IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 2 Ver. IV (Mar. – Apr. 2018), PP 01-06
www.iosrjournals.org

Ant diabetic Effects of Combinations (Ratios) Of Selected Nigerian Ant diabetic Plants

*Ezekwesili C.N[1]., Asomugha R.N[2]., Ekwealor C.C[3]., Ekwunife C.A[4]. And Adindu S.C[1]

1. *Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria.*
2. *Department of Industrial Chemistry, Nnamdi Azikiwe University, Awka,Nigeria.*
3. *Department of Applied Microbiology, Nnamdi Azikiwe University, Awka, Nigeria.*
4. *Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria.*

Corresponding Author: Ezekwesili C.N

Abstract: ***Aim:*** *Herbal preparations are produced from separate plants, various plant combinations or combinations of different species of the same plant. Nauclea latifolia root, Acalypha torta leaves, Gongronema latifolium leaves, Cinchona calisaya bark and Moringa oleifera leaves are Nigerian medicinal plants with scientifically established antidiabetic properties. The antidiabetic activities of various combinations of these plant samples were then studied in alloxan-induced diabetic wistar albino rats to determine the most efficacious combination (ratio).* ***Materials and Methods:*** *Diabetes mellitus was induced with alloxan monohydrate, 150.0 mg/kg wt. (i.p). The various plant samples were collected, washed, dried and pulverized. Various combinations (A – H) of the above plants were prepared by mixing varying ratios of the samples. Different groups of diabetic rats were treated with 50.0 and 100.0 mg/kg wt of the ethanol and aqueous extracts of each combination. Rats in the reference group were treated with glibenclamide, 20.0 mg/kg wt. Alloxan, extracts and glibenclamide were solubilized in distilled water. Phytochemical analyses and acute toxicity testing for each combination were carried out using standard experimental methods. The doses-response study was also carried out for the most effective combination.* ***Results:*** *Results indicated that all the combinations exhibited significant ($p<0.05$) antidiabetic activities and the most effective combination (aqueous extract of (D)) reduced rat blood glucose level by 52.4%. This extract was most potent at a dose of 100.0 mg/kg wt. when taken orally, and contained alkaloids (6.8%), flavonoids (9.2%), saponins (13.2%) and tannins (2.0%). Doses of the extract ≤ 5,000 mg/kg wt did not cause any behavioural changes or mortality in the animals.* ***Conclusion:*** *Therefore, these antidiabetic plants are effective either separately or in combination with other plants and may be regarded as non-toxic to the animals.*

Keywords: *Antidiabetic, Nauclea latifolia, Acalypha torta, Gongronema latifolium, Cinchona calisaya, Moringa oleifera*

Date of Submission: 22-03-2018 Date of acceptance: 05-04-2018

I. Introduction

Diabetes mellitus is a disorder of body metabolism that is characterized by hyperglycemia (increased blood glucose level). This could result from the inability of the β – cells of the pancreatic Islets of Langerhan to produce and release adequate quantity of the hormone insulin (Type 1 diabetes mellitus). It may also be caused by the inability of the body cells, especially the liver and muscles, to recognize and utilize the released insulin (Type 2 diabetes mellitus). Other symptoms of diabetes mellitus such as polyuria (excessive urination), polydipsia (increased thirst), polyphagia (excessive hunger) and weight loss are the consequences of prolonged hyperglycaemia.

Diabetes mellitus is one of the leading silent killer diseases commonly found among the world population today. The World Health Organization estimate revealed that the number of diabetics worldwide would increase to 300 million people by the year 2025 (Aravind *et al*., 2002); WHO, 2014). Nigeria has been listed among the five top ranking countries with the highest prevalence of type 2 diabetes (Onuora and Okafor, 2016).

The complications resulting from prolonged uncontrolled or poorly controlled high blood glucose level above normal include retinopathy (blindness), nephropathy (Kidney failure), neuropathy (degeneratation of the nerves (neuropathy) and cardiovascular disorders.

Current existing therapies for the management of diabetes mellitus include the use of insulin injection (for type 1), biguanides, meglitinides, alpha – amylase and alpha – glucosidase inhibitors (Keerthi *et al*., 2016).

Natural products of which plant derived products form about 25% of the total, represent more than 50% of all the drugs in clinical use today (Lawal *et al*., 2010). In South-Eastern Nigeria, the aqueous extracts of the leaves of *Gongronema latifolia, Acalypha torta*, *Moringa oleifera*, *Nauclia latfifolia* root and *Cinchona calisaya* bark are useful herbal remedies for diabetes mellitus. The antidiabetic activity of these plant extracts have been validated scientifically (Ezekwesili *et al*., 2012; Ezekwesili *et al*., 2015, Aka *et al*., 2011; Ezeigbo *et al*., 2016). These plants are widely distributed in Nigeria and other tropical African countries.

The search for a more effective and safer blood sugar-lowering plant extract that will offer a more protective and, or curative effect against diabetes mellitus and its complications is still an important area of interest in the scientific world. This research study is therefore designed to explore the possible synergistic effects of some of these antidiabetic plants commonly found in Nigeria.

II. Materials And Methods

Plant Materials

The fresh mature plant samples were collected, washed and dried at room temperature. The leaves of *Acalypha torta*, *Gongronema latifolium* and *Moringa Oleifera*, and *Nauclea latifolia* root were collected within Nnamdi Azikiwe University environment. *Cinchona calisaya* bark was collected from Abagana, Njikoka Local Government Area, Anambra State, Nigeria. All the samples were identified and anthenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka.

Experimental Animals

The experimental animal models used were male Wistar albino rats purchased from Chris Animal Farm Ltd, Awka. The animals were housed in standard animal cages in the Department of Applied Biochemistry Animal house. They were kept on Guinea animal feed pellets and drinkable water and were allowed seven (7) days for acclimatization.

Chemicals

Alloxan monohydrate was the product of Sigma, Germany. Glibenclamide was manufactured by Nigerian German Chemicals Plc, Ogun State, Nigeria. All other chemicals used were of analytical grade.

Extraction Procedures

The dried leaf, bark and root samples were pulverized using a manual Corona grinding machine. Various combinations of the plant samples were prepared by mixing them at different ratios to obtain samples A, B, C, D, E, F G,and H as described in Table 1 below.

ample	***N.latifolia* root**	***A.torta* leaves**	***G.latifolium* leaves**	***C.calisaya* bark**	***M.oleifera* leaves**
A	1	1	1	1	1
B	1	1	2	1	4
C	4	2	2	1	1
D	5	1	0	0	0
E	1	5	0	0	0
F	4	1	0	0	0
G	3	1	0	0	0
H	2	1	0	0	0

The same quantities (500.0g) of the samples were soaked in boiled distilled water (2.5L) for 24h at room temperature. At the end of 24h, the infusion was filtered through a cheese cloth and Whatman No. 1 filter paper. The filtrates were then evaporated to near dryness using a Rotary Evaporator (Rotary Evaporator RE52-2 SEARCHTECH INSTRUMENT) to obtain the aqueous extracts. The procedure outlined above for the aqueous extraction was followed using 2.5L of ethanol for each sample to obtain the various ethanol extracts of the samples. All the extracts were stored in the refrigerator prior to use.

Induction of Diabetes mellitus

Seventy (70) male Wistar albino rats weighting 120 to 150g were divided into fourteen groups of five animals each according to their weights. All the animals were fasted over-night and their baseline blood glucose levels were recorded with Accu Check Active Glucometer before the induction of diabetes mellitus. Diabetes was induced in all the test animals with a single intraperitoneal (i.p) injection of alloxan monohydrate at a dose of 150.0 mg/kg body wt. Glucose solution (50%) was given orally to the rats 2h after alloxan administration to protect against alloxan-induced hypoglycaemia. Forty-eight hours (48h) after alloxan administration, the rat blood glucose levels were measured and all the animals with blood glucose levels ≥ 200.0 mg/dl were confirmed diabetic. Alloxan was solubilized in distilled water.

Antidiabetic activity study

Single daily doses of 50.0 and 100.0 mg/kg body wt. of the ethanol and aqueous extracts of each combination (A, B, and C) were administered to different groups of the diabetic rats. Glibenclamide (20.0 mg/70kg body wt.) was given to the animals in the reference groups. Fasting blood glucose levels of the animals were recorded before the administration of both extracts and glibenclamide. The glibenclamide and extracts were solubilized in distilled water and administered orally for five days. Animals in the control group received distilled water (1.0 ml / kg wt.) orally.

The antidiabetic activities of the aqueous extracts of combinations D, E, F, G and H were also studied following the same procedure outlined above.

Effect of dose on the activity of the most effective extract

The dose-response study of the most effective extract (aqueous extract of combination D) was carried out using thirty-five (35) adult male Wistar albino rats (140.0 – 147.0 g). The animals were fasted overnight and their baseline blood glucose levels (day 0) recorded using Accu Check Active Glucometer. Diabetes mellitus was induced with a single intraperitoneal injection of alloxan (150.0mg/kg body wt.) to forty rats. The remaining five rats served as the control animals. Glucose solution (50%) was given orally 2h after alloxan administration to prevent the alloxan induced hypoglycaemia usually caused by alloxan. Forty-eight hours (48h) after alloxan injection, the blood sugar levels of the rats were measured and the diabetes was established at blood glucose level $\geq$ 200mg / dl.

The diabetic rats were then grouped into six groups of five rats each according to their body weights. The untreated diabetic rats served as the positive control group. Diabetic rats in the five test groups were treated with increasing doses of the extract (50, 100, 200, 400,and 800 mg / kg body wt.). The xtract was solubilized in distilled water. Single daily doses of extract and distilled water were administered orally to the respective groups immediately after recording their fasting blood glucose levels.

Phytochemical Analyses

The quantitative phytochemical analyses of combinations A, B, C and D were carried out according to established procedures. The cyanogenic glycosides, saponins, tannins and were determined according to the method of AOAC (1984). The amount of flavonoids present were determined using the method of Bohanaan and Kocepal (1974). The quantity of alkaloids present was determined using the method of Harbone, 1973.

Acute Toxicity

Lorke (1983) method was adopted for the determination of the median lethal dose (LD_{50}) combinations A, B, C and the most active extract (aqueous extract of *Nauclea latifolia* : *Acalypha torta* (5:1) ie sample D. In each pilot study, nine male Wistar albino rats of weight 100. 0 to 120.0 g were used. The animals were divided into three groups (A,B, and C) of three rats each according to their weights and were given the extracts (p.o) at doses of 10.0, 100.0 and 1000.0mg/kg body wt. respectively. The animals were then closely monitored for behavioral changes and mortality for 24h. When no death was recorded in any of the groups, five other groups (D,E,F,G, and H) were given 1250.0, 1500.0, 2000.0, 2500.0 and 5000.0 mg/kg body wt. of the extracts (p.o) respectively and observed for 24h for behavioural changes and mortality.

The LD_{50} was calculated as the geometric mean of the least lethal dose that killed the rat, and the highest dose that did not kill any rat.

III. Statistical Analysis

The arithmetic mean and standard error of mean (SEM) for each group were calculated and all the data obtained analyzed statistically using Analysis of Variance (ANOVA). Statistical analyses were made by a SPSS for Windows versions 13.0 packaged statistics program. All results presented were mean ± standard error of mean.

IV. Results And Discussion

Results presented in Table II show that both aqueous and ethanol extracts of the various combinations of *Nauclea latifolia* root, *Acalypha torta* leaves, *Gongronema latifolium* leaves, *Cinchona calisaya* bark and *Moringa oleifera* leaves lowered the blood glucose levels of alloxan-induced diabetic rats. At 50.0 mg/kg wt. the aqueous extracts of combinations A, B, and C reduced hyperglyceamia by 38.6%, 27.3% and 23.4% respectively. Higher dose of the extracts, 100.0 mg/kg wt., produced 41.2%, 30.8%, and 29.1% reductions respectively. These percentage decreases in blood glucose levels were statistically significant at $p \leq 0.05$. Ethanol extracts of combinations A, B, and C caused 39.7%, 20.9% and 11.8% decreases in blood glucose levels respectively, whereas at 100.0 mg/kg wt reductions of 24.5%, 36.5% and 22.1% respectively were observed. The effects of the ethanol extracts of A and C at 100.0 mg/kg were non-significant ($p>0.05$).

Table II: Effects of Combinations A, B, and C on Alloxan – Induced Diabetic Rats.

Treatment Group	Baseline Blood Glucose level (mg/dl)	Blood Glucose level (mg/dl) After alloxan	Blood Glucose level (mg/dl) on Day 2 of Treatment	Blood Glucose level (mg/dl) on Day 5 of Treatment	Change in Blood Glucose level (mg/dl) after 5 days of Treatment (%)
Control, saline	92.0 ± 2.51	94.0 ± 3.70	93.3 ± 4.17	102.0 ± 0.58	8.5 (+)
Glibenclamide,20.0mg/kg	90.5 ± 9.19	457.3 ± 14.40	374.0 ± 7.02	280.7 ± 9.63	38.6 (–)
Untreated Diabetics	78.0 ± 1.15	317.7 ± 7.98	346.8 ± 3.50	391.3 ± 18.8	23.2 (+)
EE of A, 50.0 mg/kg	61.7 ± 2.85	504.0 ± 7.20	252.7 ± 5.50	304.7 ± 8.1	20.9 (–)*
AE of A, 50.0 mg/kg	72.9 ± 4.20	436.0 ± 11.16	318.0 ± 20.40	316.7 ± 3.70	27.3 (–)*
EE of A, 100.0 mg/kg	66.0 ± 2.0	369.5 ± 9.50	277.5 ± 11.14	234.5 ± 0.50	24.5 (–)
AE of A, 100.0 mg/kg	88.0 ± 7.4	350.2 ± 10.10	301.3 ± 4.20	242.3 ± 6.12	30.8 (–)*
EE of B, 50.0 mg/kg	76.0 ± 1.80	283.0 ± 5.30	152.0 ± 3.70	224.0 ± 10.80	39.7 (–)*
AE of B, 50.0 mg/kg	84.10 ± 9.30	362.0 ± 12.70	288.0 ± 6.60	186.07 ± 4.60	48.6 (–)*
EE of B, 100.0 mg/kg	86.0 ± 12.72	510.5 ± 12.02	541.0 ± 8.32	385.0 ± 5.80	36.5 (–)*
AE of B, 100.0 mg/kg	75.0 ± 6.81	384.3 ± 7.70	260.0 ± 8.20	225.97 ± 9.30	41.2 (–)*
EE of C, 50.0 mg/kg	96.5 ± 5.01	576.0 ± 11.30	350.0 ± 9.90	508.0 ± 21.40	11.8 (–)*
AE of C, 50.0 mg/kg	68.2 ± 7.70	338.0 ± 8.90	301.0 ± 11.70	258.94 ± 6.81	23.4 (–)*
EE of C, 100.0 mg/kg	77.5 ± 0.58	570.0 ± 15.20	461.0 ± 13.13	444.0 ± 16.13	22.1 (–)
AE of C, 100.0 mg/kg	72.2 ± 3.20	319.0 ± 7.12	356.8 ± 10.01	226.17 ± 14.4	29.1 (–)*

Values are means ± SEM, n = 5, All the various combinations reduced the blood glucose levels in the diabetic rats. EE represents Ethanol extract, whereas AE is Aqueous Extract. * Significant at $p<0.05$.

This indicates that the aqueous extracts are more potent than the ethanol extracts. By implication, the bioactive ingredients responsible for amelioration of hyperglycaemia are more extractable in water than in ethanol. Combinations containing higher proportions of *G. latifolium* and *M. oleifera* leaves (B and C) were less efficacious than combination A that contained smaller quantities. Therefore combinations D – H were formulated using various ratios of *N. latifolia* root and *A. torta* leaves. Results of the antidiabetic study for combinations D to H (see Table III) revealed that combination D containing *N. latifolia* bark and *A. torta* leaves (5:1) at 100.0 mg/kg wt. produced the highest significant ($p \leq 0.05$) anti-diabetic effect (52.4%) by decreasing the blood glucose levels of the extract – treated diabetic rats from 344.3 ± 8.35 to 164.3 ± 6.90 mg / dl.

Table III: Effects of Aqueous Extracts of Various Combinations of *N. latifolia* and *A. torta* (D, E, F, G, and H) on the Blood Glucose Levels of Alloxan – Induced diabetic rats.

Treatment Group	Baseline Blood Glucose level (mg/dl)	Blood Glucose level (mg/dl) After alloxan	Blood Glucose level (mg/dl) on Day 2 of Treatment	Blood Glucose level (mg/dl) on Day 5 of Treatment	Change in Blood Glucose level (mg/dl) after 5 days of Treatment (%)
Control, Saline	65.7 ± 2.96	80.0 ± 3.71	93.3 ± 4.10	96.3 ± 3.10	-
Glibenclamide, 20.0 mg/kg	84.00 ± 6.65	457.3 ± 8.45	374.0 ± 7.03	280.7 ± 7.67	38.62 (–)*
Untreated Diabetic	78.00 ± 1.15	317.7 ± 7.98	346.7 ± 2.18	391.3 ± 2.96	23.2 (–)*
AE of D, 100.0 mg/kg	81.7 ± 4.10	344.3 ± 8.35	309.7 ± 5.45	164.3 ± 6.91	52.4 (–)*
AE of E, 100.0 mg/kg	70.0 ± 8.13	213.5 ± 5.80	267.0 ± 2.90	107.0 ± 3.82	49.9 (–)*
AE of F, 100.0 mg/kg	76.0 ± 8.60	330.0 ± 6.60	345.0 ± 10.90	217.0 ± 1.90	34.2 (–)*
AE of G, 100.0 mg/kg	82.5 ± 2.50	424.5 ± 3.85	382.4 ± 5.11	302.5 ± 2.17	28.74 (–)*
AE of H, 100.0 mg/kg	77.0 ± 4.0	408.0 ± 5.60	344.5 ± 4.25	375.5 ± 5.0	7.97 (–)*

Values are mean ± SEM, n = 5. The extracts of the combinations remarkably reduced the blood glucose levels of the diabetic rats. * Significant at $p<0.05$.

These evidences are supportive of the fact that these antidiabetic plants are effective when taken separately or in combination with other plants. Most herbal preparations are produced from various plant combinations or combinations of different species of the same plant. The antidiabetic activity of combined extracts of various *Mormodica* species has also been reported (Savila *et al*., 2012).

The study of the effect of dose on the activity of the most effective combination (D) showed that at 50.0 mg /kg wt., the extract significantly ($p \leq 0.05$) reduced blood glucose level by 12.7%, whereas 100.0 mg/kg wt. produced 61.6% decrease which was also significant at $p \leq 0.05$. Increase in dose to 200.0 mg/kg wt. caused 32.2% decline in blood glucose levels.

Very high doses of the extract above 200.0 mg/kg wt. caused statistically non-significant ($p>0.05$) increases and this inferred that the extract was optimally effective at 100.0 mg/kg wt. (Table IV).

Table IV: Effect of Dose on The Antidiabetic Activity of The Most Effective Combination (D).

Treatment Group	Baseline Blood Glucose level (mg/dl)	Blood Glucose level (mg/dl) After alloxan	Blood Glucose level (mg/dl) on Day 2 of Treatment	Blood Glucose level (mg/dl) on Day 5 of Treatment	Change in Blood Glucose level (mg/dl) after 5 days of Treatment (%)
Control, Saline	73.0 ± 4.60	79.0 ± 2.10	70.0 ± 6.00	78.0 ± 5.30	-
Untreated Diabetic	93.0 ± 3.80	323.0 ± 7.20	424.0 ± 11.15	465.3 ± 12.60*	44.1 (+)
AD, 50.0 mg/kg	96.3 ± 5.70	401.0 ± 10.40	370.5 ± 9.82	350.0 ± 8.73	12.7 (–)
AD, 100.0 mg/kg	73.5 ± 2.87	320.0 ± 11.53	281.5 ± 10.70	123.0 ± 7.70*	61.6 (–)
AD, 200.0 mg/kg	129.5 ± 1.64	416.5 ± 5.52	353.5 ± 7.63	282.5 ± 11.41*	32.2 (–)
AD, 400.0 mg/kg	87.3 ± 3.26	457.0 ± 7.29	568.5 ± 7.05	459.0 ± 9.91	0.4 (+)
AD, 800 mg/kg	80.5 ± 1.17	499.0 ± 10.05	556.5 ± 8.87	594.0 ± 10.37	19.0 (+)

Values represent mean ± SEM, n=5. AD is aqueous extract of combination D. The most effective dose was 100.0 mg/kg wt. * Significant at $p < 0.05$. (+) means Increase in blood glucose level, whereas (–) is Decrease in blood glucose level.

Results of the phytochemical analyses presented in Table V show that these extracts contain reasonable quantities of alkaloids, flavonoids, extract (combination D) contained alkaloids (6.80%), flavonoids (9.20%), saponins (13.20%) and tannins (2.00%). Comparatively, this extract contained the highest percentages of alkaloids, saponins and tannins.

Table V: Phytochemical Composition of Combinations A, B, C and D (%).

Combination	Alkaloids	Flavonoids	Tannins	Saponins	Cyanogenic Glycosides
A	4.40	8.60	1.02	12.80	2.70
B	6.40	10.60	1.40	11.26	3.24
C	4.36	10.80	1.34	12.40	2.75
D	6.80	9.20	2.00	13.20	3.07

The medicinal properties of herbal preparations have been ascribed to the presence of these secondary metabolites (Edeoga *et al*., 2005). For instance, several reports have attributed the anti-diabetic activity of many plants used in ethnomedicine for the management of diabetes mellitus to the presence of saponins and cyanogenic glycosides (Oliver and Zahnd, 1979; Omale and Haruna, 2011; Ezekwesili and Ogbunugafor, 2015). The highly water soluble polyphenolic substances, flavonoids, possess anti diabetic, antioxidant and antihypertensive properties (Bahar *et al*., 2005; Perez – Viscaino *et al*., 2009; Ramachandran *et al*., 2015; Murni *et al*., 2017).

The 24h – acute toxicity testing in rats showed that doses of the extracts of the various combinations (A to D) administered orally did not cause any behavioural alteration or mortality in the animals within the period of surveillance. This suggests that these extracts may be non-toxic to the body. These findings may also suggest that combining various plants in the preparation of herbal remedies may also reduce the toxicity. This is supported by the report by Ezekwesili (2011) that the median lethal dose (LD_{50}) of the ethanol extract of *A. torta* administered to albino mice was lower (565.4 mg/kg body wt.).

V. Conclusion

The extracts of various combinations of *N. Latifolia*, *A. torta*, *G. latifolium*, *G. calisaya* and *M. oleifera* exhibited blood glucose lowering potentials and can be employed in the management of diabetes mellitus. The most effective preparation was the aqueous extract of *N. latifolia* root: *A. torta* leaves (5:1) administered orally at a dose of 100.0 mg / kg wt. This extract when given to experimental animals such as Wistar albino rats did not produce any observable adverse effects.

Acknowledgement

The authors of this manuscript are highly indebted to Tertiary Education Trust Fund (Nigeria) for sponsoring this research work.

Reference

[1]. Aka, P.A., Uzodinma, S.U and Okolo, C.E. (2011). Antidiabetic activity of aqueous and methanol extract and fraction of *Gongronema latifolium* (Asclepidaceae) leaves in alloxan diabetic rats. Journal of Applied Pharmaceutical Science, 01 (09): 99 – 102.

[2]. Aravind, K., Pradeepa, R and Deepa, R. (2002). Diabetes and coronary artery disease. Indian Journal of Medical Research, 116:163 – 176.

[3]. Association of Offical Analytical Chemists. Analytical Oficial Methods of analysis. 14.ed. Washington DC; 1984.

[4]. Bahar A., Tawfeq, A.A., Jaber, S.M and Kehel, T. (2005). Isolation, antihypertensive activity, structure-activity relationship of flavonoids from three medicinal plants. Indian Journal of Chemistry, 44B: 400 – 404.

[5]. Bohanaam, B.A and Kocepal, A.C. (1974). Flavonoid and condensed tannins from leaves of *Hawaiian vaccinium* and *V. calycinum*. Pacific Science, 48: 458 – 463.

[6]. Edeoga, H.O., Okwu, D.E and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(79): 685 – 688.

[7]. Ezeigbo, O.R., Barrah, C.S and Ezeigbo, I.C. (2016). Phytochemical analyses and antidiabetic effect of aqueous and ethanolic extracts of *Moringa Oleifera* leaves in alloxan-induced diabetic wistar albino rats using insulin as reference drug. International Journal of Diabetes Research, 5(3): 48 – 53.

[8]. Ezekwesili, C.N., Obidoa, O., Nwodo, O. F. C and Ezekwesili – Ofili, J. O. (2011). Toxicity of *Acalypha torta* (Muell) leaves ethanolic extract in mice and rat. Animal Research International, 8(1): 1315 – 1322.

[9]. Ezekwesili, C.N., Ogunugafor, H.A and Ezekwesili –Ofili, J.O (2012). Antidiabetic activity of aqueous extracts of *Vitex doniana* leaves and *Cinchona calisaya* bark in alloxan-induced diabetic rats. International Journal of Tropical Disease and Health, 2(4): 290 – 300.

[10]. Ezekwesili, C.N and Ogbunugafor, H.A. (2015). Blood glucose lowering activity of five Nigerian medicinal plants in alloxan-induced diabetic wistar albino rats. Animal Research International, 12(2): 2150 – 2158.

[11]. Harbone, J.B. Phytochemical methods. A guide to modern techniques of plant analyses. Champman and Hall Ltd. New York; 1973.

[12]. Keerthi, K., Saraswati, M., Nyayapathi, V.B.K.S., Krishnaveni, N and Surekha, R.H. (2016). Metformin combination therapy for type 2 diabetes mellitus. Journal of Metabolic Syndrome, 5: 210. Diol: 10.4172/2167 – 0943. 1000210.

[13]. Lorke, D.A. (1983). A new approach to practical acute toxicity testing. Archives of Toxicology, 54: 275 – 287.

[14]. Murni, N.S., Qamar, U.A., Siti, Z.M., Alhassan, M.A., Suganya. M., Vikneswari, P., Sharifah, N.A. S,M., Alfi, K and Jalifah, L. (2017). Antioxidant and Antidiabetic effects of flavonoids: a structure-activity relationship based study. BioMed Research International, Vol. 2017, Article ID8386065, 14 pages.

[15]. Oliver, B and Zahnd, G.R. (1979). Plants with oral hypoglycaemic action. Quarterly Journal of Crude Drug Research, 17 (3 – 4): 139 – 196.

[16]. Omale, J., Haruna, H.U. (2011). Hypocholesterolemic effects of *Nauclea latifolia* (Smith) fruit studied in albino rats. American Journal of Tropical Medicine and Public Health, 1(1): 11 – 21.

[17]. Onuorah, E, E.O and Okafor, C.N. (2016). Efficacy of Extracts of African Eggplant and Okra leaves on Alloxan-induced Diabetes Mellitus Adult male Albino rats. Pakistan Journal of Nutrition, 15 (6): 551 – 555.

[18]. Patel, K.P., Joshi, H.M., Majmudar, F.D and Patel, V.J. (2013) New approaches in the treatment of Diabetes Mellitus. NHL Journal of Medical Sciences, 2(1): 6 – 10.

[19]. Perez-Vizcaino, F., Daurte, J., Jimez, R., Santos-Buclga, C and Osuna, A. (2009).

[20]. Antihypertensive effects of the flavonoid quercetin. Pharmacol Rep, 61(1): 67 – 75.

[21]. Ramachandran, V and Baojun, Xu (2015). Antidiabetic properties of dietary flavonoids: a cellular view. Nutrition and Metabolism, 12: 60.

[22]. Savula., Challa, S.R., Srinivas, S and Jagadeshwar, K. (2012). Antidiabetic activity of combined extracts of various *Momordica* species. International Journal of Pharm. Tech Research, 4(2): 568 571.

[23]. World Health Organization (2014). Global Health Estimates: Deaths by Cause, Age, Sex and Country 2000 – 2012. World Health Organization, Geneva.

Ezekwesili C.N. " Ant diabetic Effects of Combinations (Ratios) Of Selected Nigerian Ant diabetic Plants." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.2 (2018): 01-06.