IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 8, Issue 3 (May. – June. 2022), PP 30-35
www.iosrjournals.org

Ethnopharmacological Study of *Anthocleista vogelii*Methanol Leaves Extract on Some Biochemical Parameters in Ccl_4-Induced Liver Damage in Albino Rats.

Awote O.K[1*], Thomas-Akinwale K.O[1], Dele-osedele P.I[1], Ajiboso T.E[1], Boniwey T.S[1], Omo-okoroh M.O[1]
[1]*Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Lagos State, Nigeria.*
**Corresponding: Olasunkanmi.awote@lasu.edu.ng*

Abstract:
Background*: Anthocleista vogelii leaves extract is used traditionally in Nigeria for the treatment and management of various diseases but with very little or no scientific evidence. Herein, the ethnopharmacological potential of A. vogelii methanol extract in the treatment of carbon tetrachloride (CCl_4)-induced liver damage in albino rats was evaluated by determining different biochemical parameters in serum and tissues.*
Materials and Methods*:The activities of serum liver enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), concentrations of protein (total protein, albumin and globulin), and Lipid profile (concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides) were evaluated using standard procedures.*
Results*: The phytochemical screening of the methanol extract of A. vogelii revealed the presence of five (5) phytochemicals (tannin, saponin, flavonoid, phenol, alkaloid and fat) and absence of terpenoid and glycoside. A dose-dependent study was conducted and oral administration of A.vogelii methanol leaves extract at a dose of 1000 mg/kg body weight significantly ($P<0.05$) reduced the hepatotoxic effects of CCl_4 on the serum enzymes, proteins and lipid profile.*
Conclusion:*From the observations, it can be concluded that, the methanol leaves extract of A. vogelii stabilized the hepatic frame against the toxicity of CCl_4 by protecting the cells of the liver.*
Key Word*:Anthocleista vogelii, Liver damage, Carbon tetrachloride, Liver enzymes, Lipid profile*

Date of Submission: 07-05-2022 Date of Acceptance: 22-05-2022

I. Introduction

The liver is the largest internal organ in human body, that promotes maintenance, homeostasis, and it is the chief metabolism and excretion site[1,28], associated with biochemical pathways of nutrient supply, energy provision, fight against diseases, growth and reproduction[26]. Cellular necrosis, reduced glutathione levels and elevated biochemical markers like transaminases, alkaline phosphate, bilirubin, triglycerides and cholesterol are the results of Liver damage[30]. Till date, Liver damage and diseases still pose a serious challenge to international public health, even with the use of expensive orthodox medicine, which usually results in unwanted adverse effects after its primary effect of protecting the liver against damage or regeneration of hepatic cells[8,9,29]. Recently, as a result of these adverse effect in orthodox medicines, many medicinal plants have been employed instead and shown to play a major hepato-protective role in the management of liver diseases with little or no side effects, majorly because of their natural antioxidant levels[12,16,18,13]. *Anthocleista vogelii* (cabbage tree) is called "Sapo or Apaoro" in the Yoruba language of South-Western, Nigeria, belongs to Loganiaceae family, and is used traditionally for diverse therapeutic purposes against various metabolic diseases and non-metabolic illnesses such as, diabetes, obesity, hypertension, infertility, oxidative, fever, malaria, stomach aches, indigestion, bacterial infections (e.g typhoid and syphilis), etc[7,10]. This study aimed at evaluating the ethnopharmological potential of *A.vogelii* methanol extract in the treatment of carbon tetrachloride (CCl_4)-induced liver damage in Albino rats. Since only few of the medicinal attributes of this plant have been reported in scientific literature, yet *Anthocleista vogelii*, a medicinal plant in Nigeria, may also help in the amelioration of liver damages.

II. Material And Methods

Collection of plant materials

Fresh leaves of *A.vogelii* were purchased from a local herb seller at Iyana-iba, Ojo, Lagos State, Nigeria. The plant was identified at Lagos State University Herbarium, Department of Botany, Lagos State, Nigeria.

Preparation of plant extract

The leaves were washed, air-dried at room temperature for a week and thereafter milled with the aid of electric blender (Binatone) into a coarse powder and stored in air tight plastic container. Fifty (50g) of the powder leaves of *A.vogelii* was dissolved in 250mls of 70% methanol and stirred intermittently for 24hours to give the crude methanol extract. After 48hours, the mixture was filtered using a Whatman No.1 filter paper to remove the plant debris. The filtrate was concentrated under reduced pressure to remove the methanol. Methanol was preferred because of its greater potential for extracting medicinal phytochemicals from their crude source. The concentrated extract was kept in a refrigerator prior to analysis.

Determination of phytochemical screening

The phytochemical screening of the crude ethanol, methanol and aqueous extract of *A. vogelii* was carried out to determine the phyto-constituents present in the plant extracts as follows:

Test for tannins

0.5g of the powdered sample of each plant was mixed in 20mls of distilled water and boiled in a water bath. The boiled samples were then filtered using filter paper. A few drops of0.1%ferric chloride ($FeCl_3$) was added to the filtrate and observed for brownish green or blue black coloration which indicate the presence of tannins.

Test for saponins

This was done by weighing 0.5g of the powdered sample of each plant and mixed in 5mls of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth which indicates the presence of saponin.

Test for terpenoids

0.2mls of chloroform and 0.3mls of concentrated tetraoxosulphate (VI) acids were carefully added to 0.5mls of both plant extract. A reddish brown color was shown at the interface which indicates the presence of terpenoids.

Test for flavonoids

A sample of 0.1ml of sodium hydroxide pellet was added into 0.3mls of both plant extract. A result of yellow coloration was observed which indicate the presence of flavonoids.

Test for phenolic compounds

The extract (0.5mls) of both plant extract was dissolved in 5mls of distilled water and boiled and filtered. A few drops of 0.1% ferric chloride was added to the filtrate. A blue-black or brown coloration indicates the presence of phenol.

Test for alkaloids

0.1ml of each plant extract was treated with few drops of dragendorff's reagent. The result of an orange brown precipitate indicates the presence of alkaloids.

Test for cardiac glycosides

A sample of 0.5mls of each plant extract was treated with0.2mls of glacial acetic acid and a drop of ferric chloride. Then, 0.1ml of concentrated tetraoxosulphate (VI) acids was added. A brown ring indicates the presence deoxy-sugar as characteristics of cardenolides.

Detection of fats

2mls of the filtrate was mixed with ethanol and shaken vigorously and allowed to stand for few minutes. The solution was added into test tube containing water.

Determination of median lethal dose (LD_{50})

The median lethal doses (LD_{50}) of fresh leaves of *A. vogelii* were carried out using a modified method[17].

Experimental animals

Twenty (20) male albino rats weighing 98.58g-142.39g were purchased and housed at the Animal house of Biochemistry Department, Lagos State University, Lagos State, Nigeria. The rats were kept in clean cages and allowed to acclimatize to the laboratory conditions for about two weeks. They were fed with rat feeds and water ad libitum. Animals were maintained at 12hrs light and 12hrs darkness at room temperature.

Induction of hepatotoxicity and animal grouping

The animals were fasted overnight and then divided into four groups. Liver injury was induced in rats in groups 2-4 by intraperitoneal administration of 2 ml/kg (i.e. 2000 mg/kg) carbon tetrachloride (CCl_4) mixed with olive

oil in the ratio of 2:1 (v/v) on day 1. Until after 3 days, no treatment was administered to enable CCl_4-induced hepatotoxicity.
Group 1 (normal control) received distilled water (2ml/kg body weight) daily for 14 days
Group 2 (negative control) received CCl_4 (2ml/kg body weight) daily for 14 days.
Group 3 (positive control) received CCl_4 (2ml/kg body weight) + standard reference drug (Silymarin) at a dosage of 100mg/kg body weight daily for 14 days.
Group 4 received CCl_4 (2ml/kg body weight) + 1000mg/kg body weight methanol extract of *A.vogelii* for 14 days.

Determination of biochemical parameters

After the experimental period of 14 days, the animals were sacrificed on the 15th day after overnight fast under a mild chloroform anesthesia and blood were obtained by cardiac puncture from each animal and stored in EDTA bottles for separation. The organs such as liver, lungs, kidney, spleen and heart were collected and weighed. The bloods were centrifuged at 5000 revolution per minutes for 15minutes to obtain the serum. Serum Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT), Alkaline Phosphatase (ALP), Cholesterol, Triglyceride, Albumin, Globulin Total Protein and High Density Lipoprotein (HDL) levels were measured using Randox diagnostic kits. All samples were analyzed using spectrophotometer. The concentrations of Low Density Lipoprotein (LDL) were calculated from the formula of Friedwald, (2011). The concentrations of Globulin were calculated from the formula of Bishop and Fody, (2000).

Statistical analysis

Data are expressed as mean ± standard error of means (SEM). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using GraphPad Prism® (Version 5.0). A value of $P<0.05$ was considered to indicate a significant difference between groups.

III. Results

Toxicological studies

No weakness, aggressiveness, salivation and death seen in the mice that were treated with 100mg/kg b.wt, 500mg/kg b.wt, 1000mg/kg b.wt, 1500mg/kg b.wt, 3500mg/kg b.wt and 5000mg/kg b.wt of *A. vogelii*methanolic extracts.

Table 1: Percentage Extraction yields of *A.vogelii*methanolleaves extract after concentration

Table 1 showed the percentage yield of *A. vogelii* methanol extract as 3.68%

Extract	Initial weight (g)	Final weight (g)	Yield (%)
Methanolic A.vogelii	50	1.84	3. 68

The percentage extraction yield was calculated using the equation below:

$$\% \text{ yield} = \frac{\text{Weight of plant concentrate}}{\text{Weight of plant sample}} \times 100$$

Table 2: Qualitative phytochemical screening of *A.vogelii* methanol leavesextract

The result of the qualitative phytochemical analysis of the methanolic extract of *A. vogelii* is shown in Table 2. The result revealed the presence of five (5) phytochemicals (tannin, saponin, flavonoid, phenol, alkaloid and fat) and the absence of fat and glycoside.

Extract	Tannin	Saponin	Flavonoid	Phenol	Terpenoid	Alkaloid	Fat	Glycoside
Methanolic *A. vogelii*	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve

The signs **(**+ve) indicates detected and (-ve) indicates not detected.

Table 3: Effect of *A.vogelii*methanolleaves extract on Liver function parameters.

Elevated level of serum enzymes (AST, ALT and ALP) activities (Table 3) were seen in CCl_4 treated groups and the concomitant administration of *A. vogelii* extracts at 1000mg/kg body weight, significantly reduced the levels of these serum enzymes.

GROUPS	AST (U/I)	ALT (U/I)	ALP (U/I)
Control	33.33 ± 2.73^{b}	36.17 ± 6.28^{b}	168.36 ± 44.58^{b}
2ml/kg b.wt CCl_4	$195.2\pm18.23^{a\dagger}$	65.17 ± 16.64^{a}	$595.24\pm179.51^{a\dagger}$
2mg/kgCCl_4 + 1000mg/kg *A.vogelii* (met.)	80.00 ± 22.44^{ab}	27.00 ± 2.86^{b}	208.84 ± 58.17^{b}
2mg/kg CCl_4 + 100mg/kg silymarin	65.83 ± 9.58^{b}	43.83 ± 9.48	184.46 ± 57.00^{b}

Values are represented as mean±SEM for triplicate determination. $^{a}p < 0.05$ versus control; $^{b}p < 0.05$ versus CCl_4; $^{\dagger}p < 0.05$ Silymarin

Table 4: Effect of *A. vogelii* methanol leavesextract on Lipid profile parameters (mg/dl) and Protein concentration of CCl_4 induced rats.

Elevated level of serum cholesterol, triglycerides, LDL-C and reduced levels of HDL-C, total protein, albumin and globulin (Table 5) were seen in CCl_4 treated groups and the concomitant administration of *A. vogelii* extracts at 1000mg/kg body weight, significantly reduced the levels of cholesterol, triglycerides, LDL-C and increased HDL-C, total protein, albumin and globulin.

GROUPS	CHOL	TAG	HDL-CHOL	LDL-CHOL	TOTAL PROTEIN	ALBUMIN (mg/dL)	GLOBULIN (mg/dL)
Control	125.49± 14.93^{b}	750.80± 23.85^{b}	116.15± 11.51^{b}	91.48± 15.99^{b}	13326.66±1151.59^{b}	4796.66± 252.19^{b†}	8530.00± 1048.56^{b}
2ml/kg b.wt CCl_4	420.16± 44.11^{a†}	1157.28± 63.19^{a†}	41.45± 12.99^{a†}	230.16± 59.09^{a†}	7666.66±556.05^{a†}	2681.66± 374.90^{a†}	4985.00± 786.04^{a}
2mg/kgCCl_4 + 1000mg/kg *A.vogelii* (met.)	223.25± 24.62^{ab}	903.09± 69.52^{b}	88.27± 12.89^{b}	130.90± 27.69	10776.66±895.54†	3931.66± 267.19^{ab}	3845.00± 808.16^{ab}
2mg/kg CCl_4 + 100mg/kg silymarin	175.41± 23.04^{b}	804.28± 53.66^{b}	93.99± 11.56^{b}	108.54± 28.88^{b}	11041.66±1233.67^{b}	4128.33± 127.57^{ab}	6913.33± 1245.82

Values represent mean ± SEM of triplicate determination. $^{a}p < 0.05$ versus control; $^{b}p < 0.05$ versus CCl_4; $^{\dagger}p < 0.05$ Silymarin.

Table 5: Effect of *A. vogelii* methanol leavesextract on weight of Organs.

The liver and lungs of CCl_4 induced group showed a significant decrease and increase in size respectively compared to the control (Table 5).

GROUPS	LIVER (g)	SPLEEN (g)	KIDNEY (g)	LUNGS (g)	HEART (g)
Control	5.04±0.31^{b}	0.54±0.08	0.84±0.05	1.14±0.13^{b}	0.45±0.02
2ml/kg b.wt CCl_4	4.21±0.06^{a}	0.56±0.07	0.85±0.02	1.47±0.10^{a}	0.44±0.02
2mg/kgCCl_4 + 1000mg/kg *A.vogelii* (met.)	4.57±0.21^{a}	0.52±0.02	0.86±0.03	1.30±0.07	0.46±0.03
2mg/kg CCl_4 + 100mg/kg silymarin	4.65±0.06	0.51±0.07	0.86±0.03	1.27±0.04	0.47±0.02

Values represent mean ± SEM of triplicate determination. $^{a}p < 0.05$ versus control; $^{b}p < 0.05$ versus CCl_4; $^{\dagger}p < 0.05$ Silymarin.

IV. **Discussion**

Since the induction of hepatotoxicity in rats using CCl_4 raises the serum level of liver enzymes like AST and ALT[19] and also, represents an adequate experimental model of cirrhosis in man, hence its use for the screening of hepato-protective drugs consumed by men[3].

In the present study, methanol was preferred for the extraction process because of its greater potential for extracting medicinal phytochemicals from their crude source. The methanol extract of *A.vogelii* gave a percentage yield (3.68%), and this may indicate the quantity of varied active compounds in the plant extract. Phytochemical screening of methanol extract of *A. vogelii* showed the presence of five (5) phytochemicals (tannin, saponin, flavonoid, phenol, alkaloid and fat) and the absence of terpenoid and glycoside. Previous studies has revealed that medicinal plants rich in phenolic compounds in the form of flavonoids, tannins and phenolic acids have antioxidant properties[2], and these phytochemicals which made up the calculated extraction yields of this plant possess other biological properties such as antimicrobial, antifungal and anticancer[14] . The presence of flavonoids entails that this plant leaves extracts may be a good antioxidant, free radical scavenger and anti-lipoperoxidant, leading to their hepato-protective property. Previous study revealed that the mechanism of action of this plant extract may be compared to that of silymarin (positive control) due to their considerable amount of flavonoid content present[21].

The physical activities of the animals that were used for acute toxicity remained normal because no weakness, aggressiveness, salivation and death were seen in the rats that were treated with 100mg/kg b.wt, 500mg/kg b.wt, 1000mg/kg b.wt, 1500mg/kg b.wt, 3500mg/kg b.wt and 5000mg/kg b.wt of *A. vogelii*leaves extract. This is similar to a previous study by Rajesh and Lath[22], who reported no toxicity in the plant extracts, making it safe for oral consumption as compared to many orthodox medicines.

The serum enzymes AST, ALT and ALP activities were elevated in CCl_4 treated groups and decreased in control, standard drug (silymarin), and *A.vogelii*methanol leaves treated group. Amacher[4]and Ozer et al[20] revealed that, the elevated level of AST and ALT resulted from leakage of damaged tissues which is as a result of hepatocellular necrosis while, Ramaiah[23] revealed that, the increase in ALP level is as a result of

overproduction and release in blood due to hepatobiliary injury and cholestasis. Also, increased levels of AST, ALT and ALP in animals treated with CCl_4 indicated cellular breakage and loss of functional integrity in cell membrane of the liver. Concomitant administration of the standard drug (silymarin) at 100mg/kg body weight and the plant leaves extracts at 1000mg/kg body weight reduced the levels of these marker enzymes towards the normal value indicating stabilization of plasma and repair of hepatic tissue damage as a result of healing of the liver parenchyma and regeneration of hepatocytes. This is similar to the findings of Bilgin et al[6].

In this study, the levels of cholesterol, triglycerides, LDL-C were observed to be increased while the level of HDL-C was decreased in CCl_4 treated animals. The increase in cholesterol levels of CCl_4 induced group caused an increase in membrane fluidity and alters internal viscosity and internal chemical composition while, the increase in LDL-Cholesterol concentration of CCl_4 groups might be due to the defect in its receptor either through failure in its production or function, which is similar to the findings of Sathish et al[27]. Sabesin et al[25] reported that, the decreased level of HDL-C in CCl_4 treated group may be due to diminished lecithin-cholesterol acyl-transferase activity. Concomitant administration of the standard drug (silymarin) at 100mg/kg b.wt, and the plant leaves extract at 1000mg/kg b.wt resulted in significant decrease in triglycerides, LDL-C and increase in HDL-C which serves as protective agent, that reverses cholesterol transport by inhibiting the oxidation of LDL-Cholesterol stimulated by increasing the expression of oxidation-sensitive genes such as, Elk-1 and p-CREB.

Total protein, albumin and globulin were reduced in CCl_4treated groups which may be as a result of CCl_4 interfering with the production of protein in the liver. Administration of the standard drug (silymarin) at 100mg/kg body weight and the plant leaves extract at 1000mg/kg body weight resulted in an increase in these proteins to near normal values respectively. This result is similar to the findings reported in the previous study of Rajesh and Latha[22].

The liver and lungs of CCl_4 treated rats showed a significant decrease and increase in size respectively as compared to the normal control, silymarin and plant leaves extracts treated groups. This may be attributed to the elevated levels of AST, ALT, ALP and decreased levels of total protein, albumin and globulin as a result of fatty infiltration of the liver parenchyma.

V. Conclusion

A.vogelii methanol leaves extract possess an ethnopharmacological potential in the treatment of liver damages.

References

[1]. Ahsan, R., Islam, M., Bulbul, J.I., Musaddik, A. and Haque, E. (2009). Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Eur J Sci Res 37 (2):302–10.

[2]. Ali, S.S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A. and Bora, U. (2008). Indian medicinal herbs as sources of antioxidants. Food Res. Int., 41: 1-15.

[3]. Al-Shabanah, O.A., Alam, K., Nagi, M.N., Al-Rikabi, A.C., Al-Bekairi, A.M., 2000. Protective effect of aminoguanidine, a nitric oxide synthase inhibitor against CCl4-induced hepatotoxicity in mice. Life Sciences. 66, 265–270.

[4]. Amacher, D.E. (2002). A toxicologist's guide to biomarkers of hepatic response. Hum Exp Toxicol 21: 253-62.

[5]. Balasubramanian, S. and Viswanathan, R. (2010). Influence of moisture conent on physical properties of minor millets. J. Food Eng. Vol 47, Issue 3, Pg 279-284..

[6]. Bilgin, H.M., Atmaca, M., Obay, B.D., Zekinci, S.O., Tasdemir, E. and Ketani, A. (2011). Protective effects of coumarin and coumarin derivatives against carbon tetrachloride-induced acute hepatotoxicity in rats. Experimental. Toxicology and Pathology, 63: 325-330Biology, 3(2):280-284.

[7]. Burkill, H.M (1995). The useful plants of West Tropical Africa. Vol. 3. 2nd ed. Royal Botanic Gardens, Kew; 26-33

[8]. Chatterjee, T.K. (2000). Medicinal plants with hepatoprotective properties. In: Herbal options,3ed. Calcuta Books and Allied PublishersLimited, pp.135-137.

[9]. Chattopadhyay, R.R. (2003). Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract. Part II. Journal of Ethnopharmacology, 89(2-3): 217- 219.

[10]. Christophe, M., Frida, L., Celine, N., Zacharie, S., Emilie, N. and Paul, V.T. (2015). Evaluations of acute and subacute toxicity of stem bark aqueous extract of Anthocleista schweinfurthii (Loganiaceae). World J Pharm Pharm. Sci. 2015; 4:197-208.

[11]. de Nigris, F., Lerman, A., Ignarro, L.J., Williams-Ignarro, S., Sica, V., Baker, A.H., Lerman, L.O., Geng, Y.J. and Napoli, C.(2003). Oxidation-sensitive mechanisms, vascular apoptosis and atherosclerosis. Trends Mol. Med. 9, 351–359.

[12]. Dhuley, J.N., Naik, S.R., 1997. Protective effect of Rhinax, a herbalformulation against CCl4-induced liver injury and survival in rats.Journal of Ethnopharmacology 56, 159–164.

[13]. Girish, C. and Pradhan, S.C. (2012). Indian herbal medicines in the treatment of liver diseases: problems and promises. Fundam Clin Pharmacol 26(2):180–9.

[14]. Hossain, M.A. and Nagooru, M.R. (2011). Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant Corydylineterminalis L. Kunth. Pharmacology J: 3(24): 25-30.

[15]. Kingsly, A.R.P., Singh, D.B., Manikantan, M.R. and Jain, R.K. (2006). Moiature dependent physical properties of dried pomegranate seeds (anardana). J. Food Eng. 75: 492-496.

[16]. Latha, U., Rajesh, M.G., Latha, M.S., 1999. Hepatoprotective effect of an ayurvedic medicine. Indian Drugs 36, 470–473.

[17]. Lorke, D. (1983). A new approach to practical acute toxicity testing. Archeology of Toxicology, 54:275-287.

[18]. Mitra, S.K., Seshadri, S.J., Venkataranganna, M.V., Gopumadhavan, S., Venkatesh Udupa, U., Sarma, D.N.K., 2000. Effect of HD-03-a herbal formulation in galactosamine-induced hepatopathy in rats. Indian Journal of Physiology and Pharmacology 44, 82–86.

[19]. Naziroglu, M., Cay, M., Ustundag, B., Aksakal, M., Yekeller, H., 1999. Protective effects of vitamin E on carbon tetrachloride-induced liver damage in rats. Cell Biochemistry and Function 17, 253–259.

[20]. Ozer, J., Ratner, M., Shaw, M., Bailey, W. and Schomaker S. (2008). The current state of serum biomarkers of hepatotoxicity. Toxicology 245: 194-205.
[21]. Palanisamy, D., Kannan, E. and Bhojraj, S. (2007). Protective and therapeutic effects of the indian medicinal plant Pterocarpus santalinus on D-galactosamine-induced liver damage. Asian Journal of Traditional Medicine,2 (2):51-57.
[22]. Rajesh, M.G. and Latha, M.S. (2004). Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. Journal of Ethnopharmacology 91. pp 99–104.
[23]. Ramaiah, S.K. (2007). A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Toxicol 45: 1551-1557.
[24]. Robards, K.J. and Chromatogr, A. (2003). Strategies for the determination of bioactive phenols in plants, fruits and vegetables. 1000(1-2), 657-91.
[25]. Sabesin, S.M., Kuiken, L.B. and Ragland, J.B. (1975). Lipoprotein and lecithin: cholesterol acyltransferase changes in galactosamine induced rat liver injury. Sci., 190: 1302-1304
[26]. Sarkar, M., Sarkar, K., Bhattacharjee, R., Chatterjee, M. and Sil, P. (2005). Curative role of the aqueous extract of the herb, phyllanthus niruri, against nimesulide induced oxidative stress in murine liver. Biomedical Research, 16 (3):171-176.
[27]. Sathish, V., Kumar, K., Ebenezar, C. and Devaki, T. (2003). Effect of L-arginine and L-lysine on lysosomal enzymes and membrane bound phosphatases in isoproterenol induced myocardial infarction in rats. Molecular and Cellular. Biochem., 247: 163-169.
[28]. Simonetta, B., Sebastien, P., Thomas, F.B., Mirjam, B.Z. (2015). MiR-122-A Key factor and therapeutic target in liver disease. Journal of Hepatology. Vol 62, issue 2, pg 448-457.
[29]. Stickel, F. and Schuppan, D. (2007). Herbal medicine in the treatment of liver diseases. Dig Liver Dis 39:293–304.
[30]. Subramaniam, S., Khan, H.B.H., Elumalai, N., Lakshmi, S.Y.S. (2015). Hepatoprotective effect of ethanolic extract of whole plant of Andrographis paniculata against CCl4-induced hepatotoxicity in rats. Comp Clin Path 2015; 24:1–7.

Awote O.K, et. al. "Ethnopharmacological Study of Anthocleista Vogelii Methanol Leaves Extract on Some Biochemical Parameters in Ccl4-Induced Liver Damage in Albino Rats." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), 8*(3), (2022): pp. 30-35.