Cross Current International Journal of Medical and Biosciences
Abbreviated Key Title: Cross Current Int J Med Biosci
ISSN: 2663-2446 (Print) & Open Access
DOI: 10.36344/ccijmb.2021.v03i09.004

OPEN ACCESS

Volume-3 | Issue-9 | Dec, 2021 |

Original Research Article

Identification of Bioactive Compounds in Prebiotic Fraction of *Vernomia amydalina* Leaf Extract with Anti-Amoebic and Protective Effects in Winstar Rats

Ukwah B.N[1]*, Ezeonu I.M[2]
[1]Department of Medical Laboratory Science, Ebonyi State University, Abakaliki
[2]Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka

***Corresponding author:** Ukwah B.N
| **Received:** 16.11.2021 | **Accepted:** 22.12.2021 | **Published:** 30.12.2021 |

Abstract: This study was aimed to identify bioactive compounds and determine the anti-amoebic and organ protective potentials of *Vernonia amygdalina* prebiotic fraction of leaf extract. These were achieved by GCMS and histological analysis of the liver and large intestine of adult wistar rats. The rats were grouped into seven (I-VII.) made up of five animals each. Group I was fed with feed and water. Group II were fed with glucose, Group III were fed with rice and treated with 100mg/ml of extract per kg body weight. Group IV were fed with rice and treated with 200mg/ml of extract per kg body weight. Group V were fed with rice and treated with 300mg/dl of extract per kg body weight. Group VI was fed with rice and treated with 400mg/ml of extract per kg body weight whereas group VII were fed with rice only. Histological examination of the large intestine and liver tissues of the wistar rats in various groups revealed normal structure of the organs in all the groups with highly improved cytoarchitecture of the liver and large intestinal tissues for the group treated with 300mg/mL/kg body weight. *In vitro* evaluation of the 300mg/mL prebiotic fraction of *V. amygdalina* leaf extract and metronidazole against *Entamoeba histolytica* showed significant ($p<0.05$) anti-amoebic effects when compared with negative control (none treated). GCMS analysis identified 19 bioactive compounds which included 3 unique compounds including; phytol, Heptanediamide, N,N'-di-benzoyloxy-, and α- β-Amyrin. This study revealed that prebiotic leaf extracts of *V. amygdalina* contains bioactive compounds that are non-toxic and possess cytoprotective potentials.
Keywords: *Vernonia amygdalina*, Prebiotic fraction, Bioactive compounds, GC-MS analysis, Anti-amoebic activity, *Entamoeba histolytica*, Histopathology.

BACKGROUND

Vernonia amygdalina is commonly called bitter leaf in English because of its bitter taste. The common names in African especially southern Nigeria include: ewuro (Yoruba), etidot (Ibibio), onugbu (Igbo), ityuna (Tiv) and oriwo (Edo) (Abosi *et al.,* 2015). The Leaves of this plant are used in Nigeria as a green vegetable in soups, especially in the popular "bitter-leaf soup". Such preparation includes freshly harvested leaves which are macerated with either cold or hot water to reduce the bitterness of the leaves to a desirable taste. The leaves are then added to other condiments for the soup while the water extract may be taken as a tonic to prevent certain illnesses. The leaves can be taken as an appetizer and the aqueous extract as a digestive tonic (Akinpelu *et al.,* 2013). The leaves are widely used for fevers and also as a quinine–substitute in Nigeria and some other African countries. The young leaves are used in folk medicine as anti-helmintic, antimalarial, laxative, expectorant, worm expeller and fertility inducer in sub-fertile women. Some wild chimpanzees in Tanzania had been observed to use this plant for the treatment of parasite related diseases (Alabi *et al.,* 2016).

Rice (*Oryza sativa*) is one of the three major food crops of the world and forms the staple diet of about half of the world's population. The global production of rice has been estimated to be at the level of 650 million tones and the area under rice cultivation is estimated at 156 million hectares (Brar *et al.,* 2014). Rice occupies one quarter of the total cropped area, contributes about 40 to 43 percent of total food grain production and continues to play a vital role in the national food and livelihood security system. India is one of the leading exporter of rice, particularly basmati rice (Chang *et al.,* 2016).

Quick Response Code

Journal homepage:
https://crosscurrentpublisher.com

Citation: Ukwah B.N & Ezeonu I.M (2021). Identification of Bioactive Compounds in Prebiotic Fraction of *Vernomia amydalina* Leaf Extract with Anti-Amoebic and Protective Effects in Winstar Rats. *Cross Current Int J Med Biosci, 3*(9), 96-102.

Certain compounds contained in *Vernonia amygdalina* has been discovered to play a role in modulating some biological process in man and animals (Ezeonu *et al.,* 2016). The effects of bioactive compounds in *Vernonia amygdalina* prebiotic fraction on the large intestinal health, liver toxicity and anti-amoebic efficacy is the focus of this study. Hence, the need to identify the bioactive compounds and evaluate the anti-amoebic and digestive organ/liver cells architecture.

MATERIALS AND METHODS

Collection of Plant Materials and Identification

Fresh leaves of *Vernonia amygdalina* used for this study were obtained from a local village of Izzi land in Ebonyi state and authenticated by a botanist from the Department of Applied Biology, Ebonyi State University, Abakaliki.

Extraction of *Vernonia Amygdalina*

The leaves collected were washed in clean water and air dried in a clean surface at room temperature. The dried leaves were pulverized into a homogeneous powder using mortar and pistle. One hundred gram of the powder was soaked in a liter of boiled and cooled tap water for 24 hours with intermittent steaming. The extract was filtered using whatmann no 1 filter paper and concentrated to dryness by air drying prior to fractionation and phytochemistry. The extract was stored in the refrigerator (4-8^{0}C) in a clean sterile bottle.

Liquid-Liquid Fractionation

Fractionation of the *V. amygdalina* extract was achieved by sequential extraction with various organic solvent according to methods of Alabi *et al.,* (2016) and Ezeonu *et al.,* (2016).. The concentrated crude extract was re-extracted with chloroform. The extract was filtered with whatmann no 1 filter paper. The chloroform fraction and the residue were concentrated to dryness by air drying. The residue was again extracted with methanol for about 6 hours, washed 5 times with methanol and filtered with whatmann no 1 filter paper. The methanol extract and the residue were again concentrated to dryness by air drying. The concentrated ethanol residue was washed 3 times with ethyl-acetate. The ethyl-acetate fraction was separated using a separating funnel and concentrated to dryness by air drying. The residue was then fractionated with n-buthanol saturated with water (about 50% n-buthanol). Two layers were observed and the fractions were separated with separating funnel. The two fractions were concentrated to dryness by air drying. Four fractions were recovered with chloroform, ethyl-acetate and N-buthanol which yielded 2 fractions (lower and upper) respectively. The residue, which did not dissolve in any solvent but water only was called water residue. The fractions were designated E1 to E5 according to Ezeonu *et al.,* (2016).

Experimental Animals

Thirty five healthy white Wister rat weighing 100-200g. The rats were supplied from the Veterinary Medicine, University of Nigeria Nsukka were used for the study. The animals were kept in cages in the animal house and allowed free access to water and standard livestock pellets. The animals were examined and found to be free of wounds, swellings and infections before the commencement of the experiment. All experimental protocols were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

Experimental Design

The rats on arrival were kept for two days before grouping into the respective groups 1-7. Group1 was fed with feed and water. Group II was fed with glucose, Group III was fed with 100mg/ml of extract per body weight plus rice. Group IV was fed with 200mg/ml of extract per kg body weight and rice. Group V was fed with 300mg/dl of extract per kg body weight and rice. Group VI was fed with 400mg/ml of extract per kg body weight plus rice. Group VII was fed with rice only.

Tissue Processing & Staining

Grossing of the liver and large intestine was carried out. The tissues were carefully and appropriately fixed in 10% formol saline immediately after dissection. The fixed tissues were kept at room temperature for 7days. The tissue was passed through ascending grades of alcohol ranging from 70% to two series of Absolute ethanol. A series of increasing concentrations is used to avoid excessive distortion of the tissue. The tissue was removed from the alcohol and passed through different grades of xylene in order to clear off the tissue from alcohol because the wax and ethanol are not miscible. Another important role of the clearing agent is to remove a substantial amount of fat from the tissue which otherwise presents a barrier to wax infiltration. A typical clearing sequence for specimens not more than 4mm thick would be xylene I and xylene II for 15 minutes each. The tissue was infiltrated with a Paraffin wax for 2hrs at 56^{o}C. The tissue Leuckhart embedding mould and formed a block of tissue, which was attached to a wooden block. A section of this tissue block was cut by placing the block on a rotary microtome at (5um thickness). Section was taken to water and stained in haematoxylin solution for 5 minutes. Rinsed in water for a 30 seconds. Differentiated in 1% acid alcohol with continuous agitation for 10 seconds. Washed in tap water for 5 seconds. Stained in 1% aqueous eosin solution for 5 minutes. Washed in tap water for 30 seconds. Dehydrated, clear and mount.

Anti-Amoebic Assay

Preparation of Modified Pancreas Extract of Homemade Serum Media (PEHPS)

Freshly obtained liver was washed with distilled water and pounded to form a suspension. The medium was autoclaved at 121^{o}C for 15mins at 15rpi. After

autoclaving, 100µl chloramphenicol was added to the medium and mixed well by shaking to ensure homogenecity.

Culturing of *Entamoeba histolytica* on the Modified PEHPS medium a confirmed *Entamoeba histolytica* faecal sample was collected and a representative portion of it were inoculated into the media. The cultivated *Entamoeba histolytica* was incubated for 48hours. An aliquot of the broth was dropped on glass slide to confirm the growth of the trophozoites. The cells was counted using the improved Neubauer Counting Chamber to evaluate the exact number of cells present in one microliter of the sample.

Evaluating the effect of *Vernonia amydalina* prebiotic fraction on *Entamoeba histolytica* Ten (10 ml) of freshly prepared PHEPS medium was added into the six test tubes. Two of the tubes were added 3g (300mg/mL) *Vernonia amydalina* prebiotic extract, metronidazole was added to another pair and nothing was added to the last pair except *Entamoeba histolytica* cells (positive control). A Hundred microliter (100 µl) of the *Entamoeba histolytica* sample was added into each of the test tubes. Each of the test tubes containing the mixture was thoroughly mixed together, to attain homogeneity. The mixture were incubated at room temperature. Microscopic examinations were made using Neubauer Counting Chamber every 24hours to evaluate the response of the parasite to the extract using 1:10 saline dilution for 72hours.

Gas Chromatography/Mass Spectrometry (GCMS)

The bioactive compounds in extract were determined using Gas Chromatography Mass Spectrometry (Model 5975, Japan)

RESULTS

The photomicrograph from the histological examination of the large intestine and liver of the wistar rats in various groups were as shown below: normal structure of the large intestine and liver cells was observed in the control group, group treated with glucose, group treated with rice only and groups treated with varying concentrations of extract per kg body weight (Figures 1-6). The cyto-architecture of the large intestine and liver were better improved at 30 mg/kg body weight.

The prebiotic extract fraction of *V. amygdalina* at 300 mg/kg body weight had reduced the population of *E. histolytica* cells like metronidazole as compared with positive control (no treatment) (Figure 7).

GCMS analysis of the *V. amygdalina* prebiotic extract fraction identified 19 bioactive compounds and revealed three unique compounds including phytol (3,7,11,15-Tetramethyl-2-hexadecen-1-ol), Heptanediamide, N,N'-di-benzoyloxy- and α- β-Amyrin (Table 1).

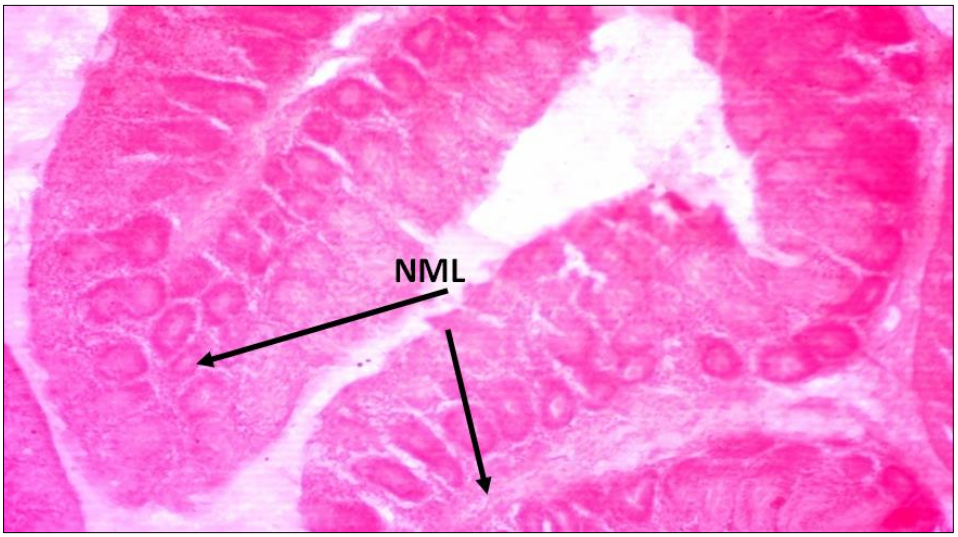

Figure 1: Photomicrograph of wister rat large intestine (groups1, 2, 3, 4 & 7) treated with glucose showing normal mucosa linning (nml). Stained with haematoxylin and eosin x200

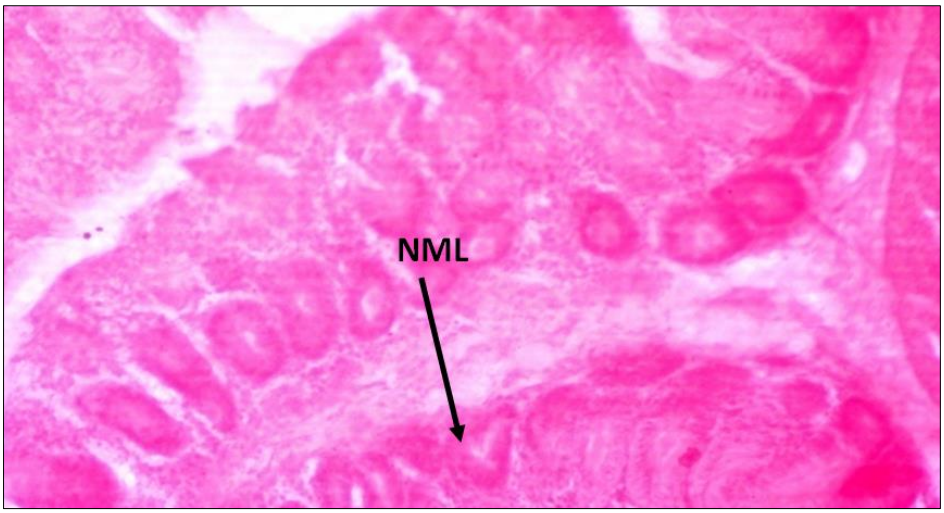

Figure 2: Photomicrograph of wister rat large intestine group 5 fed with rice and treated with 300mg/ml of extract per kg body weight showing normal mucosa lining & Improved Cyto-architecture (nml). Stain with haematoxylin and eosin x200

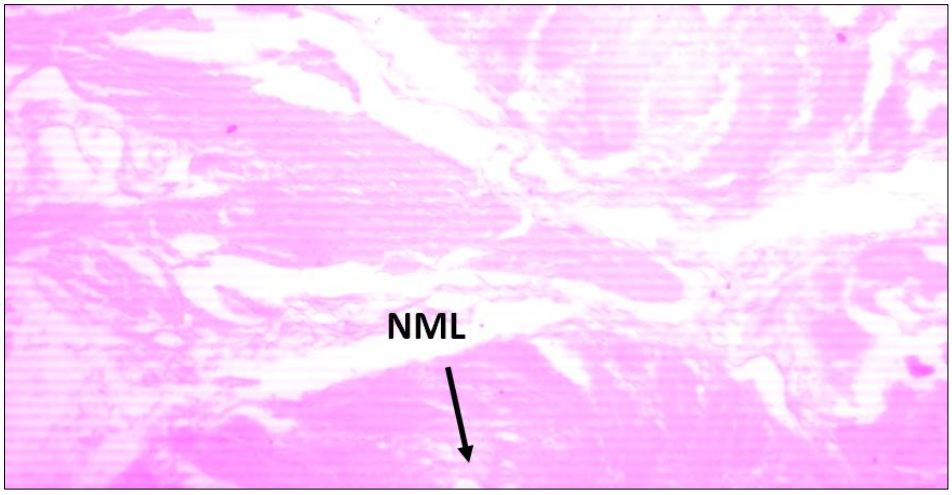

Figure 3: Photomicrograph of wister rat large intestine group 6 fed with rice and treated with 400mg/ml of extract per kg body weight showing normal mucosa lining (nml) & minor deranged Cyto-architecture. Stain with haematoxylin and eosin x200

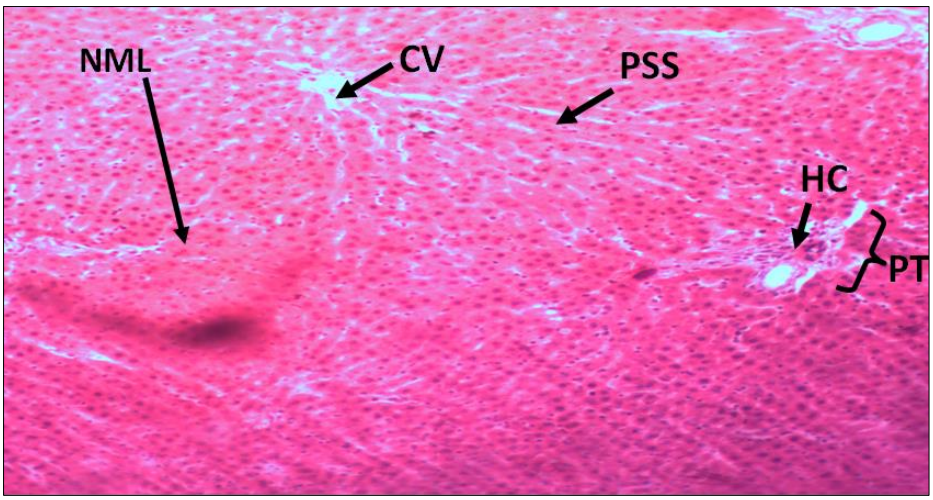

Figure 4: Photomicrograph of wister rat liver treated with feed and water showing normal central vein (cv), hepatic cells (hc), peri-sinusoidal spaces (pss) and portal triad (pt). Stain with haematoxylin and eosin. X200 (Groups 1, 2, 3, 4 & 7)

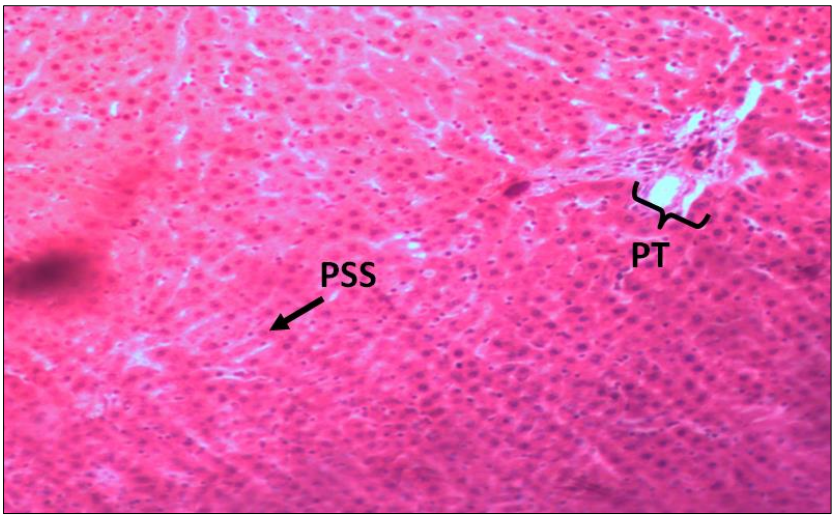

Figure 5: Photomicrograph of wister rat liver group 5 fed with rice and treated with 300mg/mL of extract showing normal peri-sinusoidal spaces (pss) and portal triad (pt) of normal hepatic cells improved Cytoarchitecture. Stain with haematoxylin and eosin. X200

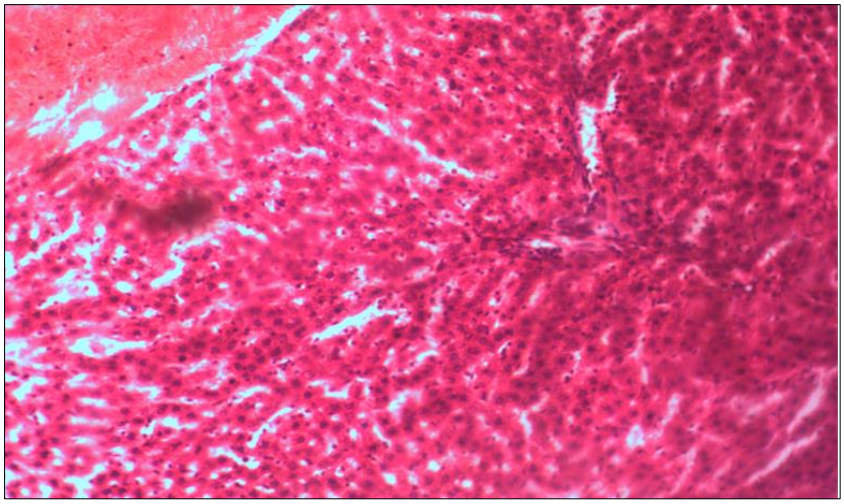

Figure 6: Photomicrograph of wister rat liver group 6 fed with rice and treated with 400mg/mL of extract showing normal distribution of hepatic cells (ndhc) &minor deranged Cyto-architecture. Stain with haematoxylin and eosin. X200

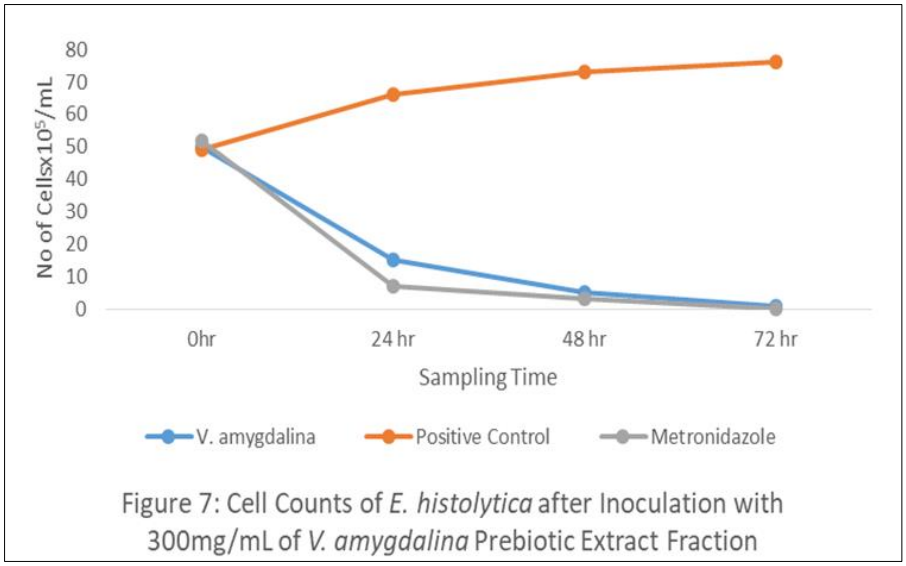

Figure 7: Cell Counts of *E. histolytica* after Inoculation with 300mg/mL of *V. amygdalina* Prebiotic Extract Fraction

Table 1: Identified Bioactive Compounds in *V. amygdalina* Prebiotic Extract Fraction

SN	Compounds Identified	Formula	Molecular Weight
1	Heptacosane	$C_{27}H_{56}$	380
2*	α- β-Amyrin	$C_{30}H_{50}O$	426
3	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358
4	2,3-Dihydroxypropyl icosanoate, 2TMS derivative	$C_{29}H_{62}O_4Si_2$	530
5	Oleic Acid	$C_{18}H_{34}O_2$	282
6	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318
7	2H-Pyran,2-(7 heptadecynyloxy) tetrahydro-	$C_{22}H_{40}O_2$	336
8	Octadecanoic acid	$C_{18}H_{36}O_2$	284
9	Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282
10*	Phytol (3,7,11,15-Tetramethyl-2-hexadecen-1-ol)	$C_{20}H_{40}O$	296
11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
12	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl) cyclohex-2-enone	$C_{13}H_{18}O_3$	222
13	3-Buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7 oxabicyclo[4.1.0] hept-2-yl)-	$C_{14}H_{24}O_2$	224
14	Tetradecanoic acid	$C_{14}H_{28}O_2$	228
15	6-(3,3-Dimethyl-oxiran-2-ylidene)-5,5-dimethyl-hex-3-en-2-one	$C_{12}H_{18}O2$	194
16	9-Tetradecen-1-ol,acetate,(E)-	$C_{16}H_{30}O_2$	254
17	Propanedioic acid, phenyl-	$C_9H_8O_4$	180
18	Tetradecane	$C_{14}H_3$	198
19*	Heptanediamide, N,N'-di-benzoyloxy-	$C_{21}H_{22}N_2O_6$	398

DISCUSSION

In this study the effects of *Vernonia amygdalina* extract fraction on the liver and large intestine of rats fed with rice was evaluated. The photomicrograph from the histological examination of the large intestine and liver of the wister rats in various groups revealed normal structure of the large intestine and liver in the control group and other groups including the group treated with varying concentrations of the leaf extract per kg body weight.

The finding of this study is in agreement with the report of Nwangwu *et al.,* (2011) who showed that histopathological changes observed in the liver and large intestine are due to the effects of *V. amygdalina* extracts on rats without evidence of lesions in the treated animals when compared with control. Histological findings further revealed that the cyto-architecture of the liver parenchyma was better organized in the groups treated with 300mg/kg extracts of *V. amygdalina*. The functions of the liver which includes detoxification, metabolic activities, synthesis of plasma protein and destruction of spent red blood cells among other functions will be better enhanced based on histological evidences from of this study. This shows that administration of extracts of *V. amygdalina* provides a protective role for the liver which is the first organ susceptible to any injurious substances in case of toxicity. This is in line with the work of Ezeonu *et al.,* (2016) who investigated the effect of *the V. amygdalina* extract fractions on immunological changes. The study of Ezeonu *et al.,* (2016) also identified galacto-oligosaccharides, which was called prebiotic fraction and contained high abundance of saponins and tannins. The renal parenchyma showed no evidence of vacuolations or distortion of any kind rather, renal corpuscles and Bowman's spaces were well preserved as compared with control groups in all the experimental groups. Also, the intestinal mucosa of the treated groups were better organized when compared with the control. There was no significant evidence of distortion in the surface epithelium, no presence of sub-mucosal edema. Studies have shown that anti-oxidants significantly strengthen the intestinal walls and protect tissue from oxidative damage (Morisse *et al.,* 2014; Elias *et al.,* 2015). Ezeonu *et al.,* (2016) reported that *V. amygdalina* aqueous extract has is very rich in anti-oxidants such as saponin and tannin. The abundance of antioxidants in the extract is possibly the major contributors to the improved organs cyto-architectural organization.

All findings in this study revealed that *V. amygdalina* is a very important plant that can be exploited for cyto-protective purposes. The cyto-protective mechanism may be by mopping up free radical there by protecting the cells of the organs from any type of assaults (Ojiako *et al.,* 2015; Elias *et al.,* 2015). Several other studies have shown that the aqueous and ethanolic leaf extracts of *V. amygdalina* is non-hazardous, even when taken for some time (Moundipa *et al*., 2015; Ezeonu *et al.,* 2016). The potency of these extracts may have been amplified on the account of increased concentration or buildup of specific phytochemicals, although the chemistry is yet unknown. Several findings showed that *V. amygdalina* has strong antioxidant activity corresponding to mitigation of the generation of hydroxyl radicals. Ezeonu *et al.,* (2016) and Igile *et al.,* (2015) postulated that this antioxidant activity may provide possible rationale for the observed therapeutic effects of *V. amygdalina*.

Polyphenolic compounds such as saponins and tannins have been reported to exhibit anti-amoebic activities against *E. histolytica* and Acanthamoeba strains (Moundipa *et al*., 2015). The finding of this study

which showed that *V. amygdalina* extract had significant anti-amoebic effect against *E. histolytica* cells by reducing the population *in vitro* is in agreement with the report Moundipa *et al*., (2015). Study by Erasto *et al.,* (2016) showed that sesquiterpene extracted from *V. amygdalina* leaves was active against microorganisms and possess anti-inflammatory properties. This report is in agreement with the findings of this study which revealed that the extract was highly effective against *E. histolytica* cells and contained alpha-beta amyrin and phytol along with other phenolic compounds (Table 1). These phenolic compounds that have been reported to have anti-inflammatory and anti-parasitic activities with cell cyto-protective and immune enhancing capabilities (Islam *et al.,* 2015 and Santos *et al.,* 2012).

Based on the results obtained, it can be concluded that leaf extracts of *V. amygdalina* are non-toxic and may possess cyto-protective potential.

CONCLUSION AND RECOMMENDATIONS

In conclusion, *V. amygdalina* possesses various bioactivities with low or absence of side effects. Based on the results obtained, it can be concluded that leaf extracts of *V. amygdalina fraction* are non-toxic and may possess cyto-protective potential with its improved health promoting effect. It may be more advantageous to incorporate *V. amygdalina* into health supplement for human benefits rather than using it solely as food.

REFERENCES

- Abosi, A. O., Kazeem, H. M. and Raseroka, B. H. (2015). *In vivo* antimalarial activity of *Vernonia amygdalina*. *British Journal of Biomedical Sciences,* 60:89–91.
- Akinpelu, D. A., Mamman, M., Kazeem, H. M. and Picard, J. (2013). Antimicrobial activity of *Vernonia amygdalina* leaves. *Fitoterapia*, 9(70):232-234.
- Alabi, D. A., Onibudo, M. Z. and Amusa, N. A. (2016). Chemicals and nutritional composition of four botanicals with fungitoxic properties. *World Journal Agricultural Sciences,* 1:84–88.
- Brar, D. S., Khush, G. S., Bhat, R. S. and Gowda, M. V. (2014). Specialty native rice (Oryza sativa L.) germplasm of Uttara Kannada, India. *Plant Gen. Res. Newsletters,* 140: 42- 47.
- Chang, T.T., Nader, G. and Forero, L., (2016). The origin, evolution, cultivation, dissemination and diversification of Asian and African rice. *Euphytica,* 25(1): 425-441.
- Ezeonu I.M, Asuquo A.E, Ukwah B.N and Ukoha P.O (2016). Immuno-Modulatory Properties of Prebiotics Extracted from *Vernonia amygdalina*. *African Journal of Traditional Complementary Alternative Medicine*, 13(6)*:* 11-17.
- Elias, H., Bengelsdorf, H. and Mark, H. (2015).The Structure of the Liver in Vertebrates. *Cells Tissues Organs,* 14(4):297–337.
- Erasto, G., Grierson, D. S. and Afolayan, A. J. (2016). Bioactive sesquiterpene from leaves of *Vernonia amygdalina*. *Journal of Ethnopharmacology,* 106:117–129.
- Igile, G.O., Oleszek, W., Burda, S. and Jurzysta, M. (2016). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. *Journal Agricultural Food Chemistry,* 43:2162–2166.
- Islam M.T, M.V.O de Alencar, K.C. Machado et al (2015). *Phyto in a Pharma-medico-stance. Chemico-Biological Interactions,* 240: 60-73.
- Morisse, J.P., Maurice, R., Boilletot, E. and Cotte, J.P. (2014). Assessment of the activity of a fructo-oligosaccharide on different caecal parameters in rabbits experimentally infected with *E.coli*. *Annals of Zootechnology,* 42:81-87.
- Moundipa, F. P., Kamini, G., Melanie, F. and Bilong, F. C. (2015). Bruchhaus I. *In vitro* amoebic activity of some medicinal plants of the Bamun region (Cameroon) *African Journal of Taditional Camonology*, 200(62):113-121.
- Ojiako, O.A., Mamman, M., Kazeem, H.M., Picard, J. and Nwajo, H.U. (2015). Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *African Journal Biotechnology,* 5:1648–1651.
- Santos F.A., JT Frota, B.R. Arruda, TS. de Melo et al (2012). Antihyperglycemic and Hypolipidemic Effects of alph-beta armyrin, a triterpenoid mixture from Protium heptaphyllum in mice. *Lipid in Health and Disease,* 11: 98.