Comparative anti-diabetic study of the leaf, stem, and root bark extracts of *Xylopia aethiopica* (*A. Dunal)* Rich

Famuyiwa, S. O.[1*], Ayoola M. D.[2], Faloye, K. O.[1] and Okewale, F. O.[1]

[1]*Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Nigeria.*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Nigeria.*
**Corresponding Author's Email: sofamuyiwa@oauife.edu.ng oluwaseyi_f@yahoo.com; Phone No: 08166020238*

Abstract

A medicinal plant is any plant containing substances that are effective therapeutically or are precursors for drug manufacture in one or more of its organs. This work was a comparative study of the hypoglycaemic and hyperglycaemia-lowering activities of the methanol extracts of the leaf, stem, and root barks of *Xylopia aethiopica* used folklorically in the management of type 2 diabetes mellitus with the view of determining its most active morphological part. The pulverized leaves, stem and root barks of the plant were separately subjected to cold extraction using methanol to obtain leaf, stem and root barks extracts respectively. The hypoglycaemic and antihyperglycaemic effects of the extracts were carried out on normoglycaemic and glucose-induced hyperglycaemic rats, respectively at 100, 200 and 400 mg/kg using glibenclamide (5 mg/kg) as positive control. The most effective antihyperglycaemic doses of the extracts were used to determine its anti-diabetic activity on streptozotocin-induced diabetic rats. The results obtained from the study were subjected to analysis of variance (ANOVA), followed by Student–Newman-Keuls post hoc tests and $p< 0.05$ was considered significant. The results of the study showed that the root bark extract of *X. aethiopica* did not cause hypoglycaemia in rats at all the tested doses while hypoglycaemia was obtained at 400 mg/kg of the leaf and stem bark extracts. In the hyperglycaemia-lowering experiment using glucose loaded rats' model, the leaf, stem and root bark extracts gave 10, 13, 10, 29; 35, 36, 38, 41 and 25, 27, 33, 44 % blood glucose levels reduction at 0.5, 1, 2 and 4 h, respectively which showed the stem bark as the most active plant part especially at 0.5-2 h. In the streptozotocin-induced hyperglycaemic rats experiment, the order of antihyperglycaemic effect of the morphological parts of *X. aethiopica* is, stem bark extract > leaf extract > root bark extract. It was concluded from the results obtained from the study that the stem bark extract of *Xylopia aethiopica* was the most active plant part both in glucose and streptozotocin-induced hyperglycaemia and it does not cause hypoglycaemia at low doses.

Keywords: Extracts, Diabetes mellitus, *Xylopia aethiopica*, hypoglycaemia, antihyperglycaemia

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by consistent hyperglycaemia that result to elevated blood glucose level which is caused by lack, insufficiency in production or action of insulin produced by the pancreas (Jayaraman *et al.,* 2018, Ayoola *et al.,* 2019, Bello *et al.,* 2022). Globally, about 415 million individuals were affected with diabetes in 2015 and this has been projected to increase to 629 million by 2045 (Kim *et al*., 2022, Bello *et al.,* 2022). Type 2 diabetes, a condition characterized by various side effects like ketoacidosis, retinopathy, renal failure, non-ketotic coma, stroke and other cardiovascular risks accounts for about 90-95 % of the various diabetic cases worldwide (Tuei *et al.,* 2010; Yazdanpanah *et al.,* 2017).

The use of expensive synthetic hypoglycaemic drugs such as insulin, biguanides, sulphonylurea in the management of type 2 diabetes is presented with clinical shortcomings and episodes of serious side effects such as flatulence, abdominal discomfort, diarrhea, brain atrophy, lactic acidosis, gastrointestinal disturbance, fatty liver, and hypoglycaemia (Krentz *et al.,* 2006; Adebajo *et al.,* 2013 a,b; Dahlen *et al.,* 2022). Medicinal plants are the cheapest source of complementary medicine in which about 70-80 % of the world population depends on as an alternative to the treatment of various diseases including diabetes (Kifle *et al.,* 2021). Therefore, plants can be explored for a cheap and effective therapeutic arsenal with little or no side effects for the management of diabetes mellitus (Liyanagamage *et al.,* 2020).

Xylopia aethiopica (A. Dunal) Rich (Annonaceae) commonly known as Negro pepper is a spice tree native to the lowland rainforest of West, Central and Southern Africa (Erhirhie *et al.,* 2014). Ethnomedicinally, different parts of the plant are used in the treatment of fever, sore, rheumatism, malaria, dysentery, skin infection and toothache (Gbadamosi and Erinoso,2016; Hwang *et al.,* 2020). The analgesic, antimicrobial, insecticidal, larvicidal, asthmatic, antidiabetic and anti-helminthic (Woode *et al*., 2012; Coli, 2013; Ito *et al*., 2018; Mohammed *et al*., 2016; Famuyiwa *et al.,* 2017) have been reported. The reported anti-diabetic activity was carried out on acetone fraction of the fruit ethanolic extract in a type 2 diabetes model of rats. The results of the report suggested that the acetone fraction showed excellent anti-diabetic effect in a type 2 diabetes model rats.

In our former work on *Xylopia aethiopica*, its fruit extract was investigated and reported for its antihyperglycaemic activity in which kaurenoic and xylopic acids were isolated from the active dichloromethane column fraction and were identified as two of its antihyperglycaemic constituents (Famuyiwa *et al.,* 2018). This study was a comparative study of the hypoglycaemic and antihyperglycaemic activities of methanolic extracts of the leaf, stem and root barks of the plant with a view to determine its most active antihyperglycaemic part.

2. Materials and methods

2.1 General experimental procedures and materials

All general-purpose solvents used were distilled. Spatula, Oral Cannula, Glucometer – (Accu-chek® level active control mg/dl) with Acuu-chek strips, measuring cylinder, Metler Analytical balance, Rotary evaporator connected to Chiller thermo-circulator and a vacuum pump.

2.2 Plant material and extraction

The morphological parts (leaf, stem and root barks) of *Xylopia aethiopica* were collected from Oke-ogbo in Ile-Ife, Osun State, Nigeria. The plant was identified and authenticated by Mr. Ademoriyo and a voucher specimen of the plant (IFE16941) was deposited at the Herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The fresh leaf, stem and root barks of *X. aethiopica* were air-dried in the Screen House, Faculty of Pharmacy, Obafemi Awolowo University. The air-dried morphological parts were pulverized at the Drug Research Production Unit, Faculty of Pharmacy, O.A.U., Ile Ife, Nigeria. The powdered morphological parts, leaf (4.5 kg), stem (3.8 kg) and root (4.5 kg) barks were extracted exhaustively with methanol and the resulting solutions were concentrated at 40 ºC under reduced pressure using rotator evaporator to obtain their respective extracts, 467.81 g, 218.84 g and 142.9 g.

2.3 Animals

Healthy albino rats between 120 – 160 g of both sexes (males and females) bred under standard conditions (temperature – 27±3 ºC, relative humidity – 65 %) at the animal house, Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile Ife, Nigeria were used for the experiment. They were fed on a standard pellet diet (Bendel Feeds, Nigeria) and water was also given to them freely.

2.4 Effect of extracts of normoglycaemic rats

Groups of 6 rats with 3-6 mMol/L blood glucose levels were fasted for 18 hours and variously treated with 1% Tween 80 in normal saline (negative control), or glibenclamide (5 mg/kg, positive control) or leaf, stem and root bark extracts (100, 200, 400 mg/kg). A drop of blood, taken from the tip of the tail of each rat, was dropped onto a Glucometer strip and the blood glucose level read off directly. All values were taken relative to blood glucose at T_o that was taken as 100% (Adebajo *et. al.* 2013a,b). The blood glucose levels of the rats were determined and recorded at 0.0, 0.5, 1.0, 2.0 and 4.0 h after administration of the extracts and the drug.

2.5 Effect of extracts on glucose-induced hyperglycaemic rats

Normoglycaemic rats weighing between 120–160 g were fasted for 18 hours and given 10 g/kg of glucose dissolved in 1% Tween 80 in normal saline. Rats with fasting blood glucose (FBG) level above 7.0 mMol/L (126 mg/dl) after 0.5 h were selected and divided into groups of six rats administered (*p.o.*) with 1 % Tween 80 in normal saline (negative control), glibenclamide (5 mg/kg, positive control) and extracts (100, 200, 400 mg/kg). The blood glucose levels of the rats were determined as shown above.

2.6 Effect of extracts on streptozotocin-induced diabetic rats

Diabetes was induced in overnight fasted normal rats by administering streptozotocin (65 mg/kg) in freshly prepared buffer solution (0.1M, pH 4.5) intraperitoneally. After 72 hours of induction, the rats' blood glucose levels were measured, and they were then left for another 5 days. Rats with Fasting Blood Sugar ≥ 11.0 mMol/L were considered diabetic and divided into four groups of five rats: negative control, administered with 1 % Tween 80 in normal saline; test groups, 200 and 400 mg/kg and positive control, glibenclamide (5 mg/kg). Blood glucose levels of rats were measured and compared to the control group nn days 1, 4, 7, 10, and 14 (Ayoola *et al.*, 2017; Bello *et al.*, 2022). This study was performed in strict accordance with the Institute for Laboratory Animal Research Division on Earth and Life Studies guidelines for the care and use of laboratory animals (National Research Council Publication, 2011) and was approved by the Institutional Animal Care and Use Committee of National Academy of Sciences (Washington, DC, USA).

3.0 Results and Discussion

3.1 Hypoglycaemic effect of the extracts

Table 1: Dose dependent hypoglycaemic effect of *X. aethiopica* leaf extract

Extract/Drug Doses (mg/kg)	Blood glucose level as percentage of T_o (reduction in blood glucose relative to negative control at T_1)				
	0 h	0.5 h	1 h	2 h	4 h
NS	100	115.60±3.69^{b}	102.95±3.69^{b}	98.36 ±10.17^{b,c}	99.23 ±3.82^{c}
XLE (100)	100	104.15±7.99^{b} (9.90 %)	93.60 ± 4.79^{a} (9.08 %)	71.58 ± 4.62^{b} (27.23 %)	75.26 ± 5.53^{b} (24.16 %)
XLE (200)	100	105.14 ±9.61^{b} (9.05 %)	104.19±7.18^{b} (-1.20 %)	82.53 ± 1.49^{c} (16.09 %)	73.45 ±3.05^{b} (25.98 %)
XLE (400)	100	92.04 ± 3.70^{a} (20.38 %)	76.75 ± 2.23^{a} (25.45 %)	69.16 ± 9.37^{b} (29.69 %)	67.81 ± 5.8^{b} (31.66 %)
GLI (5)	100	87.99 ± 5.94^{a} (23.88 %)	77.76 ± 6.91^{a} (24.47 %)	49.73 ± 6.29^{a} (49.44 %)	42.77 ± 4.88^{a} (56.90 %)

Data show the mean ±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. **NS:** 1 % Tween 80 in normal saline (Negative control); **XLE**: *Xylopia aethiopica* leaf extract, **GLI**: Glibenclamide (Positive Control, 5mg/kg).

Glibenclamide (5 mg/kg) elicited 24, 25, 49 and 57 % blood glucose levels reduction at 0.5, 1.0, 2.0 and 4 h, respectively that was significantly ($p <0.05$) higher than those elicited by 100 and 200 mg/kg of the leaf extract at all time points. This activity confirmed the hypoglycaemic side effect of

glibenclamide on normal subjects (Adebajo *et al.*, 2013a). However, the 20, 26, 30 and 32 % blood glucose levels reduction caused by 400 mg/kg of the extract that was comparable to glibenclamide at 0.5-1 h indicated that the extract may cause hypoglycaemia in normal subjects at this dose (Table 1). Similarly, 400 mg/kg of the fruit extract was reported to show comparable ($P > 0.05$) hypoglycaemic effect to glibenclamide at 2 h and 4 h (Famuyiwa *et al.*, 2018). blood glucose level reduction caused by absence of hypoglycaemic effect of plants in normoglycaemic rat is an indication of safety when consumed by normal human subjects (Adebajo *et al.*, 2013a,b).

Table 2: Dose dependent hypoglycaemic effect of *X. aethiopica* stem bark extract

Extract/Drug Doses (mg/kg)	Blood glucose level as percentage of T_o (reduction in blood glucose relative to negative control at T_1)				
	0 h	0.5 h	1 h	2 h	4 h
NS	100	115.60 ± 3.69^{b}	102.95 ± 3.69^{b}	98.36 ± 10.17^{c}	99.23 ± 3.82^{d}
XSE (100)	100	108.96 ± 6.16^{b} (5.74 %)	101.42 ± 6.08^{b} (1.49 %)	96.30 ± 4.47^{c} (2.09 %)	$86.51 \pm 8.87^{c,d}$ (12.82 %)
XSE (200)	100	106.44 ± 7.47^{b} (7.92 %)	89.98 ± 8.40^{a} (12.60 %)	73.88 ± 8.51^{b} (24.89 %)	79.61 ± 5.46^{c} (19.77%)
XSE (400)	100	96.18 ± 3.95^{a} (16.80 %)	88.95 ± 3.84^{a} (13.60 %)	65.92 ± 5.31^{b} (32.98 %)	63.84 ± 6.47^{b} (35.66 %)
GLI (5)	100	87.99 ± 5.94^{a} (23.88 %)	77.76 ± 6.91^{a} (24.47 %)	49.73 ± 6.29^{a} (49.44 %)	42.77 ± 4.88^{a} (56.90 %)

Data show the mean ±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): One-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. **NS:** 1 % Tween 80 in normal saline (Negative control); **XSE:** *Xylopia aethiopica* stem bark extract, **GLI**: Glibenclamide (Positive Control, 5 mg/kg).

Similar to the leaf extract of *X. aethiopica*, the stem bark extract also elicited a significantly lower hypoglycaemic effect than glibenclamide at 100 and 200 mg/kg at 05-4 h indicating safety at these doses (Table 2). Also, blood glucose levels reduction of 33 and 36 % caused by 400 mg/kg of the extract that was comparable ($p > 0.05$) to glibenclamide at 2-4 h showed its hypoglycaemic potential in normal subjects at this dose (Table 2). Comparable ($P > 0.05$) hypoglycaemic effect to glibenclamide at 2 h and 4 h was reported for 400 mg/kg of the fruit extract of *X. aethiopica* (Famuyiwa *et al.*, 2018).Therefore, non diabetic individuals should avoid consumption of higher doses of the stem bark of *X. aethiopica.*

Xylopia aethiopica root back extract gave a significantly ($p<0.05$) lower hypoglycaemic effect than glibenclamide (5 mg/kg) at 100-400 mg/kg and at all time points unlike its leaf and stem bark extracts

that showed high hypoglycaemic tendency at 400 mg/kg (Table 3). This result indicated a non toxic effect of the root back extract on non diabetic individual.

Table 3: Dose dependent hypoglycaemic effect of *X. aethiopica* root bark extract

Extract/Drug Doses (mg/kg)	Blood glucose level as percentage of T_o (reduction in blood glucose relative to negative control at T_1)				
	0 h	0.5 h	1 h	2 h	4 h
NS	100	115.60±3.69^{b}	102.95±3.69^{b}	98.36 ±10.17^{b}	99.23 ±3.82^{b,c}
XRE (100)	100	110.32±5.19^{b} (4.57 %)	101.20±2.23^{b} (1.70%)	99.04 ± 3.45^{b} (-0.69 %)	100.93 ±8.37^{c} (-1.71 %)
XRE (200)	100	118.43±6.18^{b} (-2.45 %)	105.82±8.45^{b} (-2.79 %)	86.19 ± 9.05^{b} (12.37 %)	84.82±9.60^{b,c} (14.52 %)
XRE (400)	100	107.86±4.80^{b} (6.70 %)	102.84±6.13^{b} (0.11 %)	91.95 ± 5.74^{b} (6.52 %)	78.36 ± 2.44^{b} (21.03 %)
GLI (5)	100	87.99 ± 5.94^{a} (23.88 %)	77.76 ± 6.91^{a} (24.47 %)	49.73 ± 6.29^{a} (49.44 %)	42.77 ± 4.88^{a} (56.90 %)

Data show the mean ±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. **NS:** 1 % Tween 80 in normal saline (Negative control); **XRE**: *Xylopia aethiopica* root bark extract, **GLI:** Glibenclamide (Positive Control, 5mg/kg).

Table 4: Dose dependent hyperglycaemia-lowering effect of *X. aethiopica* leaf extract

Extract/Drug Doses (mg/kg)	Blood glucose level as percentage of T_o (reduction in blood glucose relative to negative control at T_1)				
	0 h	0.5 h	1 h	2 h	4 h
GLU (10 g/kg)	100	97.16±4.02^{a}	81.92±2.99^{b}	75.67±2.12^{b}	72.87±2.20^{c}
XLE (100)	100	87.21 ± 1.18^{a} (10.24. %)	71.40 ± 2.74^{a} (12.84 %)	68.29 ± 2.83^{b} (9.75 %)	51.69 ± 2.31^{b} (29.07 %)
XLE (200)	100	91.17±6.88^{a} (6.17 %)	84.62 ± 2.73^{b} (-3.30 %)	82.07 ± 3.24^{b} (-8.46 %)	79.79 ± 2.67^{c} (-9.50 %)
XLE (400)	100	91.05 ± 6.68^{a} (6.29%)	72.86 ± 5.99^{a} (11.06 %)	73.87 ± 5.70^{b} (2.38 %)	69.93±4.16^{c} (4.03 %)
GLI (5)	100.00	79.37±4.0^{a} (18.31%)	66.20±2.86^{a} (19.19%)	51.32±3.87^{a} (32.18%)	34.94±3.80^{a} (52.05%)

Data show the mean ±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. **GLU:** 1 % Tween 80 in 10 g/kg glucose (Negative control); **XLE**: *Xylopia aethiopica* leaf extract, **GLI**: Glibenclamide (Positive Control, 5mg/kg).

Hyperglycaemia lowering effect of the leaf extract of *Xylopia aethiopica*

Homeostatic regulatory mechanism that suggested a healthy condition of the pancreases of the animals used was established in the rats administered with 10 g/kg glucose (negative control group) that elicited a significant time dependent blood glucose level reduction from 0.5-4 h (Kar *et al.*, 1999, Adebajo *et al.*, 2013a, b, Ayoola, 2017). Similarly, the significant time dependent glucose lowering effect up to the fourth hour given by glibenclamide (positive control) confirmed its minor extra pancreatic and major insulinotropic mechanisms of action (Luzi and Pozza, 1997). The leaf extract of *X. aethiopica* at 100 mg/kg gave a non-time dependent antihyperglycaemic effect with 29 % activity at 4 h indicating insulin stimulating effect at this dose (Table 4). At 200 mg/kg, the extract was devoid of activity from 0.5-4 h while increasing the dose to 400 mg/kg did not significantly increase the activity (Table 4). This result indicated a non dose dependent and weak antihyperglycaemic effect of the leaf extract of *X. aethiopica* with 100 mg/kg as the most effective dose (Table 4).

Table 5: Dose dependent hyperglycaemia-lowering effect of *X. aethiopica* stem bark extract

Extract/Drug Doses (mg/kg)	Blood glucose level as percentage of T_o (reduction in blood glucose relative to negative control at T_1)				
	0 h	0.5 h	1 h	2 h	4 h
GLU (10 g/kg)	100.00	97.16 ± 4.02^{c}	81.92 ± 2.99^{b}	75.67 ± 2.12^{b}	72.87 ± 2.20^{c}
XSE (100)	100.00	61.39 ± 4.50^{b} (36.82 %)	54.25 ± 3.65^{b} (33.78 %)	50.89 ± 2.06^{a} (32.75 %)	50.54 ± 4.71^{b} (30.64 %)
XSE (200)	100.00	63.69 ± 4.48^{b} (34.46 %)	52.85 ± 3.87^{b} (35.49 %)	46.58 ± 4.42^{a} (38.44 %)	$43.35\pm3.48^{a,b}$ (40.51 %)
XSE (400)	100.00	91.15 ± 5.15^{c} (6.19 %)	60.66 ± 4.25^{a} (25.95 %)	48.78 ± 4.13^{a} (35.54 %)	$41.38\pm4.39^{a,b}$ (43.21 %)
GLI (5)	100.00	79.37 ± 4.0^{a} (18.31%)	66.20 ± 2.86^{a} (19.19%)	51.32 ± 3.87^{a} (32.18%)	34.94 ± 3.80^{a} (52.05%)

Data show the mean ±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. **GLU:** 1 % Tween 80 in 10 g/kg glucose (Negative control); **XSE**: *Xylopia aethiopica* stem bark extract, **GLI**: Glibenclamide (Positive Control, 5mg/kg).

Hyperglycaemia lowering effect of the stem bark extract of *Xylopia aethiopica*

The stem bark of *X. aethiopica* at 100, 200 and 400 mg/kg gave 31, 41 and 43 % blood glucose levels reduction at 4 h, respectively indicating a dose dependent effect of the extract that was comparable to glibenclamide (5 mg/kg) only at 200 and 400 mg/kg. Also, significantly higher activity of the extract that was shown by 100 and 200 mg/kg at 0.5-1 h indicated additional extra pancreatic effect of the extract at these doses (Table 5). Highest antihyperglycaemic activity shown by the extract at 4 hours

similar to glibenclamide especially at 200 and 400 mg/kg revealed insulin stimulation as the major mechanism of action of the extract (Table 5). The results of this study showed that the stem bark of *X. aethiopica* is more active than the leaf extract (Tables 4 and 5).

Table 6: Dose dependent hyperglycaemia-lowering effect of *X. aethiopica* root bark extract

Extract/Drug Doses (mg/kg)	Blood glucose level as percentage of T_o (reduction in blood glucose relative to negative control at T_1)				
	0 h	0.5 h	1 h	2 h	4 h
GLU (10 g/kg)	100	97.16±4.02^{b}	81.92±2.99^{b}	75.67±2.12^{b}	72.87±2.20^{b}
XRE (100)	100	73.28 ± 4.8^{a} (24.58 %)	59.93 ± 4.40^{a} (26.84 %)	50.43 ± 4.33^{a} (33.35 %)	41.64 ± 4.25^{a} (43.86 %)
XRE (200)	100	78.17 ± 4.67^{a} (19.55 %)	74.58 ±3.68^{a,b} (8.96 %)	69.70 ± 4.71^{b} (7.89 %)	68.70±4.78^{b} (7.39 %)
XRE (400)	100	77.25 ± 2.9^{a} (20.49 %)	78.79 ±4.58^{b} (3.82 %)	66.71 ±4.80^{b} (11.84 %)	62.91±4.50^{b} (13.67 %)
GLI (5)	100	79.37±4.0^{a} (18.31%)	66.20±2.86^{a} (19.19%)	51.32±3.87^{a} (32.18%)	34.94±3.80^{a} (52.05%)

Data show the mean ±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. **GLU:** 1 % Tween 80 in 10 g/kg glucose (Negative control); **XRE**: *Xylopia aethiopica* root bark, **GLI**: Glibenclamide (Positive Control, 5mg/kg).

Hyperglycaemia lowering effect of the root bark extract of *Xylopia aethiopica*

The root bark extract of *X. aethiopica* at 100 mg/kg showed similar and comparable profile of activity with glibenclamide indicating similar mechanism of action with glibenclamide (Table 6). Both 200 and 400 mg/kg of the extract gave comparable effect at 0.5-4 h that was significantly lower than its 100 mg/kg and the positive control which showed that 100 mg/kg was the most active dose similar to the leaf extract (Tables 4 and 6). However, while 100 mg/kg of the leaf extract gave 10, 13, 10 and 29 % antihyperglycaemic activity at 0.5, 1, 2 and 4 h, respectively, the root bark gave 25, 27, 33 and 44 % activity which showed that the root bark was better at reducing hyperglycaemia than the leaf extract at the same dose of 100 mg/kg (Tables 4 and 6). The antihyperglycaemic effect elicited by the most active doses of the leaf, stem and root bark extracts of *X. aethiopica* in this study (Tables 4, 5 and 6) were significantly higher than the most active dose of its fruit extract (Famuyiwa *et al.,* 2018).

The dose of the plant part extract with the highest activity in the glucose loaded study (Tables 4, 5 and 6) was used in this drug-induced antihyperglycaemic experiment. The results of this study showed that the negative control group of animals were hyperglycaemic for all the period of the study which

Table 7: Anti-diabetic activity of *Xylopia aethiopica* leaf, stem and root bark extracts

Dose in mg/kg	**Day 1**	**Day 4**	**Day 7**	**Day 10**	**Day 14**
GLU	100.00	92.53±5.71^{a}	97.22±4.41^{c}	93.52±4.41^{c}	94.65±5.37^{d}
XLE (100)	100.00	80.64 ± 10.63^{a} (12.85 %)	78.83 ± 15.55^{b} (18.92 %)	52.31 ± 13.91^{b} (44.10 %)	32.59 ± 3.30^{b} (65.57 %)
XSE (200)	100.00	82.07 ± 6.81^{a} (11.30 %)	80.63 ± 15.13^{b} (17.10 %)	42.32 ± 14.18^{a} (54.75 %)	20.61 ± 4.89^{a} (78.23 %)
XRE (100)	100.00	82.82 ± 2.43^{a} (10.49 %)	67.80 ± 2.15^{b} (30.26 %)	54.64 ± 4.02^{b} (41.57 %)	46.39 ± 2.20^{c} (51.99 %)
GLI (5)	100.00	82.67±9.08^{a} (10.66%)	40.41±1.50^{a} (58.43%)	21.84±1.61^{a} (76.65 %)	17.88±1.54^{a} (81.11 %)

Data show the mean ±SEM blood glucose levels at the different time points expressed as percentage of levels at 0 h, n=6. Values with different superscripts within column are significantly different ($p<0.05$), while values with similar superscript are comparable ($p>0.05$): one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test. **GLU** (10g/kg): Glucose 10g/kg (Negative control); **XLE**: *Xylopia aethiopica* leaf extract, **XSE:** *Xylopia aethiopica* stem extract, **XRE:** *Xylopia aethiopica* leaf extract **GLI**: Glibenclamide (Positive Control, 5mg/kg).

confirmed that the diabetic state induced in the rats by the intraperitonial administration of streptozotocin was permanent (Table 7).

Glibenclamide (5 mg/kg) gave a time dependent hyperglycaemia lowering effect of 11, 58, 77 and 81 % activity on days 4, 7, 10 and 14, respectively by causing stimulation of insulin by the remaining β cells of the pancreas of the rats. Similar to glibenclamide, the antihyperglycaemic activity elicited by the extract of the various parts of *X. aethiopica* was time dependent confirming insulinotropic ability of the extracts that was suggested by the results of the glucose loaded antihyperglycaemic model (Table 4, 5, 6 and 7). The leaf, stem and root bark extracts were comparable in activity on days 4 and 7 of the experiment while the stem bark was significantly more active than others on days 10 and 14 (Table 7). Also, the leaf extract gave a significantly better activity than the root extract on day 14.

4. Conclusion

This study concluded that the leaf, stem and root bark methanolic extracts of *Xylopia aethiopica* had hyperglycaemic-lowering activity that was demonstrated in both glucose and streptozotocin induced hyperglycaemic models. The stem bark extract of *Xylopia aethiopica* was the most active plant part in the antihyperglycaemic models used. The leaf and stem bark extracts may cause hypoglycaemia at higher doses while the root extract did not show element of hypoglycaemia at all the tested doses.

Acknowledgements

The authors would like to thank the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife for providing conducive environment for the breeding of the mice used for the experiment.

References

Adebajo, A.C., Ayoola, M.D., Odediran, S.A., Aladesanmi, A.J., Schmidt, T.J.and Verspohl, E.J. (2013a). Evaluation of ethnomedical claim iiia: Anti-hyperglycaemic activities of *Gongronema latifolium* root and stem. *Journal of Diabetes,* 5(3): 336-43.

Adebajo, A.C., Ayoola, M.D., Obagbemi. O.R., Obuotor, E.M., Ogunsina, M.O. and Verspohl, E.J. (2013b). Antihyperglycaemic and antioxidant activities of *Eugenia uniflora* leaf: Evaluation of ethnomedical claims IV. *Ife Journal of Science and Technology,* 1(1): 1-18.

Ayoola, M.D., Adebajo, C.A., Zotor, F.B. and Pinkoane, M.G. (2019). Justifying Antidiabetic

Ethnomedicinal Claim of *Senecio biafrae* through its Antihyperglycemic and Antioxidant Activities. *Annals of Complementary and Alternative Medicine*, 1(2): 1-8.

Ayoola, M.D., Balogun, J.O., Famuyiwa, F.G., Yeboah, S.O. and Famuyiwa, S.O. (2017). Isolation and characterization of 2-hydroxy-3-[4-hydroxyphenyl]-2-propenoic acid and 4-bromophenol from anti-diabetic extract of the root bark of *Uvaria afzelii*. *South African Journal of Botany*, 112:527-532.

Ayyanar M, Ignacimuthu, S. (2009). Herbal medicines for wound healing among tribal people of southern India: Ethnobotanical and scientific evidences. International journal of Applied Research in Natural Product. 2(3) 29-42.

Bello, O.I, Ayoola, M.D, Obembe, O. and Akinwunmi, K.F. Antidiabetic and Toxicity Studies of the Extract of Four Nigerian Medicinal Plants (2022). *European Journal of Medicinal Plants*, 33(11):32-45.

Coli, A. U. O. E. (2013). Antimicrobial activity of Xylopia aethiopica (UDA) on Escherichia coli and Staphylococcus aureus isolates from gastroenteritic patients. *International Journal of Life Sciences Biotechnology and Pharma Research*, *2*, 330-338.

Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies, National Research Council of the National Academies, Guide for the Care and Use of Laboratory Animals, 8th edn, The National Academies Press, Washington, DC, 2011.

Dahlén, A. D., Dashi, G., Maslov, I., Attwood, M. M., Jonsson, J., Trukhan, V., and Schiöth,H. B. (2022). Trends in antidiabetic drug discovery: FDA approved drugs, new drugs in clinical trials and global sales. *Frontiers in Pharmacology*, *12*, 4119.

Erhirhie, E. O., and Moke, G. E. (2014). Xylopia aethiopica: A review of its ethnomedicinal, chemical and pharmacological properties. *Am J Pharm Tech Res*, *4*, 21-37.

Famuyiwa, F. G., Famuyiwa, S. O., and Aladesanmi, A. J. (2017). Larvicidal activities of the kaurane diterpenes from the fruit of *Xylopia aethiopica* (A. Dunal) Rich. *Nigerian Journal of Natural Products and Medicine*, *21*, 39-44.

Famuyiwa, F. G., Ayoola, M. D., Famuyiwa, S. O., and Aladesanmi, A. J. (2018). Hyperglycaemia lowering effect of Kaurane Diterpenoids from the fruits of *Xylopia aethiopica* (A. Dunal) Rich. *International Journal of Medicinal Plants and Natural Products*, *4*(3), 11-19.

Gbadamosi, I. T., and Erinoso, S. M. (2016). A review of twenty ethnobotanicals used in the management of breast cancer in Abeokuta, Ogun State, Nigeria. *African Journal of Pharmacy and Pharmacology*, *10*(27), 546-564.

Hwang, L. C., Juliani, H. R., Govindasamy, R., and Simon, J. E. (2020). Traditional botanical uses of non-timber forest products (NTFP) in seven counties in Liberia. In *African Natural Plant Products, Volume III: Discoveries and Innovations in Chemistry, Bioactivity, and Applications* (pp. 3-43). American Chemical Society.

Ito, E., Ukpohwo, R., and Okiriguo, I. (2018). Insecticidal activity of xylopia aethiopica (family; annonaceae) against callosobruchus maculatus (f)(coleoptera: bruchidae) and sitophilus oryzae (coleoptera: curculionidae). *Journal of Biological Studies*, *1*(3), 106-115.

Jayaraman, R., Subramani, S., Abdullah, S. H. S., and Udaiyar, M. (2018). Antihyperglycemic effect of hesperetin, a citrus flavonoid, extenuates hyperglycemia and exploring the potential role in antioxidant and antihyperlipidemic in streptozotocin-induced diabetic rats. *Biomedicine and Pharmacotherapy*, *97*, 98-106.

Kar A, Choudhary BK, Bandyopadhyay NG. Preliminary studies on the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. J Ethnopharmacol. 1999; 64(2):179-184.

Luzi L, Pozza G. Glibenclamide: an old drug with a novel mechanism of action? Acta Diabetol. 1997;34(4):239- 244.

Kim, J., Lee, H. J., and Jo, M. W. (2022). Health-related quality of life instrument with 8 items for use in patients with type 2 diabetes mellitus: a validation study in Korea. *Journal of Preventive Medicine and Public Health*, *55*(3), 234.

Kifle, Z. D., Bayleyegn, B., Tadesse, T. Y., and Woldeyohanins, A. E. (2021). Prevalence and associated factors of herbal medicine use among adult diabetes mellitus patients at government hospital, Ethiopia: An institutional-based cross-sectional study. *Metabolism Open*, *11*, 100120.

Krentz, A. J. (2006). Comparative safety of newer oral antidiabetic drugs. *Expert Opinion on Drug Safety*, *5*(6), 827-834.

Liyanagamage, D. S. N. K., Jayasinghe, S., Attanayake, A. P., and Karunaratne, V. (2020). Medicinal plants in management of diabetes mellitus: An overview. *Ceylon Journal of Science*, *49*(1), 3-11.

Mohammed, A., Koorbanally, N. A., Islam, M. S. (2016). Anti-diabetic effect of *Xylopia aethiopica* (Dunal) A. Rich.(Annonaceae) fruit acetone fraction in a type 2 diabetes model of rats. *Journal of Ethnopharmacology*, *180*, 131-139.

Tuei, V. C., Maiyoh, G. K., and Ha, C. E. (2010). Type 2 diabetes mellitus and obesity in sub-Saharan Africa. *Diabetes/metabolism research and reviews*, *26*(6), 433-445.

Woode, E., Ameyaw, E. O., Boakye-Gyasi, E., and Abotsi, W. K. (2012). Analgesic effects of an ethanol extract of the fruits of Xylopia aethiopica (Dunal) A. Rich (Annonaceae) and the major constituent, xylopic acid in murine models. *Journal of pharmacy and bioallied sciences*, *4*(4), 291.

Yazdanpanah, S., Rabiee, M., Tahriri, M., Abdolrahim, M., Rajab, A., Jazayeri, H. E., and Tayebi, L. (2017). Evaluation of glycated albumin (GA) and GA/HbA1c ratio for diagnosis of diabetes and glycemic control: A comprehensive review. *Critical reviews in clinical laboratory sciences*, *54*(4), 219-232.