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Comparative Studies of Hypoglycemic Potentials of Three Nigerian Medicinal Plants on Streptozotocin-Induced Diabetic Wistar Rats

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Abstract

Diabetes Mellitus and its associated complications have become a burden requiring global attention and more concerted research efforts to unravel possible treatments options. Biochemical techniques were employed in this research to study bioactive components of three plants: *Abelmoschus esculentus, Tapinanthus bagwensis and Vernonia amygdalina*. Diabetes in wistar rats was induced with streptozotocin and was treated with plants extracts at a dose of 200 mg/kg for 21 days. The levels of Glucose, Glycated hemoglobin, Insulin, Zinc and Glutathione were evaluated. *V. amygdalina* and *T. bagwensis* elucidated greater hypoglycemic potential. The hypoglycemic property of these plants vary in the order *Vernonia amygdalina>Tapinanthus bagwensis>Abelmoschus esculentus.*

Keywords: ***Comparison; Bioactive; Hypoglycemic; Flora; Streptozotocin; Wistar rats***

1. Introduction

The Niger Delta Region of Nigeria is the host to a plethora of plant species. While a few of these plants have been studied, there still remains a greater number that have not been investigated for their medicinal potentials. Several previous research efforts as shown by The World Health Organization (WHO) [1] have laid claim to their efficacy for the management and treatment of several diseases among which is Diabetes mellitus, a non-communicable disease (NCD) condition associated with constellation of metabolic derangement occasioned by chronic hyperglycemia arising from defects in insulin secretion, action or both. Major energy molecules like carbohydrates, proteins and fats are involved in these alterations. The WHO [2] has emphasized the needs to study these plants for a greater understanding of their medicinal potentials.

There is now quantum of evidence to support the claim that diabetes Mellitus is an intimidating health problem when the complications of the disease are viewed along with the mortality, morbidity and prevalence rates. It has generally been accepted that diabetes mellitus constitute a fundamental health challenge and therefore requires the scientific community to

strategize and develop comprehensive framework and setting targets through strengthening and enhancing multidisciplinary and multisectoral approach for its prevention and control through effective partnership [3,4]. There is a compendium of evidence to show that disability, depression, cognitive dysfunction, impaired vision, loss of libido, foot ulcer and possible amputation [5,6] are complications arising from diabetes mellitus.

Current estimate put the population of people suffering from Diabetes at over 382 million. In addition there are also 316 million impaired glucose tolerance at high risk from the disease- an excruciating alarming number that is set to reach 471 million by the year 2035 [7].

Previous works by some researchers have been done on *Vernonia amygdalina Delile Compositae* [8,9], *Abelmoschus esculentus(L.) Moench Malvaceae* [10,11] and *Tapinanthus bagwensis(Engl & K. Krause) Danser Loranthaceae* [12]. Each of these works published findings that support the claim of antidiabetic properties of these plants. What appear to be lacking in these works are the fact that they have not been able to clearly prove the active ingredients (principles) that controls or simulate the antidiabetic properties of these plants.

We observed that an integrated approach at investigating these plants potentials to compare their potency have been lacking. In this study, the plants were extracted with methanol and their antidiabetic potentials were evaluated using glucose, glycated hemoglobin, insulin, zinc and glutathione.

2. Materials and Method

2.1 Plant materials

Leaves of *Abelmoscus esculentus (L.) Moench* and *Vernonia amygdalina* Del. were collected in a farm in Biogbolo-Epie in Yenagoa, Bayelsa state, Nigeria. Leaves of Tapinanthus *bagwensis* (Engl. & K. Krause) Danser were collected at the Delta Park University of Port Harcourt.

All three plants were identified by Dr. Edwin-Wosu NL of Ecoland Herbarium and were given specimen No GGS/003 ID. EH/P/068, GGS/001 ID.EH/P/006, GGS/002 ID. EH/P/067 respectively and deposited at the herbarium.

2.2 Extraction

Each of the plants was separately air-dried for 4 weeks under shade and was pulverized using electric milling machine. The powdered plants were weighed into a glass jar and macerated using methanol analyte grade (a product of JHD® Gunsdong Guadgun Chemical, China) for 48 hours with periodic shaking.

It was thereafter decanted and filtered using 125 mm Whatman filter paper. The filtrate was concentrated to dryness *in vacuo* at 30°C and kept in a desiccator until use. To each rat was administered 200 mg/kg body weight of the extract.

2.3 Animals

A total of fifty- eight (58) male and female wistar albino rats aged (15-20 weeks) weighing 200 g-250 g from a colony maintained at the Animals House of the Department of Pharmacology, Niger Delta University were used for the study.

2.4 Induction

Streptozotocin (Zanosar) (a product of Sigma Aldrich, Germany) was used to induce Diabetes Mellitus. This was achieved through the intraperitoneal injection of 70 mg/kg body weight dissolved in IM citrate buffer P^H 4.5. The animals were considered Diabetic when glucose values exceeded 15.0 mmol/L after induction.

2.5 Phytochemicals analysis

Previously established methods [13] were used for qualitative and quantitative analysis of the phytochemicals in the plant.

2.6 Experimentation

A total of 58 male and female rats were used for the study which consisted of 7 groups of 8 rats each as follows:
Group 1 (normal rats on water *ad libitum)*, Group 2 (Diabetic rats untreated), Group 3 (Treatment with *Abelmuscous esculentus*), Group 4 (Treatment with *Vernonia amygdalina*), Group 5 (Treatment with *Tapinanthus bagwensis*), Group 6 (Treatment with Daonil a reference drug), Group 7 (Combined extract treatment). 200 mg/kg body weight of extract was administered for 21 days. Daonil at a concentration of 0.05 mg/ml was used as the standard reference drug.

Blood was collected from the tail vein in plain bottles, allowed to clot and later spun. The resulting serum from the samples were used for analysis.

2.7 Assessment of hypoglycemic activity

Glucose concentration was determined by GOD-PAP enzyme methodology (AGAPE, Gmbh). Measurement was taken at 540 nm with spectronic $22D^+$.

Glycated hemoglobin was determined spectrophotometrically using Glycohemoglobin Reagent Kit (a product of Teco Diagnostics, Lakeview Avenue Anaheim, CA). Measurement was taken at 415 nm [14].

Insulin was determined by the Radioimmunoassay technique, using CX9 Automated Machine (Beckman) with Beckman Assay reagent [15]. Radioactively labeled iodine ^{125}I competes with sample insulin for binding sites on anti-insulin antibodies. Separation of bound from free ligands was accomplished by double antibody precipitation.

GSH assay involved monitoring and evaluating the conjugation of CNDB (1-chloro-2-4-dinitrobenzene) with GSH at various concentration of extract at 540 nm and the optical density taken after every minute.

Zinc was measured by direct Atomic Absorption Spectroscopy using the method of JC Smith Jr and GP Bitrimovi 1979 using Perkin Elmer 403 AAS at 213 nm.

2.8 Statistical analysis

Data were analyzed using the Harrel Miscellaneous R Package Version 4.1.1 and Pairwise Multiple Comparison Package Version 1.4.1. Difference were considered significant at $P<0.05$.

3. Results

The blood glucose changes in response to a 21-day administration of the methanol extract of *Vernonia amygadalina, Abelmoschus esculentus, Tapinanthus bagwensis* leaves to STZ- induced diabetic rats are shown in TABLE 1 and 2.

TABLE 1. **Descriptives for Glucose level in Treatment.**

Treatment	N	mean	sd	Median
Control	8	4.501	0.122	4.475
Untreated	8	16.226	3.319	16.835
Ae	8	11.360	0.763	11.390
Va	8	10.819	3.487	10.965
Tb	8	11.969	3.818	13.180
Daonil	8	9.321	5.350	9.360
Ae + Va + Tb	8	12.791	1.178	12.415

Legend: *Ae, Abelmoschus esculentus, Va Vernonia amygdalina and Tb Tapinanthus bagwensis.*

TABLE 2. **Analysis of Variance for changes in glucose level among Treatments.**

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.
Treatment	6	849.3	141.55	14.49	2e-09	***
Residuals	49	478.5	9.77			
Residual standard error: 3.12						
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

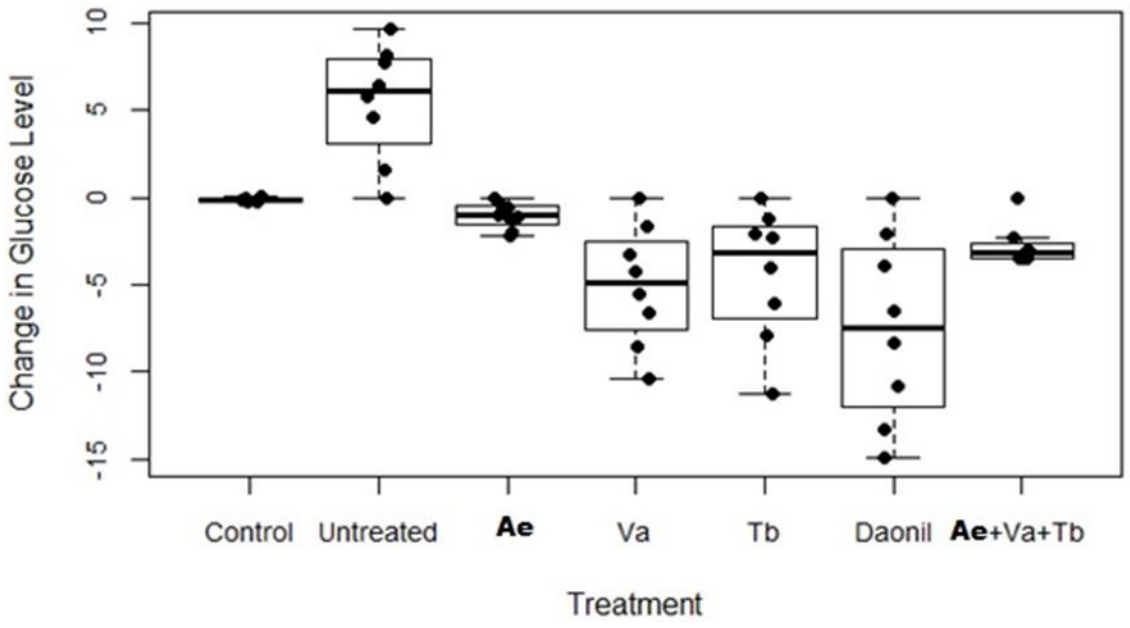

FIG. 1**. Changes in glucose level of the various treatments.**

TABLE 3. **Summary of hypoglycemic effect by rank.**

	Ranks				
	Ae	Va	Tb	Daonil	Ae + Va + Tb
Glucose	5	2	3	1	4
Glycated Haemoglobin	5	3	2	1	4
Insulin	5	1	3	2	4
GSH	1	4	2	3	5
Zinc	2	4	3	5	1
Mean Ranks	3.6	2.8	2.6	2.4	3.6

The ranks were derived from median

1 = Treatment with the greater effect relative to control

5 = Treatment with least effect relative to the control.

We observed that Daonil has the greater effect across board (mean = 2.4) while Ae and Va and Ae combination has the least effect with a tie rank of 3.6. Qualitative and Quantitative phytochemical analysis are shown in Table 4 and 5

TABLE 4. **Qualitative phytochemicals analysis.**

SAMPLE CODE	**ALK**	**TAN**	**SAP**	**FLAV**	**GLY**	**PHEN**	**TERP**
Tapinanthus bagwensis							
i i.ii	+	++	+	++	+	+++	+
ii.	+	+	+	++	+	++	+
iii.	+	++	+	+	+	++	+
Vernonia amygdalina							
i.	+++	++	++	++	+	++	+
ii.	++	++	++	+	+	++	+
iii.	+++	++	+	+	+	++	+
Abelmoschus esculentus							
i.	+	+++	+	+++	+++	+++	+++
ii.	+	+++	+	++	+++	+++	++
iii.	+	+++	+	+++	+++	++	+++

Key: ALK-Alkaloid, TAN-Tannin, SAP-Saponin, FLAV-Flavonoid, GLY-Glycoside, PHEN-Phenol**,** TERP-Terpenoid.

TABLE 5. **Quantitative phytochemicals analysis.**

SAMPLE CODE	**% ALK**	**% TAN**	**% SAP**	**% FLAV**	**% GLY**	**% PHEN**	**% TERP**
Tapinanthus bagwensis							
i.	0.52	0.94	0.68	2.38	1.52	16.86	0.48
ii.	0.48	0.90	0.6	2.40	1.57	16.80	0.56
iii.	0.50	0.92	0.70	2.36	1.60	16.88	0.54
Vernonia amygdalina							
i.	12.79	5.42	1.84	1.68	2.21	3.62	0.66
ii.	12.80	5.40	1.86	1.70	2.23	3.70	0.72
iii.	12.78	5.44	1.83	1.67	2.24	3.68	0.70

Abelmoschus esculentus							
i.	3.64	18.78	4.72	22.00	17.24	5.80	3.45
ii.	3.58	18.72	4.70	21.58	17.26	6.00	3.38
iii.	3.60	18.80	4.73	21.57	17.23	5.88	3.40

Legend: All test were triplicate determination

4. Discussion

The three plants used for this study have been reported to possess antidiabetic properties in some previous studies especially in Africa and Asia. However, the active principle responsible for this property has not been proven with certainty. To increase understanding in this area of research, the present study compared the bioactive and hypoglycemic properties of these plants simultaneously.

Five parameters which could be considered as marker analytes were used in this study: Glucose, Glycated Haemoglobin (HbA1c), Insulin, Zinc and Glutathione (GSH). Both qualitative and qualitative analysis were carried out to ascertain the phytochemicals present in these plants (see TABLE 4 and 5). We observed the presence of Alkaloids, Tannins, Saponins, flavonoids, glycosides, Phenols and Terpenoids. This observation is supported by some previous studies [16-18].

Qualitative and quantitative phytochemical data shows results that vary largely in concentration in the different plant species that were studied. A remarkable observation was the fact *Vernonia amygdalina* have a high percentage of Alkaloid, *Abelmoschus exculentus* have a high percentage of Tannins and flavonoids while presence of phenol is more marked in *Tapinanthus bagwensis.*

Some previous reports indicate that Tannins exhibit α- amylase and α- glycosidase inhibitory activities thus decreasing glucose transport through the intestinal epithelium [19].

Again [20] have reported that flavonoids and alkaloids also inhibit α- glucosidase hence their remarkable additive effect in reducing blood glucose. In addition, they have high degree of radical scavenging activity. Interestingly, saponin is known to alleviate hyperglycemia oxidative stress in type 2 Diabetes [21-23] while Terpenoids reduces blood glucose through insulin-like activity and inhibition of gluconeogenesis and glycogenolysis.

Using ranks derived from median from our statistical analysis, we noted that Daonil had the greatest effect relative to the control when compared to the three plants for glucose and glycated haemoglobin (HbA1c). *Vernonia amygdalina* had greatest effect on insulin while *Abelmoschus esculentus* had a tie rank at 3.6 with the combined treatment (See TABLE 3). It has been shown in some previous reports [24,25] that gluconate pathway precursor activities may also be accounted for in the mechanism by which Vernonia amygdalina exerts antioxidant activity with a resultant improvement in Diabetes Mellitus. It has earlier been hypothesized that insulin production is linked to the use of Vernonia amygdalina [26].

Several reports are available to assert the fact that due to diverse mechanisms of plants exhibiting these characteristics carbohydrate metabolism is enhanced along with secretion of insulin and improvement in β-cell function. The positive correlation of glucose level and glycated haemoglobin is a pointer to confirm this understanding as shown in this study. The

formation of glycosylated haemoglobin by non-enzymatically binding to glucose causes an increase in glycated haemoglobin level [27].

Streptozotocin in known to donate nitric oxide and by this action participates in derangement of pancreatic islet cells. It also generate reactive oxygen species and modulates xanthine oxidase activity. It is known that scavengers of Nitric oxide and reactive oxygen species have the potential to inhibit the damaging effect against DNA and β- cell toxicity [28]. Interestingly, we observed a significant treatment effect of *Abelmoschus esculentus* on the GSH level, a pointer to antioxidant action. *Vernonia amygdalina* also showed strength in elevation of zinc level. These findings deriving from statistical evaluation [29] as shown in this study is vital.

5. Conclusion

The present study have shown that the hypoglycemic property of these plants vary and are in the order *Vernonia amygdalina>Tapinanthus bagwensis>Abelmoschus esculentus.*

6. Competing Interests

The authors declare that they have no competing interests.

7. Acknowledgement

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