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ANTIDIABETIC EFFECT OF ETHANOLIC EXTRACT OF CLERODENDRUM VOLUBILE LEAF ON ALLOXAN INDUCED DIABETES MALE WISTAR RAT

[1]Akindele Oluwayemisi, [2]Adeogun O., [3]Olawunmi M.O.

[1]Department of Science Laboratory Technology, Moshood Abiola Polytechnic, Abeokuta, Ogun State.
akindeleoluwayemisi399@gmail.com,

[2]Department of Hospitality and Tourism Moshood Abiola Polytechnic, Abeokuta, Ogun State.
olajumokeabimbola@yahoo.com

[3]Department of Science Laboratory Technology, Moshood Abiola Polytechnic, Abeokuta,Ogun State.
Temmy07@yahoo.com .

Abstract: **This present study investigated the antidiabetic effect of ethanolic extract of clerodendrum volume on alloxan induced diabetic rats.**

Diabetes is a metabolic disorder characterized by impaired utilization and in the underlying factor for both hypoglycaemia and hyperglycaemia. Herbal medicinal plants are know emerging as substitute to drug due to their very low side effect. Clerodendrum volubile is one of such plant. This study investigated the antidiabetic activity of *clerodendrum volubile* on alloxan induced diabetics in male wistar albino rat.

The proximate analysis, phytochemical screening, mineral analysis where determined to which evaluated the chemical constituent of this plant phytomchemical analysis revealed then present of, flavoniod, tannic, saporin, terperiod cardiaglycoside which exhibited it potential to reduce blood glucose level especially present of flavorniod.

The effect of the ethanolic extract at different concentration of 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg was studied.

The test extracts showed a significant reduction of blood glucose level in which 400mg/kg demonstrated maximum blood glucose lowering potential as compared to other concentrations.

Therefore, *Clerodendrum volubile* possesses ability of lowering blood sugar.

Keywords: **Hyperglycaemia, *clerodendrum volubile,* Diabetes.**

1. INTRODUCTION

Diabetes is a metabolic disorder characterized by impaired glucose utilization and is the underlying factor for both hypoglycaemia and hyperglycaemia. Chronic hyperglycaemia results impaired function or failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Despite advances in medicine, diabetes as a major health complication seems to be growing at an alarming proportion world over and in India, in particular.

Nigeria has the highest number of people suffering from diabetics in African say WHO and Diabetes Association Nigeria (DAN) and by the end of 2030, 79.4 million Indians are expected to be affected by this metabolic disorder and this accounts for nearly one-sixth of the world's diabetics [7.] Plant drugs are frequently considered to be less toxic and more free from side effects

than synthetic ones. In the traditional system of Indian medicine plant formulation and in several cases, combined extracts of plants are used as the drug of choice rather than individual. Many of these have shown promising effects. Various herbal formulations like D-400 and Trasina are well known for their antidiabetic effects 2. Nearly 100 polysaccharides from plants have been reported to have hypoglycemic activity.

1. Clerodendrum volubile, an understudied indigenous plant, belongs to the family Lamiaceae (Verbenaceae) and it is one of the widely distributed vegetables in the warm temperate and tropical regions of the World. The plant is popularly known as "Marugbo" or "Eweta" among the Ikale, Ilaje and Apoi people found in Southern-senatorial district of Ondo State, South West Nigeria. "Obnettete", as the plant is known among the Itsekiri and Urhobo tribes in Niger-Delta, is a green climbing shrub reported to have height of 3m and possesses numerous flowers. These are averagely about 1.5cm in length Adefegha *et al*, 2011

2. and [6] The leaf of Clerodendrum volubile is commonly consumed as vegetables mostly blended with other vegetables as spice with sweet aroma and taste. Locally, the leaves can be blended either fresh or dried and applied as spices in cooking Adefegha *et al*, 2011

Interestingly, the dryer leaves produce the darker soup content commonly referred to as "Eweta" by the Ikales', the leaves of Clerodendrum volubile have great nutritional value as well as herbal and medicinal value.

The plant has been reported to contains very huge quantity of iron and zinc; elements which are important in many enzymes for their functions and for maintenance of fresh skin. The presence of phenolic compounds and other phytochemicals has also been observed . When consumed, the leaves are often noted for stimulating lost appetite as well as replenishing vitality for mothers of new born babies.

1. Proximate and nutrient analyses have been reported to play important role in assessing nutritional significance of edible plant and vegetables Adefegha *et al*, 20113. Clerodendrum volubile has continued to be an important plant in South Western Nigeria where it is widely consumed mainly as vegetables in soup. Evidence abounds that the plant is majorly grown as food. The increase in cultivation and consumption of Clerodendrum volubile may be an evidence of its rich nutritional properties. The basic nutritional value of this plant can actually be assessed by its nutrients content as determination of plants potentials, as a therapeutic agent or food, demands knowledge of its overall nutritional worth and composition.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh *C. volubile* leaves purchased from local Herbal Vendors at Itoku Market Abeokuta Ogun State, Nigeria, were identified and authenticated. The leaves were air-dried, pulverized to fine powder, and stored in air-tight containers till further analysis.

2.2 Extraction Process

Two (2) kg of the blended sample was subjected to ethanol extraction at room temperature. The resulting extract was concentrated *in vacuo* using Buchi Rotavapor (Model: R-300, Buchi, Switzerland) to yield 200g crude extract.

$$\% \text{ yield} = \frac{\text{Weight of the crude extract obtained (g)} \quad \text{X } 100}{\text{Weight of the starting pulverized dry leaf extracted (g)}}$$

2.3 Preliminary Qualitative phytochemical Analysis

The presence of Tannis, Sapons, flavonoid, Terpenoid, Anthraquinones, cyanogenetic glycosides, Sterols and Triterpene, Alkaloides, Reducing Sugar

2.4 Chemical (Proximate) Analysis

Proximate analysis was carried out using the standard procedures of the Association of Official Analytical Chemists [4]. Dry matter (DM) content was determined by drying the sample in a vacuum oven at 100°C and dried to a constant weight. Ash content was determined by incineration of 2 g of the sample in a muffle furnace at 600^0C for 8h. The percentage residue weight was expressed as ash content. Crude fat was determine by Soxhlet extraction method using petroleum ether as solvent.

Nitrogen was determine using the kjeidahl method and crude protein was calculated by multiplying the percentage nitrogen content by the conversion factor 6.25. Nitrogen Free Extract was determined by the formula: 100 – (%Moisture + %Crude protein +%Crude fat + %Ash).

2.5 Mineral analysis

Ashes obtained from the proximate analysis which was digested with concentrated nitric acid. The resulting solution was evaporated to dryness and dissolved in100 ml deionised water. The solution was analyzed for minerals using atomic absorption spectrophotometer and flame photometer.

2.6. *In Vitro* Antioxidant Profiling of *Clerodendrum voluble ethanol extract*

2.6.1. Determination of DPPH-Scavenging Activity of *Clerodendrum voluble ethanol extract*

The effect of *CVE* was estimated using the method by Liyana-Pathirana and Shahidi [9]. A solution of 0.126 mM 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich, St. Louis, USA) in methanol was prepared, and 1.0 ml of this solution was mixed with 1.0 ml of methanol containing 0.2–1.0 mg of each extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The spectrophotometric absorbance of the mixture was measured at 517 nm. The reference drug, vitamin C, used was equally prepared at the same concentration, and the experiment was conducted in triplicate. The ability to scavenge the DPPH radical was calculated by the following equation:

DPPH radical – scavenging activity %

$$= \frac{[(\text{Abs control} - \text{Abs sample0}]}{\text{Abs control}} \quad X\ 100$$

where $Abs_{control}$ = absorbance of DPPH radical + methanol and Abs_{sample} = absorbance of DPPH radical + sample extract/standard

2.7 Enzyme Inhibitory Assay

The ethanolic crude extract was evaluated for their antidiabetic potentials by assaying their inhibitory activities against α-glucosidase [12];[13]. Based on the results, the crude extract was selected for *in vivo* studies.

2.8 Animals

30 male albino rats of Wistar strain weighing about 180–200g, from University of Ibadan, Ibadan, Oyo State were used for the study. They were acclimatized on standard rat pellet chows for 1 week, with water provided *ad libitum* under standard laboratory conditions of natural photo period of 12-h light-dark cycle.

2.9 Induction of Diabetes

Diabetes was induced in 30 rats that have been fasted for 24 hours by a single intra peritoneal injection of freshly dissolved 150mg/kg alloxan monohydrate in normal Saline solution by the method used by Nigerian Journal of Pharmaceutical Research. The control rats (group 7) were given a dose of 150mg/kg normal saline alone. The rats were dose for 10 days consecutively. The blood glucose level (BGL) of the animals was monitored daily using the Glucometer and touch strips.

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2.10 Experimental Design

The 30 diabetic rats were divided into 6groups of 5 rats each. Group 1-4 received 100mg, 200mg,300mg and 400mg/kg of ethanolic extract of Clerodendrum volubile respectively. Group 5 which served as positive control received glibenclamide 5mg/kg while group 6 served as negative control received neither extract nor the standard drug. Group 7 contained 5 rats that were dose with 150mg/kg normal saline only.

Group 1 alloxan + 100mg/kg of C. Volubile Extract

Group 2 alloxan + 200mg/kg of C. Volubile Extract

Group 3 alloxan + 300mg/kg 0f C. Volubile Extract

Group 4 alloxan + 400mg/kg of C. Volubile Extract

Group 5 alloxan + Glibenclamide 5mg/kg

Group 6 Untreated alloxan

Group 7 Normal (Saline only)

3. RESULTS AND DISCUSSION

3.1. *In Vitro* Antioxidant Profiling of *Clerodendrum volubile ethanolic extract*

3.1.1. Determination of DPPH-Scavenging Activity of *Clerodendrum volubile ethanolic extract*

Table 1 shows the *in vitro* DPPH-scavenging activities of 25 μg/ml, 50 μg/ml, 70 μg/ml, and 100 μg/ml of *CVE* in comparison with those of corresponding doses of the standard antioxidant drug (vitamin C) used. As shown, the DPPH-scavenging activities of the extract were significantly (p<0.001) dose related and comparable to those of vitamin C.

Table 1: In vitro DPPH-scavenging activity of 25–100 µg/ml of CVE and vitamin C.

Drug	Graded doses (μg/ml)			
	25	50	75	100
CVE	33.41 ± 0.32	47.10 ± 0.61^c	60.69 ± 0.52^c	72.25 ± 0.32^c
Vit. C	45.05 ± 0.38	56.55 ± 0.94^c	70.45 ± 0.48^c	85.83 ± 0.36^c

cA significant increase at

Table 2: ANTIDIABETIC EFFECT RESULT (ETHANOL EXTRACT)

Blood Pre-treatment Glucose levels (mg/dl)

S/N	Group mg/kg	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	F	p
1	**100mg/kgC. volubile extract**	57±2.0	452±7.31	433±8.51	291±4.13	248±8.18	241±7.22	24.1±7.22	109±3.40	94±2.72	119±1.10	7.22	<0.05
2	**200mg/kg C. volubile extract**	64.7±1.54321 ±4.0	178 ±1.20	157±0.38	137 ±0.33	131±0.58	122±2.85	114±1.45	112±2.30	110±1.03		109.4	<0.05
3	**300mg/kg C. volubile extract**	100±7.44	273±6.16	287±6.30	419±7.73	223±4.94	266±2.52	236±5.35	238±9.77	208±7.84	147±8.07	*1.95*>0.05	
4	**400mg/kg C. volubile extract**	66±4.48	296±6.34	193±6.18	276±1.08	213±7.68	236±1.11	187±4.74	193±5.13	112±4.33	125±2.53	1.13	>0.05
5	**Glibenclamid 5mg/kg**	99±7.46	249±6.34	190±7.67	165±2.08	164±8.02	158±6.44	147±8.57	118±8.13	113±7.72	102±4.24	2.08	>0.05
6	**Untreated**	96 ±1.15202	±1.08210	±7.98430	±2.24416	±2.22402	±2.47395	±1.68390	±2.28201	±7.98250	±1.024.96	<0.05	
7	**Normal**	94±9.44	430±2.24	160±6.54	402±2.47	386±1.25	387±2.12	354±3.98	440±3.46	518±1.28	533±9.60	19.4	<0.05

value are expressed in mean±SEM of six animals one way ANOVA followed by Dunnet's t-test, (F-value denotes statistical significance at P<0.05 SEM; standard error of the mean C. Volubile.

.The results of proximate analysis, as obtained in this study, are presented in Table 3. Results showed average value of 13.97 ± 0.80, 13.86 ± 1.26, 11.26 ± 0.52, 46.69 ± 2.22 Ash, protein, fibre and carbohydrate respectively .

Carbohydrate content is in range with the values obtained for Aneilema aequinoctiale and Moringer oleifera leaves Yahaya et al,[2] but higher than the values reported for triangulare, [14]

The high carbohydrate content of this plant suggests that it provides the body with adequate source of energy and fuel for daily metabolic activities such as muscle contraction and other energy-depended processes. Usually, carbohydrates are hydrolyzed to yield glucose in the body, which can be used up immediately or stored in the liver and muscles (as glycogen) for future use. Clerodendrum volubile may be recommended solely (as vegetable) to vegetarians. It can also be mixed with other good sources of carbohydrate. A high content of carbohydrate in feeds is desirable because deficiency leads to depletion of body tissues [5]. The calorie value of the leaves of marugbo according to the study was 269.31±8.76 Kcal/100g. This analytical value is higher than the energy content of some indigenous green leafy vegetables reported by [13]

Table 3: Proximate composition (% dry weight) of Clerodendrum volubile (Marugbo) leaves Composition% by weight

Parameter	Results
Moisture	8.57 ± 0.47
Ash	13.97 ± 0.80
Crude protein	13.86 ± 1.26
Crude fibre	11.26 ± 0.52
Fat	6.14 ± 0.41
Carbohydrate	46.69 ± 2.22
Energy value (Kcal/100g)	289.31 ± 8.71

Values are means ± SD. n = 3

The results as presented in Table 4, showed phytochemical obtained from this study revealed that nearly all the bioactive compound constituents tested for were present which were terpenoid, cardiacglycoside, flavonoid ,saponin ,tannin except phlobatannin that was absent. The present of these constituents especially flavonoid indicated the potency and the reason for its antidiabetic effect this corresponded to the work reported by Dahiru, D., Onubiyi, J. A. and Umaru, H.and also absent of phlobatannin by Ojeaga *et al*.,2014 for Moriga leaf.

3.2 Phytochemical Constituent of Plant

Table 4: show, Phytochmical Constituent of Clerodendrum Leaves

Phytochemical.	Result
Terpenoids.	Present
Cardiac glycoside.	Present
Flavonoids.	Present
Saponin.	Present
Tannin.	Present
Phlobatannin.	Absent

The mineral composition presented in Table 5, the main minerals present in Clerodendrum volubile leaves were calcium, potassium, phosphorus, magnesium, zinc and sodium while lead was absolutely absent.

Lead is a known toxic element whose presence could have posed health risks. Thus, its absence in the plant may be considered as an advantage in regards to the consumption of the leaves. Potassium is the second most concentrated minerals in Clerodendrum volubile leaves. This result is contrary to the report of [1] who observed that potassium has the highest concentration of minerals in Nigerian agricultural products. Interestingly, potassium ranked second most abundant element in marugbo leaves. The Na/K ratio (0.73) of the leaves is less than one.

However, the Ca/P ratio is slightly higher than one (1.10). A Ca/P ratio of value ≥0.5 is usually considered a good source whereas < 0.5 is believed to be poor sources of food. Clerodendrum volubile leaves contained nutritionally.

Table 5: Mineral composition (mg/100g) of Clerodendrum volubile (Marugbo) leaf Minerals Concentration: g/100g

Parameter	Result
Na	22.86 ± 1.38
K	27.69 ± 3.59
Ca	30.91 ± 1.14
Mg	26.11 ± 0.85
Zn	24.27 ± 5.29
Fe	6.22 ± 0.67
Pb	-
Cu	0.04 ± 0.01
Mn	6. 25 ± 0.59
P	27.61 ± 0.71
Ca/	P**1.12
Na/K	**0.83

Values are means ± SD. n = 3. **Calculated values.

3.3 Discussion

This study was undertaken to evaluate the hypoglycernic activity of C. Volubile in alloxan induced diabetic rat. Glibenclamide was used as standard antidiabetic drug. Different concentration were employed; 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg, from the result of the study, the highest concentration of extract (400mg/kg) showed maximum significant and progressive fall rate compared to 100mg/kg. likewise the control drug gliberclamide showed the fall of blood glucose level of the greatest reduction among all.

When the test extract were administered reduction in glucose level started on day 3 but increased on day 4 and started to declined from day 5 to day 10 they all showed significant decrease in the blood glucose level. Comparison among the extract concentration 400mg/kg exhibited maximum improvement in glucose tolerance.

The hypoglycemic activity of the extract was compared with glibenclamide, a standard hypoglycemic drug. The result revealed that the extract produced significant decrease in the blood glucose level.

The blood glucose lowering ability of the test extract showed encouraging result.

4. CONCLUSION

This experimental result of this research work concluded that the ethanolic extract of C. volubile are rich with antidiabetic potential.

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