Research Article
Journal of Research and Review in Science
40-46 Volume 09, June, 2022

DOI: 10.36108/jrrslasu/2202.90.0160

ORIGINAL RESEARCH

Synergistic effect of ethanol extracts of Vernonia amygdalina and Croton zambesicus in alloxan-induced diabetic rats

Oyinlade C. Ogundare*[1], Seide M. Akoro[1], Mutiu I. Kazeem[2], Mutiat A. Omotayo[1], Adewale O. Adepoju[1], Victoria I. Oludare[3]

[1]College of Basic Science, Lagos State University of Science and Technology, Lagos State, Nigeria
[2]Department of Biochemistry, Faculty of Science, Lagos State University, Nigeria
[3]Department of Animal Production, Faculty of Agriculture, University of Ilorin, Nigeria

Correspondence
Oyinlade C. Ogundare, [1]College of Basic Science, Lagos State University of Science and Technology, Lagos State, Nigeria
Email: ogundareoyinlade@yahoo.com

Funding information
This research work is self sponsored.

Abstract:

Introduction: An important aspect of the therapeutic use of medicinal plants is their chemical components, which work synergistically in the same or different plants.

Aims: The capacity of an ethanol leaf extract from *Vernonia amygdalina* (VAMG) and *Croton zambesicus* (CZMG) to reduce blood sugar and cholesterol levels were examined in rats.

Materials and Methods: The ethanol extracts of the selected plants were screened for secondary metabolites, and the median lethal dose (LD_{50}) of the combined extracts was determined using acute toxicity tests. The anti-diabetic study used albino rats with at least fasting blood glucose of 250 mg/dL after alloxan monohydrate induction of diabetics. The blood sugar concentration, body weights, and the effects on certain hepatic indices of the diabetic rats were studied following the administration of the different extracts and the combined extracts for 28 days.

Results: The extracts contained tannins, flavonoids, saponins, phenolics, and glycosides. Furthermore, in alloxan-induced diabetic rats, the combined plant extract (VACZ) significantly ($P<0.05$) reduced fasting blood sugar concentration, serum ALT, ALP, AST, total cholesterol, triglycerides, LDL cholesterol, and VLDL cholesterol while increasing body weight, total protein, and HDL cholesterol. The activity of the combined extract was similar to that of glibenclamide's.

Conclusion: The plant extracts could be used as a supplement and a potential source of antidiabetic candidates. This further confirmed the scientific basis of the use of these medicinal plants as an antidiabetic remedy.

Keywords: Synergistic, *Vernonia amygdalina*, *Croton zambesicus*, Hepatic indices, Blood sugar

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Diabetes mellitus (DM) is a common hormonal disorder that affects both young and old, of any sex, race, or socioeconomic status worldwide. It is distinguished by an unusual rise in blood glucose levels caused by an insulin deficiency or disorder, resulting in changes in fat and protein metabolism. Although diabetes mellitus was initially considered a disorder of developed countries, recent developments indicate that the condition is now a global problem [1,2]. The causes of DM include environmental and hereditary factors, obesity, poor diets, and use of certain drugs, and may result in kidney damage, impaired vision, impotence, hardening and obstruction of blood vessels, and infections [3]. Symptoms of DM include increased urine production, increased thirst and sugar in the blood, loss of water, undue eating, and loss of memory [4, 5].

Several therapeutic paths are drifting towards the use of medicinal plants, either singly or as a combination, which is believed to have more therapeutic advantages than orthodox medicines due to low cost, easy accessibility, and ease of preparation with little or no side effects [6]. *Vernonia amygdalina* and *Croton zambesicus* are two common African plants known for their medicinal properties. *V. amygdalin*a known as "ewuro" in Southwestern Nigeria, is a common household plant where its leaves are used as vegetables in local stews and soups [7]. The stem is also used locally as a chewing stick for its antimicrobial properties [8]. Its antihepatotoxic, antimalarial, antibacterial, anti-diabetic, and anticancer activities have been well documented [9,10]. Similarly, *C. zambesicus*, known as "aje kobale" in the Southwestern part of Nigeria [8,9], is a common medicinal plant used traditionally in the treatment and management of several diseases. Some of the traditionally known uses, such as antidiabetic, antioxidant, anticancer, immunomodulatory, cytotoxic, and antileishmanial activities, have been reported [13, 2]. Akpaso *et al*. [14] investigated the effect of combined *V. amygdalina* and *Gongronema latifolium* leaf extracts on the pancreatic cells of streptozotocin-induced diabetic rats. In this study, we investigated the synergistic antidiabetic, antilipidemic, and anti-hepatotoxic effects of the ethanol leaf extracts of *V. amygdalina* and *C. zambesicus* in alloxan-induced diabetic albino rats.

2. MATERIAL AND METHODS

2.1. Chemicals

Alloxan monohydrate reagents and ethanol were obtained from Sigma Aldrich, "Accu-Chek" glucometer was used for the monitoring of the glucose levels. All other chemicals used were of analytical grades.

2.2 Sources of animals

Male Sprague-Dawley rats weighing between 160 - 200 g and Swiss mice (18- 22 g) were purchased from the Animal House of the Department of Physiology, University of Lagos, Idi-Araba, Lagos, Nigeria. These animals were allowed to acclimatize for two weeks and took water and Grower feed mash from Animal Care, Nigeria Limited *ad libitum.* The practice of animal ethic committee rules and the recommendations of National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH, 1985) were observed. Also, all experimental works involving animals were examined and approved by the Lagos State Polytechnic ethical committee on laboratory animal studies.

2.3 Collection of plant materials

Fresh leaves of *V. amgdalina* and *C. zambesicus* were obtained in a garden in Badagry, Lagos State. The two plant materials were authenticated at the University of Lagos Herbarium with the following voucher numbers: LUH 6920 and LUH 8206, respectively. The samples were taken to the laboratory and air-dried at room temperature for about two weeks. Thereafter, plants were milled into powder and stored in airtight plastic containers until future use.

2.4 Extraction of plant samples

Leaves of *V. amgdalina* and *C. zambesicus* (100 g each) were extracted separately in 80% ethanol in a ratio of 1:6 (w/v) by maceration for 72 h. The plants were stirred continuously throughout the period of extraction using a magnetic stirrer. The mixtures were filtered using filter paper and the filtrates were centrifuged at 1000 rpm for 5 min to obtain pure and clear supernatants, which were separately concentrated using a rotary evaporator at 40 °C to yield the *V. amygdalina* ethanol extract (VAMG; 9.95%) and *C. zambesicus* ethanol extract (CZMG; 9.26%), respectively. The concentrated extracts were kept in a refrigerator at -20 °C for preservation.

2.5 Preparation of Combined Extract

The combined extract (VACZ) was prepared by measuring 1 g each of the VAMG and CZMG dissolved in a normal saline solution to make 10 mL of solution.

2.6 Phytochemical analysis

A sample of each plant extract was screened for the presence of the following phytochemicals: tannins, flavonoids, saponins, phenolic and glycosides using the standard laboratory procedures of Harbone [15] and Sofowora [16].

2.7 Acute toxicity test

The protocol of Lorke [17] was adopted for the two-phase study. In the first stage, three groups of three mice each were subjected to oral administration of the combined extract of *V. amgdalina* and *C. zambesicus* at doses of 1500, 3000 and 5000 mg/kg body weight and observed for signs of toxicity and death within 24 h. In the second phase, three groups of three mice each were treated with three lower doses (100, 500, and 1000 mg/kg body weight) of the extract following the results of phase 1. The LD_{50} value was resolved by calculating the geometric mean of the lowest dose that

caused death and the highest dose for which the animals survived.

2.8 Induction of diabetes mellitus

The induction of DM was achieved according to the modified method of Ndip *et al*. [18]. Animals were fasted for about 16 h and blood samples were drawn using tail vein puncture. The fasting blood sugar was determined by means of the "Accu-Chek" glucometer. DM was induced by a single intraperitoneal injection of newly constituted alloxan monohydrate (alloxan) in cold normal saline at a dose of 150 mg/kg body weight. The animals were offered a 5% dextrose solution to drink after induction to get over drug-induced hypoglycemia. The normal control rats received normal saline at 2 mL/kg body weight via the intraperitoneal route. The animals were carefully monitored for behavioral changes, and the fasting blood glucose level was established after 48 h of alloxan injection. The animals showing hyperglycemia (fasting blood glucose of 250 mg/dL and above) were judged to be diabetic and used for the study.

2.8.1 Animal classification and treatment

The animals were arbitrarily divided into five groups of five rats each. Groups 1 (normal control) and 2 (diabetic control rats) received distilled water. Group 3 (control) received 10 mg/kg body weight glibenclamide, while groups 4 (VAMG) and 5 (CZAM) received 500 mg/kg b.w ethanol extracts of *V. amgdalina* and *C. zambesicus,* respectively, and group 6 (VACZ) received 500 mg/kg b.w of the combined extract (1:1).Blood samples of rats were collected by tail vein puncture after 12 h of overnight fasting and used for the determination of fasting blood glucose during the experiment. Animals were sacrificed by cervical dislocation after 24 h of the last treatment (Day 28). Blood samples were collected using cardiac puncture and sera were separated by centrifugation of the blood specimen at 5000 rev/min for 15 min and stored at 4 °C for biochemical analysis.

2.8.2 Determination of Serum Biochemical Parameters

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total proteins, cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDLC) concentrations were determined by colorimetric methods as described by Randox diagnostic kit procedures. The concentration of low-density lipoprotein cholesterol (LDLC) in each serum sample was calculated from the formula of Friedewald et al. [19].

2.9 Statistical Analysis

The data was presented as mean standard error of mean (SEM). Statistical difference was evaluated by means of one-way analysis of variance (ANOVA) where all data were considered statistically significant at $p< 0.05$.

RESULTS AND DISCUSSION

Table 1: Phytochemical composition of *C. zambesicus* and *V. amygdalina* extracts

Extract	Tannins	Flavonoids	Saponins	Phenolics	Glycosides
CZAM	+	+	+	+	+
VAMG	+	+	+	+	+

Table 2: Effect of administration of combined extracts of *V. amygdalina* and *C. zambesicus* on body weight and blood glucose level of alloxan-induced diabetic rats

Treatment (Group)	B. Wt. change (g)	Blood glucose conc. (mg/dL) Prior induction	Day 0	Day 14	Day 28
Normal	4.96 ± 0.25^	83.60 ± 0.53	96.80 ± 0.24^#	97.00 ± 0.96^#	94.40 ± 0.97^
DM	2.00 ± 0.05*#	79.99 ± 2.50	325.00± 1.86*	389.80± 3.16*#	467.60± 1.34*#
DM+ Glibenclamide	4.72 ± 0.57^	87.00 ± 1.03	330.40± 2.67*	254.40± 1.22*^	68.00 ± 0.76^
DM + VAMG	4.88 ± 0.15^	84.19 ± 0.23	333.22± 1.19*	278.14± 2.47*^	104.00± 0.76^
DM + CZAM	5.00 ± 0.77^	89.19 ± 0.41	303.31± 2.23*	265.32± 1.98*^	100.00± 0.76^
DM + VACZ	4.71 ± 0.03^	95.60 ± 0.90	294.19± 1.18*	260.89± 1.37*^	95.40 ± 0.45^

Data are represented as mean ± SEM (n=5). Where (*) represents significant difference at *P<.05* compared to normal rats, (^) represents significant different at $p< 0.05$ compared to diabetic rats and (#) represents significant different at *P<.05* compared to glibenclamide treated rats. DM: Diabetic animals

Table 3: Effect of administration of combined extracts of *V. Amygdalina* and *C. Zambesicus* on liver function indices in alloxan-induced diabetic rats

Groups	ALT (UL^{-1})	AST (UL^{-1})	ALP (UL^{-1})	TP (Mg/dL)
NORMAL	112.15± 0.11^#	61.26±. 0.44^	140.95± 1.19^#	12.43± 0.28^
DIABETIC	176.27± 1.34*#	96.30± 1.94*#	191.18± 1.18*#	6.10± 0.20*#
DM+ Glibenclamide	134.78± 1.14*^	65.67± 0.25^	163.44±3 .72*^	11.74± 0.21*^
DM + VAMG	129.24± 1.95*^#	67.00± 8.72*^#	152.39± 0.99*^#	11.68± 0.30*^
DM + CZAM	149.96± 1.95*^#	72.73± 0.45*^#	163.14± 1.09*^#	12.96± 0.68*^#
DM + VACZ	127.43 ± 2.52*	69.65± 6.80*^#	150.11±1 2.01*^#	11.93± 0.65*^#

Data are represented as Mean ± SEM (n=5). Where (*) represents significant difference at P<.05 compared to normal rats, (^) represents significant different at p< 0.05 compared to diabetic rats and (#) represents significant different at P<.05 compared to glibenclamide treated rats. AST: alanine aminotransferase, Ast: aspartate aminotransferase, ALP: alkaline phosphatase, DM: diabetes mellitus.

Table 4: Effect of administration combined extracts of *V. amygdalina* and *C. zambesicus* on lipid profile in alloxan-induced diabetic rats

Groups	Chol (mg/dL)	Trigs (mg/dL)	HDLC (mg/dL)	LDLC (mg/dL)
Normal	130.30 ±1.18^#	108.50± 1.32^#	42.02± 0.21#	50.14± 0.85^#
Diabetic	154.02 ±1.31*#	134.91± 0.98*#	28.23± 0.02*^#	67.12± 0.32*#
DM+Glibenclamide	136.27 ±0.60*^	120.90± 0.08*^	35.46± 0.62*^	54.71± 0.67*^
DM+ VAMG	138.14 ±1.59*^	128.00± 0.41*^#	33.74± 0.47*^	56.75± 0.01*^#
DM+ CZAM	140.39 ±0.21*^	128.07± 0.95*^#	32.72± 0.63*^#	57.87± 0.11*^#
DM+ VACZ	134.82 ±1.46^	123.71± 0.54*^	35.96± 0.01*^	53.66± 0.06*^

Data are represented as mean ± SEM (n=5). Where (*) represents significant difference at p< 0.05 compared to normal rats, (^) represents significant different at p< 0.05 compared to diabetic rats and (#) represents significant different at p< 0.05 compared to glibenclamide treated rats. HDLC: High-density lipoprotein cholesterol, LDLC: Low-density lipoprotein cholesterol, DM: DM.

DM is a metabolic disorder that features hyperglycemia, glycosuria, and hyperlipidemia [20]. It is one of the major causes of illness, death, and economic loss worldwide. The available drugs used for the management of the disease have a myriad of side effects [21]. Efficacy of medicinal plants and their phytochemicals in the treatment of DM has been reported [22,23,24]. Table 1 shows the phytochemical composition of the ethanol extracts of *C. zambesicus* and *V. amygdalina*. The result revealed that both extracts contained tannins, flavonoids, saponins, phenolics and glycosides. These substances are implicated in the treatment of different diseases and may be responsible for the observed antidiabetic activity during the study. It is also possible that these compounds might act singly or in synergy and caused the biological activity buttressed by the work reported by Okokon *et al*. [25]. This report is in agreement with the report of Ndip *et al.* [18], where the root extract of *V. amgdalina* and *C. zambensicus* was reported to contain useful phytochemicals. Also, this result is consistent with that obtained by Akoro *et al.* [2] for the leaf extracts of *C. zambensicus*.

All the animals used in this research were healthy as they were more active in the daytime than at night. They were allowed to eat food and drink water freely. The body weight of the animal was rising throughout the period of acclimatization, and there was no sign of fur loss. The combination of *V. amgdalina* and *C. zambesicus* leaf extract did not produce any form of toxicity to the mice at the tested doses. The mice in all the treatment groups were healthy throughout the period of the study, and mortality was not observed in any of the groups of mice. Their behaviour also appeared normal. Therefore, the LD_{50} for the oral administration of combined ethanol extracts of *V. amgdalina* and *C. zambesicus* leaves was more than 5000 mg/kg body weight [26]. Thus, the determination of LD_{50} was taken as the initial step in the assessment and evaluation of the toxicity testing of the extracts, which was used to regard the extracts as non-toxic and provided the basis for the evaluation of the investigated biological activity [27].

In this study, the synergistic activity of the extracts of *V. amygdalina* and *C. zambensicus* was verified against alloxan-induced diabetic rats. Alloxan administration caused a significant (*P<.05*) loss of body weight in diabetic animals when compared to the control and treated groups, as well as an increase in fasting blood glucose levels in experimental rats when compared to control rats 48 hours after alloxan monohydrate induction (Table 2).However, glibenclamide and single and *V. amgdalina* combined extracts of and *C. zambensicus* given to diabetic rats resulted in a significant (*P<.05*) reduction in blood glucose level as early as the fourth day of treatment when compared to diabetic rats until the final treatment, when normalcy was restored. Alloxan is generally known to destroy the beta cells of the pancreas, thereby hindering the secretion of insulin, liberating glucose phosphorylation by glucokinase and causing oxidation or the formation

of reactive oxygen species by Fenton reaction [28,29]. Thus, these reactions caused an increase in the blood glucose concentration in our experimental animal species in accordance with the observations made in the laboratory mouse and rat in the reports of other researchers [30,31]. Drugs such as sulfonylurea are used to treat this nutritional disorder by enhancing the release of insulin from pancreatic beta cells [32]. At the end of the 28-day experiment, fasting blood glucose levels in diabetic rats were considerably higher (P< .05) than in all the treatment groups These results were in accordance to previous scientific reports 25,33]. Conversely, the single or synergistic administration of *V. amgdalina* and *C*. *zambensicus* lowered the glucose level in the animals as they raise the secretion of insulin from the cells of the islets of langerhans in the pancreas, liberate the bound insulin, inhibit hepatic glucose synthesis, or correct insulin resistance; thereby mimicking sulfonylurea metabolic pathways [34]. The sulfonylurea used in this treatment also caused a significant reduction in the blood glucose concentration beyond normal to ensure hypoglycaemia and a substantial decline in body weight. These are common challenges associated with the antidiabetic drug [35]. Reduced body weight is a common occurrence in diabetic conditions and may be caused by unnecessary degradation of tissue proteins as well as muscle wasting.

The results of the effect of glibenclamide, single and combined extracts of *V*. *amgdalina* and *C*. *zambensicus* on liver indices are presented in Table 3. In diabetic animals, there was a significant (*P<.05*) increase in serum ALT, AST, and ALP and a decrease in total proteins, whereas these enzymes were significantly reduced as the total proteins were appreciated by the administration of the combined extracts. Furthermore, after administration of the standard drug and various forms of *V. amgdalina* and *C. zambensicus* extracts, combined extracts of V. *amgdalina* and *C. zambensicus* significantly (*P<.05*) reduced the serum ALT activity of diabetic rats to normal. After induction of diabetes mellitus, administration of glibenclamide, single and combined extracts of *V. amygdalina* and *C. zambensicus* significantly (*P<.05*) reduced serum cholesterol, triglyceride, and low-density lipoprotein cholesterol in diabetic rats, while high-density lipoprotein cholesterol increased (Table 4).Hepatic damage is characterized by increased levels of enzymes as the liver cells' membranes become damaged and release the cytosolic enzyme into the blood. The most commonly used enzymes for investigation of hepatic injury are ALT and AST. ALPs are specific for the diagnosis of hepatobilliary or cholestatic obstruction [36]. The damage caused by alloxan monohydrate was revealed as the elevated levels of AST, ALT and ALP in diabetic rats, and this increase might be due to leakage of the enzymes from the cytosol or alteration in the permeability of the membrane of the hepatocyte. Nevertheless, treatment for 7 days with glibenclamide, single and synergistic extracts of *V. amygdalina* and *C. zambensicus* lowered the activity of these enzymes within the respective treatment groups compared to the diabetic control, thereby enhancing hepatic functions. Similar observations of hepatoprotection in diabetic rats were reported by Koyaguru *et al*. [37] and Asante *et al*. [38].

On the final day of treatment, the change in lipid parameters was statistically significant (*P<.05*) when compared to the normal and diabetic rats. Moreover, the administration of combined extract enhanced a non-substantial (*P>.05*) reduction of cholesterol levels of diabetic rats in comparison to normal and glibenclamide groups. Apart from hyperglycemia, hyperlipidemia is a well-known complication associated with diabetes mellitus. An increased concentration of cholesterol, triglycerides, low-density lipoprotein cholesterol (LDLC) and decreased high-density lipoprotein cholesterol (HDLC) observed in alloxan-induced diabetic rats indicates that there is hyperlipidemia such that there is excess mobilization of fats from adipose tissues (lipolysis) when the glucose in the blood is underutilized [39]. Moreover, the administration of *V*. *amgdalina* and *C*. *zambensicus* caused a substantial reversal of these parameters in ways that the lipid metabolism is partially regulated [40]. Similarly, a combination of *V*. *amgdalina* and *C*. *zambensicus* showed similar potency to glibenclamide in lowering the serum lipid profiles in diabetic rats. As explained by Shah and Khan [41], high-density lipoprotein transports cholesterol from peripheral tissues to the liver, protects membrane damage and diminishes the tendency to develop coronary heart disease.

4. CONCLUSION

The leaf extract of *V. amygdalina* and *C. zambesicus* could be used as supplements and potential source for the development of antidiabetic candidate. Furthermore, the research results confirmed the scientific basis of the traditional application of these medicinal plants as an antidiabetic remedy.

ACKNOWLEDGEMENTS

The authors of this research article wish to acknowledge the support of the management of Lagos State University of Science and Technology (formerly Lagos State Polytechnic), Ikorodu, for granting us the facilities used for this work.

COMPETING INTERESTS

The authors declare that there is no conflict of interests concerning the publication of this article.

AUTHORS' CONTRIBUTIONS

All authors directly contributed to the analysis and compilation of this research. They have read and approved the final manuscript.

ETHICAL APPROVAL

All authors declare that our study was carried out according to the guide for laboratory animal care and use, published by National Institute of Health (NIH) No. 85-23, revised 1985. Also, all experimental works were

examined and approved by Lagos State Polytechnic ethical committee on animal studies.

REFERENCES

1. Atlas D. International diabetes federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation. 2015;33.
2. Akoro SM, Ogundare CO, Oladipupo OR. Phytochemical study and alpha-amylase inhibitory properties of leaf extracts of *C. zambesicus* (Müll. Arg.). Biotechnology Journal International. 2017;18(1):1-8.
3. Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. International Journal of Vascular Medicine. 2012.
4. Fasola T, Okeocha P, Odetola A. Screening for the Hypoglycaemic Potentials of the extract of *V. amyggalina*. Ethnobotanical Leaflets. *2010;* (7): 6.
5. Ozioma EOJ, Chinwe OAN. Herbal medicines in African traditional medicine. Herbal medicine. 2019;10: 191-214
6. Wolfsdorf JI, Glaser N, Agus M, Fritsch M, Hanas R, Rewers A et al. ISPAD Clinical Practice Consensus Guidelines 2018: ketoacidosis and the hyperglycemic hyperosmolar state. Pediatric diabetes. 2018; 19:155-177.
7. Prance G, Nesbitt M, editors. The cultural history of Plants. Routledge; 2012.
8. Adeoti OM, Adedoja SA, Adedokun EO, Olaoye OJ, Abiola AO, Okesipe FO. Efficacy of chewing sticks extract on the agent of dental carries isolates. Arch Clin Microbiol. 2020;11(1):101.
9. Taiwo O, Xu HX, Lee SF. Antibacterial activities of extracts from Nigerian chewing sticks. Phytotherapy Research: An International Journal Devoted To Pharmacological And Toxicological Evaluation Of Natural Product Derivatives. 1999;13(8):675-9.
10. Ijeh II, Ejike CE. Current perspectives on the medicinal potentials of *V. amygdalina* Del. Journal Of Medicinal Plants Research. 2011;5(7):1051-61.
11. Agishi EC, Shehu Y. Hausa, Fulfulde, and Scientific Names of Plants. Agitab Publishers Limited; 2004.
12. Odugbemi T, editor. Outlines and pictures of medicinal plants from Nigeria. Tolu Odugbemi; 2008.
13. Okokon JE, Bassey AL, Obot J. Antidiabetic activity of ethanolic leaf extract of *C. zambesicus* Muell.(thunder plant) in alloxan diabetic rats. African Journal of Traditional, Complementary and Alternative Medicines. 2006;3(2):21-6.
14. Akpaso MI, Atangwho IJ, Akpantah A, Fischer VA, Igiri AO, Ebong PE. Effect of combined leaf extracts of *V. amygdalina* (bitter leaf) and *Gongronema latifolium* (utazi) on the pancreatic [beta]-cells of streptozotocin-induced diabetic rats. British Journal of Medicine and Medical Research. 2011;1(1):24.
15. Harborne JB. The terpenoids. In Phytochemical Methods 1973 (pp. 89-131). Springer, Dordrecht.
16. Sofowora A. Screening plants for bioactive agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. 1993:134-56.
17. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54(4):275-87.
18. Ndip A, Williams A, Jude EB, Serracino-Inglott F, Richardson S, Smyth JV et al. The RANKL/RANK/OPG signaling pathway mediates medial arterial calcification in diabetic Charcot neuroarthropathy. Diabetes. 2011;60(8):2187-96.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;(6):499-502.
20. Tripathi V, Verma J. Different models used to induce diabetes: a comprehensive review. International Journal of Pharmaceutical Science. 2014;6(6):29-32.
21. Mohammed A, Ibrahim MA, Islam MS. African medicinal plants with antidiabetic potentials: A review. Planta Medica. 2014;80(5):354-77.
22. Hassan Z, Yam MF, Ahmad M, Yusof AP. Antidiabetic properties and mechanism of action of *Gynura procumbens* water extract in streptozotocin-induced diabetic rats. Molecules. 2010;15(12):9008-23.
23. Ofusori DA, Komolafe OA, Adewole OS. Ethanolic leaf extract of croton zambesicus (MÜll. Arg.) improves gastric emptying capacity and gastric mucosa integrity in streptozotocin-induced diabetic rats. International Journal of Diabetes Research. 2012;1(4).
24. Luke UO, Ebong PE, Eyong EU, Robert AE, Ufot SU, Egbung GE. Effect of ethanolic root and twig extracts of *V. amygdalina* (etidot) on liver function parameters of streptozotocin induced hyperglycaemic and normal wistar rats. European Scientific Journal. 2013;9(30).
25. Okokon JE, Bassey AL, Obot J. Antidiabetic activity of ethanolic leaf extract of *C. zambesicus* Muell.(thunder plant) in alloxan diabetic rats. African Journal of Traditional, Complementary and Alternative Medicines. 2006;3(2):21-6.
26. Sherif HB, Baba G, Abdullahi SM. Acute and sub-chronic toxicity profile of *Annona muricata* (Sour sop) on wister albino rats. Bayero Journal of Pure and Applied Sciences. 2017;10(2):57-63.
27. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Toxicological studies of hydromethanolic leaves extract of *Grewia crenata*. International Journal of Pharm Science and Drug Research. 2012;4(4):245-9.
28. Rotimi SO, Omotosho OE, Rotimi OA. Persistence of acidosis in alloxan-induced diabetic rats treated with the juice of *Asystasia gangetica* leaves. Pharmacognosy Magazine. 2011 Jan;7(25):25.
29. Usman UZ, Bakar AB, Mohamed M. A review on experimental methods of diabetic research: advantages and limitations. Annual Research and Review in Biology. 2015 May 8:100-8.
30. Sharma N, Garg V. Antihyperglycemic and antioxidative attribute of hydroethanolic extract of *Butea monosperma* (Lam.) seeds and its active constituents. 2011.
31. Sharma N, Garg V. Antihyperglycemic and antioxidant effect of hydroethanolic extract of *Butea monosperma* bark in diabetic mice. 2012.
32. Adewoye EO, Ige AO. Lipid profile and electrolyte composition in diabetic rats treated with leaf extract of *Musa sapientum*. Journal of Dietary Supplements. 2016;13(1):106-17.

33. Itelima JU, Nyam MA, Ogbonna AI, Onwuliri EA, Maiangwa DT. Comparative Hypoglycemic Effects of Three Nigerian Medical Plants, *Gongronema latifolium*, *V. amygdalina* and *Viscum album* on Alloxan-Induced Diabetic Mice. 2014.
34. Broichhagen J, Schönberger M, Cork SC, Frank JA, Marchetti P, Bugliani M, Shapiro AM, Trapp S, Rutter GA, Hodson DJ, Trauner D. Optical control of insulin release using a photoswitchable sulfonylurea. Nature communications. 2014;5(1):1-1.
35. Misra H, Soni M, Silawat N, Mehta D, Mehta BK, Jain DC. Antidiabetic activity of medium-polar extract from the leaves of *Stevia rebaudiana* Bert. (Bertoni) on alloxan-induced diabetic rats. Journal of Pharmacy and Bioallied Sciences. 2011;3(2):242.
36. Fody E. P., Schoeff L.E. and Bishop M.L. Clinical chemistry. Principles procedure and correlations (pp278-281).7Th edition Philadelphia:J. B. Linpicotts Company. 2013
37. Koyagura N, Jamadar MG, Huilgol SV, Nayak N, Yendigeri SM, Shamsuddin M. Antidiabetic and hepatoprotective activities of *Tamarindus indica* fruit pulp in alloxan induced diabetic rats. International Journal of Pharmacology and Clinical Sciences. 2013;2(2).
38. Asante D. Effah-Yeboah E, Barnes P., Abban H.A., Ameyaw E. O., Boampong J.N et al. Antidiabetic Effect of Young and Old Ethanolic Leaf Extracts of *V. amygdalina*: A Comparative Study. Journal of Diabetes Research. 2016: 1-13.
39. Lachin T, Reza H. Anti-diabetic effect of cherries in alloxan induced diabetic rats. Recent Patents on Endocrine, Metabolic and Immune Drug Discovery. 2012;6(1):67-72.
40. Ezekwesili-Ofili JO, Gwacham NC. Comparative effects of peel extract from Nigerian grown Citrus on body weight, liver weight and serum lipids in rats fed a high-fat diet. African Journal of Biochemistry Research. 2015;9(9):110-6.
41. Shah NA, Khan MR. Antidiabetic effect of *Sida cordata* in alloxan induced diabetic rats. BioMed Research International. 2014 Jul; 2014.