IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 8, Issue 1 (Jan. – Feb. 2022), PP 12-22
www.iosrjournals.org

Antidiabetic and hypolipidemic properties of *Vernonia* amygdalina aqueous, ethanol, methanol, toluene and benzene extracts in Alloxan-Induced diabetic rats

Alozie, E. U.[1]*, Iheanacho, K. M.[1], Alisi, C. S.[1], Asiwe, E. S[1]. Nwosu, C. J.[2], Iheanacho, J. N[3].

[1]*Department of Biochemistry, Federal University of Technology Owerri, Imo State*
[2]*National Universities Commission, Abuja, Nigeria.*
[3]*National Verterinary Research Institute Vom, Jos, Nigeria.*
**Corresponding author: E-mail: ugochukwusmile@gmail.com,*

ABSTRACT
The present study evaluated the antidiabetic and hypolipidemic properties of Vernonia amygdalina (VA) aqueous, ethanol, methanol, toluene and benzene extracts in Alloxan-Induced diabetic rats. The experimental plot involved eight groups of five animals each (35 alloxan-induced diabetic rats and five normal rats) in which Group 1 and Group 2 served as Negative Control (NC) and positive control group (Diabetic control (DC); non-diabetes and diabetes-induced respectively. Groups 3-8 were diabetes induced with 120mg/Kg body weight alloxan. These groups were treated with 200mg kg^{-1} b.wt. toluene, benzene, methanol, ethanol, aqueous extracts of VA leaf extract respectively, group 8, standard (STD) was treated with metformin (200mg kg^{-1} b.wt.) using an intra-gastric tube for 14 days; All animals were fed with normal feed and clean water ad libitum. Blood glucose, serum total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol levels in plasma were determined by specific standard colorimetric methods. The results of the present study indicate that VA leaf extracts significantly ($P<0.05$) reduced blood glucose concentration in diabetic rats. The efficacy of the extract was in the order methanol > ethanol >benzene > aqueous> toluene. The ethanol and methanol extract reduced baseline blood glucose concentration by 33.19% and 43.66% respectively; and significantly ($P<0.05$) reduced total cholesterol concentration, comparing favorably with metformin. Furthermore, ethanol, methanol, and aqueous extracts of VA leaf resulted in a significant ($P<0.05$) reduction of cardiovascular risk ratio; and compared favorably with normal control and metformin. Also, administration of the aqueous and methanol extract alone resulted in a significant ($P<0.05$) restoration of altered HDL-cholesterol concentration. The administration of methanol extract of V. amygdalina leaf caused a significant homeostatic restoration of deranged lipid profile indices of the diabetic rats; lowered LDL cholesterol level, hypercholesterolemia significantly ($P<0.05$) increased HDL-cholesterol concentration and lowered cardiovascular risk ratio compared to other solvents extract studied.

Keywords: *Diabetes mellitus, Alloxan, Vernonia amygdalina, Antidiabetic and hypolipidemic*

Date of Submission: 13-02-2022 Date of Acceptance: 28-02-2022

I. Introduction

Diabetes milletus (DM) is a metabolic disorder that affects carbohydrate, protein, and fat metabolism; marked with chronic hyperglycemia resulting from insulin deficiency or insulin inaction (Porth, 1998; Dilworth *et al.,* 2021). Globally, diabetes is estimated to affect 9.3% (463 million people) and prevalence may increase to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (Saeedi *et al.,* 2019). It is more prevalent in urban and high-income countries than rural areas in low-income economies (Saeedi *et al.,* 2019). However, the developing economies of Africa and Asia contribute a significant fraction to the diabetes burden. In Nigeria, it is reported that approximately 5.8% (about 6 million) of adult Nigerians are living with DM (Uloko *et al.,* 2018). It cuts across all regions of the country; with the highest prevalence seen in the south-south geopolitical zone. Urban dwelling, physical inactivity, advanced age, and unhealthy diet are important risk factors for DM among Nigerians (Uloko *et al.,* 2018). Diabetes Mellitus's high prevalence and potential deleterious effect on physical and psychological state make it a major medical concern and a leading cause of death in the world (Bertoldi *et al.,* 2013).

Diabetes mellitus can be classified into type 1 and type 2; with type 1 resulting from autoimmune destruction of β-cells of the pancreas, while in type 2 diabetes, the peripheral tissues resist the effects of insulin

or they are reduced secretion of insulin (Paschou *et al.,* 2018). The impaired insulin pathway may generate an array of disturbances in glucose and lipid homeostasis resulting in inflammation, hyperglycemia, and dyslipidemia (Newairy *et al.,* 2002). Diabetic microvascular complications such as antipathies, cardiovascular disorders, blindness, renal failure, neuropathies, and cancers are caused by persistent exposure to high glucose levels, leading to increased production of oxygen free radicals from autoxidation of glucose and glycosylation of protein (Giacc and Brownlee, 2010).

Recent research has focused on medicinal plants as precursors for the synthesis of useful drugs, or crude formulation for the treatment of diabetes mellitus and other diseases. An estimated 80% of the people of developing countries especially Asia and Sub-Saharan Africa rely on traditional medicines, mostly plant-derived drugs, for their primary health needs (Oyebode *et al*., 2016).

Vernonia amygdalina (VA) belongs to the Asteraceae family, it is a shrub common in tropical Africa (Ijeh and Ejike, 2011). It is commonly known as bitter leaves due to its bitter taste, attributable to rich antinutritional components such as alkaloids, saponins, glycosides, and tannins (Adewole et *al*., 2019). In Nigeria, it is known locally as ‘’Ewuro’’, ‘’Onugbu’’, and “Chusar doki or fatefate” by the Yoruba, Igbo, and Hausa-speaking tribes respectively (Igile *et al.,* 1994). The use of bitter leaves ranges from culinary to medicinal; the roots and leaves decoction of VA are commonly used in ethno-medicine to treat fevers, stomach discomfort, diabetes, malaria, hiccups, and as a laxative, etc. (Hamowia and Safran, 1994; Argheore *et al.,* 1998). Numerous documented experiments by various workers abound on the phytochemical and antioxidant properties of the plant parts (Iwalokun *et al.,* 2006; Ayoola *et al.,* 2008; Akah *et al.,* 2009). The medicinal properties such as antibacterial, antioxidant, antimalarial activity, gastroprotective, and wound healing properties of VA have been reported (Ogbulie *et al.,* 2007, *SweeKeong et al., 2010*). Chemopreventive properties (Izevbigie, 2003, Izevbigie *et al.,* 2003) and anti-diabetic properties (Ebong *et al.,* 2008; Taiwo *et al.,* 2009) have also been reported. It is known that differences like the polarity of the solvent can affect the number of extractive substances dissolved in the sample extract tested (Wakeel *et al.,* 2019; Nawaz *et al.,* 2019; Nawaz *et al.,* 2020). In this study, we evaluated the antidiabetic and hypolipidemic properties of VA leaf crude extract using aqueous, ethanol, methanol, toluene and benzene extract in alloxan-induced diabetic rats.

II. Materials and methods

Chemicals

The chemicals Alloxan and Metformin were purchased from Fluka-Chemie, Switzerland and Ranbaxy Chemicals (P) Ltd (Mumbai, India) respectively, Toluene and Benzene (BDH, England), Methanol and Ethanol (JHD, China). Cholesterol, Triglycerides, and HDL-cholesterol test kit were from Biosystems, Spain. All other reagents were of analytical grade.

Plant materials

The fresh leaves of *Vernonia amygdalina Delile* was collected in May 2021 from Anguldi, Zawan Bukuru metropolis, Plateau State, Nigeria. The sample was then authenticated by Mr. O.E. Agyeno at the Herbarium Unit of the Department of Plant Science and Biotechnology, Faculty of Natural Science, University of Jos, Nigeria with voucher number (JUHN21000359).

Experimental Animals:

Fifty (50) apparently healthy male albino rats (*Rattus norvegicus*) weighing 120-150g, averaging about 6 weeks old was used for the study. The animals were purchased from Animal friend farms Owerri, Imo State; and housed in stainless steel cages under standard laboratory conditions of light, temperature (25 ± 2^{o}C), and relative humidity (55 ± 5%). The animals were fed standard rat pellets (Vital finisher, Nigeria) and portable water *ad libitum.*

Preparation of extracts

The fresh leaves of the plants were collected, dried under a shed for 7 days at ambient temperature (25^{0}C). The dried leaves were crushed to a fine powder in a commercial mill.

The coarse powder of *V. amygdalina* leaves (1kg) was extracted with 2.0 L polar solvents (Toluene, Methanol, Ethanol) in a Soxhlet extractor, while an equivalent amount of the plant powder was extracted in benzene and water by cold maceration for 48 hours. The filtrates were evaporated to dryness in a water bath and stored in a freezer ($\leq 4.0\ ^{0}$C) until when needed.

Induction of diabetes mellitus in rats

Diabetes was induced in 12hrs fasted rats by intraperitoneal injection of alloxan monohydrate dissolved in sterile normal saline (120 mg/kg). The alloxanized rats were kept for 7 days with free access to food and water. The rats fasted on the 8th day for 12 hours and their blood glucose levels were determined in blood withdrawn from the animals through the tail veins using a Glucometer (Accucheck Active, Roche Diagnostics, Mannheim, Germany). Rats with glucose levels above < 250 mg/dl or 13.89mmol/L were considered diabetic and used for the study (Cam *et al.,* 2003).

Experimental design and treatment

In the anti-diabetic study, the animals have divided into eight (8) groups of five (5) rats per group. The study consisted of 40 rats (35 alloxan-induced diabetic rats and five normal rats). The experimental plot was as follows:

Group 1– Negative Control (NC); This group was fed with normal feed and clean water *ad libitum*, It includes normal rats, with no alloxan and plant extracts treatment. Group 2– Diabetic control (DC); This group is also called the positive control group and was fed with normal feed and clean water *ad libitum*, It includes induced diabetics, with no plant extracts treatment. Group 3 (TOL): This group includes diabetic rats treated with toluene extract of VA (200mg kg^{-1} b.wt.) using an intra-gastric tube for two weeks. Group 4 (BENZ): This group includes diabetic rats treated with benzene extract of VA (200mg kg^{-1} b.wt.) using an intra-gastric tube for two weeks. Group 5 (METH): This group includes diabetic rats treated with methanol extract of VA (200mg kg^{-1} b.wt.) using an intra-gastric tube for two weeks. Group 6 (ETH): This group includes diabetic rats treated with ethanol extract of VA (200mg kg^{-1} b.wt.) using an intra-gastric tube for two weeks. Group 7 (AQE): This group includes diabetic rats treated with an aqueous extract of VA (200mg kg^{-1} b.wt.) using an intra-gastric tube for two weeks. Group 8 (STD): This group includes diabetic rats treated with Metformin (200mg kg^{-1} b.wt.) using an intra-gastric tube for 14 days. All treatments were given orally to experimental rats using a gavage tube at a single dose daily; and also fed with normal feed and clean water *ad libitum*. After 14 days of treatment, the animals fasted overnight; rats were sacrificed by light anesthesia using dichloromethane. Blood was collected by cardiac puncture into plain containers; the sera were obtained by allowing the blood sample to clot for 15 min and centrifuging at 3500 rpm at 30 ^{o} C for 10 min. The clear sera were aspirated into plain tubes and stored in the refrigerator for biochemical evaluation.

Determination of serum lipid profile

The serum lipid profile including serum total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) was determined using Biosystem test Kits. Low-density lipoprotein cholesterol (LDL-C), LDL/HDL-cholesterol ratio, and cardiovascular risk ratio (CRR) were calculated (Perini *et al.,* 2019).

LDL cholesterol = Total cholesterol – Triglyceride/5 – HDL cholesterol (mg/dl)

LDL/HDL-cholesterol ratio = LDL-C/HDL-C

Cardio Risk Ratio (CRR) = TC/HDL-C

Statistical Analysis

The results for this study were expressed as mean ± standard deviation (n=5) and a test of statistical significance was employed using one-way analysis of variance (ANOVA). The results were analyzed using the Statistical Package for social sciences (SPSS), version 22, and p values < 0.05 were considered significant.

III. Results

Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on fasting blood glucose concentration in alloxan-induced diabetic rats

Table 1 shows the effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on Fasting blood glucose concentration in alloxan-induced diabetic rats. The result shows that alloxan-induced diabetes resulted in a significant ($p<0.05$) increase in blood glucose in exposed rats as compared to normal control animals. After the administration of the VA leaf extract to diabetic rats during a 7days period, it resulted in a varying effect on blood glucose concentration. The extracts demonstrated glucose reducing effect, the blood glucose monitored over 7 days showed a 16.40%, 33.19%, 43.66%, 12.65%, 17.12%, 60.17% decrease in blood glucose relative to baseline values in the groups treated with VA aqueous, ethanol, methanol, toluene, benzene extracts, and Glucophage respectively. The glucose reducing effect of the extract was in the order Methanol > Ethanol>Benzene> Aqueous> toluene. The ethanol and methanol extract yielded 33.19% and 43.66% of baseline blood glucose reduction; compared favorably with the standard drug glucophage.

Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum total cholesterol concentration.

Figure 1 shows the effect of VA leaf aqueous, ethanol; methanol, toluene, and benzene extract administration on serum total cholesterol concentration in alloxan-induced diabetic rats. Results obtained show that diabetes induction resulted in a significant ($p<0.05$) elevation of total cholesterol concentration. However, the ethanol and methanol extracts caused a significant ($p<0.05$) reduction of total cholesterol concentration when compared to the diabetic control group. Furthermore, the result presented in figure 1, serum total cholesterol concentration was 61.82 ± 3.41, 91.74 ± 3.34, 83.68 ± 6.29, 75.94 ± 5.13, 69.87±2.51, 80.96±6.42,

80.75±4.95, and 87.66±6.46 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ, and STD respectively.

Table 1.0 Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on Fasting blood glucose concentration in alloxan-induced diabetic rats

	Glucose Concentration (mg/dl)					
	Day 0	Day 1	Day 3	Day 5	Day 7	Glucose Reduction(%)
NC	104.40 ± 10.62	98.80 ± 8.41	112.80 ± 16.63	119.20 ± 3.42	103.60 ± 13.83	0.77
DC	333.00 ± 38.42	371.75 ± 50.86	402.25 ± 74.50	427.75 ± 59.29	349.50 ± 23.30	-4.95
AQE	338.20 ± 29.76	427.00 ± 49.00	387.75 ± 12.26	321.25 ± 10.28	282.75 ± 30.74	16.40
ETH	374.20 ± 43.46	329.00 ± 56.83	314.80 ± 37.19	280.20 ± 32.67	250.00 ± 40.98	33.19
METH	361.00 ± 53.92	306.80 ± 55.65	270.40 ± 43.37	225.20 ± 38.98	203.40 ± 26.08	43.66
TOL	371.50 ± 39.95	398.75 ± 29.55	422.25 ± 26.96	391.75 ± 59.43	324.50 ± 20.82	12.65
BENZ	366.50 ± 15.72	397.25 ± 53.77	335.00 ± 41.00	327.00 ± 31.21	303.75 ± 21.91	17.12
STD	287.25 ± 42.86	242.50 ± 35.54	228.00 ± 29.74	197.80 ± 9.15	114.40 ± 13.91	60.17

Values are Mean ± standard deviation of 4 determinations.

Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum triacylglycerol concentration.

Figure 2 shows the effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum triacylglycerol concentration in alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) elevation of triacylglycerol concentration. Administration of the extracts produced a varying degree of triacylglycerol reduction. The methanol extracts of VA leaf caused normalization of TG similar to those of normal control and the standard. The extracts of ethanol, toluene, benzene, and aqueous also caused a significant ($p<0.05$) reduction of TG but did not normalize the concentration. The result presented in figure 2 serum triacylglycerol concentrations was 51.85±4.61, 84.36±7.41, 68.93±5.80, 70.58±8.75, 50.82±4.67, 65.84±3.98, 63.79±3.19, and 39.92±7.13 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ, and STD respectively.

Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum HDL-cholesterol concentration.

Figure 3 shows the effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum HDL-cholesterol concentration in alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) decrease in HDL-cholesterol concentration. However, administration of the aqueous and methanol extract resulted in a significant ($p<0.05$) restoration of altered HDL-cholesterol concentration. Result presented in figure 3, serum HDL-cholesterol concentration was 45.09±1.30, 26.25±1.92, 41.71 ±2.89, 35.09±3.22, 44.18±3.00, 30.93 ±4.02, 27.68±2.66, and 47.69±2.89 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ, and STD respectively.

Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum LDL-cholesterol concentration.

Figure 4 shows the effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum LDL-cholesterol concentration in alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) increase in LDL-cholesterol concentration. However, administration of the ethanol, methanol toluene, and benzene extract resulted in a significant ($p<0.05$) restoration of altered LDL-cholesterol concentration. Result presented in figure 4, serum LDL-cholesterol concentration was 40.95±4.37, 64.31± 3.45, 56.39±5.03, 46.31±2.92, 36.55±6.42, 35.44±8.02, 46.97±7.48, and 41.69±4.36 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ, and STD respectively.

Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum LDL/HDL-cholesterol ratio.

Figure 5 shows the effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum LDL/HDL-cholesterol ratio in alloxan-induced diabetic rats. The different solvent

extracts of VA leaf resulted in a significant ($p<0.05$) reduction of LDL/HDL ratio. This reduction in the groups receiving Methanol and toluene extracts was comparable to those of normal control and standard. In the result presented in figure 5, serum LDL/HDL ratio was 0.910±0.119, 2.462±0.255, 1.350±0.027, 1.330±0.161, 0.834±0.188, 1.135±0.113, 1.690 ±0.146 and 0.878±0.119 in the groups NC, DC, AQE, ETH, METH, TOL, BENZ, and STD respectively.

Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on cardiovascular risk ratio (CRR).

Figure 6 shows the effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on CRR in alloxan-induced diabetic rats. Results obtained showed that diabetes induction resulted in a significant ($p<0.05$) increase in cardiovascular risk. The different solvent extracts of VA leaf resulted in a significant ($p<0.05$) reduction of CRR. This reduction was significant in the groups receiving ethanol, Methanol and aqueous extracts were comparable to those of normal control and standard. Furthermore, the result presented in figure 6, the cardiovascular risk ratio was 1.37±0.11, 3.51±0.25, 2.01 ± 0.10, 2.19 ± 0.31, 1.58±0.06, 2.63±0.21, 2.93±0.14, and 1.84±0.17 in the groups NC, DC, AQE, ETH, METH, TOL, BENZ, and STD respectively.

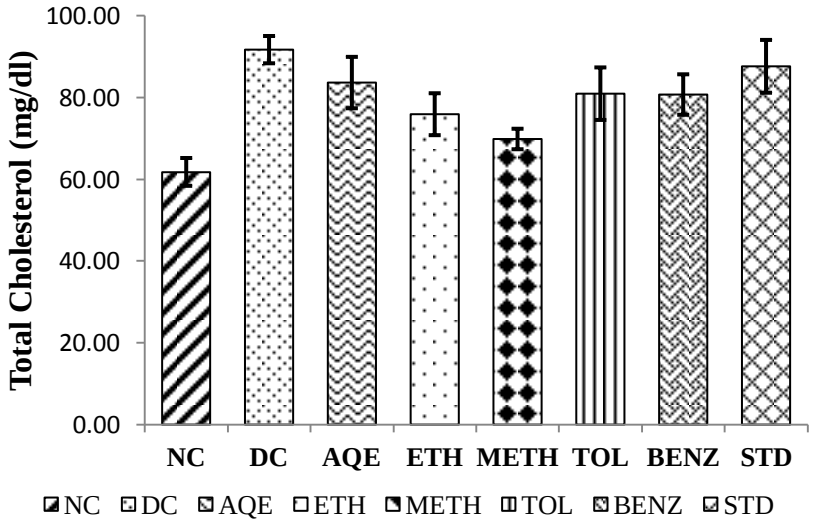


Figure 1: Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum total cholesterol concentration in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscripts across groups are significantly different ($p<0.05$).

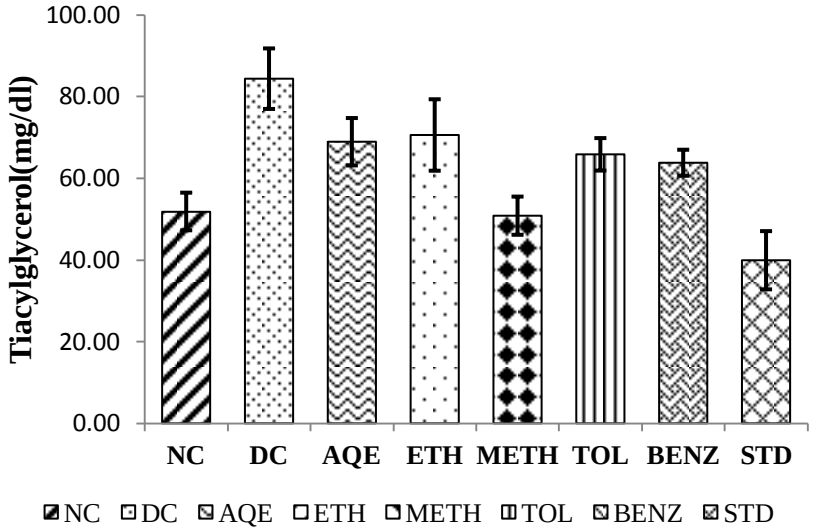


Figure 2: Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum triacylglycerol concentration in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscripts across groups are significantly different ($p<0.05$).

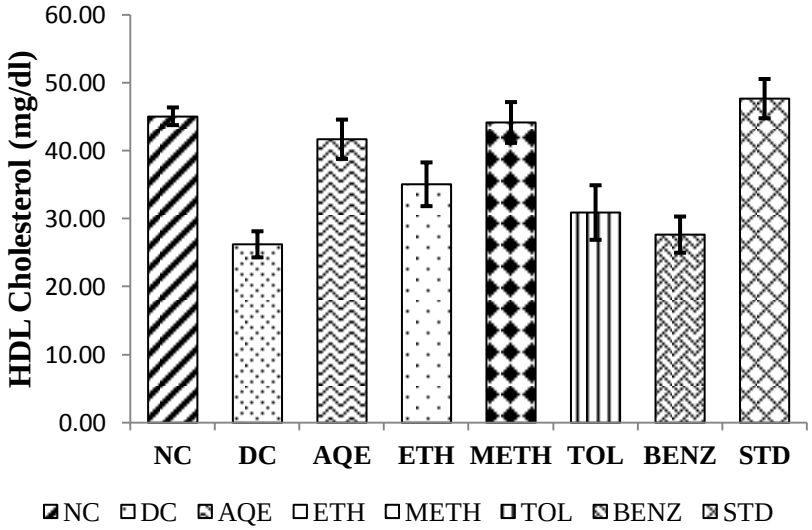
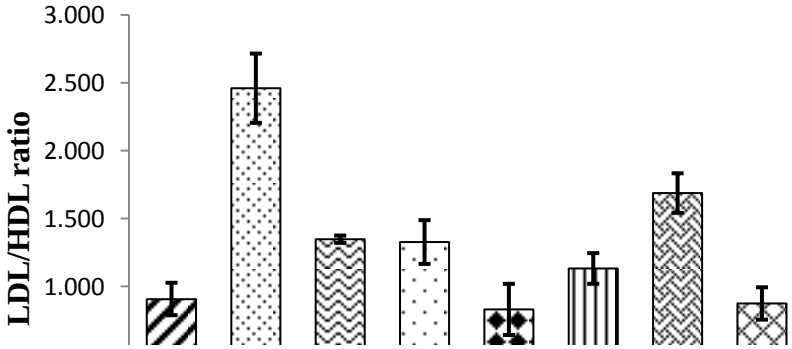


Figure 3: Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum HDL-cholesterol concentration in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscripts across groups are significantly different ($p<0.05$).



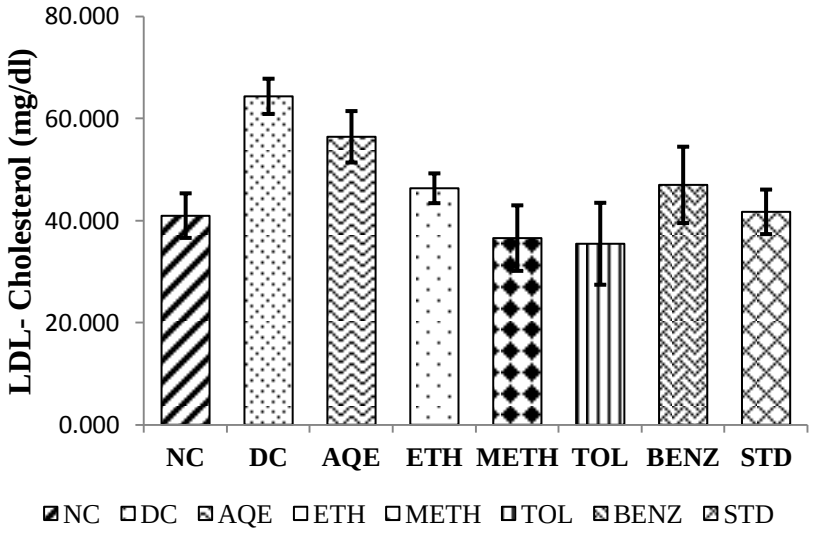


Figure 4:Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum LDL-cholesterol concentration in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript across groups are significantly different ($p<0.05$).

Figure 5: Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on LDL/HDL ratio in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscripts across groups are significantly different ($p<0.05$)

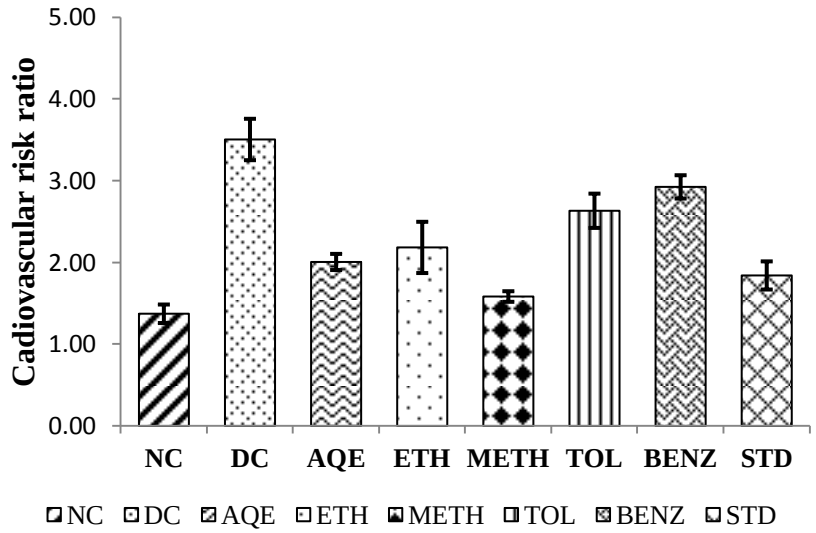


Figure 6: Effect of VA leaf aqueous, ethanol, methanol, toluene and benzene extract administration on cardiovascular risk ratio in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscript across groups are significantly different ($p<0.05$).

Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum VLDL-cholesterol concentration.

Figure 7 shows the effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum VLDL-cholesterol concentration in alloxan-induced diabetic rats. Results obtained show that diabetes induction resulted in a significant ($p<0.05$) elevation of VLDL-cholesterol concentration. However, only the methanol extract resulted in a normalization of VLDL-cholesterol concentration. Furthermore, the result presented in figure 7, serum VLDL-cholesterol concentrations was 10.37 ± 0.92, 16.87 ± 1.48, 13.79 ±1.16, 14.12 ±1.75, 10.16 ± 0.93, 13.17 ± 0.80, 12.76 ± 0.64, and 7.98 ± 1.43 mg/dl in the groups NC, DC, AQE, ETH, METH, TOL, BENZ, and STD respectively.

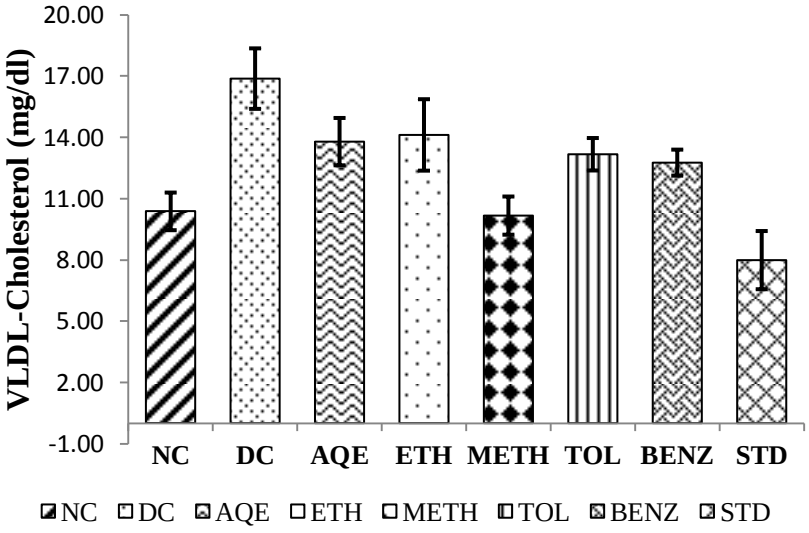


Figure 7: Effect of VA leaf aqueous, ethanol, methanol, toluene, and benzene extract administration on serum VLDL-cholesterol concentration in alloxan-induced diabetic rats. Results are Mean ± SD of 4 determinations. Values with different superscripts across groups are significantly different ($p<0.05$).

IV. Discussion

The present study assessed the antidiabetic and hypolipidemic properties of *Vernonia* amygdalina leaf solvent extracts in alloxan-induced diabetic rats. Administration of different solvent extracts of VA leaf to diabetic rats resulted in varying hypoglycemic effects. The extracts reduced blood glucose monitored over 7 days in the order methanol > ethanol >benzene> aqueous> toluene. The ethanol and methanol extract yielded 33.19% and 43.66% of baseline blood glucose reduction and were compared favorably with the standard drug Glucophage. The effectiveness of the ethanol and methanol extracts may be attributed to polarity differences. Ngo *et al.,* 2017 showed that extraction solvents play an important role in the extraction of phenolic compounds from samples. Extraction yield, phytochemical content, and antioxidant properties have also been shown to be significantly influenced by the polarity of extracting solvents (Ngo *et al.,* 2017; Nawaz *et al.,* 2020). The polarity of the extracting solvents relative to water is Water > methanol > ethanol > benzene > toluene; a mixture of 50% (v/v) water with methanol, ethanol, and acetone is reported to be the best solvents for maximum extraction of phenolics (Ngo *et al.,* 2017; Nawaz *et al.,* 2020). The observed antidiabetic properties of the different solvent extracts of VA leaf may be tenable to many possible mechanisms accountable to the diversity of chemical classes present. It is known that oxygen-free radicals contribute to the development of diabetes and complications, especially through β-cell cytotoxicity. The extracts may be acting through its reported antioxidant properties which counter alloxan-induced free radicals mediated destruction of pancreatic β-cells (Ayoola *et al.,* 2008; Akah *et al.,* 2009). It may be also acting through other mechanisms which involve the induction of increased insulin release by the remaining pancreatic β-cells, increase sensitivity of insulin receptors in cells, and/or reduction of gluconeogenesis (Akhtar *et al.,* 2011).

Polyphenol compounds such as flavonoids, tannins, and saponins inhibit the enzyme activities of α-amylase and α-glucosidase, Glucose-6-phosphatase (G6Pase), and fructose-1,6-bisphosphate (Emejulu *et al.,* 2014; El Barky *et al.,* 2016, Li *et al.,* 2018; Laddha and Kulkarni, 2019; Koshy and Vijayalakshmi, 2001). The repression of gluconeogenic enzyme activity decreases glucose absorption, producing insulin-like effects in insulin-sensitive tissues; reducing blood glucose levels, and regulating the antioxidant environment in cells (Asante *et al.,* 2016; Kunyanga *et al.,* 2011; Laddha and Kulkarni, 2019). Our report is consistent with the findings of Asante *et al.,* (2016) and Fatmawaty *et al.* (2020) in which VA leaf extract was shown to reduce blood glucose levels in diabetic rats. Healthy control of the blood glucose levels in diabetics is important in averting or delaying the progression of diabetic co-morbidities which may lead to premature disability or death. Our findings showed that diabetes induction resulted in a significant elevation of total cholesterol, triacylglycerol, LDL-cholesterol, and VLDL-cholesterol concentration. The buildup of these lipoproteins and triglycerides are implicated as risk factors in the progression of coronary heart disease (CHD) (Ye, 2019). However, the ethanol and methanol extract caused a significant reduction of total cholesterol concentration; while the ethanol, methanol toluene, and benzene extract resulted in a significant reduction of elevated LDL-cholesterol concentration. In addition, only the methanol extract normalized VLDL-cholesterol concentration. It is also notable that the aqueous and methanol extract resulted in significant restoration of altered HDL-cholesterol concentration. Insulin exerts a modulatory effect on the metabolism of carbohydrates, lipids, and proteins (Cabezas *et al.,* 2018); thus diabetes mellitus is characterized by dyslipidemia. The reduced HDL-cholesterol levels, hypercholesterolemia, and hypertriglyceridemia are commonly associated with diabetes (Shepherd, 2005). This is accounted for by insufficiency in fatty acid metabolism, increased gluconeogenesis, and high production of ketone bodies in the diabetic state. Cardiovascular risk ratio and LDH/HDL ratio assessment indicated a significant reduction of cardiovascular risk in the diabetic groups treated with ethanol, methanol, and aqueous extracts; which compared favorably to those of normal control and standard. Previous studies show that a high LDL-c/HDL-c ratio is linked with coronary plaque progression, whereas a lower LDL-c/HDL-c ratio that is achieved by pharmacological interventions may be linked with coronary plaque regression (Wan *et al.,* 2015; Kunutsor *et al.,* 2017). This study indicated a dependence of hypolipidemic property to extracting solvent polarity; thus the more polar solvents may have extracted the dominant active polyphenol components of the plant. In this study, VA leaf methanol extract administration showed a significant homeostatic effect on deranged lipid profile indices of diabetic rats; lowered LDL cholesterol level, hypercholesterolemia significantly increased HDL-cholesterol concentration and lowered cardiovascular risk ratio compared to other solvents extract studied.

V. Conclusion

The present study has shown that *V. amygdalina* leaf extract reduced hyperglycemia and dyslipidemia resulting from alloxan-induced diabetes milletus. However, the potency of this benefit was found to be partly dependent on the polarity of the extracting solvents. The methanol and ethanol extracts of *V. amygdalina* leaf were most effective compared to the less polar solvents toluene and Benzene.

References

[1]. Adefisayo, M. A., Akomolafe, R. O., Akinsomisoye, S. O., Alabi, Q. K., Ogundipe, O. L., Omole, J. G., & Olamilosoye, K. P. (2017). Gastro-protective effect of methanol extract of *Vernonia amygdalina* (del.) leaf on aspirin-induced gastric ulcer in Wistar rats. *Toxicology reports*, *4*, 625–633.

[2]. Adewole, E., Ojo, A., & Oluwafemi, O. (2019). Antioxidant Activities and Nutritional Compositions of Vernonia Amygdalina. *International Journal of Basic & Applied Sciences*, 4, 9-16.

[3]. Akah, P. A., Alemji, J. A., Salawu, O. A., Okoye, T. C., & Offiah, N. V.(2009). "Effects of Vernonia amygdalina on biochemical and hematological parameters in diabetic rats,"*Asian Journal of Medical Sciences*, 1(3), 108–113.

[4]. Akhtar, N., Khan, B.A., Majid, A., Khan, H.M., Mahmood, T., Gulfishan, S.T. (2011). Pharmaceutical and biopharmaceutical evaluation of extracts from different plant parts of indigenous origin for their hypoglycemic responses in rabbits. *Acta Poloniae Pharmaceutica*, 68(6), 919-925.

[5]. Argheore, E.M., Makkar, H.P.S., & Becker, K. (1998). Feed value of some browse plants from the central zone of Delta State Nigeria. *Tropical Science*, 1998, *38*, 97-104.

[6]. Asante, D., Effah-Yeboah, E., Barnes, P., Abban, H.A., Ameyaw, E. O., Boampong, J.N., Ofori, E. G., & Dadzie, J.B. (2016). Effect of Young and Old Ethanolic Leaf Extracts of *Vernonia amygdalina*: A Comparative Study. *Journal of Diabetes Research*, 1-13.

[7]. Ayoola, G., Coker, H., Adesegun, S. et al. (2008). "Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria," *Tropical Journal of Pharmaceutical Research*, 7(3), 1019–1024.

[8]. Bertoldi, A. D., Kanavos, P., França, G. V., Carraro, A., Tejada, C. A., Hallal, P. C., Ferrario, A., & Schmidt, M. I. (2013). Epidemiology, management, complications and costs associated with type 2 diabetes in Brazil: a comprehensive literature review. Globalization and health, 9, 62.

[9]. Cabezas, C. M., Burggraaf, B., & Klop, B. (2018). Dyslipidemias in clinical practice, *Clinca Chimica Acta*, 487, 117–125.

[10]. Diabetes Atlas Committee (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes research and clinical practice*, *157*, 107843. https://doi.org/10.1016/j.diabres.2019.107843

[11]. Dilworth, L., Facey, A., & Omoruyi, F. (2021). Diabetes Mellitus and Its Metabolic Complications: The Role of Adipose Tissues. *International journal of molecular sciences*, *22*(14), 7644.

[12]. Ebong, P.E., Atangwho, I.J., Eyong, E. U., & Egbung, G. E. (2008). The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African bitter leaf). American Journal of Biochemistry and Biotechnology, 4, 239-244.

[13]. El Barky, A. R., Hussein, S. A., Alm-Eldeen, A. A., Hafez, Y. A., & Mohamed, T. M. (2016). Anti-diabetic activity of Holothuria thomasi saponin. *Biomedicine & pharmacotherapy Biomedecine & pharmacotherapie*, *84*, 1472–1487.

[14]. Emejulu, A. A., Alisi, C. S., Asiwe, E. S. and Iheanacho, K. M. and Onwuliri, V. A. (2014). Hypolipidemic effect of Irvingia gabonensis fruits juice on sodium fluoride induced dyslipidemia in rats. African Journal of Biochemistry Research, 8(8), 151-157.

[15]. Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation Research*, *107*(9), 1058–1070. https://doi.org/10.1161/CIRCRESAHA.110.223545

[16]. Hamowia, A.M., & Safran, A.M. (1994) Pharmacological Studies on Vernonia amygdalina (Del) and Tithonia Diversifolia (Gray). Journal of Vetrinary Medicine. 42, 91-97.

[17]. Igile, G.O., Oleszek, W., Jurzysta, M., Burda, S., Fafunso, M. et al. (1994). Flavonoids from Vernonia amygdalina and their antioxidant activities. *Journal of Agricultural and Food Chemistry*, 42, 2445–2448.

[18]. Ijeh, I.I., & Ejike,C.E. (2011). Current perspectives on the medicinal potential of Vernonia amygdalina Del, *Journal of Medicinal Plant Research*, 5 (7), 1051–1061.

[19]. Iwalokun, B.A., Efedede, B.U., Alabi-Sofunde, J.A., Oduala, T., Magbagbeola, O.A., & Akinwande, A.I. (2006). Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. *Journal of Medicinal Foods*, 9, 524-530.

[20]. Izevbigie, E.B. (2003). Discovery of water-soluble anticancer Agents (Edotides) from a vegetable found in Benin City, Nigeria. *Experimental Biology and Medicine*, *228*, 293-298.

[21]. Izevbigie, E.B., Bryant, J.L., Walker, A. (2003). Edible Vernonia amygdalina leaf extract inhibits extracellular signal-regulated kinases and human breast cancer cell growth. *Journal of Nutrition*, 133, 3860S.

[22]. Koshy, A. S., & Vijayalakshmi, N.R. (2001). Impact of certain flavonoids on lipid profiles potential action of Garcinia cambogia flavonoids. *Phyotherapy Research*, 15(5), 395-400.

[23]. Kunutsor, S. K., Zaccardi, F., Karppi, J., Kurl, S., & Laukkanen, J. A. (2017). Is High Serum LDL/HDL Cholesterol Ratio an Emerging Risk Factor for Sudden Cardiac Death? Findings from the KIHD Study. *Journal of atherosclerosis and thrombosis*, *24*(6), 600–608.

[24]. Kunyanga, C. N., Imungi, J. K., Okoth, M., Momanyi, C., Biesalski, H. K., & Vadivel, V. (2011). Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected raw and processed indigenous food ingredients from Kenya. Journal of food science, 76(4), C560-C567.

[25]. Laddha, A. P., & Kulkarni, Y. A. (2019). Tannins and vascular complications of Diabetes: An update. *Phytomedicine : international journal of phytotherapy and phytopharmacology*, *56*, 229–245.

[26]. Li, K., Yao, F., Du, J., Deng, X., & Li, C. (2018). Persimmon tannin decreased the glycemic response through decreasing the digestibility of starch and inhibiting α-amylase, α-glucosidase and intestinal glucose uptake. *Journal of agricultural and food chemistry*, 66(7), 1629-1637.

[27]. Nawaz, H. Shad, M., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. *Brazilian Journal of Pharmaceutical Sciences*, 56. 10.1590/s2175-97902019000417129.

[28]. Nawaz, H., Aslam, M., & Tul Muntaha, S. (2019). Effect of Solvent Polarity and Extraction Method on Phytochemical Composition and Antioxidant Potential of Corn Silk, *Free Radicals and Antioxidants*, *9*(1), 5–11.

[29]. Newairy, A.-S. A., Mansour, H. A., Yousef, M. I., & Sheweita, S. A.(2002). Alterations of lipid profile in plasma and liver of diabetic rats: effect of hypoglycemic herbs. *Journal of Environmental Science and Health—Part B: Pesticides, Food Contaminants, and Agricultural Wastes*, 37(5), 475–484.

[30]. Ngo Van, T., Scarlett, C., Bowyer, M., Ngo, P., & Vuong, Quan. (2017). Impact of Different Extraction Solvents on Bioactive Compounds and Antioxidant Capacity from the Root of Salacia chinensis L.. *Journal of Food Quality*, 1-8, 10.1155/2017/9305047.

[31]. Ogbulie, J.N., Ogueke, C.C., & Nwanebu, F.C. (2007). Antibacterial properties of Uvaria chamae, Congronema latifolium, Garcinia kola, Vernonia amygdalina and Aframomium melegueta. *African Journal of Biotechnology*, 6, 1549-1553.

[32]. Oyebode, O., Kandala, N. B., Chilton, P. J., & Lilford, R. J. (2016). Use of traditional medicine in middle-income countries: a WHO-SAGE study. *Health policy and planning*, *31*(8), 984–991.
[33]. Paschou, S. A., Papadopoulou-Marketou, N., Chrousos, G. P., & Kanaka-Gantenbein, C. (2018). On type 1 diabetes mellitus pathogenesis. *Endocrine connections*, *7*(1), R38–R46.
[34]. Perini, W., Snijder, M. B., Peters, R. J., Kunst, A. E., & van Valkengoed, I. G. (2019). Estimation of cardiovascular risk based on total cholesterol versus total cholesterol/high-density lipoprotein within different ethnic groups: The HELIUS study. European journal of preventive cardiology, 26(17), 1888–1896. https://doi.org/10.1177/2047487319853354
[35]. Porth, C. M. (1998). *Pathophysiology: Concepts of Altered Health States*, Lippincot-Raven Publishers, Philadelphia, Pa, USA, 5th edition.
[36]. Pottathil, S., Nain, P., Morsy, M.A., Kaur, J., Al-Dhubiab, B.E., Jaiswal, S., & Nair, A.B. (2020). Mechanisms of Antidiabetic Activity of Methanolic Extract of *Punica granatum* Leaves in Nicotinamide/Streptozotocin-Induced Type 2 Diabetes in Rats, *Plants*, 9(11),1609.
[37]. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., Williams, R., & IDF
[38]. Shepherd, J.(2005). "Does statin monotherapy address the multiple lipid abnormalities in type 2 diabetes?" *Atherosclerosis Supplements*, 6(3), 15–19.
[39]. SweeKeong, Y., WanYong, H., BoonKee, B., WoonSan, L., Huynh, K., Hadi, N.A., & Alitheen, N.B. (2010). Vernonia amygdalina, an ethnoveterinary and ethnomedical used green vegetable with multiple bio-activities. *Journal of Medicinal Plants Research*, 4, 2787-2812.
[40]. Taiwo, I.A., Odeigah, P.G.C., & Ogunkanmi, L. A. (2009). The glycaemic effects of *Vernonia amygdalina* and *Vernonia tenoreana* with tolbutamide in rats and the implications for the treatment of diabetes mellitus, *Journal of Science Research and Development*, 11,122-130.
[41]. Uloko, A. E., Musa, B. M., Ramalan, M. A., Gezawa, I. D., Puepet, F. H., Uloko, A. T., Borodo, M. M., & Sada, K. B. (2018). Prevalence and Risk Factors for Diabetes Mellitus in Nigeria: A Systematic Review and Meta-Analysis. *Diabetes therapy : research, treatment and education of diabetes and related disorders*, *9*(3), 1307–1316.
[42]. Wakeel, A., Jan, S.A., Ullah, I., Shinwari, Z.K., and Xu, M.(2019). Solvent polarity mediates phytochemical yield and antioxidant capacity of Isatis tinctoria, *PeerJournal*, 7, e7857
[43]. Wan, K., Zhao, J., Huang, H., Zhang, Q., Chen, X., et al. (2015). The Association between Triglyceride/High-Density Lipoprotein Cholesterol Ratio and All-Cause Mortality in Acute Coronary Syndrome after Coronary Revascularization. *PLOS ONE*, 10(4), e0123521.
[44]. Yang, Q., Vijayakumar, A., & Kahn, B.B. (2018). Metabolites as regulators of insulin sensitivity and metabolism, *Nature Reviews Molecular Cell Biology*, 19(10), 654–672.
[45]. Yazid, F., Bonita, N., Rosmalena, H., Hanafi, M. and Dewi, V. (2020). Antidiabetic and Antioxidant potential of *Vernonia amygdalina* eeaf extract in alloxan-induced Sprague-Dawley rats. *Online Journal of Biological Sciences*, 20 (4): 190-200
[46]. Ye, X., Kong, W., Zafar, M.I. *et al.* (2019). Serum triglycerides as a risk factor for cardiovascular diseases in type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. *Cardiovascular Diabetology*, 18**,** 48.

Alozie, E. U, et. al. "Antidiabetic and hypolipidemic properties of Vernonia amygdalina aqueous, ethanol, methanol, toluene and benzene extracts in Alloxan-Induced diabetic rats." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), 8*(1), (2022): pp. 12-22.