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EFFECT OF AQUEOUS LEAF EXTRACT OF *CNIDOSCOLUS ACONITIFOLIUS* ON LIPID PROFILE AND HAEMATOLOGY OF CARBON TETRACHLORIDE TREATED RATS

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Abstract: Effect of aqueous extract of *Cnidoscolus aconitifolius* on lipid profile and haematology of carbon tetrachloride (CCl_4) treated rats were investigated using standard analytical methods. Forty two adult male Wistar rats (91-185 g) were housed in plastic cages of seven groups with six animals each. Group I received normal rat feed (control). CCl_4 (prepared 1:5 (v:v) in olive oil) was administered subcutaneously to groups II – VI on day twenty-one. In addition, vitamin C was administered orally to group III on daily basis for twenty-one days; groups IV, V and VI took 50 mg/KgBW, 75 mg/KgBW and 100 mg/KgBW extract dose orally for twenty-one day while olive oil was administered to group VII. Phytochemical analysis of the leaf extract revealed a highest saponin content (7.84±0.09%) while tannin levels was slightly decreased (1.01±0.20%). Hydrogen cyanide was absent in all investigated samples. In comparison to group II and I, administration of the extract dose (50 mg/KgBW, 75 mg/KgBW and 100 mg/KgBW) dependently lowered total cholesterol, triglycerides, plasma LDL, VLDL, non-HDL and atherogenic indices but increased plasma HDL cholesterol levels of treated rats. There was no significant difference in the WBC and lymphocytes concentrations between untreated and treated rats with the leaf extract of *C. aconitifolius*. The 50 mg/KgBW and 75 mg/KgBW extract doses provided better results than 100 mg/KgBW. The result of this study expressed positive effect on the lipid profile and haematological parameters by using *Cnidoscolus aconitifolius* leaf extract against carbon tetrachloride treated rats.

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Introduction

The use of plants in medicine goes back to the beginnings of human civilization. Substantial evidence has been found on the use of plants for preventive and/or therapeutic purposes in ancient cultures.[1] According to the World Health Organization (WHO),[2] a medicinal plant is one that contains substances that can be used for therapeutic purposes and/or can serve as active ingredients for the synthesis of new drugs. Medicinal plants cover a wide range of metabolic activities and have many functions in the body which include antioxidant, anti-inflammatory, platelet aggregation inhibitory and as immune response triggers.[3] Studies have been carried out on the ability of *C. aconitifolius* to regulate the levels of lipids. The authors reported the ability of this extract to normalize the appearance of haematological and oxidative indicators caused by high level of fats in rat's diet.[4] Furthermore, research has shown that the vitamin A content in the leaf extract of *C. aconitifolius* helps to offer resistance to malaria.[5] Vitamins A, C and zinc are capable of controlling immune functions and impaired growth related to malaria and sickle cell disease.[6,7,8,9,10] Plants usage in traditional medicines has been widely observed in most developing countries where they are seen as therapeutic agents for the maintenance of good health.[11]

For several decades, various fields of research have centered on medicinal plants and their components. The high rate of ingestion of chemicals and substances in form of drugs and herbs is a potential hazard to the body. Inhalation of carbon tetrachloride affects the central nervous system, kidney and liver organs with prominent symptoms as nausea,

weakness of the bones and muscles, vomiting, lethargy and headache.[12] The quest for alternative approach to prevention of diseases by using natural plant extracts is one of the current requirement. This has given rise to the need to investigate the claims on use of *Cnidoscolus aconitifolius* leaf extract for its pharmacological importance. This study was aimed to investigate the effects of an aqueous leaf extract of *Cnidoscolus aconitifolius* on lipid profile and haematological indices on carbon tetrachloride treated rats.

Methodology

Method of leaf Extraction

Cnidoscolus aconitifolius leaves were freshly harvested, washed, pounded with a mortar and pestle and pressed for aqueous extraction.[13] Suction filtration (vacuum) was employed on the extract and the process repeated several times for discoloring in order to obtain a soluble mixture. The total extract of the leaves was lyophilized at the pharmacognosy laboratory of the Faculty of Pharmaceutical Sciences, University of Port Harcourt. The residue obtained was measured and used for analysis.

Phytochemical Screening

Primary components of *C. aconitifolius* leaves were phytochemically analyzed for flavonoid, saponin, tannin, oxalate, cyanogenic glycosides, alkaloids or phenol using[14] suitable method for bioactive substances determination in food samples.

Experimental Design

Forty two adult male Wistar rats weighing 91-185 g were purchased from the Animal House of the Department of Physiology University of Port Harcourt. The animals were housed in plastic cages of seven groups with six animals each per group. After one-week acclimatization period on normal

rat feeding, the treatment commenced. The extract was administered orally on daily basis for twenty one days. The dosage of administration of the extract was adapted, with modification.[15,16] The carbon tetrachloride was prepared in a ratio of 1:5 (v:v) in olive oil, and administered subcutaneously at 0.17 mL/kg body weight of rats, on day twenty one after administration of leaf extract. The dosage and method of administration of carbon tetrachloride was adapted with modification.[17] The experimental groups were as follow:

- Group I: received daily normal feed and water.
- Group II: received daily normal feed and water + subcutaneous dose of CCl_4 (0.17 mL/KgBW) on day twenty one.
- Group III: received daily normal feed and water + a daily oral dose of vitamin C + subcutaneous dose of CCl_4 (0.17 mL/KgBW) on day twenty one.
- Group IV: received daily normal feed and water + a daily oral dose of *C. aconitifolius* extract (50 mg/kg) + subcutaneous dose of CCl_4 (0.17 mL/KgBW) on day twenty one.
- Group V: received daily normal feed and water + a daily oral dose of *C. aconitifolius* extract (75 mg/kg) + subcutaneous dose of CCl_4 (0.17 mL/KgBW) on day twenty one.
- Group VI: received daily normal feed and water + a daily oral dose of *C. aconitifolius* extract (100 mg/kg) + subcutaneous dose of CCl_4 (0.17 mL/KgBW) on day twenty one.
- Group VII: received daily normal feed and water + subcutaneous dose of olive oil.

Twenty four hours after administration of carbon tetrachloride, the rats were weighed and anaesthetized by exposure to chloroform. While under anesthesia, they were painlessly sacrificed after 8 hrs fasting and blood was collected from each rat into heparin and EDTA (ethylenediamino

tetra-acetic acid) sample bottles via cardiac puncture for biochemical and hematological analyses. The heparin anti-coagulated blood samples were centrifuged at 1000 rpm for 10 minutes, after which the plasma was collected and stored for subsequent analysis.

Biochemical Parameters

The kits used for the determination of lipid profile are products of Randox Laboratories (Crumlin, UK).

Determination of lipid profile parameters

Total cholesterol, triglyceride (TAG), high density lipoprotein (HDL) cholesterol were determined according with previously reported methodology.[18-20] Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were extrapolated from equation.[21] Cardiac risk ratio (CRR), atherogenic coefficient (AC) and atherogenic index of plasma (AI) was obtained using strategies.[22]

Haematological Indices

Haematological indices were done according to method.[23] Packed cell volume (PCV) was measured with micro-haematocrit and capillary tubes filled with blood and centrifuged at 3000 g for 5 minutes. Plasma haemoglobin (Hb) concentration was measured with DTH Haemoglobinometer™ while the total white blood cell (WBC) and red blood cell (RBC) counts were visually estimated. Differential white blood cell count (lymphocytes) was determined by Leishman staining technique. The mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration were obtained using the following equations:

$$\text{Mean Cell Volume} = \frac{\text{Packed cell volume (\%)}}{\text{Red blood cell count (cell/L)}} \times 10$$

$$\text{Mean Cell Haemoglobin (pg/cell)} = \frac{\text{Haemoglobin concentration (pg/L)}}{\text{Red blood cell count (cell/L)}}$$

$$\text{Mean Cell Haemoglobin concentration (g/dL)} = \frac{\text{Haemoglobin concentration (g/dL)}}{\text{Packed cell volume (\%)}}$$

Method of Data Analysis

Statistical Package for Biological and Social Sciences (SPSS) Inc. 21.0 Software program was used. Mean values (M) ± SD were calculated and one-way analysis of variance (ANOVA) test was performed. Values (P) that was less than 0.05 ($P<0.05$) was considered statistically significant.

Results

Table 1. Phytochemical content of *Cnidoscolus aconitifolius* leaf.

Phytochemical content	(%)
Tannins	1.01±0.20
Saponins	7.84±0.09
Alkaloids	3.32±0.09
Flavonoids	5.70±0.28
HCN	0.00±0.00
Oxalate content	3.49±0.05
Phenol content	3.13±0.04

Values are Mean ± SD of triplicate determinations (n=3).

Table 2. Effect of the leaf extract of *C. aconitifolius* on the plasma lipid profiles of normal and CCl_4-induced hepatotoxicity in rats.

Groups	Lipid profile concentrations (mmol/L)					
	TAG	Total chol	HDL chol	LDL chol	VLDL chol	Non-HDL chol
Group I (Normal control)	2.42 ± 0.47^{a}	3.31 ± 3.21^{a}	3.09 ± 1.18^{a}	3.42 ± 0.29^{a}	1.10 ± 0.21^{a}	2.27 ± 1.20^{a}
Group II (Disease control)	2.64 ± 0.38^{a}	5.74± 0.48^{b}	3.07 ± 0.74^{a}	2.06 ± 0.67^{b}	1.20 ± 0.17^{b}	4.73 ± 0.36^{b}
Group III (Vitamin c)	1.98 ± 0.30^{b}	5.31 ± 0.67^{b}	3.13 ± 1.49^{a}	1.69 ± 0.84^{b}	0.90 ± 0.14^{c}	2.18 ± 1.29^{a}
Group IV (50 mg/Kg BW extract)	2.15 ± 0.32^{b}	3.99 ±1.08^{ab}	3.83 ±1.55^{a}	0.83 ± 0.47^{c}	0.98 ± 0.15^{a}	1.89 ± 0.43^{a}
Group V (75 mg/Kg BW extract)	1.78 ± 0.33^{b}	4.76 ±1.04^{ab}	5.07 ± 0.52^{b}	0.21 ± 0.00^{c}	0.81 ± 0.15^{c}	0.71 ± 0.20^{ac}

Table 2. continued

Group VI (100 mg/Kg BW extract)	2.26 ± 0.20[a]	4.63 ±0.60[ab]	4.33 ± 1.22[b]	0.19 ± 0.08[c]	1.03 ± 0.09 [a]	0.70 ± 0.48[ac]
Group VII (Olive oil)	2.77 ± 0.49[a]	6.06 ± 2.53[b]	3.48 ± 0.82[a]	2.17 ±1.22[b]	1.26 ± 0.22[b]	2.99 ± 1.21[a]

Values are Mean ± SD of six determinations (n = 6). Values in the same column bearing same superscript letters show no significant differences between the groups while those with different superscript letters (a, b, c...) show significant differences between the groups (P<0.05).
mg/KgBW = mg/Kg body weight.

Note: TAG- triglyceride, Total chol- total cholesterol, HDL chol- high density lipoprotein cholesterol, LDL chol- low density lipoprotein cholesterol, VLDL chol- very low density lipoprotein cholesterol, Non-HDL chol- non-high density lipoprotein cholesterol.

Table 3. Effect of the leaf extract of *C. aconitifolius* on the atherogenic indices of normal and CCl_4-induced hepatotoxicity in rats.

Groups	Cardiac risk ratio	Atherogenic coefficient	Atherogenic index of plasma
Group I (Normal control)	1.81 ± 0.98[a]	0.78 ± 0.68[a]	-0.11 ± 0.13[a]
Group II (Disease control)	2.61 ± 1.00[a]	3.24 ± 1.52[b]	-0.10 ± 0.11[a]
Group III (Vitamin c)	1.78 ± 0.68[a]	1.61 ± 1.00 [a]	-0.12 ± 0.14[a]
Group IV (50 mg/KgBW extract)	1.51 ± 0.59[a]	1.38 ± 0.46[a]	-0.20 ± 0.11[a]
Group V (75 mg/KgBW extract)	0.94 ± 0.19[a]	0.14 ± 0.04[a]	-0.46 ± 0.09[b]
Group VI (100 mg/KgBW extract)	1.09 ± 0.21[a]	0.18 ± 0.12[a]	-0.28 ± 0.05[a]
Group VII (Olive oil)	2.23 ± 1.70[a]	1.23 ± 0.69[a]	-0.04 ± 0.08[a]

Values are Mean ± SD of six determinations (n = 6). Values in the same column bearing same superscript letters show no significant differences between the groups while those with different superscript letters (a, b,...) show significant differences between the groups (P < 0.05).
mg/KgBW = mg/kg body weight.

Table 4. Effect of the leaf extract of C. aconitifolius on the haematological parameters of normal and CCl_4-induced hepatotoxicity in rats.

Groups	HB (g/dl)	RBC (10^{12}/L)	WBC (10^9/L)	PCV (%)	LYM (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)
Group I (Normal control)	13.40± 0.97 [a]	7.15± 0.54[a]	5.14± 1.71 [a]	39.10± 3.28 [a]	61.06± 30.54[a]	54.68± 2.62[a]	18.80± 0.79[a]	34.40± 0.67[a]
Group II (Disease control)	8.70± 1.87[b]	3.65± 0.68[b]	5.70± 1.07 [a]	26.17± 5.56[b]	71.33± 4.55 [a]	73.17± 2.64[b]	24.40± 0.97[b]	33.33± 0.07[b]
Group III (vitamin c)	9.93± 1.31[b]	4.07± 0.64[b]	5.58± 1.56 [a]	29.83± 3.97[b]	70.00± 6.32 [a]	74.67± 1.75[b]	24.52± 0.76[b]	33.30± 0.10[b]
Group IV (50 mg/KgBW extract)	9.23± 2.33[b]	3.92± 0.84[b]	5.33± 1.88 [a]	27.67± 7.00[b]	70.00± 4.47 [a]	70.83± 4.36[b]	23.41± 1.24[b]	33.38± 0.07[b]
Group V (75 mg/KgBW extract)	10.00± 1.47[b]	4.10± 0.61[b]	4.38± 0.69 [a]	30.00± 4.43[b]	65.83± 4.92 [a]	71.17± 3.19[b]	23.76± 1.13[b]	33.24± 0.11[b]
Group VI (100 mg/KgBW extract)	9.90± 1.84[b]	4.05± 0.71[b]	5.50± 1.29[a]	29.83± 5.49[b]	68.00± 6.93 [a]	72.67± 3.08[b]	24.42± 0.73[b]	33.19± 0.55[b]
Group VII (Olive oil)	13.02± 2.17 [a]	6.87± 1.24[a]	4.53± 1.7[a]	37.58± 6.91 [a]	68.00± 34.36[a]	54.70± 0.98 [a]	19.02± 0.90 [a]	34.80± 1.26[a]

Values are Mean ± SD of six determinations (n=6). Values in the same column bearing same superscript letters show no significant differences between the groups while those with different superscript letters (a, b) show significant differences between the groups ($P<0.05$).
mg/KgBW = mg/kg body weight.

Discussion

Phytochemicals confer medicinal relevance on plants. The phytochemical screening in this study revealed the presence of tannins, saponins, flavonoids, oxalate, alkaloid and phenol in substantial amounts which implies *C. aconitifolius* leaves may have antimicrobial, antioxidant and anti-inflammatory potential (Table 1). Natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols. The flavonoids show antioxidant activity and have strong anti-cancer activity.[24] Flavonoids exhibit their antioxidative properties through several mechanisms, such as scavenging of free radicals,

chelation of metal ions, such as iron and copper, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent.[25,26] Tannin is a complex moiety with wide pharmacological activities and is produced by majority of plants as protective substance. Tannin has astringent property, hastens the healing of wounds and inflamed mucous membrane and has been used as tanning agents. Tannin has received considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory properties.[27,28] Saponins protect against hyperglycaemia, hypercholesterolaemia, hypertension,[29] have antibiotic properties and anti-inflammatory property and aid healing.[30] Alkaloids have been reported to be powerful pain relievers, exert an anti-pyretic, antihypertensive, antifungal, anti-inflammatory, antifibrogenic effect,[31] stimulating, anaesthetic action[32] and inhibiting activity against most bacteria.[33]

The effect of the leaf extract of *C. aconitifolius* on the plasma lipid profiles of normal and CCl_4-induced hepatotoxicity in rats are shown in Table 2. Plasma levels of very low density lipoprotein (VLDL) and non-high density lipoprotein cholesterol (non-HDL-C) were significantly higher ($P<0.05$) in rats administered with CCl_4 when compared to normal control but were decreased significantly upon treatment with the leaf extracts at all doses administered. Plasma LDL was significantly decreased ($P<0.05$) in rats administered with CCl_4 and Vitamin C when compared to normal control but was further decreased significantly upon treatment with the leaf extract at all doses administered when compared to both the normal and disease control. There was no significant difference in the plasma levels of high density lipoprotein cholesterol (HDL-C) and triglyceride (TAG) of disease control rats when compared to normal control. The Vitamin C group

and the extract at 50 mg/KgBW and 75 mg/KgBW dose significantly decreased ($P<0.05$) the plasma levels of TAG when compared to the normal control and disease control. Also, the extract at 75 mg/KgBW and 100 mg/KgBW dose significantly increased ($P<0.05$) plasma levels of HDL-C when compared to normal control and as well as when compared to disease control. Plasma total cholesterol was significantly higher ($P<0.05$) in the disease control rats when compared to normal rats. Treatment with the extract decreased total cholesterol level at a rate that is dose dependent with the lowest dose more efficient. Higher levels of plasma cholesterol is an established risk factor for atherosclerosis and other heart diseases,[34] hence a decrease in the plasma cholesterol level minimizes risk of heart diseases. Furthermore, treatment with the leaf extract of *C. aconitifolius* reduced the plasma total cholesterol levels at a rate that is dose dependent with 50 mg/KgBW dose being more effective. This implies that the leaf extract has the ability to protect against cardiovascular diseases. This might have been brought about by saponins, tannins and flavonoids[35,36] which are known to be atheroprotective and cholesterol-lowering.[37,38,39,40] Also, these components put together or singly could have brought about the hypocholesterolemic effect of the extract. Increase in plasma triglyceride signifies synergistic and independent risk factors for cardiovascular diseases.[41,42,43] It may also lead to hypertension[44,45] and may be the result of an abnormal lipoproteins' metabolism.[42,46,47] Treatment with 50 mg/KgBW and 75 mg/KgBW dose of the leaf extract significantly ($P<0.05$) reduced the plasma triglycerides levels. These results can be explained by the fact that flavonoid[35] and tannins content decrease plasma triglyceride levels.[48,49] High levels of plasma HDL cholesterol reduces the risk of cardiovascular complications.[50,51] Results obtained signified that treatment of rats

administered with CCl_4 with the extracts slightly increased the levels of HDL-C at 50 mg/KgBW dose of the extract and significantly ($P<0.05$) increased it at 75 mg/KgBW and 100 mg/KgBW doses of the extract. This further highlights the potency of the *C. aconitifolius* leaf extract against cardiovascular diseases. Elevated levels of plasma LDL connotes cardiovascular complications;[34,52] invariably reduced levels of plasma LDL reduces the risk of coronary heart disease.[50,47] From this study, treatment with the leaf extract at all doses administered significantly reduced plasma LDL cholesterol levels of rats administered with CCl_4 which further supports the cardio-protective ability of *C. aconitifolius* leaf extract. Increased levels of plasma VLDL cholesterol increased the tendency to cardiovascular disease.[34,52] Findings from this study indicated that treatment with extract at all doses of the leaf extract decreased significantly the plasma VLDL cholesterol levels when compared to the disease control. This proves the ability of the leaf extract to protect against cardiovascular diseases. Several studies have indicated that non-HDL cholesterol is a better indicator of cardiovascular complications than LDL cholesterol.[53,54,55] In light of the above, this study showed a significant decrease of non-HDL cholesterol in rats treated with the leaf extract in a dose dependent manner when compared to disease control rats which in turn connotes a reduced cardiovascular risk.

The effect of the leaf extract of *C. aconitifolius* on the atherogenic indices of normal and CCl_4-induced hepatotoxicity in rats is shown in Table 3. There was no significant difference in all comparisons on the cardiac risk ratio, however, disease control rats had higher cardiac risk ratio which was further reduced by treatment with the leaf extract. There was a significant increase ($P<0.05$) in the atherogenic coefficient of the disease control rats

when compared to the normal but treatment with the leaf extract significantly decreased atherogenic coefficient at all administered doses. There was no significant difference ($P<0.05$) in the atherogenic index of plasma of the disease control group and those administered with Vitamin C when compared to the normal but treatment with 75 mg/KgBW extract significantly decreased atherogenic index of plasma when compared to both the normal and disease control. The higher the atherogenic indices value, the higher the risk of developing cardiovascular disease and vice versa.[41, 56,57,43] These atherogenic indices are very potent and powerful indicators that show the risk of heart disease and low levels implies protection against coronary heart disease.[57] This study has shown that the treatment dose reduced the cardiac risk ratio of the rats at all extract doses though not significantly and decreased significantly the atherogenic index of plasma in rats administered with the extract at 75 mg/KgBW. Also, the atherogenic coefficient was decreased significantly ($P<0.05$) in rats administered with the extract at all doses. Similar findings on the atherogenic indices were previously reported[58,59] by studying the effects two leaves extracts (from *Tridax procumbens* and *S. liberica*) had on the plasma lipid profile and atherogenic indices of normal and alloxan treated rats.

Table 4 shows the effect of the leaf extract of *Cnidoscolus aconitifolius* on the haematological parameters of normal and test rats administered with CCl_4. The extract had a dose dependent effect on the haemopoietic system of the test rats. There was a significant decrease ($P<0.05$) in the packed cell volume, haemoglobin, red blood cell count, and mean cell heamoglobin concentrations of disease control rats and those administered with vitamin C and treated with the extract when compared to the normal rats but a slight increase in packed cell volume, haemoglobin and red blood cell count of the rats administered with vitamin C and rats

treated with the extract at all doses when compared with the disease control. On the other hand, there was a significant increase ($P<0.05$) in the mean cell haemoglobin and mean cell volume of the disease control rats and those administered with vitamin C and treated with the extract when compared to the normal rats. White blood cell count and lymphocytes were not significantly different in treated rats when compared to normal and disease control indicating that the immune systems of the rats were not at risk. Results obtained here differ from previously reported data.[58] Lower PCV levels often results from lower red cell mass. This is often reflected by decrease in the levels of RBC and Hb which is in line with what was obtained here. There was no significant difference in the white blood cell count of the disease and treated rats when compared to the normal. According to other studies,[60,61,62,63] white blood cells (WBC) play important roles in destabilization of coronary artery plaques at the onset of acute coronary syndrome, however, increased white blood cell count in peripheral blood is a pointer to coronary artery diseases and inflammation.[63,64,65] Hence, the lower WBC observed in rats treated with the leaf extract of *C. aconitifolius* at all doses indicates the potential of this plant's extract to protect against CCl_4-induced elevation in WBC which also signifies a reduction in tendency of suffering coronary artery disease.

Conclusion

From this study, it can be concluded that the protective effect on lipid profile and haematological indices against carbon tetrachloride-induced rats by using *Cnidoscolus aconitifolius* leaf extract was clearly demonstrated.

Contributions to Knowledge

This study has shown that *Cnidoscolus aconitifolius* leaf extract dose dependently reduced low density lipoprotein (LDL), very low density lipoprotein (VLDL), non- high density lipoprotein-cholesterol (NON-HDL-C), total cholesterol, triglyceride and atherogenic indices and increased high density lipoprotein-cholesterol (HDL-C); thus, it may be used in management of cardiovascular disease.

References

1. Mwine, J. T.; Van Damme P. Why do Euphorbiaceae tick as medicinal plants? A review of *Euphorbiaceae* family and its medicinal features. *J. Med. Plants Res.* **2011**, *5(5)*, 652-662.
2. World Health Organization. WHO monographs on selected medicinal plants. Volume 4, **2005**. http://apps.who.int/iris/bitstream/10665/42052/4/9789241547055_eng.pdf;
3. Gutiérrez-Grijalva, E.P.; Ambriz-Pérez, D. L.; Leyva-López, N.; Castillo-López, R. I.; Heredia, J. B. Review: Dietary phenolic compounds, health benefits and bioaccessibility. *Arch. Latinoam. Nutr.* **2016**, *66(2)*, 87-100.
4. Obichi, E. A.; Monago, C. C.; Belonwu, D. C. Effect of *Cnidoscolus aconitifolius* (Family *Euphorbiaceae*) aqueous leaf extract on some antioxidant enzymes and haematological parameters of high fat diet and streptozotocin induced diabetic wistar albino rats. *J. Appl. Sci. Environ. Manage*. **2015**, *19(1)*, 201-209.
5. Serghides, L.; Kain, K. C. Mechanism of protection induced by vitamin A in *falciparum* malaria. *Lancet*, **2002**, *359*, 1404-1406.
6. Shankar, A. H.; Prasad, A. S. Zinc and immune function: The biological basis of altered resistance to infection. *Am. J. Clin. Nutr.* **1998**, *68*, 4478-4463.
7. Muriel, P.; Rivera-Espinoza, Y. Beneficial drugs for liver diseases. *J. Appl.Toxicol.* **2001**, *28*, 93-103.
8. Garba, I. H., Ubom, G. A., Haruna, M. Falciparum malaria infection is associated with increased mobilization of tissue stores of ascorbic acid: evidence that ascorbate plays a significant role in the acute onset of this disease. *Informedia*. Paving the way to the global e-Health. 2nd Ibero

American Congress of Medical Informatics on the Internet, Nov. 4-30th, 2002.

9. Duggen, C., Macleod, W. B.; Krebs, N. F.; Westcott, J. L.; Fawzi, W. W.; Premji, Z.G.; Mwanakasale, V.; Simon, J. L.; Yeboah-Antwi, K.; Kamer, D. H. Plasma Zinc concentrations are depressed during the acute phase response in children with Falciparum Malaria. *J. Nutr.* **2005**, *135*, 802-807.

10. Berkelaar, D. Chaya. *Echo Technical Note*, **2006**; www.echonet.org.

11. Soetan, K. O.; Aiyelaagbe, O. O. The need for bioactivity-safety evaluation and conservation of medicinal plants - A review. *J. Med. Plants Res.* **2009**, *3(5)*, 324-328.

12. United State Environmental Protection Agency. Integrated Risk Information System (IRIS) on Carbon tetrachloride. National Center for Environmental Assessment, Office of Research and Development, Washington, DC, 1999.

13. Sofora, A. *Medicinal plants and traditional medicine in Africa*. 3rd edition; University of Ife Press: Ile-Ife, Nigeria, 2008; pp. 196-203.

14. Association of Official Analytical Chemists. Method of analysis. Washington DC, U.S.; 1990, pp. 1250-1255.

15. Ikewuchi, J. C.; Ikewuchi, C. C. Hypocholesterolaemic effect of aqueous extract of *Acalypha wilkesiana* 'Godseffiana' Muell Argon rats fed egg yolk supplemented diet: Implications for cardiovascular risk management. *Res. J. Sci. Tech*. **2010**, *2(4)*, 78-81.

16. Ikewuchi, J. C. Changes in the weight, plasma lipid profile, and atherogenic indices of salt-loaded rats by aqueous extract of *Acalypha wilkesiana* Muell Arg: Potential for cardiovascular risk reduction. *Pac. J. Sci. Technol*. **2010**, *11*, 516-523.

17. Obi, F. O.; Uneh, E. pH dependent prevention of carbon tetrachloride - induced lipid peroxidation in rats by ethanolic extract of *Hibiscus rosasinensis* petals. *Biokemistri*, **2003**, *13(1)*, 42-50.

18. Richmond W. Preparation of cholesterol oxidase from *Nacordia ssp* and its application to the enzymatic assay of total cholesterol in serum. *Clin. Chem.* **1973**, *19*, 1350-1356.

19. Abell, L. L.; Levy, B. B.; Brodie, B. B.; Kendall, F. E. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **1952**, *195*, 357-366.

20. Lopes-Virella, M. F.; Stone, P.; Ellis, S.; Colwell, J. A. Cholesterol determination of HDL separated by three different methods. *Clin. Chem.* **1977**, *23*, 882-884.

21. Friedewald, W. T.; Levy, R. I.; Friedrickson, D. S. Estimation of the concentration of Low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **1972**, *18*, 499-502.

22. Ikewuchi, J. C.; Ikewuchi, C. C.; Ifeanacho, M. O.; Igboh, N. M.; Ijeh, I. I.; Enuneku, E. C. Aqueous extract of the rhizomes of *Sansevieria senegambica* Baker (*Agavaceae*) moderated plasma biochemical and haematological indices in salt-loaded rats. *Pac. J. Sci. Technol.* **2013**, *14(1)*, 370-379.

23. Cheesbrough, M. *District laboratory practice in tropical countries*, Part 1. UK: Cambridge University Press; 2006.

24. Salah, N.; Miller, N.J.; Paganga, G.; Tijburg, L.; Bolwell, G. P.; Rice. E.; Evans, C. Polyphenolic flavonols as scavenger of aqueous phase radicals and chain breaking antioxidants. *Arch. Biochem. Biophys*. **1995**, *322(2)*, 339-346.

25. Frankel, E. N.; Andrew, L. W.; Pierre, L. T. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43(4)*, 890-894.

26. Kessler, R. C.; Berglund, P.; Demler, O.; Jin, R.; Koretz, D. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *J. Am. Med. Assoc.* **2003**, *289*, 3095-3105.

27. Asquith, T. N.; Butter, L. G. Interaction of condensed tannin with selected proteins. *Phytochemistry* **1986**, *25(7)*, 1591-1593.

28. Killedar, S. G.; More, H. N. Estimation of tannins in different parts of *Memecylon umbellatum Burm*. *J. Pharm. Res.* **2010**, *3(3)*, 554-556.

29. Trease, G. E.; Evans, W.C. *Pharmacognosy*, 14th edition, London: W.B. Sanders company; 1985.

30. Krishnaiah, D.; Devi, T.; Bono, A.; Sarbatly, R. Studies on phytochemical constituents of six Malaysian medical plants. *J. Med. Plants Res.* **2009**, *3(2)*, 67-72.

31. Awoyinka, O. A.; Balogun, I. O.; Ogunnowo, A. A. Phytochemical screening and invitro bioactivity of *Cnidoscolus aconitifolius* (*Euphorbiaceae*). *J. Med. Plants Res.* **2007**, *1(3)*, 63-65.

32. Edeoga, H. O.; Enata, D. O. Alkaloids, tannins and saponin content of some medicinal plants. *J. Med. Aromat. Plant Science* **2001**, *23*, 344-349.

33. Al-Bayati, F. A.; Sulaiman, K. D. In-vitro antimicrobial activity of *Salvadora persica L*. extracts against some isolated oral pathogens in Iraq. *Turk. J. Biol.* **2008**, *32(1)*, 57-62.

34. Ademuyiwa, O.; Ugbaja, R. N.; Idumebor, F.; Adebawo, O. Plasma lipid profiles and risk of cardiovascular disease in occupational lead exposure in Abeokuta, Nigeria. *Lipids Health Dis.* **2005**, *4*, 19.

35. Ikewuchi, C. C.; Ikewuchi, C. J.; Ayalogu, O. E.; Onyeike, N. E. Proximate and phytochemical profile of *Sansevieria liberica* Gérôme and Labroy. *J. Appl. Sci. Environ. Manag.* **2010**, *14(2)*,103-106.

36. Ikewuchi, J. C.; Onyeike, E. N.; Uwakwe, A. A.; Ikewuchi, C. C. Effect of aqueous extract of the leaves of *Acalypha wilkesiana*' Godseffiana' Muell Arg on blood pressure components and pulse rates of sub chronic salt loaded rats. *Res. J. Sci. Technol.* **2011**, *3*, 264-269.

37. Dillard, C. J.; German, J. B. Phytochemicals: nutraceuticals and human health. *J. Sci. Food Agric.* **2000**, *80(12)*, 1744-1756.

38. 38. Berhow, M. A.; Cantrell, C. L.; Duval, S. M.; Dobbins, T. A.; Mavnes, J.; Vaughn, S. F. Analysis and quantitative determination of group B saponins in processed soybean product. *Phytochem. Anal.* **2002**, *13(6)*, 343-348.

39. Basu, S. K.; Thomas, J. E.; Acharya, S. N. Prospects for growth in global nutraceutical and functional food markets: A Canadian perspective. *Aust. J. Basic Appl. Sci.* **2007**, *1(4)*, 637-649.

40. Soetan, K. O. Pharmacological and other beneficial effects of antinutritional factors in plants - A review. *Afr. J. Biotechnol*. **2008**, ***7(25)***, 4713-4721.

41. Dobiásová, M. Atherogenic index of plasma [log (triglyceride/ HDL-Cholesterol)]: theoretical and practical implications. *Clin. Chem.* **2004**, *50*, 1113-1115.

42. McBride, P. E. Triglycerides and risk for coronary heart disease. *J. Am. Med. Assoc*. **2007**, *298*, 336-338.

43. Martirosyan, D. M.; Miroshnichenko, L. A.; Kurokawa, S. N.; Pogojev, A. V.; Zolotov, V. I. Amaranth oil application for heart disease and hypertension. *Lipids Health Dis.* **2007**, *6*, 1.

44. Lopes, F. H.; Bernardes, R.; Silva, H.; Soares, J. A.; Filho, B.; Consolim, B.; Colombo, F. M.; Giorgi, D. M. A. Lipid metabolism alterations in normotensive subjects with positive family history of hypertension. *Hypertension* **1997**, *30*, 629-631.

45. Zicha, J.; Kunes, J.; Devynck, M. A. Abnormalities of membrane function and lipid metabolism in hypertension: a review. *Am. J. Hypertens*. **1999**, *12*, 315-331.

46. Franz, M. J.; Bantle, J. P.; Beebe, C. A.; Brunzell, J. D.; Chiasson, J. L.; Garg, A. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *Diabetes Care* **2002**, *25*, 148-198.

47. Shen, G. X. Lipid disorders in diabetes mellitus and current management. *Curr. Pharm. Anal.* **2007**, *3*, 17-24.

48. Middleton, E. Jr.; Kandaswami, C.; Theoharides, T. C. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacol. Rev.* **2000**, *52*, 673-751.

49. Park, S. Y.; Bok, S. H.; Jeon, S. M.; Park, Y. B.; Lee, S. J.; Jeong, T. S. Effect of rutin and tannic acid supplements on cholesterol metabolism in rats. *Nutr. Res.* **2002**, *22*, 283-295.

50. Assmann, G.; Gotto, A. M. Jr. HDL cholesterol and protective factors in atherosclerosis. *Circulation* **2004**, *109*, III8-III14.

51. Rang, H. P.; Dale, M. M.; Ritter, J. M.; Moore, P. K. *Pharmacology*, 5th edition. India: Elsevier, 2005; pp 14.

52. Lichtennstein, A. H.; Appel, L. J.; Brands, M.; Carnethon. M.; Daniels, S.; Franklin, B. Diet and lifestyle recommendations revision 2006.A Scientific Statement from the American Heart Association Nutrition Committee. *Circulation* **2006**, *114(1)*, 82-96.

53. Liu, J.; Sempos, C.; Donahue, R.; Dorn, J.; Trevisan, M.; Grundy, S. M. Joint distribution of non-HDL and LDL cholesterol and coronary heart disease risk prediction among individuals with and without diabetes. *Diabetes Care* **2005**, *28(8)*, 1916-1921.

54. Pischon, T.; Girman, C. J.; Sacks, F. M.; Rifai, N.; Stampfer, M. J.; Rimm, E. B. Non-high density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation* **2005**, *112(22)*, 3375-3383.

55. Brunzell, J. D.; Davidson, M.; Furberg, C. D.; Goldberg, R. D.; Howard, B. V.; Stein, J. H. Lipoprotein management in patients with cardio metabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. *J. Am. Coll. Cardiol.* **2008**, *51(15)*, 1512-1524.

56. Brehm, A., Pfeiler, G.; Pacini, G.; Vierhapper, H.; Roden, M. Relationship between serum lipoprotein ratios and insulin resistance in obesity. *Clin. Chem.* **2004**, *50(12)*, 2316-2322.

57. Usoro, C. A. O.; Adikwuru, C. C.; Usoro, I. N.; Nsonwu, A. C. Lipid profile of postmenopausal women in Calabar, Nigeria. *Pak. J. Nutr*. **2006**, *5(1)*, 79-82.

58. Ikewuchi, J. C. Alteration of plasma biochemical, haematological and ocular oxidative indices of alloxan induced diabetic rats by aqueous extract of *Tridax procumbens Linn* (*Asteraceae*). *EXCLI Journal* **2012**, *11*, 291-308.

59. Ikewuchi, J. C.; Ikewuchi, C. C. Moderation of Haematological Indices, Plasma Electrolytes and Markers of Hepato-Renal Function in Sub-Chronic Salt-Loaded Rats by an Aqueous Leaf Extract of *Tridax procumbens Linn* (*Asteraceae*). *Pac. J. Sci. Technol.* **2013**, *14(1)*, 362-369.

60. Moreno, P. R.; Falk, E.; Palacios, I. F.; Newell, J. B.; Fuster, V.; Fallon, J. T. Macrophage infiltration in acute coronary syndromes: Implications for plaque rupture. *Circulation* **1994**, *90(2)*, 775-778.

61. van der Wal A.C.; Becker, A. E.; van der Loos C. M.; Das, P. K. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* **1994**, *89(1)*, 36-44.

62. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* **2001**, *104(3)*, 365-372.

63. Takeda, Y.; Suzuki, S.; Fukutomi, T.; Kondo, H.; Sugiura, M.; Suzumura, H. Elevated white blood cell count as a risk factor of coronary artery disease: Inconsistency between forms of the disease. Japanese Heart Journal **2003**, *44(2)*, 201-211.

64. Chillaci, G.; Pirro, M.; Pucci, G.; Ronti, T.; Vaudo, G.; Mannarino, M. R. Prognostic value of elevated white blood cell count in hypertension. *Am. J. Hypertens*. **2007**, *20(4)*, 364-369.

65. Kho, A. N.; Hui, S.; Kesterson, J. G.; McDonald, C. J. Which observations from the complete blood cell count predict mortality for hospitalized patients? *J. Hosp. Med*. **2007**, *2(1)*, 5-12.