Brazilian Journal of Biological Sciences, 2016, v. 3, no. 6, p. 293-298.
ISSN 2358-2731
http://dx.doi.org/10.21472/bjbs.030606

Phytochemical screening and hypoglycaemic activity of *Lasianthera africana* Beauv. (Aquifoliales: Stemonuraceae) leaf extract on diabetic rats

Norah Godwin Ekanem[1,3,*], Herbert Orji Chidi Mbagwu[2] and Gamaliel Ibiama Harry[1]

[1]Department of Crop Science. Faculty of Agriculture. University of Uyo. P.M.B.1017, Uyo, Nigeria. *Email: norahgodwin@uniuyo.edu.ng.
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, P.M.B. 1017, Uyo, Nigeria.
[3]Plant Genetics Resources and Cell and Tissue Research Laboratory, Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.

Abstract. *Lasianthera africana* Beauv. (Aquifoliales: Stemonuraceae) is a native leafy vegetable used in traditional medicine for control and management of problems associated with complications of diabetes mellitus. The study was aimed to evaluate the phytochemical and hypoglycaemic properties for effective control of diabetes. Phytochemical analyses were conducted using standard methods. The rats were divided into four groups of six rats each of which Group 1 received distilled water (1 mL); Group 2 received aqueous extract (doses of 500 mg/kg, 1,000 mg/kg and 1,500 mg/kg); Group 3 received ethanolic extract (doses of 500 mg/kg, 1,000 mg/kg and 1,500 mg/kg); Group 4 received synthetic drug (dose of 10 mg/kg) for seven consecutive days. The phytochemical showed the presence of alkaloids, glycosides, saponins, tannins, phlobatannins, flavonoid and terpenes. Hypoglycaemic studies of 1,000 mg/kg body weight of the ethanolic extract administered intraperitoneally significantly reduced the blood sugar level ($p < 0.05$) from 239.71 ± 4.26 mg/dL (fasting hour) to 51.42 ± 2.19 mg/dL within 4 h post administration. The aqueous extract also significantly lowered the blood sugar from 230.29 ± 5.54 mg/dL to 88.57 ± 4.88 mg/dL within 3 h. The percentage decrease in blood sugar level of ethanolic extract at 1,000 mg/kg was more significant ($p < 0.05$) than glibenclamide. Acute toxicity showed that *L. africana* was non toxic because 5,000 mg extract/kg body weight did not produce visible toxic sign or mortality within 24 h. The study has shown that *L. africana* leaf possesses some useful medicinal potential for therapeutic purposes and that the hypoglycaemic activity of the leaf could be utilized by diabetic patients as supplement. This may contribute greatly to the management of their health conditions. The evaluation of hypoglycaemic activity in this study validates the claims that the plant leaf is useful in controlling and management of diabetes mellitus.

Keywords: *Lasianthera africana*; Phytochemicals; Hypoglycaemic activity; Diabetes mellitus; Intraperitoneal.

Received
November 11, 2016

Accepted
December 27, 2016

Released
December 31, 2016

Open Acess
Full Text Article

ORCID
0000-0003-4615-2512
Norah Godwin Ekanem
0000-0003-1159-9779
Herbert Orji Chidi Mbagwu
0000-0002-5664-0870
Gamaliel Ibiama Harry

Introduction

Crop plants generally represent an extraordinary reservoir of novel molecules. However, hundreds of thousands of metabolites may be present in medicinal crop plant. There is currently a resurgence of interest in the use of vegetables as a possible source of new lead compounds for therapeutic screening (Hostettma et al., 1997). *Lasianthera africana* Beauv. (Aquifoliales: Stemonuraceae) is a vegetable crop which has promising potentials for curative uses. It is commonly known as "editan" in Efik and Ibibio communities of Akwa Ibom and Cross River States. It is monospecific genus located in South Eastern Nigeria and extending towards Camerouns (Bassey et al., 2004). They are used in traditional concoction for the treatment of constipation, stomach aches/ulcer and prevention of miscarriage in pregnant women (Sofowora, 1993). Folklore information revealed that the decoction of the plant is used as a remedy for internal heat as well as antihelmintic agent. Chatterjee (1963) reported that bitter principles from plants were associated with management and control of diabetes mellitus. Prevalence of diabetes in some countries has risen up to 1% to 2% of the population with African countries on the increase (Barnett, 1991). It is estimated that about 140 million people worldwide are affected by diabetes and it is quite shocking to note that in our teeming population almost one million Nigerians are suffering from this traumatic disease (Ike, 2001).

Despite considerable progress in the management of diabetes mellitus by conventional synthetic drugs, the search for natural anti-diabetic plant for the control of diabetes is crucial. This calls for the evaluation of the therapeutic potential of *L. africana* leaf in the control and management of diabetes mellitus using experimentally induced diabetic rats.

Materials and methods

Plant material

L. africana leaves were collected from Essien Udim Local Government Area of Akwa Ibom State, Nigeria.

Extraction and analysis

600 g each of ground air-dried leaf sample were extracted with 70% of ethanol at room temperature while aqueous extraction was carried out with the cold extraction method. The extracts were concentrated under pressure to yield 46.0 g and 28.0 g ethanol and aqueous extract, respectively.

Phytochemical analysis

Phytochemical screening of the leaf was conducted on the two extracts according to the methods described by Trease and Evans (2002). All experimental procedures for phytochemical screening were replicated 3 times to establish the reproducibility of the extract.

Experimental animals

Twenty-four (24) male albino rats of body weight range of 120-200 g were purchased from Animal Unit of The Department of Pharmacology and Toxicology, University of Uyo, Uyo, Nigeria. The rats had access to pellets (product of Feeds Grower Nigeria Ltd.) and drinking water before they were fasted overnight.

Diabetes induction

The animals were fed with standard pellet and after 12 h fasting overnight, the animals were made to be diabetic by a single dose of alloxan (150 mg/kg) administered as 5% w/v in distilled water injected by intraperitoneal (i.p) route. Diabetes developed after 72 h and confirmation was carried out by testing glucosuria using indicator sticks (Bayer Diagnostics Basingstoke, UK). Rats with fasting blood sugar at 200 mg/dL and above

were considered diabetic (Gidado et al., 2005).

Hypoglycaemic evaluation

The 24 experimental rats were divided into four groups with six animals per group. Group 1 served as normal control and received (1 mL) distilled water; Group 2 received 500 mg/kg, 1,000 mg/kg and 1,500 mg/kg of ethanolic extract and Group 3 received 500 mg/kg, 1,000 mg/kg, 1,500 mg/kg of aqueous extract, respectively. Group 4 received 10 mg/kg of reference control drug Daonil (glibenclamide) (Resmi et al., 2001; Ghosh et al. 2004). Blood samples were collected between and after treatments.

Toxicity

The LD_{50} of the extract was done on the rats by oral and intraperitoneal (i.p) route using the method of Dietrich (1983). In the first phase, 24 male albino rats randomly divided into four groups of six rats per group were given 500, 1,000, 1,500 mg extract/kg body weight orally (via a cannula), respectively. The rats were observed for signs of adverse effects and death for 24 h and then weighed daily for 14 days. In the second phase of the study, the procedure was repeated using six rats randomly divided into four groups each given 500, 1,000, 1,500 mg extract/kg body weight intraperitoneally. The rats were also observed for signs of toxicity, mortality and weighed for 14 days.

Statistical analysis

Statistical analysis were performed by ANOVA, using SPSS (Naggar, 2005).

Results and discussion

Phytochemical screening revealed the presence of alkaloids, philobatannis, flavonoids, glycosides, terpenes, tannins and saponnins (Table 1). This shows that *Lasianthera africana* contains important class of bioactive substances, frequently employed as starting material for the synthesis of some useful drugs. The

Table 1. Qualitative phytochemical screening result of aqueous and ethanolic leaf extracts of *L. africana.*

Tests	Conclusion	
	Aqueous extract	Ethanol extract
Alkaloids		
(i) Dragendorff's reagent	+++	+++
(ii) Mayer's reagent	++	+++
(iii) Wagner's reagent	+	++
Philobatannins		
Hydrochloric Acid	+	++
Flavonoids		
Magnesium Metal	++	+++
Anthrquinones		
Borntrager	-	-
Glyosides		
(i) Salkowski	++	+++
(ii) Keller Killiani	++	+++
(iii) Sodium Picrate	+	+
Terpenes		
Lieberman's	+	++
Tannins		
(i)Ferric Chloride	+++	+++
(ii)Bromine Water	+++	+++
Saponins		
(i) Frothing	+++	+++
(ii) Fehling Solution	+++	+++
(iii) Sodium bicarbonate	+++	+++

+++ = Strongly present; ++ = moderately present; + = trace; - = absent.

hypoglycaemic studies as shown with the ethanolic and aqueous extracts of the plant significantly lowered blood sugar levels ($p < 0.05$) which compared favourably with the synthetic reference drug. This study implies that *L. africana* leaf possesses adequate anti-diabetic properties which may be useful for diabetic patients in the management and control of this complex disorder (Table 2 and Figure 1). This study corroborates with the findings of Delfan et al. (2014) who reported that anti-diabetic herbs have phytochemicals that can inhibit facilitated diffusion and glucose uptake through active transportation. The decrease in blood sugar could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetic patients (Cho et al., 2002). Toxicity results showed that the leaf is non-toxic and confirmed its safety for consumption (Etukudo, 2003) (see Table 3 and 4).

Table 2. Comparative analysis of blood glucose levels (mg/dL) of alloxan-induced diabetic rats treated with different concentrations of aqueous and ethanolic *L. african* leaf extracts.

Treatment		Blood glucose level before alloxan	Blood glucose level after alloxan induction	Blood glucose level after treatment with the leaf extracts						
				1 h	2 h	3 h	4 h	5 h	6 h	7 h
Diabetic control (distilled water) (1 mL)	1	62.67±3.48	245.28±10.01	253.57±9.44	259.0±8.05	261.46±7.92	260.94±9.79	261.37±8.00	260.65±6.74	261.35±7.96
Diabetic rats+ aqueous extract										
(a) 500 mg/kg	2	59.67±3.18	204.0±3.06	201.0±2.65	195.33±3.18	188.33±2.60	176.0±5.03	179.24±4.08	183.34±8.04	189.05±5.14
(b) 1,000 mg/kg		69.0±0.58	230.28±5.54	196.85±14.48	150.71±14.94	88.57±4.87	111.71±4.06	129.71±7.40	144.85±7.22	178.00±7.22
(c) 1,500 mg/kg		65.57±2.85	264.33±22.92	163.67±8.76	116.67±14.53	104.67±13.48	76.0±8.08	74.56±15.4	69.374±21.25	64.81±14.6
Diabetic rats + ethanolic extracts										
(a) 500 mg/kg	3	57.33±1.86	287.33±9.91	250.33±4.70	217.67±8.82	187.67±2.33	162.33±4.91	165.55±7.63	170.06±8.04	177.46±10.36
(b) 1,000 mg/kg		59.67±3.38	239.71±4.25	182.00±8.30	147.57±7.30	77.28±2.29	51.42±2.19	83.42±4.21	121.85±5.06	169.14±17.12
(c) 1,500 mg/kg		51.33±3.76	222.0±10.21	167.0±12.34	109.33±4.64	86.67±3.18	38.67±3.53	36.42±10.24	33.53±7.65	28.26±16.36
Diabetic + glibenclamide (10 mg/kg)	4	57.33±1.45	246.0±7.11	181.0±12.75	129.0±11.36	81.0±2.30	101.42±3.05	116.0±5.83	128.0±10.06	136.71±8.54

Values are expresssed as mean±SEM in 3 replicates; statistical significance ($p < 0.05$).

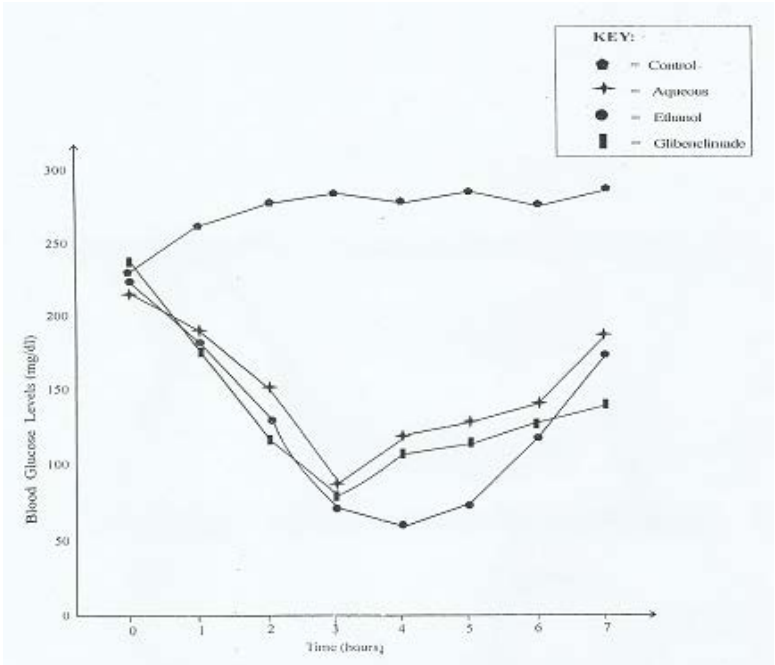

Figure 1. Effect of 1,000 mg/kg of aqueous and ethanol leaf extracts of *L. africana* on diabetic rats.

Table 3. Toxicity test trials of ethanol extract on oral administration.

Dose (mg/kg)	Number of dead mice (in 24 h)	Percentage Death	Time range of death (h)
1,000	Nil	Nil	Nil
2,000	Nil	Nil	Nil
3,000	Nil	Nil	Nil
4,000	Nil	Nil	Nil
5,000	Nil	Nil	Nil

Table 4. Toxicity test trials of ethanol extract after intraperitoneal administration of the extract.

Dose (mg/kg)	Number of dead mice (in 24 h)	Percentage death	Time range of death (h)
1,000	Nil	Nil	Nil
2,000	Nil	Nil	Nil
3,000	Nil	Nil	Nil
4,000	Nil	Nil	Nil
5,000	Nil	Nil	Nil

Conclusion

The study has established for the first time, that *Lasianthera africana* leaf possesses hypoglycaemic and anti-hyperglycaecmic properties and could serve as anti-diabetic regimen. It also validates the claims of the plant's use as an anti-diabetic supplement. It is necessary for researchers to find out the active substances responsible for hypoglycaemic activity, so that it would be possible to produce drugs which are useful in management and control of diabetes.

Acknowledgement

The authors appreciate the efforts of Messrs Bala Danladi and Malachy Nsikan for all the technical and laboratory assistance. We acknowledge the contributions of late Dr. (Sir) C.S. Odoemena, late Mr. E. B. Oforah and Prof. Kingsley Akpabio for their encouragement.

Conflicts of interest

Authors declare that they have no conflict of interests.

References

Barnett, A. H. Diabetes mellitus in the tropics. **Trans. R. Soc. Trop. Med. Hyg**., v. 85, p. 324-335, 1991.

Bassey, M. E.; Etuk, U. I.; Ekpo, J. U. Morphological diversity in the macrophyte genus *Lasianthera* (Icacinaceae) and the tanonomic implications. **Liv. Sys. Sus. Dev.**, v. 2, p. 1-5, 2004.

Cho S. Y.; Park, J. P.; Park, E. M.; Choi, M. S.; Lee, M. Y.; Jeon, S. M.; Jang, M. K.; Kim, M. J.; Park, Y. B. Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. **Clin. Chem. Acta**, v. 317, p. 109-117, 2002.

Delfan, B.; Saki, K.; Bahmani, M.; Rangsaz, N.; Delfan, M.; Mohseni, N.; Shirzad, H.; Babaein, Z. A study of anti-diabetic and anti-hypertension herbs used in Lorestan Province, Iran. **J. HerbMed. Pharmacol.**, v. 3, no. 2, p. 71-76, 2014. Available from: <http://www.herbmedpharmacol.com/PDF/JHP-3-71.pdf>. Accessed on: Oct. 15, 2016.

Dietrich, L. A new approach to practical acute toxicity testing. **Arch. Toxicol.**, v. 54, p. 275-287, 1983.

Etukudo, I. **Ethnobotany**: conventional and traditional uses of plants. Uyo: The Verdict Press, 2003.

Ghosh, R.; Sharatchandra, K. H.; Rita S.; Thokchom, S. I. Hypoglycaemic activity of *Ficus hispida* bark in normal and diabetic albino rats. **Indian J. Pharmacol**., v. 36, no. 4, p. 222-225, 2004.

Gidada, A.; Ameh, D. A.; Atawodi, S. E. Effect of *Naucla latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. **African J. Biotech**., v. 4, no. 1, p. 91-93, 2005.

Hostettman K.; Wolfender, J. I.; Rodrigues, S. Rapid detection and subsequent isolation of bioactive constituents of crude extract. **Planta Medica I**, v. 63, no. 2, p. 2-10, 1997.

Ike, V. One million Nigeria diabetes. North Director, Vanguard Profile. South-East-News. 19th November, 2001. p. 22.

Naggar, E. M. B.; Bartosikova, L.; Zemlicka, M.; Svajdlenka, E.; Rabiskova, M.; Strnedova, V.; Necas, J. Antidiabetic effect of *Cleome droserifolia* aerial parts: lipid peroxidation - induced oxidative stress in diabetic rat. **Acta Vet. Brno.**, v. 74, p. 347-352, 2005.

Resmi, C. R.; Fathima, A.; Sinilal, B.; Latha, M. S. Anti-diabetic effect of herbal drug in alloxan-diabetic rats. **Indian Drugs**, v. 38, no. 6, p. 319-322, 2001.

Sofowora, A. **Medicinal plants and traditional medicine in Africa**. Ibadan, Nigeria: Spectrum Books, 1993.

Trease, G. E.; Evans, W. C. **A textbook of Pharmacognosy**. 15. ed. London: Bailliere Tindall, 2002. p. 1150-1159.