



Antidiabetic potentials of ethanolic extract of *Aristolochia ringens* (Vahl.) roots



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ABSTRACT

Ethnopharmacological importance: Oral administration of alcoholic decoctions of *Aristolochia ringens* is used extensively by Traditional Medicine Practitioners (TMP) of Yoruba heritage in South west, Nigeria for the management of diabetes and its associated complications.

Aim of the study: To assess the potentials of root ethanolic extract of *Aristolochia ringens* V. (REAR) in the management of diabetes using chemical induced experimental animal model.

Materials and methods: Qualitative and quantitative analyses were carried out to elucidate chemical constituents of the REAR extract using phytochemical analyses and gas chromatography-mass spectrometry (GC–MS) technique. Streptozotocin (STZ)-induced diabetic rats were orally administered with 75, 150 and 300 mg/kg body weight of the REAR, once a day and the blood glucose (GLU) levels were monitored for 14 days. Mechanisms of GLU lowering effect were investigated by performing oral glucose tolerance test (OGTT) and modulation of diabetes associated biomarkers including hepatic glycogen (GLY), GLU, alpha amylase (α AMY) and glucose-6-phosphate dehydrogenase (GPDH) by the extract.

Results: Extraction from *A. ringens* roots using ethanol/water (70/30) yielded 10.25% w/w REAR extract. Preliminary phytochemical profiling of REAR extract revealed the presence of flavonoids (23.03%), phenols (19.15%), alkaloids (16.13%), tannins (4.21%), saponins (1.44%) and GC–MS analyses showed that bulk of the extract was constituted by aristolone (92.3%). Oral administration of 300 mg/kg b. wt. REAR extract caused significant reduction ($> 90\%$) in hyperglycemia to normal GLU level (≤ 120 mg/dl) after 11 days of treatment. Lower doses of 75 and 150 mg/kg b. wt. caused similar effect after 13 days. The extract also normalized postprandial GLU to baseline level within 90–120 min. Also, GLY concentrations and GPDH activity were significantly increased, whereas GLU levels and α AMY activity were reduced in the liver of diabetic rats treated with REAR extract compared to values in non-treated diabetic group.

Conclusion: These findings revealed that ethanolic extract from *A. ringens* root possess antihyperglycemic activity and the data established its usage in folkloric decoctions for management of diabetes.

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1. Introduction

Diabetes Mellitus (DM) is a non-communicable disease, which is considered one of the five principal causes of death in the world affecting more than 382 million people (IDF, 2013; WHO 2014). It is rightly recognized as a global public health issue, considered the

Abbreviations: REAR, Root Ethanolic Extract of *Aristolochia ringens*; GC–MS, Gas chromatography-mass spectrometry; STZ, Streptozotocin; GLU, Glucose; GLY, Glycogen; OGTT, Oral Glucose Tolerance Test; α AMY, Alpha amylase; GPDH, Glucose-6-phosphate dehydrogenase; DM, Diabetes Mellitus; MET, Metformin; CHO, Carbohydrate; TMPs, Traditional Medicine Practitioners.

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most challenging metabolic endemic of the 21st century, because of its multifactorial facets affecting essential biochemical processes in almost every cell in the body (Gupta et al., 2007). This metabolic endemic scourge is characterized by persistent hyperglycemia due to lack of insulin production by the pancreas or the inability of the insulin produced to control blood glucose (Shoback et al., 2011). A number of modern medicines like biguanides, sulphonylureas and thiozolidinediones are available for the management of DM (Fowler, 2007; Dineshkumar et al., 2010). These synthetic drugs have undesired side effects associated with their usage (Broadhurst et al., 2000). Alternative medicines particularly herbal remedies are available and should be explored. Medicinal plants used by Traditional Medicine Practitioners (TMPs) in Nigeria for management of DM have been reported and documented by various authors/researchers in ethnobotanical reports and reviews

(Abo et al., 2008; Udenta et al., 2014; Ezuruike and Prieto, 2014). Extract from these plants especially those widely used such as *Aristolochia* sp needs to be investigated.

Aristolochia ringens (Vahl.) Aristolochiaceae is a bushy climber native to tropical America and introduced to most West African countries such as Sierra Leone and Nigeria. The plant is generally referred to as “Dutchman’s pipe” and “Snake work” but in Nigeria, the local names include “Ako-igun” (Yoruba; South west Nigeria) and “Dumandutsee” (Hausa; North Nigeria). The root of this plant has been reported in South west Nigeria for the treatment of asthma and diarrheal (Sonibare and Gbile, 2008; Adeyemi et al., 2012). The use of *A. ringens* in the management of DM by TMPs in Nigeria has also been reported in ethnobotanical study (Olabanji et al., 2008). However, there is paucity of information on its scientific role in the management of diabetic complications, thus a detailed ethno-pharmacology investigation needs to be carried out to establish such claim.

The lack of research in this niche may be due to the complications and toxicity arising from ingestion of extracts from *Aristolochia* sp. specifically the aristolochic acid chemotype. Aristolochic acid has been isolated from *Aristolochia* species (Pacheco et al., 2009) and aristolochic acid-induced toxicity and nephropathy have been reported (Izotti et al., 2005; Heinrich et al., 2009). However, ethnobotanical survey and personal communication with TMPs in South west Nigeria revealed non-existent of *Aristolochia* sp induced toxicity in patients using *A. ringens* decoctions for management of diabetes. Xiao et al. (2009) indicates that further research on promising species of these plants may allow for the optimization of their medicinal value.

Thus, this study was designed to investigate the antidiabetic potentials of root ethanolic extract of *A. ringens* (REAR), in order to determine and establish the pharmacological bases for its usage by TMPs for the management of diabetes by elucidating its mechanism of antihyperglycemic actions and phytochemical constituents.

2. Materials and methods

2.1 Experimental animals

A total of thirty albino rats (*Rattus norvegicus*) weighing 130 ± 5.02 g were obtained from the animal holding unit of the Department of Biosciences and Biotechnology (Biochemistry Unit), College of Pure and Applied Sciences, Kwara State University Malete, Nigeria. They were acclimatized for a week to standard housing condition. Water and rat pellets were provided ad-libitum. The research adhered strictly and conforms to the Principles of Laboratory Animal Care (NIH Publication, No. 85-23).

2.2 Plant materials, chemicals and reagents

Aristolochia ringens roots were collected from Idumota, Lagos State, South west Nigeria. It was authenticated at the herbarium unit of the Botany Department, University of Lagos, where a voucher specimen (LUH 6234) was deposited. Metformin (MET; Qianjin Pharm. Co. Ltd., China), streptozotocin (SCBT, Heidelberg, Germany), ethanol and sucrose (SIGMA, St Louis, MO, USA), assay kits for G6PDH and GLU (Randox Laboratories, Antrim, UK) and other chemicals used were of analytical/research grades.

2.3 Preparation of the extracts

Extracts were prepared based on simulation from the local method as described by traditional medicine practitioners in Lagos, Nigeria. The powdered root of *A. ringens* (200 g) was soaked with 2000 ml of (70%) ethanol for 24 h with intermittent shaking,

filtered and evaporated using vacuum-assisted rotary evaporator at 40 °C to give a yield of 20.5 g (10.25% w/w). The dried extract was labeled REAR and stored in air-tight container at 4 °C.

2.4 Phytochemical analyses

The REAR extract was preliminary screened for alkaloids (Harbone, 1973), steroids, anthraquinones, and cardenolides (Trease and Evans, 1989), phenols and flavonoids (Awe and Sodipo, 2001), tannins and triterpenes (Odebiyi and Sofowora, 1978), glycosides (Sofowora, 1993) and saponins (Wall et al., 1954). Quantitative analyses were then carried out for total flavonoids (Chang et al., 2002), phenols (McDonald et al., 2001), tanins (Van-Burden and Robinson, 1981), alkaloids (Manjunath et al., 2012) and saponins (Brunner, 1984).

2.5 Gas chromatography and mass spectroscopy (GC–MS)

A Hewlett-Packard ITP5890A GC, interfaced with a VG analytical 70–250 s double focusing mass spectrometer was used. Helium was the carrier gas at 1.2 ml/min. The MS operating conditions were: ionization Voltage 70 eV, ion source 230 °C. The GC was fitted with a 25 m × 0.25 mm, fused silica capillary column coated with CP-sil 5. The MS data were acquired and processed by on-line desktop computer equipped with this memory. The percentage composition of the plant extracts were computer in each case from GC peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the retention time of series of *n*-alkanes) and mass spectral with those of authentic samples and with data from literature (Jennings and Shibamoto, 1980; Adams, 1995; Joulain and Koenig, 1998).

2.6 Induction of diabetes

The animals were subjected to 18 h fast (6 p.m–12 a.m) prior to the induction of diabetes. Streptozotocin (STZ) freshly prepared in citrate buffer (0.01 M, pH 4.5) was administered intraperitoneally at a single dose of 65 mg/kg b. wt. (Milani et al., 2005) according to individual body weight of the rats. Rats in the non-diabetic group were injected with 0.5 ml citrate buffer to serve as control. Blood glucose (GLU) was determined prior to induction and after 48 h, using a glucose oxidase-based commercial glucometer (AccuChek active, Roche Diagnostic). Animals with blood glucose ≥ 250 mg/dl after 48 h and sustained hyperglycemia for the next 72 h (5 days after STZ induction) were considered diabetic and were used in the study.

2.7 Animal grouping, dose determination and extract administration

Preliminary studies were carried out to determine the therapeutic doses. Various groups of hyperglycemic rats ($n=3$ in each; ≥ 250 mg/dL glucose) were treated with oral administration of 5–500 mg/kg b.wt REAR) once a day for 7 days and FBG levels were determined on day 7.

The results revealed that ≤ 50 mg/kg b.wt REAR failed to show any significant glucose lowering effect. Relative reduction was observed in rats treated with 75 and 100 mg/Kg, whereas significant reduction was observed in those treated with ≥ 150 mg/kg b.wt. Thus, half the therapeutic dose (75), therapeutic dose (150) and twice the dose (300) were selected for the study.

For the main study, rats were completely randomized into six group of five rats each (A–F).

- NC (Non-treated non-diabetic rats) received 0.5 ml of distilled water.
- DC (non-treated diabetic rats) received 0.5 ml of distilled water.

- DS (diabetic rats treated with standard drug) received 0.5 ml of 14.2 mg/kg b.wt. of MET.
- DT1 (diabetic rats treated with extract) received 0.5 ml of 75 mg/kg b.wt. of REAR.
- DT2 (diabetic rats treated with extract) received 0.5 ml of 150 mg/kg b.wt. of REAR.
- DT3 (diabetic rats treated with extract) received 0.5 ml of 300 mg/kg b.wt. of REAR.

2.8 Determination of blood glucose

The method of Ortiz-Andrade et al. (2009) was used with slight modifications. The animals were subjected to a 12-h overnight fast with free access to water (feeds were withdrawn from 7 pm to 7 am). The rats were treated as shown above. On day 1 of treatment, blood samples were obtained from their caudal vein, GLU levels were determined at 0 h before treatment and 24 h after treatment. Feeds were returned and left ad libitum until 7 pm. GLU levels were also determined every other day and on the 14th day of treatment.

2.9 Oral glucose tolerance test (OGTT)

An oral glucose tolerance test (OGTT) was performed on day 9 of administration. Test animals were fasted for 4 h (from 7:00 am) and then administered with the REAR extracts, after thirty minutes a dose of 2 g/kg of glucose solution was administered to each rat orally. Blood samples were collected from the tail tip at 0 (before substrate administration), 0.5, 1, 1.5, 2, and 2.5 h after administration. Blood glucose concentration was estimated as previously described (Atte et al., 2002; Ortiz-Andrade et al., 2009).

2.10 Preparation of tissues homogenate

At the end of the 14 days experimental period, the rats were anaesthetized, sacrificed by simply incising the jugular vein and then quickly dissected; the liver were excised and a known weight rinsed and then homogenized in ice cold 50 mM Tris/HCl buffer pH 7.5 (Ugochukwu and Babady, 2002) containing Triton x-100 (Muhammad et al., 2006) at a final concentration of 0.5% to maintain the integrity of the tissues. All operations were carried out at between 0 and 4 °C. The homogenate were stored in the freezer (each in a labeled specimen bottle) and used for analysis within 24 h (Muhammad and Oloyede, 2010).

2.11 Biochemical analysis

Glycogen measurement was carried out, as described by Pas-soneau and Lauderdale (1974) with slight modifications. Glucosyl units were determined by enzymatic oxidation by the method described by Barham and Trinder (1972). G6PDH activity (Lohr and Waller, 1974) was measured spectrophotometrically by using commercially available diagnostic kits and reagents obtained from Randox Laboratories (UK). Alpha amylase activity (McCue et al., 2004) and protein concentration (Lowry et al., 1951) were also determined.

2.12 Statistical analysis

All data are expressed as the mean of five replicates $\pm$ standard error of mean. One way analysis of variance with Dunnett's post hoc test was used for multiple comparisons of treatment groups using GraphPad prism version 5.02. Values were considered statistically significant at the 95% confidence level.

3. Results

3.1. Characterization of chemical constituents

Extraction from *A. ringens* roots using ethanol/water (70/30) yielded 10.25 g of REAR extract per 100 g of dried plant sample (10.25% w/w). Preliminary phytochemical profiling of the extract revealed the presence of some diverse classes of plant secondary metabolites such as saponins, tannins, terpenoids, cardenolides, phenolics, triterpenes, flavonoids, cardiac glycosides and alkaloids (Table 1). Table 2 shows the quantitative analyses of some of the detected phytochemicals; flavonoids (23.03%), phenols (19.15%), alkaloids (16.13%), tannins (4.21%) and saponins (1.44%). GC–MS chromatogram (Fig. 1) and mass spectra data showed that the bulk of the REAR extract was constituted by sesquiterpenes and the major compound was aristolone (92.33%; Table 3). Other constituents quantified were dihydroresorcinol (3.35%), eicosane (3.32%) and a bicyclic alkene ester (3.20%).

3.2. Glycemic studies

The effects of oral administration of 75, 150 and 300 mg/kg b. wt. REAR extracts and 14.2 mg/kg b. wt MET on blood GLU (mg/dl) in STZ-induced diabetic rats are shown on Fig. 2. In this study, all doses of REAR extracts administered to diabetic rats showed significant [F (5, 50)=80.75; P < 0.0001] dose dependent reduction in elevated blood GLU level over the course of treatment. Significant [F (5, 30)=30.84; P < 0.0001] reductions in hyperglycemia were observed 72 h after single daily dose administration of the extracts and the effect was maintained until the end of the treatment. Normoglycemic (≤ 120 mg/dl) level was observed in diabetic rats treated with 300 mg/kg b. wt. of REAR after 11 days of treatment. The lower doses of 75 and 150 mg/kg b. wt. caused similar reduction to normal GLU level after 13 days. Above 90% reduction in hyperglycemia were recorded after 14 days of treatment with the extract (Table 4).

3.3. Oral glucose tolerance test

Table 5 shows the effect of oral administration of 75, 150 and 300 mg/kg b. wt. REAR extracts and 14.2 mg/kg b. wt. MET on oral glucose tolerance in STZ-diabetic rats evaluated on 9th day of treatment. As shown on the table, normoglycemic and diabetic rats demonstrated normal fasting glycemia and increased basal hyperglycemia respectively and this was exacerbated by the oral glucose loading. Pre-treatment with 300 mg/kg b. wt REAR stalled exacerbation in postprandial GLU following loading, whereas 75 and 150 mg/kg b. wt. REAR as well as 14.2 mg/kg b. wt MET attenuated the increase in the post-prandial glucose concentration

Table 1
Qualitative phytochemical profiling of root ethanolic extract of *Aristolochia ringens*.

Phytochemicals	REAR extract
Alkaloids	+
Tannins	+
Anthraquinones	–
Flavonoids	+
Glycosides	+
Cardenolides	+
Phenolics	+
Terpenoids	+
Steroids	–
Saponins	+
Triterpenes	+

*+ = detected; – = not detected.

Table 2Quantitative phytochemical profiling of root ethanolic extract of *Aristolochia ringens*.

Phytochemicals	% Composition
Alkaloids	16.1 ± 0.1
Tannins	4.2 ± 0.4
Flavonoids	23.0 ± 1.3
Phenolics	19.2 ± 1.7
Saponins	1.4 ± 0.1

within 90 min, whereas. Further significant [$F(5, 30)=69.39$; $P < 0.0001$] time related decreases in the post-prandial GLU levels were recorded at 120 min with the most profound [$F(5, 30)=75.33$; $P < 0.0001$] decrease recorded at the 150 min post-glucose loading.

3.4. Hepatic glucose and glycogen

Table 6 shows the glycogen content and glucose concentration in the liver of diabetic rats treated with REAR extracts. The non-treated diabetic control rats showed significant [$F(5, 30)=2911$; $P < 0.0001$] increase in hepatic GLU levels and decrease [$F(5, 30)=94.75$; $P < 0.0001$] in GLY content when compared to the non-treated non-diabetic control rats. Elevated GLU levels were significantly lower in diabetic rats treated with REAR and values were within range recorded in non-diabetic control rats. Also, REAR extracts significantly increased GLY contents in diabetic rats in a dose dependent manner. When compare to values in non-diabetic rats, Hepatic GLY values determined in diabetic rats treated with metformin and 150 mg/kg b. wt. of REAR were not significantly different whereas the other groups treated with lower 75 and higher 300 mg/kg b. wt. of REAR recorded significantly lower and higher GLY contents respectively.

3.5. Alpha amylase and Glucose-6-phosphate dehydrogenase

The activities of hepatic α AMY and G6PDH in diabetic rats treated with REAR were shown on Table 7. The α AMY and G6PDH activities in the liver were significantly [$F(5, 30)=34.58$;

Table 3Chemical composition of root ethanolic extract of *Aristolochia ringens*.

S/N	RT	Compounds	% Composition
1	15.3	Eicosane	3.2
2	19.2	Dihydroresorcinol	3.4
3	19.8	Cis-Bicyclo[4.3.0]nonene ester	3.2
4	22.7	Aristolone	90.2

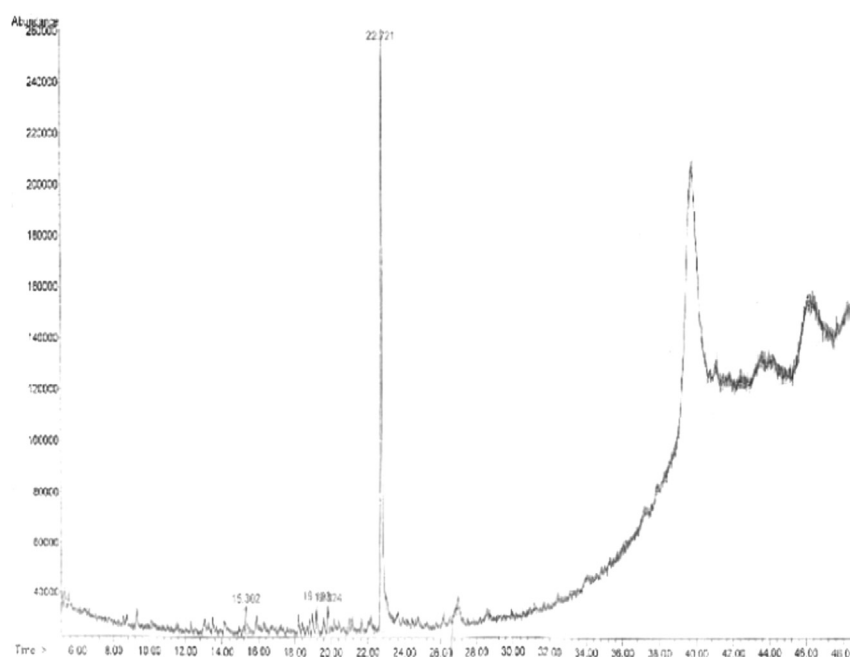
RT=Retention Time

$P < 0.0001$ and $F(5, 30)=27709$; $P < 0.0001$] increased and reduced respectively in non-treated diabetic control group compared to values in the non-diabetic control group. Treatment of diabetic rats with REAR extracts significantly decreased liver α AMY and increased G6PDH activities compared to values in non-treated diabetic or non-diabetic groups except for α AMY levels in rats treated with 150 mg/kg b. wt. of REAR, which was not significantly different from the non-diabetic group (Table 7).

4. Discussion

Aristolochia sp are widely used in by Traditional Medicine Practitioners (TMPs) in Nigeria for management of DM and this health benefit has been documented in ethno-botanical reports and reviews (Olabanji et al., 2008; Ezurike and Prieto, 2014). One of its specie, *Aristolochia indica* had been shown to possess GLU lowering effects in animal models (Karan et al., 2012). However, there is no detailed scientific data to substantiate the antidiabetic properties of *A. ringens*, the common specie used among TMPs in the South west region of Nigeria.

In this study, chemical induction of DM was performed in rats using STZ to establish and sustain hyperglycemia prior and throughout the experimental period. STZ-induced hyperglycemia resulted from deficient insulin production and release caused by near absolute destruction of the pancreatic β -cells (Szkudelski, 2001). Data from this present study revealed that administration of REAR extracts for 14 days reduced the elevated blood GLU levels of STZ-induced diabetic rats to random fasting baseline GLU range in non-diabetic conditions. This study established the antidiabetic

**Fig. 1.** Chromatogram of root ethanolic extract of *Aristolochia ringens*.

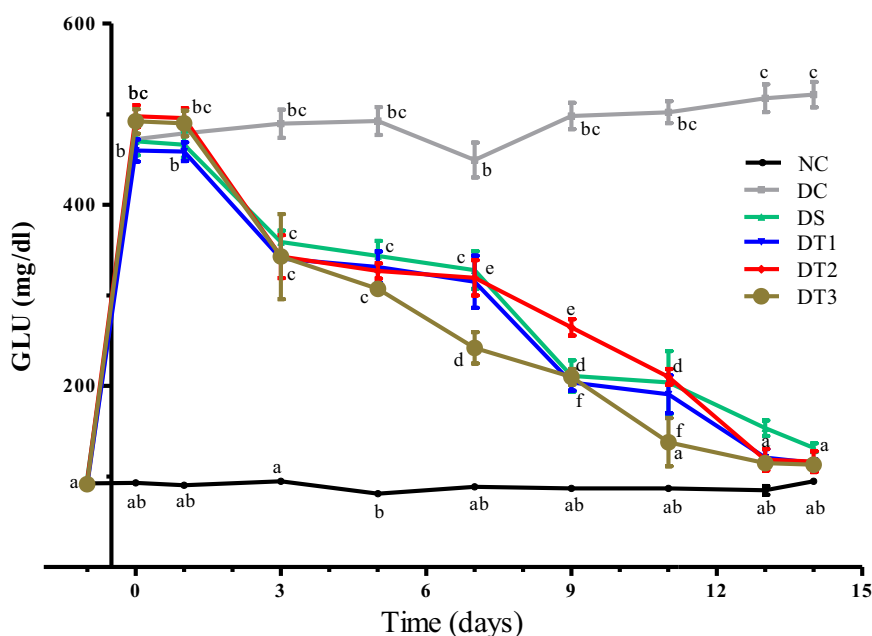


Fig. 2. Antihyperglycemic effect of oral administration of root ethanolic extracts of *Aristolochia ringens* and metformin on glycemia (mg/dl) in streptozotocin induced diabetic rats. NC=normal control; DC=diabetic control; DS=diabetic+standard drug (metformin) DT1=diabetic+75 mg/kg b.wt REAR; DT2=diabetic+150 mg/kg b.wt REAR; DT3=diabetic+300 mg/kg b.wt REAR. REAR=Root Ethanolic Extract of *Aristolochia Ringes*. Points on graph are values expressed as mean of five replicates $\pm$ SEM. Points with different superscript are significantly different ($p < 0.05$) from each other.

Table 4

Glucose (mg/dl) lowering effect of oral administration of root ethanolic extracts of *Aristolochia ringens* and metformin before and after 14 days of treatment in streptozotocin induced diabetic rats.

	Day-5 Before STZ induction	Day 0 Before treatment	Day 14 After treatment	% Glucose reduction
NC	92.4 $\pm$ 2.1 ^a	93.1 $\pm$ 1.0 ^a	94.7 $\pm$ 0.9 ^a	ND
DC	88.5 $\pm$ 1.4 ^a	472.5 $\pm$ 13.9 ^{bd}	521.6 $\pm$ 13.9 ^d	–12.8*
DS	97.1 $\pm$ 1.9 ^a	470.2 $\pm$ 15.5 ^b	131.7 $\pm$ 4.8 ^c	90.7
DT1	90.8 $\pm$ 1.9 ^a	459.9 $\pm$ 12.1 ^b	115.2 $\pm$ 4.1 ^{ac}	93.4
DT2	94.1 $\pm$ 2.2 ^a	497.7 $\pm$ 12.3 ^b	114.8 $\pm$ 3.2 ^{ac}	94.9
DT3	91.6 $\pm$ 1.7 ^a	492.1 $\pm$ 13.5 ^b	113.1 $\pm$ 1.8 ^{ac}	94.6

Values are expressed as mean of five replicates $\pm$ SEM.

Values with different superscripts along either a row or a column are significantly different ($p < 0.05$) from each other.

NC=normal control; DC=diabetic control; DS=diabetic+standard drug (metformin) DT1=diabetic+75 mg/kg b.wt REAR; DT2=diabetic+150 mg/kg b.wt REAR; DT3=diabetic+300 mg/kg b.wt REAR. REAR=Root Ethanolic Extract of *Aristolochia Ringes*.

% Glucose reduction = [(Day0–Day14)/(Day0–Day–5)] $\times$ 100.

* Persistent or exacerbation in hyperglycemia.

Table 5

Effect of oral administration of root ethanolic extracts of *Aristolochia ringens* and metformin on glycemia (mg/dl) after a single oral administration of glucose (2 g/kg b.wt.) in streptozotocin-induced diabetic rats.

Treatment	0 min	30 min	60 min	90 min	120 min	150 min
NC	88.6 $\pm$ 2.3 ^a	197.2 $\pm$ 12.5 ^c	175.4 $\pm$ 10.3 ^c	140.4 $\pm$ 8.0 ^b	104.0 $\pm$ 3.2 ^a	87.20 $\pm$ 3.2 ^a
DC	493.8 $\pm$ 15.4 ^a	519.0 $\pm$ 18.0 ^a	506.4 $\pm$ 14.4 ^a	507.0 $\pm$ 6.9 ^a	510.6 $\pm$ 8.9 ^a	501.0 $\pm$ 17.7 ^a
DS	397.8 $\pm$ 20.8 ^a	503.0 $\pm$ 17.5 ^b	484.4 $\pm$ 17.5 ^b	447.0 $\pm$ 17.7 ^{ab}	418.6 $\pm$ 19.2 ^a	397.2 $\pm$ 20.6 ^a
DT1	314.5 $\pm$ 28.8 ^a	439.4 $\pm$ 21.8 ^b	421.6 $\pm$ 20.5 ^b	368.2 $\pm$ 24.3 ^{ab}	337.4 $\pm$ 26.6 ^{ab}	316.4 $\pm$ 27.5 ^a
DT2	349.4 $\pm$ 19.5 ^a	506.6 $\pm$ 8.9 ^c	466.0 $\pm$ 8.7 ^{bc}	408.0 $\pm$ 13.6 ^{ab}	377.0 $\pm$ 16.6 ^a	354.4 $\pm$ 18.0 ^a
DT3	242.0 $\pm$ 17.3 ^b	269.4 $\pm$ 18.6 ^a	264.0 $\pm$ 18.4 ^a	254.0 $\pm$ 17.4 ^a	250.4 $\pm$ 17.2 ^a	221.0 $\pm$ 15.8 ^a

Values are expressed as mean of five replicates $\pm$ SEM.

Values with different superscripts along either a row (time of treatment) are significantly different ($p < 0.05$) from each other.

NC=normal control; DC=diabetic control; DS=diabetic+standard drug (metformin) DT1=diabetic+75 mg/kg b.wt REAR; DT2=diabetic+150 mg/kg b.wt REAR; DT3=diabetic+300 mg/kg b.wt REAR. REAR=Root Ethanolic Extract of *Aristolochia Ringes*.

Table 6

Effect of oral administration of root ethanolic extracts of *Aristolochia ringens* and metformin on glucose and glycogen in the liver homogenate (mg/g) of streptozotocin-induced diabetic rats.

Treatment	Glucose	Glycogen
NC	15.5 $\pm$ 0.7 ^d	7.8 $\pm$ 1.0 ^c
DC	35.9 $\pm$ 0.1 ^e	0.9 $\pm$ 0.2 ^a
DS	14.1 $\pm$ 0.1 ^c	7.5 $\pm$ 0.7 ^c
DT1	15.3 $\pm$ 0.3 ^d	4.7 $\pm$ 0.8 ^b
DT2	12.9 $\pm$ 0.4 ^b	7.1 $\pm$ 0.9 ^c
DT3	10.4 $\pm$ 0.4 ^a	10.1 $\pm$ 0.4 ^d

Values are expressed as mean of five replicates $\pm$ SEM.

Values with different superscripts along a column (among the groups) are significantly different ($p < 0.05$) from each other.

NC=normal control; DC=diabetic control; DS=diabetic+standard drug (metformin) DT1=diabetic+75 mg/kg b.wt REAR; DT2=diabetic+150 mg/kg b.wt REAR; DT3=diabetic+300 mg/kg b.wt REAR. REAR=Root Ethanolic Extract of *Aristolochia Ringes*.

potentials and usage of the *A. ringens* root decoctions by Nigerian TMPs for management of DM.

Oral daily administration of REAR extracts caused dose

Table 7

Effect of oral administration of root ethanolic extracts of *Aristolochia ringens* and metformin on alpha amylase and glucose-6-phosphate dehydrogenase in the liver homogenate (nm/min/mg protein) of streptozotocin-induced diabetic rats.

Treatment	α AMY	G6PDH
NC	6.1 $\pm$ 0.4 ^a	115.8 $\pm$ 1.7 ^b
DC	12.7 $\pm$ 0.1 ^d	36.9 $\pm$ 8.0 ^a
DS	9.3 $\pm$ 0.9 ^b	827.8 $\pm$ 2.4 ^d
DT1	9.3 $\pm$ 0.7 ^c	157.6 $\pm$ 8.3 ^c
DT2	7.5 $\pm$ 1.5 ^{ab}	917.9 $\pm$ 2.0 ^e
DT3	8.8 $\pm$ 0.8 ^{bc}	107.9 $\pm$ 5.3 ^b

Values are expressed as mean of five replicates $\pm$ SEM.

Values with different superscripts along a column (among the groups) are significantly different ($p < 0.05$) from each other.

NC=normal control; DC=diabetic control; DS=diabetic+standard drug (metformin) DT1=diabetic+75 mg/kg b.wt REAR; DT2=diabetic+150 mg/kg b.wt REAR; DT3=diabetic+300 mg/kg b.wt REAR. REAR=Root Ethanolic Extract of *Aristolochia ringens*.

dependent GLU lowering effects, higher doses caused lower blood GLU reduction. Also, OGTT results revealed that administration of REAR and the drug MET used as standard prior to GLU loading normalized post-prandial GLU to baseline level within 120 min. This suggests that REAR extract improved GLU homeostasis through peripheral uptake of GLU in a similar fashion to the mechanism of action of the antidiabetic drug (MET).

Furthermore, REAR extracts also caused reduction in hepatic GLU and dose dependent increase in hepatic GLY contents suggesting other possible mechanism of antidiabetic actions. Glycogen is the primary intracellular storage form of glucose in various tissues. Since STZ causes destruction of β pancreatic cells that produce insulin, it may be deduced that hepatic GLY level will be low in diabetic rats due to reduced or lack of insulin release to trigger or stimulates conversion of GLU into GLY. Abnormal increase in hepatic GLU production is also a key biochemical pathology condition in DM and contributes significantly to sustain hyperglycemia (Consoli, 1992; Reaven, 1997; Beck-Nielsen et al., 2002). REAR extracts may had exerted its antihyperglycemic effects either through hepatic GLU reduction as evident from the enhanced conversion of GLU to GLY in liver homogenate of diabetic rats treated with the extracts or through modulation of enzymes involved in carbohydrate (CHO) metabolism.

α AMY activity in the tissue homogenate of diabetic rats were inhibited by the extract (REAR) as well as the standard drug (MET). α AMY is a prominent CHO degrading enzyme that breakdowns CHO especially starch polymeric molecules into smaller ≤ 6 molecular units and eventually into absorbable maltose monomers. α AMY inhibitors delay the breakdown of CHO, thus improving postprandial GLU excursion levels in diabetic conditions resulting in reduction in hyperglycemia (Kazeem et al., 2013). In contrast to it inhibitory effects on the action of α AMY, REAR extracts enhanced the activities of G6PDH, a cytoplasmatic enzyme affecting the production of the reduced form of the extra-mitochondrial NADPH by controlling the rate limiting step involving conversion of glucose-6-phosphate to 6-phosphogluconate in the pentose phosphate pathway (PPP). In this study, the activity of hepatic G6PDH was reduced in non-treated diabetic rats correlating with previous studies on the effect of antidiabetic agents and alternative botanical extracts (Gupta et al., 1997; Zhang et al., 2000; Panneerselvam and Govindaswamy, 2002). It may be suggested that the REAR extract seems to increase influx of GLU into the alternative PPP in an attempt to reduce hyperglycemia, thus activating, stimulating and enhancing G6PDH activity. This hypothesis is further supported by decreased hepatic glucose levels observed in diabetic rats treated with the extract.

Phytochemical analyses of the REAR extract revealed the

presence of diverse classes of secondary metabolites that have been implicated by numerous researchers to be responsible for antihyperglycemic activities of medicinal plant extracts and these include tannins, flavonoids, phenolics, terpenoids and triterpenes (Hsu et al., 2009; De et al., 2011; Akolade et al., 2014; Oloyede et al., 2015). Although the phytochemical analysis revealed a number of secondary metabolites, while further characterization by GCMS of hexane effluent of the extract only detected few compounds. This variation may be due to differences in analytical procedure, principle and sensitivity. The sesquiterpene lactone, aristolone was the major compound detected by GCMS. Although there are other plants that contain aristolone such as *Asarum canadense* (Bauer et al., 1967) and *Nardostachys chinesis* (Wang et al., 2010), the terpene derived biomolecule is mainly found in and the name derived from *Aristolochia* sp (Kostalova et al., 1991; Lajide et al., 1993). Terpenoids of *Aristolochia* sp have been implicated as the bioactive principles responsible for pharmacological actions of the plant such as antitumour, antibacterial, anticancer and anti-inflammatory in a detailed review by Wu et al. (2004).

Aristolone has not been shown or previously documented in literature to possess antihyperglycemic activities; however, Karan et al. (2012) implicated the terpene derived β -sistosterol isolated from chloroform extracts of *Aristolochia indica* aerial parts grown in India as the bioactive molecule responsible for its GLU lowering effect in alloxan-induced diabetic mice. One or synergistic effects of two or more of the chemical constituents may be responsible for such actions. This suggests that other constituents of the REAR extract such as dihydroresorcinol or eicosane detected and quantified in minor quantities may also possess GLU lowering effect.

Variations observed in chemical composition of *Aristolochia* sp from the different part of the world are not only due to the type of specie but also differing agro-climatic conditions, extraction processes, time of harvest and characterization techniques (Usman et al., 2010; Akolade et al., 2014). Thus, absence of aristocholic acid that is associated with toxicity in both the Indian *A. indica* characterized by Karan et al. (2012) and the Nigeria *A. ringens* (investigated in this study) may be attributed to the one or more of the above stated factors.

Research are ongoing in our laboratory to isolate and investigate antihyperglycemic effects of individual constituents of the extracts from *A. ringens* grown in Nigeria especially dihydroresorcinol and aristolone as well as their combinations, in order to ascertain which of the compound(s) is or are actually responsible for the observed antidiabetic properties.

5. Conclusion

In summary, findings from this study revealed that the ethanolic extract from *A. ringens* root (REAR) was an aristolone chemotype and possessed marked antihyperglycemic effects. The data established its usage by TMPs in Nigeria for treatment of DM. The extracts may have exerted the GLU lowering effects by excursion of postprandial GLU either through enhancing peripheral uptake or conversion to GLY as well as increasing utilizing of GLU by stimulating activities of the alternative PPP rate limiting G6PDH enzyme. Overall, the plant is a good candidate for further exploration as alternative therapy for the management of DM.

Declaration of conflicting interests

No potential conflicts of interest with respect to this research, authorship, and/or publication of this article.

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