International Journal of Medicinal Plants and Natural Products (IJMPNP)
Volume 4, Issue 3, 2018, PP 11-19
ISSN No. (Online) 2454-7999
DOI: http://dx.doi.org/10.20431/2454-7999.0403002
www.arcjournals.org

Hyperglycaemia Lowering Effect of Kaurane Diterpenoids from the Fruits of *Xylopia aethiopica* (A. Dunal) Rich

Funmilayo Gladys Famuyiwa[1*], Marcus Durojaye Ayoola[1], Samson Oluwaseyi Famuyiwa[2], Adetunji Joseph Aladesanmi[1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria*

[2]*Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria*

Corresponding Author:** ***Funmilayo Gladys Famuyiwa, *Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria*

Abstract: *This study evaluated the hyperglycaemia lowering activity of the methanolic extract, partition, column fractions and two isolated compounds from the fruit of Xylopia aethiopica in glucose-induced hyperglycaemic rats using glibenclamide (5 mg/kg) as positive control. The extract showed highest activities at 400 mg/kg while its dichloromethane and aqueous fractions showed comparable activity to glibenclamide (5 mg/kg). Kaurenoic and xylopic acids were isolated from active column fraction of dichloromethane and they showed comparable activity to glibenclamide (5 mg/kg) at 10 and 20 mg/kg. The study justified the anti-diabetic claim of Xylopia aethiopica fruits and showed kaurenoic acid as a lead drug.*

Keywords: *Xylopia aethiopica, extract, hypoglyceamia, partition, column chromatography, kaurenoic acid, xylopic acid.*

1. INTRODUCTION

Plants have been used for medicinal purposes long before recorded history in different cultures of the world (1). The World Health Organization (WHO) gave a list of about 21,000 plants used for medicinal purposes around the world (21). Plants have the ability to synthesize a wide variety of chemical compounds that possess pharmacological properties and defend against attack from predators such as insects, fungi and herbivorous animals (25,40). About 12,000 of such compounds, representing less than 10 % of the total, have been isolated. These compounds have been found to exert their effects on the human body through mechanisms that are similar to those already established in conventional drugs revealing that herbal medicines may be as effective as the latter (25,41). Furthermore, the plant kingdom is believed to hold many new drug templates; hence, the continued investigation into ethno-medicinal plants (22).

Xylopia aethiopica is a slim, tall, evergreen aromatic tree; widely distributed in the humid forest zones of West Africa especially along rivers in the drier area of the region (39) and West African rainforest from Senegal and to Sudan in Eastern Africa down to Angola in Southern Africa (12). The dark brown, small and twisted bean-pods fruits are an important item of trade and the most widely used of its various morphological parts. Its parts are used in ethno-medicine to treat various ailments including skin infections, candidiasis, dyspepsia, cough, dysentery, biliousness, bronchitis, rheumatism, malaria, uterine fibroid, amenorrhea, boil, sore, fever, respiratory ailments, lumbago and neuralgia. It is also used as a mouthwash to treat toothaches (12,19,28,44). It is also used in the management of diabetes (16,15,23,37). Additionally, there are some anti-diabetic recipes such as ‘‘Okudiabet’’ in Nigeria and Togo that contained *X. aethiopica* fruit as one of their ingredients (34). Pharmacological activities such as insecticidal (6), antioxidant (5,24,27), anti-helminthic (38), anti-asthmatic (32), ocular effect (43), analgesic (45), anti-diabetic and associated complications (29,34), effect on reproductive hormones (7), antimicrobial (8,11,14,31,35,42) and influence on some hematological and biochemical profiles (30) have been reported for the plant. These activities were attributed to a wide variety of secondary metabolites it contains (18). This study reports the hyperglycaemia lowering activity of the methanolic extract and the compounds isolated from its active partitioned fraction.

2. MATERIALS AND METHODS

2.1. General Experimental Procedures

IR spectra (film) were registered on a Shimadzu FT-IR 8300 spectrophotometer. Melting points were determined in a capillary on a Galenkamp melting point apparatus. 1D (^{1}H, ^{13}C, DEPT135) and 2D NMR spectra (COSY, HSQC, HMBC) were recorded using a BrukerAvance DPX 300 instruments using $CDCl_3$, as solvent and internal standard. Chemical shifts are given in δ and coupling constants (*J*) in Hz. The HREIMS were determined on a Waters GCT-premier spectrometer. The fragments were described as a relation between atomic mass units and charge (*m/z*) and the relative abundance in a percentage of the base peak intensity. TLC analyses were carried out on 0.25 mm thick pre-coated Merck TLC silica gel plates (Silica 60 F_{254}). Spots on TLC plates were visualized under UV light (254 and 365 nm) and further sprayed with 20% sulphuric acid, followed by heating. Column chromatography was performed using silica gel (60-200 mesh).

2.2. Plant Collection, Extraction and Isolation

The fruits were collected at Ifewara via Ile-Ife, Osun State, Nigeria. Specimen was deposited at the Herbarium of Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife under the reference number IFE 16926. The fruits were air-dried and powdered using an electric mill (Christy Norris). The powdered fruit (1.70 kg) was extracted with methanol at room temperature for 3 days, with agitation. The solution was filtered and the marc was re-extracted twice. The filtrate was bulked and concentrated *in vacuo* to obtain the methanolic extract (**MXA**, 475.06 g).

The extract (450.00 g) was suspended in water (150 mL) and partitioned into petroleum ether (60-80^{o}C, 6 x 300 mL) and dichloromethane (9 x 300 mL). Each fraction was concentrated *in vacuo* to give petroleum ether (**PXA**) 129.84 g, DCM (**DXA**) 248.78 g and aqueous (**AXA**) 55.04 g as their final weight respectively. The dichloromethane fraction was subjected to column chromatography with gradient elution using solvent systems of n-Hex, DCM, EtOAc and MeOH. The separation was monitored by TLC and 77 fractions collected were bulked to give 8 column fractions **DX_1** (58.13 g), **DX_2** (7.39 g), **DX_3** (32.85 g), **DX_4** (55.02 g), **DX_5** (19.92 g), **DX_6** (3.41 g), **DX_7** (9.69 g) and **DX_8** (1.05 g). Fraction 1 (**DX_1**, 58.13 g) eluted with *n*-Hex/DCM 75:25 to *n*-Hex/DCM 25:75 was further subjected to chromatographic separations and it afforded kaur-16-en-19-oic acid **(DC_1,** 3.99 g) and 15-acetoxy kaur-16-en-19-oic acid (**DC_2**, 8.26 g).

2.3. Animals

Healthy Wistar albino rats of either sex (180 g, average weight) that were bred under standard conditions (temp. 27±3° C, relative humidity 65 %, natural 12 h day–night) and housed in different cages in the animal house, Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile-Ife, Nigeria, were used for the experiments. They were acclimatized for at least 5 days before experiments commenced and fed on a standard pellet diet (Bendel Feeds, Benin-City, Nigeria), with water given *ad libitum*. All animal experiments conformed to the *Guide for the Care and Use of Laboratory Animals* (13).

2.4. Effect of the Extract on Normal Rats

Groups of five normoglycaemic rats were fasted for 24 hours and ingested (*p.o.*) with either 1 % Tween 80 in normal saline (negative control), or the methanolic fruit extract (100, 200, 400 mg/kg) or glibenclamide (5 mg/kg, positive control). A drop of blood, taken from the tip of the tail of each rat at 0.0, 0.5, 1.0, 2.0 and 4.0 h after administration of the test agents, was dropped onto a glucometer strip and the blood glucose (bg) levels were read off directly. The blood glucose levels at 0.0 h (T_o) were taken as 100 %, while those at other times were expressed as percentages of these values (4,9).

2.5. Hyperglycaemia Lowering Assay of the Extract, Fractions and Isolates

A glucose tolerance test was performed by giving glucose (10 g/kg, *p.o.*) to 24 hour fasted normal rats and those that were hyperglycaemic [blood glucose level ≥ 7.0 mmol/L (126 mg/dL)] after 0.5 hour (T_o) were divided into groups of five and administered (*p.o.*) with 1 % Tween 80 in normal saline (negative control), glibenclamide (5 mg/kg, positive control) and extract (100, 200, 400 mg/kg). The bg 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 extract and the drug.

The partition fractions were subjected to glucose-lowering assay as described for the extract. Column chromatography fractions obtained from the dichloromethane fraction (**DX_1-DX_8**) were also tested. The column fraction **DX_8** could not be tested due to its low weight. The isolated compounds **DC_1** and **DC_2** were subjected to hyperglycaemia lowering assay as described for the extract.

2.6. Statistical Analysis

Data represent mean ± SEM and n = 5 for the animals in the group. They were analysed with One Way Analysis of Variance (ANOVA), followed by Student-Newman-Keuls post-hoc tests, using Graph Pad®Instat, version 5.0 (GraphPad Software Inc., San Diego, USA). $P < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1. Hypoglycaemic Effect of the Extract

The extract at all the tested doses (100, 200 and 400 mg/kg), gave a non-time dependent hypoglycaemic activity that was significantly lower ($p<0.05$) than that of glibenclamide (5 mg/kg) at all-time points apart from 400 mg/kg that gave comparable activity ($p>0.05$) at 4 h (Table 1). This suggested that *X. aethiopica* fruit and its extract may not precipitate hypoglycaemic coma in non-diabetic subjects. However, 31 % and 44 % blood glucose level reduction caused by 400 mg/kg of this extract at 2 h and 4 h respectively may dictate caution in its use at higher doses by normal human subjects (Table 1). The leaf aqueous and methanolic extracts of *Nauclea latifolia* and *Eugenia uniflora*, respectively that similarly lacked significant hypoglycaemic effect in normal rats have been reported safe (3,20).

Table1. *Dose related hypoglycaemic effect of X. aethiopica fruit extract*

Doses of Extract /Drug (mg/kg)	0 h	0.5 h	1 h	2 h	4 h
NSA	100.00	115.64 ± 12.08^{b}	108.22 ± 8.62^{c}	101.25 ± 10.30^{d}	102.81 ± 5.23^{c}
MXA (100)	100.00	91.83 ± 2.11^{b} (20.59%)	$91.69\pm1.53^{b,c}$ (15.27%)	$81.52\pm1.44^{c,d}$ (19.49%)	83.66 ± 1.14^{b} (18.63%)
MXA (200)	100.00	87.10 ± 3.03^{b} (24.68%)	85.50 ± 1.85^{b} (20.99%)	$72.07\pm4.93^{b,c}$ (28.82%)	84.58 ± 1.66^{b} (17.73%)
MXA (400)	100.00	89.39 ± 4.22^{b} (22.70%)	84.89 ± 3.78^{b} (21.56%)	70.40 ± 4.17^{b} (30.47%)	57.85 ± 1.14^{a} (43.73%)
GLI (5)	100.00	68.04 ± 6.88^{a} (41.16%)	50.22 ± 4.14^{a} (53.59%)	40.02 ± 2.36^{a} (60.47%)	57.76 ± 4.41^{a} (43.82%)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_0), n = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$), One-way analysis of variance followed by the Student–Newman–Keuls' test). ***NSA****: 1 % of Tween 80 in normal saline (negative control);* ***MXA:*** *Xylopia aethiopica extract:* ***GLI****: Glibenclamide (positive control).*

3.2. Hyperglycaemia Lowering Effect of the Extract

The glucose-induced hyperglycaemic rats administered with normal saline (negative control) demonstrated time dependent blood glucose level reduction up to the fourth hour (Table 2) due to the homeostatic regulatory mechanism and confirmed that their pancreases were functioning well (4,9). Glibenclamide (5 mg/kg) similarly showed a time dependent hyperglycaemia lowering activity that was highest at 4 h confirming its major insulin stimulating and minor extrapancreatic modes of action (26). The extract was devoid of activity at 100 mg/kg while its 200 mg/kg gave a comparable activity to glibenclamide at 0.5-2 h. At 400 mg/kg however, its activity was comparable to the positive control at 0.5-4 h suggesting similar mechanism of action as glibenclamide at this dose (Table 2).

Table2. *Dose related hyperglycaemia lowering effect of X. aethiopical fruit extract*

Doses of Extract /Drug (mg/kg)	0 h	0.5 h	1 h	2 h	4 h
GLU (10 g/kg)	100.00	83.79±3.81^{a}	85.89±0.50^{b}	76.45±1.71^{b}	74.18±1.97^{c}
MXA (100)	100.00	85.74±4.59^{a} (-2.33%)	80.32±2.77^{a,b} (6.49%)	76.16±0.74^{b} (0.38%)	74.36±3.65^{c} (-0.24%)
MXA (200)	100.00	83.10±1.60^{a} (0.82%)	70.47±4.88^{a} (17.95%)	66.85±3.57^{a} (12.56%)	65.74±3.08^{b,c} (11.38%)
MXA (400)	100.00	84.34±1.37^{a} (-0.66%)	76.84±2.55^{a} (10.54%)	68.87±2.21^{a} (9.91%)	59.86±2.50^{a,b} (19.30%)
GLI (5)	100.00	75.64±6.73^{a} (9.73%)	70.68±6.86^{a,b} (17.71%)	58.32±6.44^{a} (23.72%)	45.27±6.88^{a} (38.97%)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_0), n = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$), One-way analysis of variance followed by the Student–Newman–Keuls' test). ***GLU:*** *1 % of Tween 80 in glucose (negative control);* ***MXA:*** *Xylopia aethiopica extract;* ***GLI:*** *Glibenclamide (positive control).*

3.3. Hyperglycaemia Lowering Effect of the Partition Fractions

The most active hyperglycaemia lowering dose (400 mg/kg) of the extract (Table 2) was used in testing this effect in its partition fractions. Petroleum ether fraction (**PXA**) lacked significant activity at 0.5-2 h but gave 24 % blood glucose level reduction at 4 h that was comparable to the activity of its extract and significantly lower than that of glibenclamide, 5 mg/kg, (Table 3). The activity of glibenclamide (5 mg/kg), dichloromethane (**DXA**) and aqueous (**AXA**) fractions were comparable at 0.5-4 h indicating that the constituents that were responsible for the activity observed in the extract were mostly concentrated in these fractions and were insulinotropic in action (Table 3).

Table3. *Hyperglycaemia lowering effect of the partition fractions (400 mg/kg) of X. aethiopica fruit extract*

Doses of Extract /Drug (mg/kg)	0 h	0.5 h	1 h	2 h	4 h
GLU (10 g/kg)	100.00	83.79±3.81^{a}	85.89±0.50^{b}	76.45±1.71^{c}	74.18±1.97^{c}
MXA	100.00	84.34±1.37^{a} (-0.66%)	76.84±2.55^{b} (10.54%)	68.87±2.21^{b,c} (9.91%)	59.86±2.50^{b} (19.30%)
PXA	100.00	89.25±2.23^{a} (-6.52%)	82.43±2.50^{b} (4.05%)	75.99±2.40^{c} (0.60%)	55.83±1.17^{b} (24.74%)
DXA	100.00	90.21±1.42^{a} (-7.66%)	75.23±7.24^{a,b} (12.41%)	58.48±4.99^{a,b} (23.51%)	46.60±1.62^{a} (37.18%)
AXA	100.00	79.20±2.19^{a} (5.48%)	62.04±1.43^{a} (27.77%)	56.63±3.15^{a} (25.93%)	46.02±1.53^{a} (37.96%)
GLI (5)	100.00	75.64±6.73^{a} (9.73%)	70.68±6.86^{a,b} (17.71%)	58.32±6.44^{a,b} (23.72%)	45.27±6.88^{a} (38.97%)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_0), n = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$), One-way analysis of variance followed by the Student–Newman–Keuls' test). ***GLU:*** *1 % of Tween 80 in glucose (negative control);* ***MXA:*** *Xylopia aethiopica extract;* ***PXA:*** *Petroleum ether fraction;* ***DXA:*** *Dichloromethane fraction****;*** ***AXA:*** *Aqueous fraction;* ***GLI:*** *Glibenclamide (positive control).*

3.4. Hyperglycaemia Lowering Effect of the Column Fractions

Glibenclamide (5 mg/kg) and column fractions **DX_1-DX_3** (400 mg/kg), had similar profile of activity that was time dependent (Table 4). Hence, they may have the same little extrapancreatic and high insulinotropic activities as glibenclamide (26). However, highest hyperglycaemia lowering activity at 4 h for all the fractions and glibenclamide, may suggest insulin release as their main mechanism of action (3,4). Fractions **DX_1**, **DX_2**, **DX_3** and **DX_7** were significantly more active than the negative control and gave comparable ($p > 0.05$) activity with glibenclamide (5 mg/kg) especially at 1-4 h with **DX_3** showing the highest percentage glucose lowering effect of 50 % at 4 h while **DX_7** showed the

least effect (17 %) at the same hour (Table 4). Fractions **DX_4** and **DX_5** with percentage blood glucose level reductions of 8 and 10 respectively, were the least active while **DX_6** showed similar extra-pancreatic effect with glibenclamide but lacked its time dependent activity. Therefore, the order of hyperglycaemia lowering effect at 4 h based on their percentage blood glucose level reductions therefore is:**DX_3> DX_1> DX_2> GLI >DXA> DX_6> DX_7> DX_5> DX_4.** This indicated that the constituents responsible for the activity in the dichloromethane fraction were concentrated in column fractions **DX_1**, **DX_2** and **DX_3** (Table 4). Due to the low weight of **DX_2** and comparable activity of **DX_3** with **DX_1**, only **DX_1** was further purified with a view to isolate the active constituents.

Table4. *Hyperglycaemia lowering effect of the column fractions of **DXA** (400 mg/kg) of X. aethiopica fruit*

Doses of Extract /Drug (mg/kg)	0 h	0.5 h	1 h	2 h	4 h
GLU (10 g/kg)	100.00	83.79±3.8^{b}	85.89±0.50^{b}	76.45±1.71^{b}	74.18±1.97^{b}
DXA	100.00	90.21±1.42^{a,b} (-7.66%)	75.23±7.24^{a,b} (12.41%)	58.48±4.99^{a} (23.51%)	46.60±1.62^{a} (37.18%)
DX_1	100.00	87.36±1.40^{a,b} (-4.26%)	74.39±5.08^{a,b} (13.39%)	60.16±1.41^{a} (21.31%)	41.90±1.19^{a} (43.52%)
DX_2	100.00	86.41±6.12^{a,b} (-3.13%)	63.79±2.80^{a} (25.73%)	50.48±8.66^{a} (33.97%)	44.63±1.60^{a} (39.84%)
DX_3	100.00	82.07±1.34^{a,b} (2.05%)	68.63±0.92^{a} (20.10%)	54.02±1.12^{a} (29.34%)	37.46±2.43^{a} (49.50%)
DX_4	100.00	112.58±15.09^{a,b} (-34.36%)	94.45±10.07^{a,b} (-9.97%)	71.36±9.73^{a,b} (6.66%)	68.36±4.94^{b} (7.85%)
DX_5	100.00	88.20±1.13^{a,b} (-5.26%)	78.53±2.27^{a} (8.57%)	66.83±1.60^{a} (12.58%)	66.67±3.19^{b} (10.12%)
DX_6	100.00	78.60±3.18^{a,b} (6.19%)	70.97±4.70^{a} (17.37%)	67.75±4.01^{a,b} (11.38%)	56.48±1.27^{a} (23.86%)
DX_7	100.00	87.76±0.70^{a,b} (-4.74%)	82.70±1.38^{a,b} (3.71%)	65.91±3.15^{a} (13.79%)	61.67±2.04^{a} (16.86%)
GLI (5)	100.00	75.64±6.73^{a} (9.73%)	70.68±6.86^{a} (17.71%)	58.32±6.44^{a} (23.72%)	45.27±6.88^{a} (38.97%)

*Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_0), n = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test). GLU: glucose in < 1 % of Tween 80 in normal saline (negative control); **DXA**: Dichloromethane fraction; **DX_1-DX_7**: Column fractions of **DXA**; **GLI**: Glibenclamide.*

3.5. Structural Elucidation of Isolated Compounds

Compound **1** (**DC_1**, 3.99 g) with melting point of 170-172 °C was isolated from the fraction eluted with n-Hex/DCM 40:60 as a white powder. The infrared spectrum showed bands at 3368 cm^{-1}, 1705 cm^{-1} and 1689 cm^{-1} indicated OH, C=O and C=C respectively. The HREIMS showed a molecular ion peak at *m/z* 302.2252 (calc 302.2246), consistent with a molecular formula $C_{20}H_{30}O_2$, which was supported by the analyses of the ^{13}C and DEPT 135 NMR spectra. The mass spectrum revealed the base peak as the molecular ion at *m/z* 302.2252 (100 %). This indicated a stable molecular ion. The loss of methyl group from the base peak resulted in *m/z* 287.2023 (50 %) and subsequent loss of carbon dioxide gave *m/z* 243.1405 (50 %). Loss of two methyl groups from *m/z* 243.1405 was responsible for *m/z* 213.1697 (60 %) observed.

Compound **2** (**DC_2**, 8.26 g) with melting point of 230-232 °C was isolated from the fraction with n-Hex/DCM 40:60 as a white powder. The infrared spectrum showed bands at 3280 cm^{-1}, 1705 cm^{-1} and 1723 cm^{-1} indicated OH, C=O (acid) and C=O (ester) respectively. The band at 1272 cm^{-1} indicated C–O–C (ester). The HREIMS showed a molecular ion peak at *m/z* 360.2312 (calc 360.2301) that is consistent with a molecular formula $C_{22}H_{32}O_4$ which was supported by the analyses of the ^{13}C and DEPT 135 NMR spectra. The mass spectra showed a base peak at *m/z* 300.2113(100 %) due to the loss of methyl and carboxylic groups (acetic acid moiety) from the molecular ion 360.2312 (15 %). The subsequent loss of methyl group from the base peak resulted in *m/z* 285.1875 (70 %).

The NMR spectroscopic data of the compounds compared very well with the published data (17,36). Thus compound **1** (**DC_1**) was identified as kaur-16-en-19-oic acid (kaurenoic acid) while compound **2** (**DC_2**) was identified as 15-acetoxy kaur-16-en-19-oic acid (xylopic acid).

3.6. Hyperglycaemia Lowering Effects of the Isolated Compounds

The compound **1**, **DC_1**, gave time and dose dependent hyperglycaemia lowering activity up to the fourth hour at both 10 and 20 mg/kg while compound **2**, **DC_2**, gave time dependent activity only at 20 mg/kg (Table 5). Compound **2**, **DC_2**, showed activity up to the fourth hour only at 20 mg/kg and gave comparable activity to glibenclamide (5 mg/kg) at the fourth hour while compound **1**, **DC_1**, gave comparable activity to glibenclamide (5 mg/kg) at all times for both tested doses with significantly better activity than that of the glibenclamide (5 mg/kg) at the fourth hour for 20 mg/kg (Table 5). This indicated that **DC_1** had similar mechanism of action as glibenclamide while **DC_2** may have slightly different mechanism of action from glibenclamide. Their highest activity at 4 h indicated insulin stimulation as their main mechanism of action. Furthermore, they were twenty to forty times more active than the extract, dichloromethane and column bulked fraction.

Table5. *Hyperglycaemia-lowering effect of the isolates (**DC_1** and **DC_2**)*

Doses of Extract /Drug (mg/kg)	0 h	0.5 h	1 h	2 h	4 h
GLU (10 g/kg)	100.00	83.79±3.8^{b}	85.89±0.50^{b}	76.45±1.71^{b}	74.18±1.97^{b}
DC_1 (10)	100.00	74.66±3.99^{a,b} (10.90%)	69.68±3.75^{a} (18.87%)	58.71±4.21^{a} (23.20%)	42.84±2.68^{a} (42.25%)
DC_1 (20)	100.00	79.30±3.93^{a,b} (5.36%)	66.75±7.83^{a} (22.28%)	51.08±6.81^{a} (33.19%)	36.28±5.24^{a} (51.09%)
DC_2 (10)	100.00	76.17±5.22^{a,b} (9.09%)	65.94±4.21^{a} (23.23%)	63.77±5.05^{a} (16.59%)	54.25±4.82^{a} (26.87%)
DC_2 (20)	100.00	88.67±2.05^{a,b} (-5.82%)	77.73±2.43^{a} (9.50%)	63.70±4.03^{a} (16.68%)	46.37±4.62^{a} (37.49%)
GLI (5)	100.00	75.64±6.73^{a} (9.73%)	70.68±6.86^{a} (17.71%)	58.32±6.44^{a} (23.72%)	45.27±6.88^{a} (38.97%)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_0), n = 5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test). ***GLU**: glucose in < 1 % of Tween 80 in normal saline (negative control);* ***DC_1**: kaurenoic acid;* ***DC_2**: xylopic acid;* ***GLI**: Glibenclamide.*

4. Conclusion

This study established the hyperglycaemia lowering effect of kaurane diterpenoids from the fruits of *Xylopia aethiopica*. It was shown that purification increased activity in this case. Kaurenoic acid (**1**) showed a comparable reduction of blood glucose level of 42.25 % at 10 mg/kg as against 38.97 % of glibenclamide at 5 mg/kg and better than xylopic acid (**2**) at doses tested. Kaurenoic acid (**1**) at 20 mg/kg demonstrated significantly and considerable reduction of blood glucose than glibenclamide (5 mg/kg). This suggested that kaurenoic acid (**1**) could be a lead drug in the management of diabetes mellitus (10).

Acknowledgements

Authors are very grateful to Network for Analytical and Biological Assay for Southern Africa (NABSA) for three month research visit of SOF to University of Botswana.

References

[1] Abel, C. and K. Busia. 2005. An exploratory ethnobotanical study of the practice of herbal medicine by the Akan peoples of Ghana. *Alternative Medicine Review,* **10**(2): 111–122.

[2] Adaramoye, O.A., Sarkar, J., Singh, N., Meena, S., Changkija, B., Yadav, P.P., Kanojiya, S. and S. Sinhar. 2011. Antiproliferative action of *Xylopia aethiopica* fruit extract on human cervical cancer cells. *Phytotherapy Research,* **25**(10): 1558–1563.

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

[4] Adebajo, A.C., Ayoola, M.D., Odediran, S.A., Aladesanmi, A.J., Schmidt, T.J. and E.J. Verspohl. 2013a. Evaluation of ethnomedical claim III: Antihyperglycaemic activities of *Gongronema latifolium* root and stem. *Journal of Diabetes*, **5**: 336-343.

[5] Adefegba, S. and G. Oboh. 2012. Effect of diets supplemented with Ethiopian pepper [*Xylopia aethiopica* A. Rich (Annonaceae)] and Ashanti pepper [*Piper guineense* Schumach. etThonn (Piperaceae)] on some biochemical parameters in normal rats. *Asian Pacific Journal of Tropical Biomedicine*, **2**(2): 558-566.

[6] Adewoyin, F.B., Odaibo, A.B. and C.O. Adewunmi. 2006. Mosquito repellent activity of *Piper guineense* and *Xylopiaa aethiopica* fruits oils on *Aedes aegypti*. *African Journal of Traditional Complementary and Alternative Medicines*, ***3***: 79-83.

[7] Adienbo, M.H, Nwafor, A. and S. Iwuji. 2011. Effect of the aqueous fruit extract of *Xylopia aethiopica* on the reproductive hormones in male guinea pig. *Global Journal of Pure and Applied Sciences*, ***17***(2): 137-139.

[8] Asekun, O.T. and B.A. Adeniyi. 2004. Antimicrobial and cytotoxic activities of the fruit essential oil of *Xylopia aethiopica* from Nigeria. *Fitoterapia*, ***75***(3-4): 368-370.

[9] Ayoola, M.D., Balogun, J.O., Famuyiwa, F.G., Yeboah, S.O. and S.O. Famuyiwa. 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 (In press).

[10] Bresciani, L.F.V., Yunes, R.A., Bürger, C., Oliveira, L.F.D., Bóf, K.L. and V. Cechinel-Filho. 2004. Seasonal variation of kaurenoic acid, hypoglycemic diterpene present in *Wedelia paludosa*. *Z. Naturforsch*, **59c**: 229-232.

[11] Boakye-Yiadom, K., Fiagbe, N.I.Y. and J. Ayim. 1977. Antimicrobial properties of some West African Medicinal Plants IV. Antimicrobial activity of xylopic acid and other diterpenes from the fruits of *Xylopia aethiopica* (Annonaceae). *Llyodia*, ***40***: 543-545.

[12] Burkill, H.M. The useful plants of west tropical Africa. Kew Publishing, Royal botanical gardens, Kew. 1985, pp857.

[13] 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.

[14] David, O., Ojo, O., Olumekun, V. and O. Famurewa. 2014. Antimicrobial activities of essential oils from *Huracrepitans* (L.), *Monodora myristica* (Gaertn Dunal) and *Xylopia aethiopica* (Dunal A. Rich) Seeds. *British Journal of Applied Science and Technology*, **4**(23): 33-39.

[15] Diallo, A., Traore, M.S., Keita, S.M., Balde, M.A., Keita, A., Camara, M., Miert, S.V., Pieters, L. and A.M. Balde. 2012. Management of diabetes in Guinean traditional medicine: an ethnobotanical investigation in the coastal low lands. *Journal of Ethnopharmacology*, **144**: 353–361.

[16] Diéye, M.A., Sarr, A., Diop, S.N., Ndiaye, M., Sy, G.Y., Diarra, M., Gaffary, I.R., Sy, A.N. and B. Faye. 2008. Medicinal plants and the treatment of diabetes in Senegal: survey with patients. *Fundamental Clinical Pharmacology*, **22**: 211–216.

[17] Ekong, D. E. U. and A.U. Ogun. 1968. Chemistry of the constituents of *Xylopia aethiopica*. The structure of xylopic acid, a new diterpene acid. *Journal of Chemical Society C*: *Organic*, **1**: 311.

[18] Fleischer, T.C. 2003. *Xylopia aethiopica* (Dunal) A. Rich.: A chemical biological perspective. *Journal of Science and Technology*, ***23***(2): 24-31.

[19] Ghana Herbal Pharmacopoiea.. Policy research and strategic planning institute (PORSPI). The Advent Press, Accra. 1992, pp. 150-152.

[20] Gidado A., Ameh D.A. and S.E. Atawodi. 2005. Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan induced diabetic rats. *African Journal of Biotechnology.***4**: 91-93.

[21] Grover, J.K., Yadav, S. and V. Vats. 2002. Medicinal plants of India with anti-diabetic potential. *Journal of Ethnopharmacology*, ***81***: 81–100.

[22] Gurib-Fakim, A. 2006. Medicinal plants: Traditions of yesterday drugs of tomorrow. *Molecular Aspects of Medicine*, **27**(1): 1–93

[23] Karou, S.D., Tchacondo, D., Tchibozo, M.A.D., Abdoul-Rahaman, S., Anani, K., Koudouvo,Khan, M.R.I., Islam, M.A., Hossain, M.S., Asadujjaman, M., Wahed, M.I.I., Rahman, B.M., Anisuzzaman, A.S.M., Shaheen, S.M. and M. Ahmed. 2011. Antidiabetic effects of the different fractions of ethanolic extracts of *Ocimum sanctum* in normal and alloxan induced diabetic rats. *Journal of Scientific Research*, ***2***(1): 158-168.

[24] Konan, N., Kouame, B.A., Mamyrbekova-Bekro, J.A., Nemlin, J. and B. Yves-Alain. 2009. Chemical composition and antioxidant activities of essential oils of *Xylopia aethiopica* (Dunal) A. Rich. *European Journal of Scientific Research*, ***37***(2): 311-318.

[25] Lai, P.K. and J. Roy. 2004: Antimicrobial and chemopreventive properties of herbs and spices. *Current Medicinal Chemistry*, ***11***(11): 1451–1460.

[26] Luzi, L. and G. Pozza. 1997. Glibenclamide: an old drug with a novel mechanism of action. *Acta diabetologica*, **34** (4): 239-244.

[27] Molyneux, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for the antioxidant activity. *Songklanakarin Journal of Science and Technology*, **26**(2): 211-219.

[28] Mshana, N.R., Abbiw, D.K., Addae-Mensah, I., Adjanouhoun, E., Ahyi, M.R.A., Ekpere, J.A., Enow-Orock, E.G., Gbile, Z.O., Noamesi, G.K., Odei, M.A., Odunlami, H., oteng-Yeboah, A.A., Sarpong, K., Sofowora, A. and A.N. Tackie. Traditional medicine and pharmacopoeia contribution to the revision of ethnobotanical and floristic studies in Ghana.OAU/STRC Technical Report, 2000, pp 67.

[29] Muhammed, A., Aminu, M. and K. Neil. 2016. Anti-diabetic effect of *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae) fruit acetone fraction in type 2 diabetes model of rats. *Journal of Ethnopharmacology*, **10**(16): 3-7.

[30] Nnodim, J., Emejulu, A., Amaechi, A. and E.C. Nwosu Njoku. 2011. Influence of *Xylopia aethiopica* fruits on some hematological and biochemical profile. *Al Ameen Journal of Medical Sciences*, ***4***(2): 191-196.

[31] Nwaiwu, M. Y. and E.O. Imo. 1999. Control of food-borne fungi by essential oil from local spices in Nigeria. *Actaphytopathologicaet Entomologica Hungarica*, ***34***: 91-97.

[32] Obiri, D.D., Osafo, N., Ayande, P.G. and A.O. Antwi. 2014. *Xylopia aethiopica* (Annonaceae) fruit extract suppresses Freund's adjuvant induced arthritis in Sprague-Dawley rats. *Journal of Ethnopharmacology*, **152**(3): 522-531.

[33] Ogbonna, A., Abuajah, C. and E. Hart. 2015. Preliminary evaluation of physical and chemical properties of *Piper guineense* and *Xylopia aethiopica* seed oils. *International Food Research Journal*, **22**(4):1404-1409.

[34] Okpashi, V.E., Bayim, B.P. and A.M. Obi. 2014. Comparative effects of some medicinal plants: *Anacardium occidentale, Eucalyptus globulus, Psidium guajava*, and *Xylopia aethiopica* extracts in alloxan-induced diabetic male Wistar albino rats. *Biochemistry Research International*, **20**(3): 51-67

[35] Oloyede, A. M. and A.O. Aduramigba-Modupe. 2011. Antimicrobial activities of crude ethanolic extract of *Xylopia aethiopica*. *International Journal of Current Research*, ***3***(10)*:* 5-7

[36] Pacheco A.G, De Oliveira P.M, Piló-Veloso D. and A.F.D. Alcântara. 2009. ^{13}C-NMR data of diterpenes isolated from Aristolochia species. *Molecules*, **14**: 1245-1262.

[37] Soladoye, M.O., Chukwuma, E.C. and F.P. Owa. 2012. An 'Avalanche' of plant species for the traditional cure of diabetes mellitus in South-Western Nigeria. *Journal of Natural Product and Plant Resources*, **2:** 60–72.

[38] Suleiman, M. M., Mamman, M., Aliu, Y. O. and J.O. Ajanusi. 2005. Anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats. *Veterinarski Arhiv*, ***75***(6): 487-495.

[39] Tairu, A.O., Hofman, T. and P. Scieberle. 1999. Characterisation of the key aroma compounds in dried fruits of the West African pepper tree *Xylopia aethiopica* (Dunal) A. Rich. (Annonacae) using aroma extract dilution analysis. *Journal of Agriculture and Food Chemistry, **47:*** 3285-3287.

[40] Tan, A.C., Konczak, I., Sze, D.M. and I. Ramzan. 2010. Towards the discovery of novel phytochemicals for disease prevention from native Australian plants: an ethnobotanical approach. *Asian Pacific Journal Clinical Nutrition*, **19**(3): 330–334.

[41] Tapsell, L.C., Hemphill, I., and Cobiac, L., Patch, C.S., Sullivan, D.R., Fenech, M., Roodenrys, S., Keogh, J.B., Clifton, P.M., Williams, P.G., Fazio, V.A. and K.E. Inge. 2006. Health benefits of herbs and spices: the past, the present, the future. *Medicinal Journal of Australia*, **185**(4): S4–24.

[42] Tatsadjieu, L.N., Essia, N.J.J., Ngassoum, M.B. and F.X. Etoa. 2003. Antibacterial and antifungal activity of *Xylopia aethiopica, Monodora myristica, Zanthoxylum xanthoxyloides* and *Zanthoxylum leprieurii* from Cameroon. *Fitoterapia*, ***74***: 469-472.

[43] Uzodike, E.B. and I.N. Onuoha. 2010. The Effect of *Xylopia aethiopica* (Uda) on intraocular pressure. *Journal of the Nigerian Optometric Association*, ***16:***21-25.

[44] Woode, E., Alhassan, A. and C.S. Abaidoo. 2012a. Effect of xylopic acid on sex hormones and spermatogenesis in male rats. *Al Ameen Journal of Medical Sciences*, **5**(3): 28-297.

[45] Woode, E., Ameyaw, E.O, Boakye, G.E. and W.K. Abotsi. 2012b. 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): 291-301.

Citation: *F. Famuyiwa et al., "Hyperglycaemia Lowering Effect of Kaurane Diterpenoids from the Fruits of Xylopia aethiopica (A. Dunal) Rich", International Journal of Medicinal Plants and Natural Products (IJMPNP), vol. 4, no. 3, pp. 11-19, 2018. http://dx.doi.org/10.20431/2454-7999.0403002*