethyl acetate, n-butanol and aqueous fractions of *G. braunii* on Alloxan-induced hyperglycaemia in adult Wistar rats (Acute pharmacological study)

A group of six diabetic rats each were treated with 500 mg/kg single administration of the ethanol leaf extract, petroleum ether, ethyl acetate, n-butanol, aqueous fractions given through the oral route using a gastro enteral cannula compared with control groups of rats treated with 1 mL/kg of normal saline and 500 mg/kg metformin. Their blood was collected through the caudal vein and glucose levels were determined with a glucometer- Accu-Chek Active (GC Roche Mannheim Germany) after 1 h, 2 h, 4 h and 24 h of treatments. Rats were denied access to food during 1 h, 2 h and 4 h post treatment but were allowed freely to drink water and eat pellet diet after 4 h.

2.7 Statistical Analysis

Data obtained from the hypoglycemic study were expressed as Mean ± Standard Error of Mean (mean±SEM). Data of Statistical differences between the treatments and the control were analyzed by one-way and two-way Analysis of Variance (ANOVA) followed by Bonferroni Posttest. Values of P =.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Preliminary Investigation and Phytochemical Screening

The physical properties of *G. braunii* leaf and Ethanol leaf extract of *G. braunii* (GBE) is shown in Table 2. The percentage yield of the ethanol leaf extract was 10.22% w/w while the percentage yields of the fractions are shown in Fig. 2. Phytochemical screening of the ethanol leaf extract revealed the presence of alkaloids, anthraquinones, flavonoids, saponins, steroids, phenols/tannins and triterpenoids as shown in Table 3. The phytoconstituent were redistributed among the fractions of the extract as shown in Table 4 using different visualization reagents. The retention factor values of the phytoconstituents for the redistributed fractions are shown in Table 5 with PEF having the greatest non-polar plant constituents (PEF>EAF>NBF>AQF) but NBF and AQF contained higher polar constituents compared with PEF and EAF. The hypoglycaemic effect of the ethanol leaf extract and fractions may be linked to their phytoconstituents especially the flavonoids and phenols because of their antioxidant properties [31,32]. The other plant constituents may act as a synergist in the significant reduction of blood glucose levels [33]. Triterpenoids and saponins present in the extract and fractions may also be responsible for their antidiabetic effect. Literature suggests that the pharmacological effects of triterpenoids occur through mechanisms such as modulation of incretin activity, stimulation of insulin secretion and release, regeneration of β-endocrinocyte Langerhans islets, activation of enzymes responsible for glucose utilization, reduction of glucose and fatty acid assimilation in the small intestine, and interference with the sensation of sweetness [34].

Table 2. Physical properties of *G. braunii* leaf and GBE

Properties	Result
Colour of leaves	Green
Colour of extract	Greenish-brown
Smell	Pungent
Taste	Bitter
Texture	Sticky/Gummy
Percentage yield of extract (% w/w)	10.22

Table 3. Preliminary phytochemical screening results for GBE [27]

Phytochemical constituents	Inference
Anthraquinones(Bontrager's Test)	+
Flavonoids	+
Saponins (foam Test)	+
Steroids (Salkowski Test)	+
Tannins (Braymer's Test)	+
Triterpenoids	+

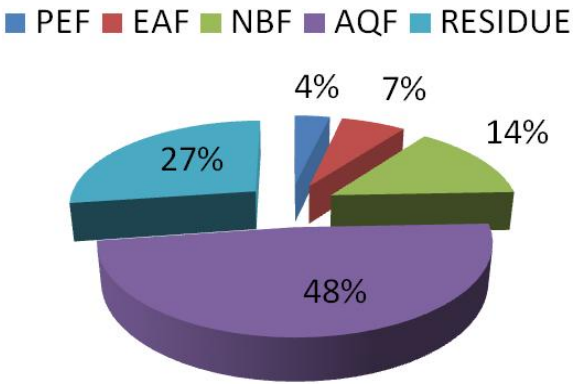

Fig. 2. Percentage yield of each fraction from the GBE

PEF, EAF, NBF, AQF represents Petroleum ether, ethyl acetate, n-butanol and aqueous fractions respectively.

Table 4. TLC profile of plant constituents present in fractions of GBE

Plant constituents	Visualization reagent	Inferences			
		P.E.	E.A.	BUT.	AQUEOUS
Anthraquinones	Bonstrager's	–	+	+	–
Phenol/Tannins	$FeCl_3$	–	+	+	+
Flavonoids	$AlCl_3$	–	+	+	+
Alkaloids	Dragendorff	+	+	+	–
Steroids/Triterpenes	L.B.	+	+	+	+

Key: L.B.- Liberman Burchard; P.E- Petroleum ether; E.A- Ethyl acetate; BUT- N-butanol; + - Present; - - Absent

Table 5. TLC analysis of EAF, PEF, NBF and AQF of GBE

Fraction	Developing system	Visualization reaction	Number of spots	Rf values of the spots
Ethyl acetate	Hexane: Ethyl acetate: Formic Acid (9:9:2 v/v)	P-anisaldehyde	9	0.09, 0.17, 0.28, 0.43, 0.53, 0.68, 0.81, 0.89, 0.96
		Bonstragers	2	0.20, 0.40
		$FeCl_3$	6	0.08, 0.26, 0.40, 0.72, 0.84, 0.88
		$AlCl_3$	3	0.10, 0.30, 0.90
		Dragendorff	3	0.06, 0.28, 0.44
		Liberman Burchard	5	0.28, 0.44, 0.62, 0.74, 0.86
Petroleum Ether	Hexane: Ethyl acetate (8:2 v/v)	P-anisaldehyde	8	0.13, 0.27, 0.44, 0.52, 0.67, 0.76, 0.82, 0.91
		Dragendorff	1	0.27
		Liberman Burchard	6	0.08, 0.16, 0.37, 0.43, 0.56, 0.87
n-butanol	Butanol: Acetic Acid: Water (10:1:1 v/v)	P-anisaldehyde	10	0.06, 0.24, 0.31, 0.39, 0.59, 0.71, 0.78, 0.82, 0.88, 0.94
		Bonstragers	2	0.14, 0.53
		$FeCl_3$	5	0.06, 0.61, 0.73, 0.82, 0.88
		$AlCl_3$	3	0.08, 0.61, 0.80
		Dragendorff	1	0.90
		Liberman Burchard	3	0.27, 0.59, 0.84
Aqueous	Butanol: Acetic Acid: Water (10:1:1 v/v)	P-anisaldehyde	6	0.07, 0.21, 0.32, 0.46, 0.61, 0.89
		$FeCl_3$	3	0.31, 0.45, 0.94
		$AlCl_3$	1	0.07, 0.59, 0.80
		Liberman Burchard	4	0.09, 0.40, 0.57, 0.88

GBE, PEF, EAF, NBF, AQF represents Ethanol leaf extract, petroleum ether, ethyl acetate, n-butanol and aqueous fractions respectively

3.2 Pharmacological Studies

3.2.1 Acute toxicity test- median lethal dose (LD_{50})

GBE was practically non-toxic when administered orally (LD_{50}>5000 mg/kg) as shown in Table 6. The Median Lethal Dose (LD_{50}) studies showed that the ethanol extract of *G. braunii* leaves administered orally was relatively safe even at a higher value of LD_{50} of >5000 mg/kg.

Table 6. Median lethal dose (LD_{50}) of GBE given by oral administration [28]

Dose (mg/kg)	Oral administration
First phase	
10	0/3
100	0/3
1000	0/3
Second phase	
1200	0/1
1600	0/1
2900	0/1
5000	0/1
LD_{50}	**>5000 mg/kg**

3.2.2 Effect of ethanol leaf extract of *G. braunii* on Alloxan-induced hyperglycaemia in adult Wistar rats

The outcome of the effect of Ethanol leaf extract *of G. braunii* (GBE) on blood glucose levels is presented in Table 7. The study revealed that after one hour of single administration, GBE and metformin (500 mg/kg) had maximal significant (*P*<.001) reduction effect on blood glucose levels, while the other doses of GBE (250 mg/kg and 1000 mg/kg) did not produce significant effect (*P*≠.05) when compared from the base line. At two hours post administration, metformin 500 mg/kg and GBE at 250 mg/kg and 500 mg/kg had maximal significant (*P*<.001) reduction effect on blood glucose levels. At four hours post administration, metformin 500 mg/kg and GBE at 250 mg/kg and 500 mg/kg had maximal significant (*P*<.001) reduction effect on blood glucose levels. The dose of 1000 mg/kg GBE had no significant (*P*≠.05) reduction in blood glucose level. After twenty-four hours of administration, none of the doses significantly reduced blood glucose levels.

3.2.3 Effect of ethanol leaf extract, Petroleum ether, ethyl acetate, n-butanol and aqueous fractions of *G. braunii* on Alloxan-induced hyperglycaemia in adult wistar rats (Acute pharmacological study)

The effect of GBE, EAF, PEF, NBF and AQF fractions on blood glucose levels are shown in Table 8 and Table 9. The Two-way ANOVA analysis presented in Table 8 revealed that at one hour post administration with various treatments at 500 mg/kg of GBE, EAF, PEF, NBF and AQF fractions, compared with metformin as positive control, only metformin had a maximal significant (*P*<.001) reduction effect in blood glucose level when compared from the baseline (zero hours). After two hours of administration, metformin, petroleum ether fraction and crude extract at 500 mg/kg had a maximal significant (*P*<.001) reduction effect in blood glucose levels. The n-butanol fraction, the ethyl acetate and aqueous fractions did not produce significant (*P*≠.05) reduction in blood glucose levels. At four hours post administration, metformin, petroleum ether fraction and crude extract at 500 mg/kg had a maximal significant (*P*<.001) reduction effect in blood glucose levels. The ethyl acetate, n-butanol and aqueous fractions had no significant (*P*≠.05) reduction in blood glucose level. After twenty hours of administration, none of the treatment groups significantly reduced blood glucose levels.

Table 7. Effect of GBE on blood glucose levels of Alloxan induced hyperglycaemic albino wistar rats

Group	Dose (mg/kg)	0 hr	Mean 1 hr	Blood glucose 2 hr	Level (mg/dL) 4 hr	24 hr
Normal saline	1 mL/kg	114.8±1.16	115.0±1.05	114.2±1.58	113.2±0.97	115.2±1.02
Alloxan	150	358.6±34.51	361.4±33.99	343.8±33.06	349.6±38.66	464.4±53.96
Metformin	500	401.4±46.39	240.4±32.97***	156.8±28.06***	163.4±24.42***	467.6±90.54
GBE	250	484.0±27.96	436.4±39.82	373.8±36.92***	334.6±35.17***	465.0±22.47
GBE	500	473.0±23.17	366.6±30.80***	308.4±21.14***	293.4±28.31***	488.8±24.77
GBE	1000	385.4±51.12	354.8±40.32	324.0±45.73	310.0±48.92	407.0±38.10

*Data were analysed by Two-way ANOVA followed by Bonferroni Posttest and presented as mean±SEM; superscript *** denotes the maximal level of significant at P<.001, compared with normal saline and metformin. n=6. GBE represents Ethanol leaf extract*

However, the One-way ANOVA analysis presented in Table 9 revealed that at one hour post administration with various treatments at 500 mg/kg of GBE, EAF, PEF, NBF and AQF fractions, compared with metformin as positive control, only metformin (positive control) and n-butanol fraction had a maximal significant (P<.001) reduction effect in blood glucose level compared with the group treated with 1 mL/kg of normal saline. After two hours of administration, metformin, petroleum ether fraction and n-butanol fraction at 500 mg/kg had a maximal significant (P<.001) reduction effect in blood glucose levels. At four hours post administration, only metformin and petroleum ether fraction at 500 mg/kg had a maximal significant effect (P<.001) reduction effect in blood glucose levels. The other treatment groups had no significant (P≠.05) reduction in blood glucose level. After twenty hours of administration, none of the treatment groups significantly reduced blood glucose levels.

This study has revealed the presence of important medicinal phytochemical constituents in ethanol leaf extract, petroleum ether, ethyl acetate, n-butanol and aqueous fractions of *G. braunii*. The petroleum ether fraction contained the highest concentration of steroids/triterpenoids because it has the greatest number of spots as seen in Table 5. The ethyl acetate, n-butanol and aqueous fractions contained a low concentration of steroids/triterpenoids. The ethyl acetate, n-butanol and aqueous fractions appeared to have a high concentration of phenol/tannins and flavonoids. The petroleum ether fraction contained none.

Alloxan induced diabetes by destroying the insulin-secreting cells of the pancreas leading to hyperglycemia. Alloxan is known to damage the beta cells of the islets of the pancreas that function in the regulation of insulin secretion which then leads to an increase in the blood concentration of glucose [35]. Metformin was used as a standard drug to compare the efficacy of the extract and fractions. It acts by increasing fatty acid oxidation, decreasing hepatic glucose production and intestinal absorption, increasing peripheral glucose uptake and insulin sensitivity [36].

Table 8. Effect of ethanol leaf extract of GBE, EAF, PEF, NBF and AQF fractions on blood glucose levels of alloxan induced hyperglycaemia in albino wistar rats

Group	Dose (mg/kg)	0 hr	Mean blood 1 hr	Glucose 2 hr	Level (mg/dL) 4 hr	24 hr
Normal saline	1 ml/kg	114.8±1.16	115.0±1.05	114.2±1.58	113.2±0.97	115.2±1.02
Alloxan	150	358.6±34.51	361.4±33.99	343.8±33.06	349.6±38.66	464.4±53.96
Metformin	500	401.4±46.39	240.4±32.97***	156.8±28.06***	163.4±24.42***	467.6±90.54
GBE	500	473.0±23.17	366.6±30.80***	308.4±21.14***	293.4±28.31***	488.8±24.77
EAF	500	351.0±91.83	282.0±65.16	309.6±90.29	270.8±91.59	371.0±88.59
PEF	500	344.4±76.77	255.8±58.74	192.6±38.37***	161.4±42.45***	298.4±101.80
NBF	500	337.2±49.30	251.4±36.82	224.8±44.05	240.6±73.09	399.8±60.24
AQF	500	399.8±56.39	346.4±34.17	342.2±47.74	328.6±46.20	305.8±26.84

*Data were analyzed by Two-way Analysis of Variance (ANOVA) followed by Bonferroni Posttest and presented as mean±SEM; Superscript *** denotes the level of significance at P<.001, compared with normal saline and metformin. n=6. GBE, PEF, EAF, NBF, AQF represents Ethanol leaf extract, petroleum ether, ethyl acetate, n-butanol and aqueous fractions respectively*

Table 9. Effect of ethanol leaf extract of GBE, EAF, PEF, NBF and AQF fractions on blood glucose levels of alloxan induced hyperglycaemia in albino wistar rats

Group	Dose (mg/kg)	0 hr	Mean blood 1 hr	Glucose 2 hr	Level (mg/dL) 4 hr	24 hr
Normal saline	1 ml/kg	114.8±1.16	115.0±1.05	114.2±1.58	113.2±0.97	115.2±1.02
Alloxan	150	358.6±34.51	361.4±33.99	343.8±33.06	349.6±38.66	464.4±53.96
Metformin	500	401.4±46.39	240.4±32.97***	156.8±28.06***	163.4±24.42***	467.6±90.54
GBE	250	484.0±27.96	436.4±39.82	373.8±36.92	334.6±35.17	465.0±22.47
GBE	500	473.0±23.17	366.6±30.80	308.4±21.14	293.4±28.31	488.8±24.77
GBE	1000	385.4±51.12	354.8±40.32	324.0±45.73	310.0±48.92	407.0±38.10
EAF	500	351.0±91.83	282.0±65.16	309.6±90.29	270.8±91.59	371.0±88.59
PEF	500	344.4±76.77	255.8±58.74	192.6±38.37***	161.4±42.45***	298.4±101.80
NBF	500	337.2±49.30	251.4±36.82***	224.8±44.05***	240.6±73.09	399.8±60.24
AQF	500	399.8±56.39	346.4±34.17	342.2±47.74	328.6±46.20	305.8±26.84

*Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Bonferroni Posttest and presented as mean±SEM; Superscript *** denotes the level of significance at P<.001, compared with normal saline and metformin. n=6. GBE, PEF, EAF, NBF, AQF represents Ethanol leaf extract, petroleum ether, ethyl acetate, n-butanol and aqueous fractions respectively*

The oral administration of 500 mg/kg bodyweight of the extracts and fractions of *G. braunii* and 500 mg/kg bodyweight metformin on rats as shown in Tables 7, 8 and 9 caused a significant (*P*=.05) reduction in the blood glucose concentration of diabetic rats. The hypoglycemic effects experienced may be due to the greater presence of phytochemical constituents in the extract and fractions. The flavonoids and phenols are responsible for their hypoglycemic property because of their antioxidant properties [31,32]. The other plant constituents may act in synergism towards the significant reduction of blood glucose levels [33]. Triterpenoids and saponins present in the extract and fractions may also be responsible for their antidiabetic effect. The pharmacological effects of terpenoids occur through mechanisms such as modulation of incretin activity, stimulation of insulin secretion and release, regeneration of β-endocrinocyte Langerhans islets, activation of enzymes responsible for glucose utilization, reduction of glucose and fatty acid assimilation in the small intestine, and interference with the sensation of sweetness [34].

4. CONCLUSION

The phytochemical screening of the ethanol extract showed the presence of alkaloids, anthraquinones, flavonoids, saponins, steroids, phenols/tannins and triterpenoids which were redistributed among the fractions of the extract. Pharmacological study of the extract and fractions of *G. braunii* leaves has been proven to be safe with significant blood glucose reduction effects compared with metformin. The petroleum ether fraction had the most significant effect (*P*<.001). Their hypoglycemic property may be linked to their phytoconstituents especially the flavonoids, phenols and triterpenoids.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors declare that “principle of laboratory animal care” (NIH publication No. 85-23, revised 1985) was followed as well as specific national laws where applicable. All experiments have been examined and protocol approved by Ahmadu Bello University Animal Ethics committee. Animals that died as a result of experimentation were put in bio-hazard bags before being incinerated. After the study, euthanasia was performed on all the rats by suffocating them with chloroform in a closed chamber and incinerated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adesina SK, Illoh HC, Johnny II, Jacobs IE. African mistletoes (*Loranthaceae*); Ethnopharmacology, chemistry and medicinal values: An update. Afri J of Trad Compl Alter Med. 2013;10(3):161-170.
2. Burkill AM. The useful plants of West Tropical Africa. Royal Botanical Gardens, Kew. 1985;3:548-560.
3. Bright EO, Okunsanya BA. Infestation of economic plants in Badeggi by *Tapinanthus dodoneifolius* (DC) Danser and *Tapinanthus globiferus* (A. Rich) Van Tiegh Nig J. Weed Sci. 1998;11:51-56.
4. Overfield D, Riches C, Samoah AM, Sarkodie O, Baah F. A farming system analysis of the mistletoe problem in Ghanian cocoa. Cocoa Grower's Bulletin. 1998;51:42-50.
5. Gill LS, Onyibe HI. Mistletoes on rubber trees in Nigeria. Haustorium. 1990;23:1-2.
6. Begho ER, Omokhafe KO, Omo-Ikerodah EE, Akpaja EO. Some observations on the fruit set and incidence of mistletoes on rubber trees in Nigeria. Am-Eurasian J. Sustain Agric. 2007;1(1):13-18.
7. Sridhar TS, RaO VR. *Dendrophthoe falcata* a menace to fruit orchards. Current Sci. 2007;38:908.
8. Zewdie K, Eshetu T. Preliminary observation on feasibility of mechanical control of parasitic plants on trees. Proceedings of the 7th annual conference of the Ethiopian weed science committee, Rezene Fesehale (IAR, Ethiopia): EWSC. 1993:41-42.
9. Jiofack RT, Dondjang JP, Nkongmeneck BA. The *Loranthaceae* of the Bafou area in Cameroun: Identification, distribution, biology and eradication strategies. In: Systematics and conservation of African plants. Proceedings of the 18th AETFAT Congress, Yaounde, Cameroom 26th February – 2nd March, 2007 (Burgt, X. van

der; Maesen, J. vonder: Onana, J.M., Editors). 2010;229-235.

10. Dibon SD, Taffouo VD, Ndiang Z, Ngotta B, Mony R, Engone Obiang NL, Din N, Priso JR, Issaka BJ, Amougou A. The study of sodium and potassium distribution in five host species of *Phragmanthera capitata* (sprengel), S. Balle in the littoral region of Cameroon. J Appl. Bio sci. 2010;30:1839-1844.
11. Adodo A. Nature power: A christian approach to herbal medicine. Benedictine publication Nigeria, 3rd Edition. Edo state: 7th printing by Generation Press Ltd, Surulere Lagos. 2004;103-11.
12. Jadhav N, Patil CR, Chaudhari KB, Wagh JP, Surana SJ, Jadhav RB. Diuretic and natriuretic activity of two mistletoes species in rats. Phcog. Research. 2010;2(1):50-57.
13. Dibong SD, Priso RJ, Taffouo VD, Fankem H, Salle G, Amougou A. Parasitism of host trees by *Loranthaceae* in the region of Douala (Cameroon). Afri J Environ. Sci. Toxicol. 2010;2:371-378.
14. Oboh IE, Nworgu ZAM. Oxytocic properties of the aqueous extract of *Globimetula braunii* (*Loranthaceae*). Pak. J. Pharm. Sci. 2008;21:356-360.
15. Le O, Zam N. Oxytocic properties of the Aqueous extract of *Globimetula braunii* (*Loranthaceae*). Pak. J. Pharm. Sci. 2008;21(4):356-360.
16. Fred-Jaiyesimi A, Onabanjo T, Ademuyiwa O. Phytochemical and laxative studies of *Globimetula braunii* (Engle) van tiegh growing on *Cola acuminata* (Schott and Endl); 2008.
17. Okpuzor J, Ogbunugafor H, Kareem GK. Antioxidative properties of ethyl acetate fraction of *Globimetula braunii* in normal albino rats. J. Biol Sci. 2009;9(5):470.
18. Eurkainure OL, Abovwe JA, Adefegha AS, Egwuche RU, Fafunso MA. Antilipemic and hypocholesteremic activities of *Globimetula braunii* in rats. Exp. Toxicol. pathol. 2011;63(7-8):657-661.
19. Okpuzor J, Ogbunugafor H, Kareem GK. Hepatic and hematologic effects of fractions of *Globimetula braunii* in normal albino rats. Excli Journal. 2009;8:182-189.
20. Muhammad HL, Kabiru AY, Mann A, Adefolalu FS, Busari MB, Raji RO, Shekown SM, Iwegbulam B. Effects of petroleum ether and n-Hexane extracts of *Globimetula braunii* on glucose, lipids and some biochemical parameters of diabetic rats. Int J Sci Res Pub. 2014;4(11):1-9.
21. Erah PO. Herbal medicines: Challenges. Trop J Pharm Res. 2002;1(2):53-54.
22. Pal SK, Shukla Y. Herbal medicine current status and the future. Asian Pac J Can Prev. 2002;4(4):281-8.
23. Shri JNM. Ginger: Its role in Xenobiotic metabolism. ICMR Bullettin. 2003;33(6): 57-63.
24. Okogun JI. Drug production from plant extracts. Pharmanews. 2015;37(2):1.
25. Rocha RP, Melo EC, Radunz LL. Influence of drying process on the quality of medicinal plants. A review. J Med Plants Res. 2011;5(33):7076-7084.
26. Woo MG, Shin HY, Kang KS. Chemistry and pharmacology of flavone-Glycosides from *Ziziphus* seeds. The korean J of Pharmacognosy. 1980;11(34)141-148.
27. Sofowora A. Medicinal plants and traditional medicine in Africa. Supreme Books Limited. 1993;4:50-63.
28. Lorke D. A new approach to tropical acute toxicity testing. Arch. Toxicol. 1983;53: 275-287.
29. Isah AB. Tableting and Hypoglycaemic properties of *Stachytarpheta augustifolia (Verbanaceae)* extracts. A Ph.D dissertation submitted to the department of pharmaceutics and pharmaceutical microbiology, Ahmadu Bello University, Zaria, Nigeria; 2005.
30. Alarcon-Aguilar FJ, Calzada-Bermejo F, Hernendez-Galicia E, Ruiz- Angeles C, Roman-Ramos R. Acute and chronic hypoglycemic effect of *Ibervillea sonorae* root extracts-II. J of Ethnopharmacology. 2005;97(3):447-452.
31. Nkirote CK, Kathenya IJ, Micheal O, Clare M, Konrad BH, Vellingiri V. Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected raw and processed indigenous food ingredients from Kenya. J Food Sci. 2011;76(4): C560-C567.
32. Pari L, Amarnath S. Antidiabetic activity of *Boerhavia diffusa* L. effect on hepatic key enzymes in experimental diabetic rats. J of ethno. 2004;9:109-113.
33. Ruiz GG, Nelson EO, Kozin AF, Turner TC, Walters RF, Langland JO. A lack of bioactive predictability for marker

compounds commonly used for herbal medicine standardization. PloS ONE. 2016;11(7):1-10.

34. Di Fabio G, Romanucci V, De Marco A, Zarrelli A. triterpenoids from *Gymnema sylvestre* and their pharmacological activities. Molecules. 2011;19: 10956-10981.

35. Eleazu CO, Okafor PN, Ahamefuna I. Total antioxidant capacity, nutritional composition and inhibitory activity of unripe plantain (*Musa paradisiacae*) on oxidative stress in alloxan induced diabetic rabbits. Pakistan Journal of Nutrition. 2010;9: 1052-1057.

36. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: An update. Annals of Internal Medicine. 2002;137:25-33.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24145