

Original Research Article

Anti-hyperglycaemic Effects of the Ashes of some Nigerian Anti-diabetic Ethnomedicinal Plants

ABSTRACT

Aims: To evaluate blood glucose-lowering ability of leaf ashes and compare anti-hyperglycaemic activities of *Annona muricata* leaf extract and ash.

Study design: Ashes and extract of medicinal plants were assayed using glucose-loaded rats model.

Place and Duration of Study: Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria, between March 2017 and February 2020.

Methodology: Adequately prepared ashes of seven plants and *A. muricata* methanol extract were assayed for anti-hyperglycaemic potentials, using glucose-loaded (10 g/kg, *p.o.*) Wistar rats that were hyperglycaemic [blood glucose levels ≥ 7.0 mmol/L] thirty minutes thereafter ($T_{0.0}$). Groups of 5 rats each, were administered 100, 150, 200 mg/kg of different ashes and *A. muricata* extract (200 mg/kg). Normal saline and glibenclamide (5 mg/kg) were negative and positive controls, respectively. Their blood glucose levels were determined at 0-4 hours post-extract/ash/drug administration; results analysed using ANOVA followed by the Student-Newman-Keuls' and Dunnett post-hoc tests. $P < .05$ was considered significantly different.

Results: 100 mg/kg of *Momordica charantia*, *Azadirachta indica* and *Eugenia malaccensis* leaf ashes was their most active dose, indicating significantly higher extrapancreatic activity. 32, 37, 54, 59 and 36, 43, 50, 48 % reductions elicited by *Chromolaena odorata* (COLA) and *A. muricata* (AMLA) ashes (200 mg/kg) at 0.5, 1, 2 and 4 hours, respectively made them the most active ashes. Also, blood glucose levels in glibenclamide (5 mg/kg)-, COLA-, AMLA- and its extract (200 mg/kg)-treated rats were comparable ($P > .05$) and their anti-hyperglycaemic activity was suggested to be due to the pancreatic (insulinotropic) and extra-pancreatic actions of their constituents.

Conclusion: Study justified anti-diabetic ethnomedicinal use of plant-ashes in Nigeria, while doses were recommended for the optimum folkloric usage of these leaf ashes in managing diabetes in the rural areas of Nigeria. The leaf ashes may contain elements with glucose postprandial tolerance factor and insulin stimulating properties.

Keywords: *Diabetes mellitus*, *anti-hyperglycaemic activity*, *insulin stimulation*, *plant ashes*, *plant extract*

1. INTRODUCTION

As at 2006 and in her third edition of the Diabetes Atlas, International Diabetes Federation, declared Diabetes mellitus (DM) as a fast growing public health concern and a world-wide epidemic [1]. It is considered a pathological condition characterized by hyperglycaemia due to partial or total loss of insulin production, secretion and/or its action [2,3]. It is a metabolic disorder of carbohydrate, fat and protein metabolism, leading to several complications, such as cardiovascular, nephro-, neuro- and retinopathies, and possibly death [2-4].

Type 2 diabetes had changed from being a disease of the affluent countries and the elderly to plaguing all ages, with a greater proportion of its sufferers located in low- and middle-income countries, whose meager resources are already stretched thin with battling contagious and chronic diseases [1]. Increasing "Western-style" diet and sedentary life styles and other urbanization lifestyle changes of citizens in the developing countries have been blamed for its projected 400 million world-wide sufferers by the year 2030 [2]. This is in spite of the availability of novel drugs, techniques, and surgical interventions, including increased use of herbs, nutraceuticals and supplements that have improved the survival rate of individuals with diabetes [1-5]. All types of diabetes are treatable and last a lifetime. Type 1 diabetes has no known cure, while special diets have been reported to help sufferers of type 2 diabetes control the condition [3]. Type 2 diabetes is a result of chronic insulin resistance and loss of β -cell mass and function [1,3-4].

For several thousands of years, plants have been used in traditional medicines of the various continents. Therefore, the knowledge of these medicinal plants that are used in the different medicinal systems, such as Ayurveda, Unani, Siddha, Chinese, Asian, African, Americans, Australian, etc has accumulated over these centuries. Hence, during the last few decades, there has been an increase in the study of medicinal plants and their traditional uses in different parts of the world [2-5]. In our laboratory and together with collaborators, we have reported the anti-hyperglycaemic activities of plants' organic extracts, their partitioned and column fractions [6-10], identified their anti-hyperglycaemic constituents [2,11-15] and established the *in vivo* and/or *in vitro* insulinotropic properties of extracts, fractions and/or isolated compounds [8,10-15], in a bid to justify their antidiabetic ethnomedicinal usage. In addition, that the extract and isolated carbazole alkaloids of *Murraya koenigii* leaf inhibited insulin release *in vitro* [16], was suggested as a justification for the slow acting anti-hyperglycaemic activity of this leaf [9].

Moreover, rutin (quercetin-3-O-rhamnosylglucoside) and quercetin were reported as the major and minor active constituents of *Bauhinia monandra* leaf, respectively [12,13]. Rutin was suggested to be a pro-drug that needed to be metabolized in the human gut by α -rhamnosidase into isoquercitrin (quercetin 3-glucoside), which in turn must be hydrolysed by β -glucosidase to release quercetin for the plant's anti-hyperglycaemic action [12]. Therefore, the reported insulinotropic and/or free radical-scavenging properties of both quercetin and isoquercitrin [17] gave further justification for the folkloric use of *B. monandra* leaf in managing diabetes in Nigeria [12,13].

Herbalists and traditional medicine practitioners use a lot of black, powdery, burnt and ashed herbal, animal and mineral drugs, in addition to decoctions, infusion, teas, etc in treating diseases of their clients and patients in Nigeria [18]. Furthermore, incinerating plants at about 450°C would have consumed all their organic constituents, leaving only ashes and their inorganic elements/constituents [19]. Also, trace and minor elements implicated in the regulation of insulin and control of the blood sugar levels in the human body [19] have been reported in medicinal and food plants used to treat diabetes in some Nigerian and Asian communities [19-27]. Furthermore, ashes of some plants have been investigated for α -glucosidase inhibitory effect [23] and anti-hyperglycaemic activity [19,23,27].

Since reports of anti-hyperglycaemic activity of plant ashes appear to be limited to the Asian countries [19,23,27], we evaluated the blood glucose-lowering ability of ashes of seven plants that are used ethnomedicinally as Nigerian antidiabetics and whose organic extracts have been reported to possess anti-hyperglycaemic activities, as well as compared anti-hyperglycaemic activities of *A. muricata* leaf extract and ash [28].

2. MATERIAL AND METHODS

2.1 Equipment

Electric muffle furnace (Supertek, Serial No 1025, India), Mettler analytical balance (Golden-Mettler, U.S.A. Model: 2003), Oral canula, Glucometer and Test strips (Accu-Chek Active, Roche, Germany, Model: GC), Vitreosil crucibles and Dessicator.

2.2 Solvents and materials

Glucose powder (Glucose-D, Brian Munro, Nigeria), Methanol BDH, normal saline, Glibenclamide (Daonil Swiss Pharma, Sanofi-Aventis, Nigeria), gelatin, syringes and needles.

2.3 Animals

Male and female healthy Wistar rats (100-150 g) were separately kept in cages in the animal house of Department of Pharmacology, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. They were acclimatized for 5 days, under standard conditions (temp. 27 ± 3 °C, relative humidity 65 %). All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed.

2.4 Anti-hyperglycaemic assay of ashes and extract of plants

Wistar rats that were fasted for 18 hrs were given (*p.o.*) 10 g/kg of glucose and rats with blood glucose level (bgl) > 7.0 mmol/L (126 mg/dL) after 0.5 hr (time point $T_{0.0}$) were presumed to be hyperglycaemic and divided into groups of five rats. They were thereafter fed (*p.o.*) with either normal saline (negative control), or glibenclamide (5 mg/kg, positive control), or ashes of the seven plants (100, 150, 200 mg/kg), or organic extract of *A. muricata* (200 mg/kg). A drop of blood, taken from tip of the tail of each rat at 0.0, 0.5, 1.0, 2.0 and 4.0 hrs, representing $T_{0.0}$, $T_{0.5}$, $T_{1.0}$, $T_{2.0}$ and $T_{4.0}$, respectively after administering extract/ash/drug was dropped onto glucometer strip that was inserted into the glucometer. The bgl's were read off directly from the glucometer display [2,6-8,11,14,15].

2.5 Plant materials

Leaves of *Annona muricata* L. (Annonaceae), *Azadirachta indica* A. Juss. (Meliaceae), *Chromolaena odorata* L. (Asteraceae), *Eugenia malaccensis* L. (Myrtaceae), *Mangifera indica* L. (Anacardiaceae), *Momordica charantia* L. (Curcubitaceae) and *Murraya koenigii* (L.) Spreng. (Rutaceae) were collected between November and December, 2016 from various locations in O.A.U. Campus, Ile-Ife and its environs. After authentication, their herbarium vouchers IFE-17644, -17647, -17645, -17649, -17648, -17646 and -17651, respectively were deposited in IFE Herbarium, Botany Department, O.A.U., Ile-Ife. The leaves were thereafter air-dried, powdered and kept in black polythene bags, until needed [28].

2.6 Preparation of plants' ashes

Dry ashing method was used for the collected medicinal plants [19,23,28-29]. A 10 g each of the powdered leaves was weighed into a vitreosil crucible and incinerated (450-500°C) for 3-4 hours in an electric muffle furnace. The ashes obtained were taken out of the furnace, cooled in a desiccator and weighed. The procedure was repeated (2-3 times) until constant weights were obtained and their percentage ash values were calculated.

2.7 Methanol extract of *Annona muricata* leaf

A 5 kg of powdered *A. muricata* leaves was extracted by soaking in methanol (2.5 L) at room temperature for 72 hours, with occasional agitation. The resulting extract was filtered through cotton wool and Whatman filter paper and concentrated *in vacuo* to dryness. Extraction was

repeated for another two times (3 x 72 hours) to obtain 660 g (13.2 % w/w) of *A. muricata* leaf methanol extract.

2.8 Statistical analysis

The bgl of all rats at time ($T_{0,0}$) was taken as 100 % and those at other time points (T_t) were relative to the value at $T_{0,0}$. The percentage blood glucose reductions at all time points (T_t) in the tested rats were calculated relative to those of the negative control group and represented the glucose-lowering activities of the ashes/extract/drug. The results were statistically analysed using One way Analysis Of Variance (ANOVA), followed by Student-Newman-Keul's and Dunnett range *post-hoc* tests (GraphPad Instat, GraphPad Software Inc, 11452 El Camino Real, #215, San Diego 92130, USA). $P < .05$ was taken as being statistically significant.

3. RESULTS AND DISCUSSION

Some people claimed to have managed to rid themselves of their diabetic symptoms without medication by using lifestyle measures, including exercise, diet and body weight control [3]. However, trained medical personnel, including the authors, would advise immediate consultation with physicians and use of prescribed orthodox medicine in acute and chronic cases. Once the patient is stabilized, the authors believe that traditional and/or alternative medicines can thereafter be added to manage this health condition. Currently, the treatment/management of DM entails the use of insulin injectables, oral hypoglycaemic agents (OHA) typified by sulfonylureas and biguanides, plant supplements, nutraceuticals, e.g. vitamins and minerals supplements, strict dietary regimen (including functional foods and therapeutic fasting) and exercise [26].

With the rising global prevalence of diabetes, especially in the African continent [1,2,26], high cost, unavailability, toxicity (undesirable side effects) of synthetic anti-diabetic drugs and the avalanche of literature and information on Traditional, Complementary and Alternative Medicines (TCAM) have caused a paradigm shift to greater interest and use of herbs and herbal drugs to treat diabetes [26,30]. Also, World Health Organization recommending safe and effective use of herbs [26], cheapness, ready availability of TCAM are other factors encouraging their increased use and patronage. Integrative Medicine that makes use of the gains of all these medical/medicinal practices have been suggested by incorporating TCAM into the orthodox primary health care systems in the developing countries [18].

In the 1970-1980s, wives of the Nigerian nomadic herdsmen transformed in the south from sellers of local dairy products into traditional medical consultants on women and children diseases [Adebajo, AC, Personal communication, 2022]. These charlatans also prescribed and dispensed blackish or greyish powders of burnt natural products or their ashes. Percentage yield of ashes of the Nigerian ethnomedicinal antidiabetic leaves used in this study was 6.3-18.7 % w/w (Table 1), indicating that their inorganic constituents varied widely.

Table 1. Percentage yield of ashes of some Nigerian ethnomedicinal anti-diabetic leaves

S/No	Name of plant	Codes	Percentage yield (% w/w)
1	<i>Annona muricata</i>	AMLA	6.34
2	<i>Azadirachta indica</i>	AILA	12.36

S/No	Name of plant	Codes	Percentage yield (% w/w)
3	<i>Chromolaena odorata</i>	COLA	11.36
4	<i>Eugenia malaccensis</i>	EMLA	12.72
5	<i>Mangifera indica</i>	MILA	18.74
6	<i>Momordica charantia</i>	MCLA	17.69
7	<i>Murraya koenigii</i>	MKLA	13.30

3.1 Anti-hyperglycaemic assay model used

Oral Glucose Tolerance Test (OGTT) model with glibenclamide and other insulin-stimulating drugs as references/standards have been reported to mimic type 2 diabetes state in humans and reportedly used in anti-hyperglycaemic assays of medicinal plants [2,6-8,11,14,28,31-34]. Therefore, the glucose-loaded rat model that is often used in our laboratory [2,6-8,11,14] was employed in this study.

Negative control group of glucose-induced hyperglycaemic rats gave significant ($P < .05$) time dependent reductions in their bgl's, up to the 4th hour (Table 2, Figs. 1-4), a phenomenon adduced to the homeostatic regulatory mechanism in normal rats. They also confirmed that the rats' pancreases were functioning well [2,6-8,11,12,14,31,32]. At 0.5-4.0 hours, glibenclamide (5 mg/kg) elicited significant ($P < .05$) blood glucose-lowering activity, when compared with this negative control group (Table 2). This time dependent profile of activity agreed with the early mild extrapancreatic and late major pancreatic mechanisms of action reported for glibenclamide [2,6-8,11,13-15,32-34].

Table 2. Dose related anti-hyperglycaemic activities of some plant ashes in glucose-loaded rats

Ash/Drug (mg/kg)	Blood glucose levels* (percentage reduction in blood glucose levels**)				
	0.0 h	0.5 h	1.0 h	2.0 h	4.0 h
Glucose (10,000)	100.0	103.5±3.4 ^c	100.1±2.1 ^d	91.6±6.7 ^c	67.0±5.3 ^{c,d}
AMLA (100)	100.0	83.0±6.9 ^b (19.8%)	72.0±7.2 ^{b,c} (28.1%)	70.5±9.1 ^{b,c} (23.0%)	57.7±6.1 ^{b,c} (13.9%)
AMLA (150)	100.0	83.5±3.9 ^b (19.3%)	75.6±3.2 ^{b,c} (24.5%)	71.5±2.9 ^{b,c} (21.9%)	69.4±3.1 ^c (-3.6%)
AMLA (200)	100.0	66.7±4.9 ^a (35.6%)	57.5±4.4 ^{a,c} (42.6%)	45.5±4.7 ^{a,b} (50.3%)	34.8±3.4 ^{a,b} (48.1%)
COLA (100)	100.0	86.6±3.5 ^b (16.3%)	70.7±5.0 ^{b,c} (29.4%)	52.8±4.8 ^{a,b} (42.4%)	42.5±3.7 ^{a,b} (36.6%)

Ash/Drug (mg/kg)	Blood glucose levels* (percentage reduction in blood glucose levels**)				
	0.0 h	0.5 h	1.0 h	2.0 h	4.0 h
COLA (150)	100.0	91.5±2.4 ^{b,c} (11.6%)	80.3±3.3 ^{b,c,d} (19.8%)	72.7±5.6 ^{b,c} (20.6%)	49.2±1.6 ^{b,c} (26.6%)
COLA(200)	100.0	71.1±5.9 ^a (31.3%)	62.9±2.9 ^{b,c} (37.2%)	41.9±4.0 ^a (54.0%)	27.5±2.7 ^a (59.0%)
MKLA (100)	100.0	89.2±1.0 ^{b,c} (13.8%)	84.7±2.3 ^{b,d} (15.4%)	74.5±3.9 ^{b,c} (18.7%)	64.8±2.3 ^c (33.3%)
MKLA (150)	100.0	93.7±2.7 ^{b,c} (9.5%)	80.9±2.9 ^{b,c,d} (19.2%)	64.6±1.9 ^{a,b,c} (29.5%)	41.0±1.9 ^{a,b} (38.8%)
MKLA(200)	100.0	88.9±2.8 ^{b,c} (14.2%)	70.8±5.8 ^{b,c} (29.3%)	65.4±6.4 ^{a,b,c} (28.6%)	52.7±5.7 ^{b,c} (21.3%)
MCLA (100)	100.0	87.3±4.8 ^{b,c} (15.7%)	73.7±3.9 ^{b,c} (26.4%)	53.2±3.4 ^{a,b} (41.9%)	47.8±3.0 ^{b,c} (28.7%)
MCLA (150)	100.0	88.5±3.1 ^{b,c} (14.5%)	77.2±3.9 ^{b,c} (22.9%)	73.4±3.7 ^{b,c} (19.9%)	63.8±4.3 ^c (4.8%)
MCLA(200)	100.0	91.4±1.5 ^{b,c} (11.7%)	79.5±3.2 ^{b,c,d} (20.6%)	67.7±5.0 ^{a,b,c} (26.1%)	53.4±4.7 ^{b,c} (20.3%)
AILA (100)	100.0	89.1±2.7 ^{b,c} (13.9%)	65.8±7.3 ^{b,c} (34.3%)	55.7±5.1 ^{a,b} (39.2%)	51.5±4.5 ^{b,c} (23.1%)
AILA (150)	100.0	92.9±1.9 ^{b,c} (10.2%)	82.8±5.7 ^{b,d} (17.3%)	66.0±8.7 ^{a,b,c} (28.0%)	54.8±5.6 ^{b,c} (18.2%)
AILA(200)	100.0	87.3±3.6 ^{b,c} (15.7%)	82.8±3.1 ^{b,d} (17.3%)	73.5±8.4 ^{b,c} (19.8%)	53.6±4.3 ^{b,c} (20.0%)
MILA (100)	100.0	88.2±4.4 ^{b,c} (14.8%)	70.6±3.2 ^{b,c} (29.5%)	63.4±4.2 ^{a,b,c} (30.8%)	55.9±3.7 ^{b,c} (16.6%)

Ash/Drug (mg/kg)	Blood glucose levels* (percentage reduction in blood glucose levels**)				
	0.0 h	0.5 h	1.0 h	2.0 h	4.0 h
MILA (150)	100.0	88.1±3.7 ^{b,c} (14.9%)	68.4±5.4 ^{b,c} (31.7%)	55.2±5.3 ^{a,b} (39.7%)	46.5±3.1 ^{b,c,d} (30.6%)
MILA (200)	100.0	93.7±2.0 ^{b,c} (9.5%)	72.1±7.6 ^{b,c} (28.0%)	64.2±8.5 ^{a,b,c} (30.0%)	57.5±8.0 ^{b,c} (14.2%)
EMLA (100)	100.0	89.5±2.1 ^{b,c} (13.5%)	55.3±5.0 ^{a,b} (44.8%)	45.4±6.8 ^{a,b} (50.4%)	40.9±6.5 ^{a,b} (39.0%)
EMLA (150)	100.0	92.3±1.9 ^{b,c} (10.8%)	90.5±2.9 ^{c,d} (9.6%)	74.9±5.6 ^{b,c} (18.2%)	59.6±2.6 ^{b,c} (11.0%)
EMLA (200)	100.0	92.7±2.1 ^{b,c} (10.4%)	85.9±4.9 ^{c,d} (14.2%)	74.4±5.1 ^{b,c} (18.8%)	67.8±4.9 ^c (-1.2%)
GLIB (5)	100.0	77.5±4.3 ^{a,b} (25.1%)	70.6±6.0 ^{b,c} (29.5%)	55.5±5.1 ^{a,b} (39.4%)	39.4±6.4 ^{a,b} (41.2%)

Data show the mean ± SEM blood glucose level (bgl)* at the different time points (T_i) expressed as percentages of level at 0.0 hr (T₀), percentage reductions** in the bgl's relative to negative control for each time point (T_i), N = 5. Values with different superscripts within columns are significantly different ($P < .05$, one-way analysis of variance followed by the Student-Newman-Keuls' post-hoc test). **Glucose (10,000)**: Negative control group of rats ingested with 10 g/kg of glucose; **AMLA, COLA, MCLA, AILA, MILA, EMLA, MKLA**: Test groups of rats ingested with leaf ashes of *Annona muricata*, *Chromolaena odorata*, *Momordica charantia*, *Azadirachta indica*, *Mangifera indica*, *Eugenia malaccensis*, *Murraya koenigii*, respectively; (100, 150, 200): 100, 150 and 200 mg/kg doses of plant ashes administered, respectively; **GLIB (5)**: Positive control group of rats ingested with glibenclamide (5 mg/kg).

3.2 Anti-hyperglycaemic activities of ashes of seven Nigerian medicinal plants

To the best of our knowledge, there is no anti-hyperglycaemic report for ashes of Nigerian plants [19,23,27,35]. Therefore, having reported anti-hyperglycaemic activities of African and Asian antidiabetic plants [2,6-16], and considering the use of plant ashes enumerated above by Nigerian traditional doctors and charlatans, we investigated Nigerian plant ashes for similar effects [28].

Generally, *A. muricata* (AMLA) and *C. odorata* (COLA) leaf ashes gave dose dependent blood glucose-lowering effects, as their activities at 200 mg/kg were significantly ($P < .05$) the highest at each time point. However, 150 mg/kg of these two plant ashes demonstrated reduced anti-hyperglycaemic activities. Activity of AMLA at 100 and 150 mg/kg was comparable ($P > .05$) at all time points. Also, that of 100 and 150 mg/kg of COLA was comparable at 0.5-1.0 hours, but activity of its 100 mg/kg was significantly ($P < .05$) higher at 2-4 hours. Although COLA and AMLA (200 mg/kg) gave higher percentage blood glucose reductions than the standard drug at 0.5-4 hours, they however gave bgl's that were comparable to those given by glibenclamide (Table 2, Fig. 1).

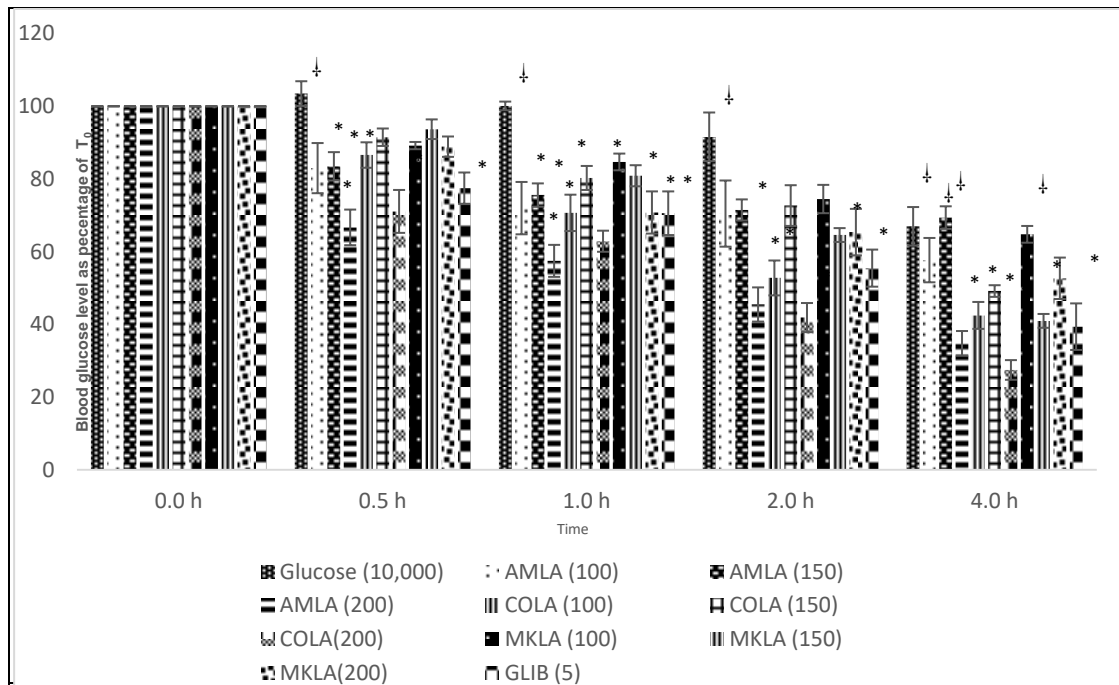


Fig. 1: Dose related anti-hyperglycaemic activities of some plant ashes in glucose-loaded rats.

Data show the mean \pm SEM blood glucose level (bgl) at the different time points expressed as percentages of level at 0.0 hr (T_0), $N = 5$. *†: Values are significantly different ($P < .05$, one-way analysis of variance followed by the Dunnett post-hoc test) from negative and positive controls, respectively. Glucose (10,000 mg/kg): Negative control group of rats ingested with glucose (10 g/kg); AMLA, COLA, MKLA: Test groups of rats ingested with leaf ashes of *Annona muricata*, *Chromolaena odorata*, *Murraya koenigii*, respectively; (100, 150, 200): 100, 150, 200 mg/kg doses of plant ashes administered, respectively; GLIB(5): Positive control group of rats ingested with glibenclamide (5 mg/kg).

At 0.5 hour, 100, 150, 200 mg/kg of *M. koenigii* leaf (MKLA) ash were inactive. Effects of glibenclamide at this time would normally be due to its well known mild extrapancreatic activity [2,6-8,11,13-15,32-34]. Since its 100 mg/kg gave lowered percentage blood glucose-reduction than glibenclamide at all hours and significantly lower anti-hyperglycaemic activity at 2-4 hours (Table 2, Fig. 1), this dose was considered inactive, similar to lack of significant ($P < .01$) change in bgl's reported in rats given 90 mg/kg of Indian *M. koenigii* leaf ash [19]. This inactivity [19] was interpreted as inability of her inorganic constituents to play the role of glucose-tolerance factor [8,23] or have an indirect effect in managing DM [19]. Therefore, *M. koenigii* leaf ash at 90 and 100 mg/kg lacked respective hyperglycaemia-preventing [19] and hyperglycaemia-lowering (Table 2, Fig. 1) activities and confirmed its no-use in folkloric treatment of diabetes in India and Nigeria.

Furthermore, 150 mg/kg of this MKLA and 200 mg/kg of AMLA and COLA, with the highest blood glucose reducing abilities at 4 hour (Fig. 1), are hereby suggested as the optimum doses for their use in managing diabetes, ethnomedicinally. The anti-hyperglycaemic activity of COLA (200 mg/kg) and MKLA (150 mg/kg) was significantly time-dependent, reaching the maximum of 59 and 39 %, respectively at 4.0 hours, similar to glibenclamide (Table 2). Therefore, activity of MKLA was significantly lower than those of AMLA and COLA. Also, similar profile of blood glucose-lowering effects (Fig. 1) of glibenclamide, MKLA (150 mg/kg),

COLA and AMLA (200 mg/kg) suggested they probably acted through extra-pancreatic and pancreatic (insulin stimulating) mechanisms that glibenclamide is reputed for [2,6-8,11,13-15,32-34].

Moreover, significantly higher anti-hyperglycaemic activities of 36, 43, 50 %, and 31, 37 and 54 % given by AMLA and COLA (200 mg/kg) at 0.5, 1 and 2 hours, respectively (Table 2) may indicate probable presence of elemental constituents with additional extra-pancreatic activity. Therefore, combination of major extra-pancreatic and pancreatic activities in these 2 ashes (Fig. 1) may suggest that they either contained elements that have both major extra-pancreatic and pancreatic activities or had one set of anti-diabetic elements with extrapancreatic activity and another set with pancreatic effects. Similar high extra-pancreatic and pancreatic activities of AMLA and COLA ashes of this study (Table 2, Fig. 1) have been suggested for root extract of *M. koenigii* regimen grown alone (MNC, 400 mg/kg), leaf extract of *M. koenigii* regimen (TTM, 100 mg/kg) that was grown watered with the aqueous extract of *Tithonia diversifolia* [6], as well as 400 mg/kg of *Senecio bialfrae* whole plant extract and its C₁₂ column fraction [2]. Hence, detailed investigation of mechanism(s) of action of COLA and AMLA leaf ashes (200 mg/kg), using other standard drugs, should similarly be carried out [2,6] to determine if their mechanisms of action are different from that of glibenclamide [2,7-8,32-34]. Additionally, it may be needful to identify, if possible, which of the elements of these ashes were responsible for these actions.

The significant difference between moderate anti-hyperglycaemic activity demonstrated by the MKLA (150 and 200 mg/kg) of this study (Table 2, Fig. 1) and the non-activity given by 90 mg/kg of *M. koenigii* leaf ash [19] may be due to the different doses and anti-hyperglycaemic models used in the two investigations. Kar et al. [19] used an OGTT model made of concomitant administration of *M. koenigii* leaf ash (90 mg/kg) and glucose (1.5 g/kg) to normoglycaemic rats. Ashes and extracts with hyperglycaemia-preventing activity using Kar's model [19] were suggested to play the role of glucose tolerance factor [8,19,23] probably by stimulating insulin release from β -cells of islets of Langerhans [19]. Conversely, this current study (Table 2, Fig. 1) entailed feeding of rats made hyperglycaemic 30 minutes after glucose load (10 g/kg) with 150 and 200 mg/kg of MKLA, and had established hyperglycaemia-lowering ability of the MKLA ash that probably occurred through extra-pancreatic or pancreatic (insulinotropic) activities or a combination of both. Although hyperglycaemia-preventing and hyperglycaemia-lowering activities are both anti-hyperglycaemic actions, they should have different mechanism of action. Therefore, in relating their current research studies with literatures on anti-hyperglycaemic activities or postprandial blood glucose homeostatic properties of plants, scientists may need to consider details of methods of investigations and doses used in the studies.

The mild anti-hyperglycaemic activity of all the doses of *M. charantia* leaf ash was comparable to that of glibenclamide at 0.5-1.0 hours, while only that of 100 mg/kg was comparable at the second and fourth hours (Fig. 2). Thus, 100 mg/kg was the most active dose of MCLA, *A. indica* and *E. malaccensis* ashes. On the other hand, activity of 100-200 mg/kg of *M. indica* ash was comparable to that of glibenclamide at 0.5-1 hour, while only 150 mg/kg gave the highest percentage blood glucose reductions at 2-4 hours, making it its most active dose (Fig. 2). Additionally, that COLA, MCLA, AILA, EMLA (100 mg/kg) and MILA (150 mg/kg) had their highest (≥ 40 %) blood glucose reducing activity at 2.0 hour (Table 2, Figs. 1,2) indicated that they had an activity profile and a mechanism of action different from that of glibenclamide [2,6-8,13,32-34]. This may support the earlier suggested detailed investigation of the mechanism of action of some plant extracts reported by our group [2,6] and ashes of this current study, using more appropriate standard drugs. The moderate activities of MCLA and AILA in this study (Table 2, Fig. 2) agreed with those reported for *M.*

charantia fruit and *A. indica* leaf ashes that were suggested to be due to stimulation of insulin release from Langerhan's islets [19].

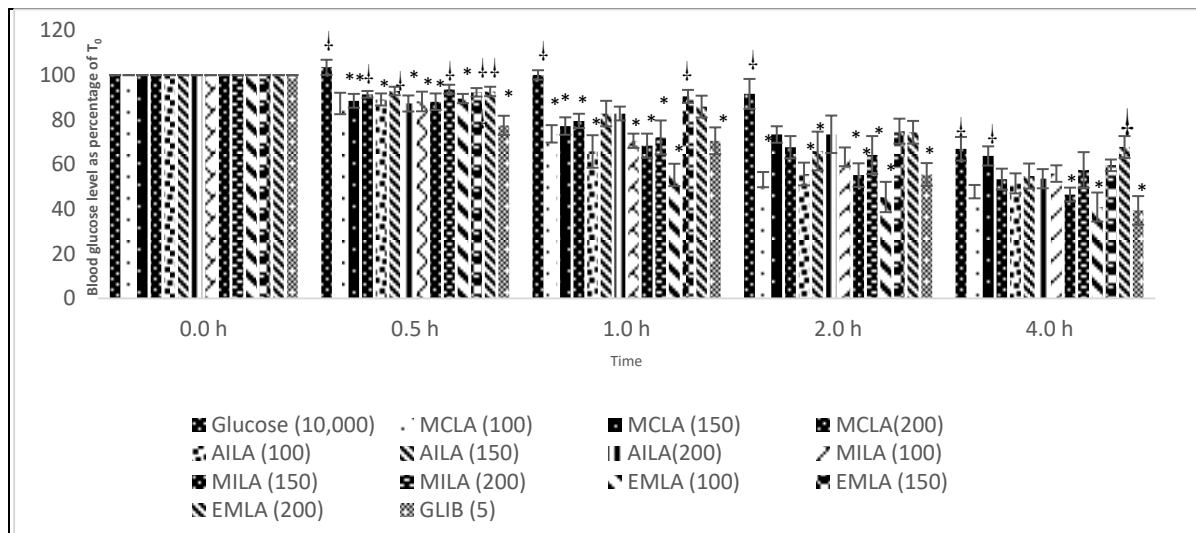


Fig. 2: Dose related anti-hyperglycaemic activities of some plant ashes in glucose-loaded rats.

Data show the mean \pm SEM blood glucose level (bgl) at the different time points expressed as percentages of level at 0.0 hr (T_0), $N = 5$. *†: Values are significantly different ($P < .05$, one-way analysis of variance followed by the Dunnett post-hoc test) from negative (Glucose, 10 g/kg) and positive (Glibenclamide, 5 mg/kg) controls, respectively. Glucose (10,000 mg/kg): Negative control group of rats ingested with 10 g/kg of glucose; MCLA, AILA, MILA, EMLA: Test groups of rats ingested with *Momordica charantia* leaf, *Azadirachta indica* leaf, *Mangifera indica* leaf, *Eugenia malaccensis* leaf, respectively; (100, 150, 200): 100, 150 and 200 mg/kg doses of plant ashes administered, respectively; GLIB (5): Positive control group of rats ingested with 5 mg/kg of glibenclamide.

3.3 Comparison of anti-hyperglycaemic activities of the tested plant ashes

For AMLA and COLA, the most active dose was 200 mg/kg, while it was 150 mg/kg for MILA and MKLA, and 100 mg/kg for MCLA, EMLA and AILA (Fig. 3). Only doses of 90 and 100 mg/kg of plant ashes have been previously tested [19,23,27]. Therefore, it is imperative that the toxicity profiles of these ashes at 150 and 200 mg/kg be determined to establish if they are equally safe.

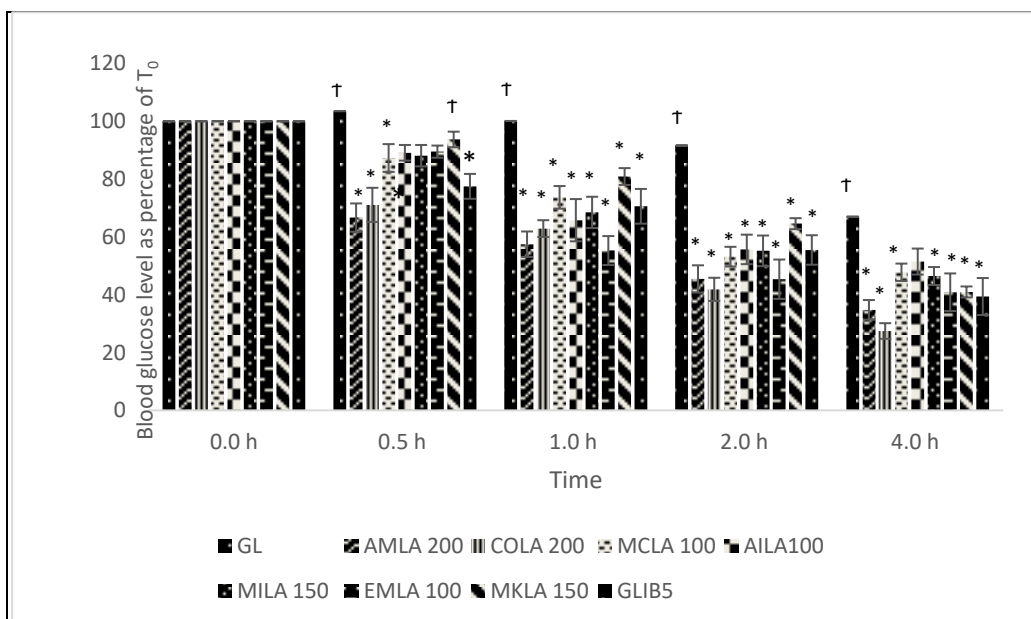


Fig. 3: Comparison of anti-hyperglycaemic activities of the tested plant ashes.

Data show the mean \pm SEM blood glucose level (bgl) at the different time points expressed as percentages of level at 0.0 hr (T_0), $N = 5$. $^* \dagger$: Values are significantly different ($P < .05$, one-way analysis of variance followed by the Dunnett post-hoc test) from negative (Glucose, 10 g/kg) and positive (Glibenclamide, 5 mg/kg) controls, respectively. Glucose (10,000 mg/kg): Negative control group of rats ingested with 10 g/kg of glucose; AMLA, COLA, MCLA, AILA, MILA, EMLA, MKLA: Test groups of rats ingested with *Annona muricata* leaf, *Chromolaena odorata* leaf, *Momordica charantia* leaf, *Azadirachta indica* leaf, *Mangifera indica* leaf, *Eugenia malaccensis* leaf *Murraya koenigii* leaf ashes, respectively; 100, 150, 200: 100, 150 and 200 mg/kg doses of plant ashes administered, respectively; GLIB (5): Positive control group of rats ingested with 5 mg/kg of glibenclamide.

Reducing orders of significant blood glucose-lowering ability of these ashes were *C. odorata* > *A. muricata* = *E. malaccensis* > *M. charantia* = *M. indica* = glibenclamide = *A. indica* > *M. koenigii*, and *C. odorata* > *A. muricata* = glibenclamide = *E. malaccensis* = *M. koenigii* > *M. indica* = *M. charantia* = *A. indica* at 2.0 and 4.0 hours, respectively (Fig. 3). Hence, AMLA and COLA (200 mg/kg) were confirmed the two topmost active ashes (Fig. 3). At 4 hour, activity of glibenclamide, MKLA and EMLA were comparable (Fig. 3), indicating that they similarly stimulated insulin release [2,6-8,11,13-15,32-34] and may further help the diabetics with controlled blood sugar from relapsing and preventing postprandial glucose excursion in the prediabetics [30,36]. However, it remains to be determined if *E. malaccensis* (100 mg/kg) and *A. muricata* and *C. odorata* (200 mg/kg) leaf ashes could fulfill this role in Nigerian ethnomedicine.

That selenium and vanadium were reported probably lost in the ashing process of *Phyllanthus amarus* seeds may not be beneficial for the diabetics who are also cancer patients. However, absence of arsenic and cadmium in the ash, compared to its powdered plant [23], may indicate that the ashes were probably safer.

3.4 Enhancement of anti-hyperglycaemic potentials by ash-combinations

Herbal drugs are mostly multi-component in nature, containing many bioactive-compounds and operating through different mechanisms of actions for enhanced abilities to cure/manage diseases. They are intended to give synergistic or additive combination of their

chemical constituents in treating diseases. Combinations of two or more medicinal plants or of different parts of the same plant in polyherbal formulations are common in African traditional medicine and are believed to have minimal side effects [37-39]. These are prepared by traditional healers and some industries for use in herbal clinics and sale in supermarkets/shops [38].

Binary, ternary and quaternary combinations of Nigerian anti-malarial plants possessed higher anti-plasmodial activities than their components plants [37-39]. *Vernonia amygdalina*, *Ocimum gratissimum* and *Gongronema latifolium* are used to treat diabetes in Akwa-Ibom State of Nigeria [38]. 100 mg/kg of their varied combinations gave activity that was either comparable to or significantly higher than glibenclamide (10 mg/kg) at 7th hour and 15th day in acute and sub-chronic studies, respectively. They were reported to be working in synergy by potentiating insulin effect through increased pancreatic secretion or its release from bound insulin, inhibition of hepatic glucose production, intestinal glucose absorption, or correction of insulin resistance. Other hepatoprotective, antioxidant and hypolipidaemic activities of the composite plants were suggested to complement their anti-diabetic activities [38].

Therefore, combination of highest insulin-stimulating *A. muricata* or *C. odorata* (200 mg/kg) leaf ashes with highly extrapancreatic active *M. charantia*, or *A. indica*, or *E. malaccensis* (100 mg/kg) should give herbal products with improved post-prandial glucose management property and enhanced insulin secreting ability, which may improve self-management and quality of life of the controlled diabetics, as well as reduce risk of diabetes complications [38]. However, the assumption of improved anti-hyperglycaemic activities and safety of these ash-combinations need to be ascertained.

3.5 Comparison of anti-hyperglycaemic activities of *A. muricata* leaf extract and ash

The anti-hyperglycaemic activities of glibenclamide, *A. muricata* leaf ash (Table 2, Fig. 3) and its methanol extract (200 mg/kg) were comparable at all hours and had similar profile of activity. Also its extract elicited blood glucose reductions of 43, 47 % at 1-2 hours, respectively that were insignificantly ($P > .05$) higher than 30, 39 % of glibenclamide (Fig. 4). Antihyperglycaemic activity of *A. indica* leaf extract and ash was also reported comparable, while that of *E. jambolana* seed ash was significantly higher [19]. Hence, glucose tolerance action demonstrated by these ashes were suggested to be due to their inorganic elements that probably enhanced glucose utilization and inhibited intestinal glucose absorption, and thus playing a prominent role in the management of DM [19,27].

Since the results (Fig. 4) indicated that both *A. muricata* leaf extract and ash have extrapancreatic and pancreatic mechanisms of action of glibenclamide, as well as an additional potent extra-pancreatic activity [2,6-8,11,13-15,32-34], either its extract or ash would serve as an ethnomedicinal anti-diabetic drug. Traditional usage of medicinal plants are often in powdered or paste forms [19,27]. Hence, eating powdered whole drug, such as *A. muricata* leaf, in soup or with pap would offer the diabetics anti-hyperglycaemic properties of both the organic and inorganic constituents, similar to reports on *P. amarum* seeds [23]. In addition, the low yields of 6.34 and 12 % for *A. muricata* leaf ash and methanol extract, respectively (Table 1) may allay fear of over dosage when its whole leaf is prescribed or compounded as herbal powdered-drug.

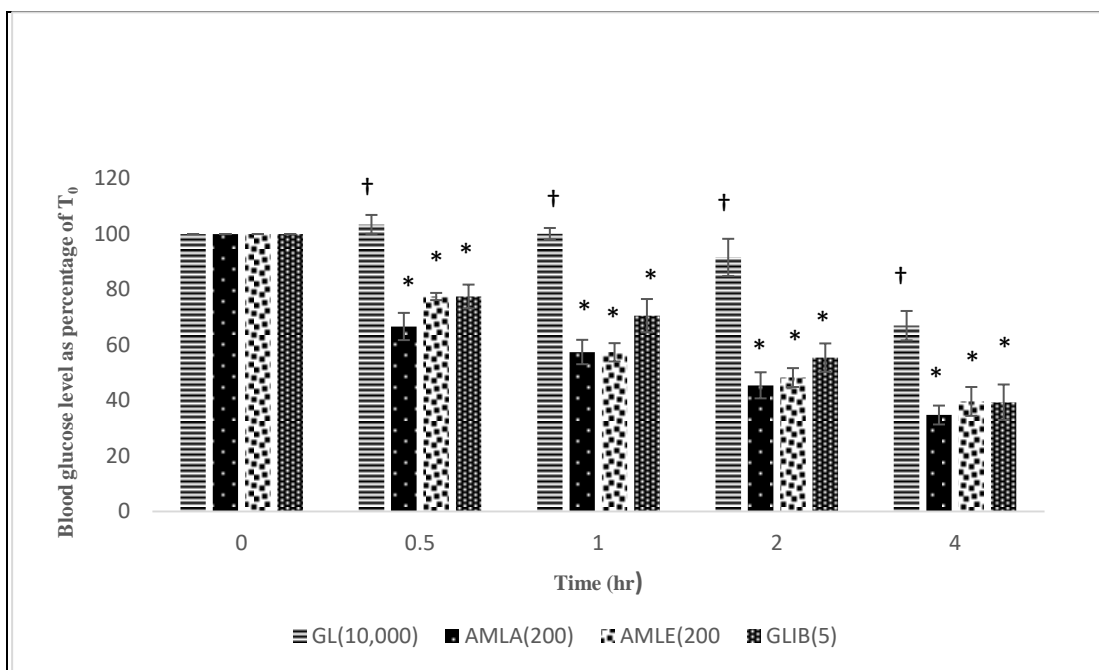


Fig. 4: Anti-hyperglycaemic activities of ash and methanol extract (200 mg/kg) of *Annona muricata* leaf in glucose loaded rats.

Data show the mean \pm SEM blood glucose level (bgl) at the different time points expressed as percentages of level at 0.0 hr (T_0), $N = 5$. *: Values are significantly different ($P < .05$, one-way analysis of variance followed by the Bonferroni post-hoc test) from negative control (Glucose, 10 g/kg); †: Values are significantly different ($P < .05$, one-way analysis of variance followed by the Bonferroni post-hoc test) from positive control (Glibenclamide, 5 mg/kg). Glucose (10,000 mg/kg): Negative control group of rats ingested with 10 g/kg of glucose; AMLA(200), AMLE(200): Test groups of rats ingested with 200 mg/kg of *Annona muricata* leaf ash or methanol extract, respectively; GLIB (5 mg/kg): Positive control group of rats ingested with 5 mg/kg of glibenclamide.

3.6 Mechanisms of action of herbal ashes and roles of their elements

Orally administered glucose gets absorbed from the gut in 0.5 hour [2,7,8,11,15,19,32] and attains maximum blood glucose level in 1.0 hour [19]. This largely represents a period of extra-pancreatic mechanism of hyperglycaemia-lowering action of this study (Table 2, Figs. 1-4). Increased peripheral utilization of glucose was reported as an extra-pancreatic anti-diabetic activity for β -stigmasterol, a plant's constituent [2]. Inhibition of decomposition of carbohydrates and fats, and glucose absorption in the gut are also extra-pancreatic activities [8]. Primary factors determining post-prandial blood glucose profile are carbohydrate absorption, insulin and glucagon secretion, and their coordinated effects on glucose metabolism in the liver and peripheral tissues [23]. Others are action of insulin secretion in stimulating glucose uptake, glucose and glucagon suppression, glucose homeostasis, gastric emptying and incretin hormones. Defects in any of these could give underlying post-prandial hyperglycemia in pre-diabetes and overt diabetes [36].

Trace elements detected in plant materials and their ashes were considered to be their active hypoglycaemic or anti-hyperglycaemic agents acting through varied mechanisms of actions, such as optimal insulin secretion and storage, and stimulation of insulin release from β -cells [19,23-26,40]. Traditionally, ashes or juice of *Cucumeropsis mannii* is ingested. Zinc, manganese, chromium, vanadium and magnesium reported to help in improving insulin

resistance, impaired glucose tolerance and oxidative stress in DM were identified in *C. mannii* seeds [40]. *Phyllanthus amarus* seed-ash (90 mg/kg) decreased glucose absorption in the gut and its role in post-prandial blood glucose homeostasis were probably due to its *in vitro* α -glucosidase inhibitory activity, which was comparable to that of acarbose (40 mg/kg). Trace elements of copper, selenium, cobalt, nickel, iron, Zn, V, Mn and Cr, as well as potassium, calcium and Mg that were also detected in this seed-ash were suggested to be responsible for their insulin-stimulating effects on the islets of Langerhans' β -cells, insulin receptor binding and signaling pathway, and as co-factors of many glycolytic enzymes [23]. Vanadium is also known for its insulin-mimetic effects [19,27].

4. CONCLUSION

The study justified ethnomedicinal use of plant ashes in managing diabetes in rural regions of Nigeria. It also confirmed that both ash and organic extract of *A. muricata* leaf have significant anti-hyperglycaemic activity that was comparable to that of glibenclamide. In such cases, anti-diabetic herbal drugs made from the powdered plant parts may be more beneficial than one made from either its organic extract or ash. *Chromolaena odorata* and *A. muricata* leaf ashes (200 mg/kg), 150 mg/kg of *M. koenigii* and *M. indica*, and 100 mg/kg of *M. charantia*, *A. indica* and *E. malaccensis* leaf ashes are hereby recommended as doses for their optimum anti-diabetic folkloric usage. However, it should be ascertained that 150 and 200 mg/kg doses used in this study are safe for internal use. Hence, the leaf ashes may contain elements with glucose post-prandial tolerance factor and insulin stimulating properties, while some had significantly high extrapancreatic activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist and that the work is solely for the advancement of knowledge. The study was not funded by any company or funding agency but by individual efforts of the authors.

AUTHORS' CONTRIBUTIONS

As the MSc research student, AAA did the research work, while ACA was the main supervisor and MDA the co-supervisor. All the authors wrote the manuscript and approved for publication, while ACA processed for publication.

Ethical Approval:

All experiments have been examined and approved by the appropriate ethics committee.

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