Asian Journal of Research in Biochemistry

7(4): 12-20, 2020; Article no.AJRB.59205
ISSN: 2582-0516

Protective Effects of Bi-Herbal Formulation of Aqueous Extracts of *Vernonia amygdalina* and *Gongronema latifolium* against Gentamicin Induced Nephrotoxicity and Liver Injury in Rats

Nwadike Constance[1*], Dike-Ndudim Joy[1], Oly-Alawuba Nkeiruka[2], Ezekwe Ahamefula[3], Akanazu Chidimma[4] and Aloy-Amadi Oluchi[1]

[1]Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria.
[2]Department of Nutrition and Dietetics, Imo State University, Owerri, Nigeria
[3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Rivers State University, Nkpolu Oroworokwo, Port Harcourt, Nigeria.
.[4]Department of Public Heath, Federal University of Technology, Owerri, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors NCDNJ and EA designed the study. Authors OAN and AC performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAO and NC managed the analyses of the study. Authors NCEA, OAJ and AAO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2020/v7i430144
Editor(s):
(1) Dr. Mohamed Fawzy Ramadan Hassanien, Zagazig University, Egypt.
Reviewers:
(1) Suryakant Somabhai Parikh, Junagadh Agricultural University, India.
(2) Jasmeet Singh, Chhattisgarh Kamdhenu Vishwavidyalaya, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59205

Original Research Article

Received 10 May 2020
Accepted 16 July 2020
Published 22 October 2020

ABSTRACT

The study was carried out to investigate the protective effects of bi-herbal formulation of aqueous extracts of *Vernonia amygdalina* and *Gongronema latifolium* against gentamicin induced nephrotoxicity and liver injury in rats. Forty (n=40) male Wistar albino rats were procured and separated into five groups. Groups I and II served as normal control and experimental control respectively. Groups III to V served as test groups. Rats of experimental control (group II) and test groups were induced with lethal dose of gentamicin. Test groups III and IV were placed on herbal formulation of aqueous extracts of *V .amygdalina* and *G. latifolium* respectively, whereas rats in test

**Corresponding author: E-mail: nwadikeconstanceimsu@gmail.com;*

group V received bi-herbal formulation of aqueous extracts of *V. amygdalina* and *G. latifolium*. Nephrotoxic indices such as urea reduced significantly ($p<0.05$) in test groups (III, IV, and V) when compared to experimental control (group II) and normal control (group I). Creatinine also reduced significantly ($p<0.05$) in test groups III and V against group II (experimental control), and increased insignificantly ($p>0.05$) in test group V when compared to normal control (group I). Rats induced with gentamicin had upsurge in liver enzymes indicating possible compromise of hepatocellular integrity but the ameliorating effects of the herbal formulations were seen clearly in test groups in this study as they tried to protect the hepatocellular integrity. The bi-herbal formulation of aqueous extract of 5% v/v each of *V. amygdalina* and *G. latifolium* offered the best protection as observed in this study. This study has revealed the protective effects of bi-herbal formulation of aqueous extract of *V. amygdalina* and *G. latifolium* against gentamicin induced nephrotoxicity and liver injury in rats.

Keywords: Aminoglycosides; Gongronema latifolium; gentamicin; neprotoxicity; Vernonia amygdalina.

1. INTRODUCTION

For years now, the use of plants in the art of folkric medicine has been on the increase [1-7]. Since the acceptance of plants as agent to remedy disease conditions in primary and healthcare, greater part of the world has been relying on them [8-11]. The universal role of plants against diseases has been accepted and it is applicable to almost all major aspects of medicine and healthcare system [7,12-13]. The use of folkric medicine is prevalent in the traditional African Society and most of the Asian countries. The plants used in folkric medicine are known as medicinal plants [14-21]. Medicinal plants have been extensively defined by different authors [16-21]. It has been reported that medicinal plants are biologically and physiologically active against disease conditions due to their constituents [3-4,7,12,14,21-32]. These constituents dissolve in extracts, infusions, concoctions and decoctions made from medicinal plants, which are effective against disease inducing microorganisms.

V. amygdalina (Del.) and *G. latifolium* (Benth) are among those medicinal plants that are bioactive in nature and are physiologically active against diseases. *V. amygdalina* popularly known as bitter leaf, is a common shrub that grows in tropical Africa [10,18]. It belongs to the Asteraceae family. In Nigeria, the Igbos of Southeast call it “Onugbu”, the Yorubas of Southwest call it “Ewuro”, while the Hausas of North call it “Chusar-doki”. *V. amygdalina* is also known as “‘Ilo”, “Ityuna” and “Oriwo” in Igala, Tiv and Edo respectively. The leaves of *V. amygdalina* have characteristic bitter taste and green colouration [10,18]. They are used in the preparation of the popular “bitter leaf soup” commonly consumed in Nigeria. They are also used as spice for “Ndole”, a Cameroon dish [33]. The plant is used in the production of extracts, infusion, concoction, decoction or tonic that are traditionally used in folkric medicine against ailments such as infertility, diabetes, malaria, gastrointestinal problems and sexually transmitted diseases [34-35]. The use of *V. amygdaline* is not limited to humans alone. Studies have also implicated its bioactivity to others animals such as horse, chimpanzee as well as minute organisms [36-37]. The fresh leaves of the plant contain high moisture, protein and carbohydrate, with low fibre and ash [38].

G. latifolium (Benth), known commonly as amaranth globe, is a glaborous plant that grows in tropical Africa. It is a climber with characteristics greenish yellow flowers [10]. It can inhabit secondary forest, deciduous forest, rainforest or mangrove forest. It can be propagated by seed, stem cutting or softwood [10,17-18]. The flower grows around July and August. Aside Nigeria, the plants can also be found in Senegal, Chad, and DR Congo [17-18,39-40]. In Nigeria, the Igbos, Efik/Ibibios call *G.latifolium* “utasi”, the Yorubas call it “arokeke” or “madumaro”. According to Edim et al. [39], the Akan-asantes in Ghana, the Serers in Senegal and the Kissis in Sierra leone call it “kurutu nsurogya”, “gasub” and “ndondo-polole” respectively. *G. latifolium* has sweetly bitter in taste. It is used as in soups, salads and sauces as spice [41]. It is also used in the local brewing of beer. The stem is sometimes used as chewing stick [10,17-18,39].The plant has wide application in folkric medicine against abnormal blood glucose levels, diarrhoea, and tussive [10,17-18,42]. Its bioactive constituents are effective against disease inducing microorganisms and certain diseases [43-45].

Gentamicin is an aminoglycoside antibiotic approved for use in the United States since

1970. It has excellent against many Gram-negative bacterial organisms. Nicholas et al [46]. noted that gentamicin in synergy with β-lactams, is effective against *Staphylococcus* and *Enterococcus*. The same authors noted that gentamicin remains a first-line antibiotic for many severe infections with low rates of resistance and inexpensive [47]. Gentamicin can be taken through the muscle or vein and has multiple generic parental formulations. The antibiotic acts by binding to bacterial ribosomes and inhibiting protein synthesis [47]. It has been reported that dose related adverse effects are common to all aminoglycosides include oto- and nephrotoxicity [46-51]. Different studies have reported as low as 1.2% to 56% of such adverse effects on patients post administered the drug [46-51]. Generally, the uptake of aminoglycosides by the hepatocytes is highly limited and they are rapidly excreted in the urine with high concentrations found in the urine though there are isolated cases reports of acute injury on the liver coupled with jaundice on associated aminoglycosides therapy [47].

Different studies have observed the aminoglycosides had oto- and nephrotoxicity [46-51], but not much has been done to seek a possible protection to avert such toxicity occurring following the therapy of aminoglycosides. This study looked at such area and evaluated the protective effects of bi-herbal formulation of aqueous extract of *V. amygdalina* and *G. latifolium* against gentamicin induced injury on kidney and liver in rats.

2. MATERIALS AND METHODS

2.1 Sample Collection, Identification and Preparation

V. amygdalina and *G. latifolium* used in the present study were collected in November-2019, from Imo State University School farm in Owerri. The samples were identified in the Department of Plant Science and Biotechnology, Imo State University and the voucher specimens deposited at the Herbarium unit of Imo State University, Owerri. The leafy parts of the freshly harvested and identified samples were cut, rinsed, and air-dried for seven days before they were milled and packaged in sterile well labelled polyethene bags for storage and for further use.

2.2 Preparation of Aqueous Extracts

Two hundred gram (200 g) each of the milled sample was soaked separately in 2000 mL of distilled H_2O for a day, filtered, and freeze-dried to obtain the dried extract. 250 mL of the each of the filtrate was freeze dried to yield approximately 12.72 mg/kg *V.amygdalina* and 13.87 mg/kg for *G.latifoilum*. The extracts were re-dissolved in distilled water for further use.

2.3 Gentamicin

The gentamicin used in this study was commercially purchased and formulated into 80 mg/kg bod weight capable of inducing nephrotoxicity.

2.4 Experimental Design and Experimental Animals

Forty male Wistar albino rats weighing between 110 to 200 g were procured from the animal colony of Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. The rats were placed on drinking water, food and room temperature of 25 ºC for the initial acclimatization of four days, before they were separated into five groups of 8 rats each and induced with the gentamicin. The grouping was done on weight basis. The treatments of the rat groups were designated as follows.

Group I: Rats with no treatments with gentamicin, no aqueous extract of *V.amygdalina* and *G.latifoilum* (Normal control 1).

Group II: Rats treated with only 80 mg/kg body weight of gentamicin for 8 days (Experimental control 2).

Group III: 10% v/v of aqueous extract of *V.amygdalina* and 80 mg/kg body weight of gentamicin for 8 days.

Group IV: 10% v/v of aqueous extract of *G.latifoilum* and 80 mg/kg body weight of gentamicin for 8 days.

Group V: Bi formulation (5% v/v) each of aqueous extract of *V.amygdalina* and *G.latifoilum* and 80 mg/kg body weight of gentamicin for 8 days.

Groups III, IV and V were test rats. The extracts were given to the rats orally on daily basis as the experiment completed (8 days). The groups were placed on the same feed and water. The floors of the cages were constantly cleaned at on daily basis, their feed consumption and body weights were taken on daily basis.

Experimental handling of animals was in accordance with international guidelines on animal care and uses of National Institute of Health [52].

2.5 Sample Collection for Analysis

After 8 days of treatment, the rats were sacrificed after subjecting them to overnight fast and weighed. The rats were anaesthetized with chloroform and blood samples were collected by cardiac puncture into clean tubes for nephrotoxicity and liver enzyme studies.

2.6 Nephrotoxicity and Liver Enzyme Analysis

Urea, creatinine, sodium ion, potassium ion, chloride, and bicarbonate were analysed for nephrotoxicity. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin were for liver status. Urea was analysed using the Bethlot Searcy's method [53]. creatinine was determined by the method described by Larsen [54]. Sodium ion (Na^+) and chloride (Cl^-) ion levels were determined according to the instructions on their diagnostic kits purchased from Randox laboratories (UK). Potassium ion (K^+) was determined by direct spectrophotometric method. Bicarbonate (HCO_3^-) was determined using Forrester et al [55]. method. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using Reitman and Frankel [56]. methods; alkaline phosphatase (ALP) was carried out using the phenolphthalein monophosphate method [57]. total bilirubin was measured using Doumas et al [58]. method.

2.7 Statistical Analysis

Results are presented as mean and standard deviations of triplicate determinations. Group comparisons were done using least significant difference (LSD). Significant difference was established at 5% level as described by Onu and Igwemma [59].

3. RESULTS AND DISCUSSION

Fig. 1 shows the weight changes in rats placed on bi-herbal formulation of aqueous extract of *V.amygdalina* and *G. latifolium*. From the results Group I rats (Normal control) had the highest increase in weight (8.81 g). It was followed by group III (6.38 g), group IV and group V. Group II rats (Experiment control) had reduced weight of -3.09 g. Gentamicin induced in the rats could be behind the reduction.

From Table 1, urea ranged from 34.66 to 71.20 mg/dl. Creatinine ranged from 0.89 to 2.06 mg/dl. Sodium (Na^+) ion values ranged from 135.02 to 153.31 mEq/L, Potassium (K^+) ranged from 3.98 to 4.87 mEq/L. Chloride (Cl^-) ranged from 81.29 to 104.00 mEq/L and bicarbonate (HCO_3^-) ranged from 25.02 to 32.30 mmol/L. Urea is mainly a nitrogenous waste product of metabolism generated by protein break down [5,16]. It is normally eliminated exclusively through the urine by the kidneys [15,16,60]. Reduced urea excretion and consequent rise in blood concentration indicates possible kidney disease. Urea reduced significantly ($p<0.05$) in test rats (Groups III, IV and V) when compared to group II (Experimental control) rats but increased significantly ($p<0.05$) against group I (Normal control) rats. Creatinine has been described as the waste product of normal wear and tear on the muscles of the body. Different authors have noted that creatinine is a waste production of muscle metabolism [5,11,60]. It has important and interesting properties that make it very critical in assessing the failure of the renal system [5,27,60]. The creatinine levels of groups III to V rats reduced significantly ($p<0.05$) against group II rats. However, the increase observed in creatinine level of group V rats against group I rats was insignificant ($p>0.05$). Sodium ion, potassium ion and bicarbonate levels of groups III to V reduced significantly ($p<0.05$) when compared to group II rats but only groups II and IV had creatinine levels that increased significantly ($p<0.05$) against group I. Chloride in groups III to V increased significantly ($p<0.05$), when compared to the normal control (Group I) and experimental control (group II).

Aspartate aminotransferase (AST) ranged from 21.43 to 33.42 U/L. Alanine aminotransferase (ALT) ranged from 31.71 to 46.90 U/L. ALP ranged from 47.90 to 68.34 U/L while bilirubin total ranged from 0.75 to 1.51 mg/dl. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are excellent markers of liver damage brought about by exposure to toxic substances [16].

According to Duru et al [16]. Moss and Henderson [61]. when the integrity of the

hepatocellular membrane is comprised, there is extrusion of the enzymes into the plasma. Levels of AST and ALT in test rats reduced significantly ($p<0.05$) against the experimental control rats (group II) but test groups III and IV rats had significantly ($p<0.05$) increased AST levels against normal control rats (group I). Alkaline phosphatase (ALP) levels in test rats (groups III, IV, and V) reduced significantly ($p<0.05$) when compared to experimental control rats (group II) and increased significantly ($p<0.05$) against normal control (group I) rats. The observed enzyme reduction in test rats as the case maybe in this study against the control in the present study, could be indication of the extracts trying to salvage the integrity of the liver. Levels of bilirubin reduced significantly ($p<0.05$) in test groups (III, IV and V) when compared to the control. However, only test groups III and IV rats significantly increased ($p<0.05$) bilirubin against normal control rats (group I).

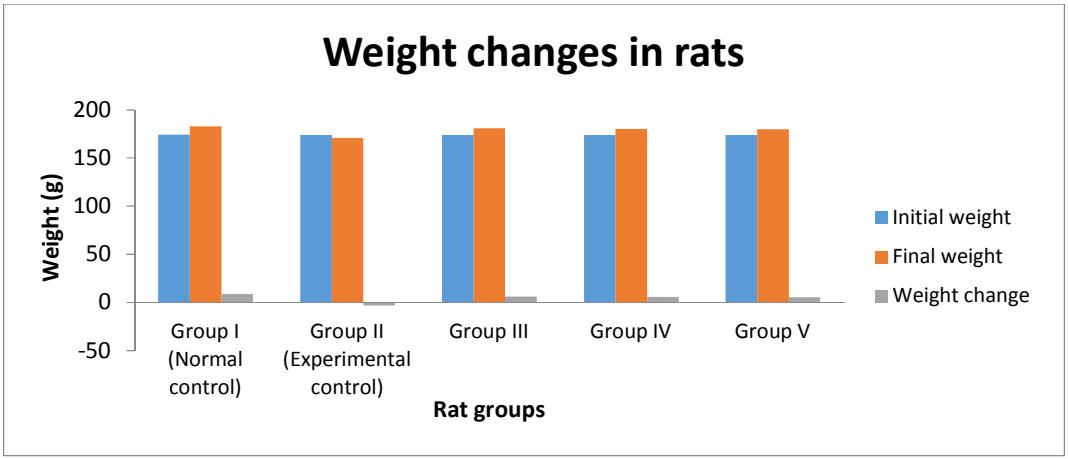

Fig.1. Weight changes in rats

Table 1. Nephrotoxicity indices

Parameters	Groups				
	I	II	III	IV	V
Urea (mg/dl)	34.66 ± 1.40^{b}	71.20 ± 2.77^{e}	40.40 ± 2.51^{d}	38.90 ± 3.00^{c}	36.10 ± 2.00^{a}
Creatinine (mg/dl)	0.89 ± 0.10^{a}	2.06 ± 1.11^{d}	1.22 ± 0.48^{c}	1.08 ± 0.19^{b}	0.97 ± 0.16^{a}
Na^{+} (mEq/L)	137.68 ± 0.80^{b}	153.31 ± 2.00^{d}	142.06 ± 1.48^{c}	135.02 ± 1.78^{a}	138.00 ± 1.24^{b}
K^{+} (mEq/L)	4.28 ± 0.75^{c}	3.98 ± 0.50^{a}	4.87 ± 1.05^{d}	4.08 ± 0.75^{b}	4.24 ± 0.70^{c}
Cl^{-}(mEq/L)	95.40 ± 5.36^{b}	81.29 ± 4.44^{a}	100.70 ± 4.69^{c}	104.00 ± 3.77^{d}	101.60 ± 2.30^{c}
HCO_3^{-}(mmol/L)	26.20 ± 3.00^{b}	32.30 ± 2.03^{d}	27.76 ± 2.41^{c}	25.02 ± 2.73^{a}	26.44 ± 2.70^{b}

Results are mean and standard deviation if triplicate determinations. Values with different letters of alphabets along the same row are statistically significant at 5% significant levels

Table 2. Liver enzyme of rats

Enzyme	Groups				
	I	II	III	IV	V
AST (U/L)	21.43 ± 1.13^{a}	33.42 ± 1.54^{c}	27.37 ± 0.23^{b}	29.18 ± 1.19^{b}	23.09 ± 2.01^{a}
ALT (U/L)	31.71 ± 0.15^{a}	46.90 ± 0.25^{e}	41.20 ± 2.81^{d}	38.40 ± 1.23^{c}	35.62 ± 2.38^{b}
ALP (U/L)	47.90 ± 0.00^{a}	68.34 ± 2.14^{e}	54.12 ± 2.90^{c}	57.43 ± 1.65^{d}	49.30 ± 0.00^{b}
Bilirubin (mg/dl)	0.75 ± 0.04^{a}	1.51 ± 0.08^{d}	0.93 ± 0.01^{c}	0.86 ± 0.04^{b}	0.79 ± 0.05^{a}

Results are mean and standard deviation if triplicate determinations. Values with different letters of alphabets along the same row are statistically significant at 5% significant levels

4. CONCLUSION

There was observed ameliorating effects on nephrotoxicity and liver damage in rats given bi-herbal formulation of aqueous extract of *V. amygdalina* and *G. latifolium* induced with gentamicin in this study. From the study, bi-herbal formulation of aqueous extract (5% v/v) each of *V. amygdalina* and *G. latifolium* offered better protection than aqueous extract (10% v/v) of *V.amygdalina* and aqueous extract (10% v/v) of *G. latifoilum.* This study has revealed the protective effects of bi-herbal formulation of aqueous extract of *V. amygdalina* and *G. latifolium* against gentamicin induced nephrotoxicity and liver injury in rats.

ETHICAL APPROVAL

This study was approved by Ethical Committee of the Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria**.**

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Duru MKC, Agomuo EN, Amadi BA. Biochemical studies on "udu" an antimalarial concoction used in Umunchi village, Isiala Mbano L.G.A of Imo State, Nigeria. Continental J. Pharmacology and Toxicology Research. 2012;5(2):28-34.
2. World Health Organization (WHO). Promotion and development of traditional medicine. Tech. Rep. Series. 1978;622.
3. Duru M, Amadi B, Agomuo E, Adindu E. Chemical profile of an antimalarial concoction "Udu" used in Umunchi autonomous community in Isiala Mbano L.G.A of Imo State, Nigeria. Journal of Emerging Trends in Engineering and Applied Sciences. 2012;3(3):444-447.
4. Duru MKC, Arukwe U, Amadi, BA. Bioactive constituents and macronutrients composition of anti-malarial concoction used in Umunchi village in Isiala Mbano L.G.A of Imo State, Nigeria. International Science Research Journal. 2011;3:61–64.
5. Monago-Ighorodje C, Duru M, Adindu E, Nwauche K, Ezekwe A, Nosiri I, Odika P, Onyeabo C, Ogar I, Berezi EP, Ugoh AI, Eboagwu I, Otta E. Effect of ethanolic leaf extract of *Vinca major* L. on biochemical parameters and glucose level of alloxan induced diabetic rats. African Journal of Biotechnology. 2019;18(32):1054-1068.
6. Duru M, Amadi B, Ugbogu A, Eze A. Effect of "udu" an antimalarial herbal preparation on visceral organ weight and blood lipid profiles in wistar rats. Journal of Pharmacy and Clinical Sciences. 2014;8:1-7.
7. Ibegbulem CO, Eyong EU, Essien EU. Biochemical effects of drinking *Terminalia catappa* Linn. decoction in Wistar rats. African Journal of Biochemistry Research. 2011;5(8):237-243.
8. Nwachukwu MI, Duru MKC, Nwachukwu IO, Obasi CC, Uzoechi AU, Ezenwa CM, Anumodu CK. *In-vitro* phytochemical characterization and antibacterial activity of *Newbouldia laevis* (boundary tree) on *Escherichia coli* and *Staphylococcus aureus*. Asian Journal of Microbiology and Biotechnology. 2017;2(1):30-36.
9. Amadi BA, Arukwe U, Duru MKC, Adindu EA, Ufornwa EC, Odika PC. The effect of fermentation on anti-nutrients, carbohydrates and vitamin contents of *Pentaclethra macrophylla* Seed. International Science Research Journal. 2011;3:74-77.
10. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Limited, Ibadan; 2006.
11. Duru MKC, Amadi BA, Eze AE, Ugbogu AE, Onuoha N. *In vivo* studies of *Solanum aethiopicum* fruit on some biochemical parameters using rats. Journal of Chemical and Pharmaceutical Research. 2013;5(2): 1-4.
12. Okwu DE. Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. J. Sustain. Agric Environ. 2004;6:30-34.
13. Amadi B, Onuoha N, Amadi C, Ugbogu A, Duru M. Elemental, amino acid and phytochemical constituents of fruits of three different species of eggplants. International Journal of Medicinal and Aromatic Plants. 2013;3(2):200-202.
14. Nwachukwu MI, Duru MKC, Amadi BA, Nwachukwu lO. Comparative evaluation of phytoconstituents, antibacterial activities and proximate contents of fresh, oven-dried uncooked and cooked samples *of Buchholzia coriacea* s*eed* and their effects on hepatocellular integrity International. Journal of Pharmaceutical Science Invention. 2014;3(6):41-4.
15. Nwachukwu MI, Duru MKC, Nwachukwu IO. Antifungal properties and effect of

fresh, oven dried uncooked and cooked seeds *of Buchholzia coriacea* on haematology and kidney. Elixir Food Science. 2013;64:19350-19356.

16. Amadi BA, Agomuo EN, Duru, MKC. Toxicological studies of Asmina triloba leaves on haematology, liver, kidney using rat model. International Science Research Journal. 2013;4(2)11-17.
17. Neuwinger HD. African traditional medicine, a dictionary of plants application, Medpharm GmbH Publishers, Stuttgart, Germany. 2000;589.
18. Gill LS. Ethnomedical uses of plants in Nigeria. University of Benin Press, Nigeria. 1992;215.
19. Adebajo AO, Adewumi, CO, Essein EE. Antiinfective agents of higher plants. International Symposium of Medicinal Plants, 5th Edn. University of Ife, Nigeria. 1983;152–158.
20. Okoli RI, Aigbe O, Ohaju-obodo JO, Mensah JK. Medicinal Herbs used for managing some common ailments among Esan people of Edo State, Nigeria. Pak. J. Nutr. 2007;6(5):490-696.
21. Duru M, Ugbogu A, Amadi B, Odika P, Chima-Ezika R, Anudike J, Osuocha K. Chemical constituents *of Buchholzia coriacea* se*ed.* Proceedings of the 35th Annual International Conference, Workshop & Exhibition of Chemical Society of Nigeria*.* 2012;*2:*39-45.
22. Okoroh PN, Duru MKC, Onuoha SC, Amadi BA. Proximate composition, phytochemical and mineral analysis of the fruits of *Ficus Capensis*. International Journal of Innovative Research & Development. 2019;8(9):83-88.
23. Amadi BA, Arukwe U, Duru MKC, Amadi CT, Adindu EA, Egejuru L, Odika PC. (2012): Phytonutrients and antinutrients screening *of D. edulis* fruits at different maturation stage*s.* Journal of Natural Product Plant Resourse. 2(4):530-533.
24. Duru M, Amadi C, Ugbogu A, Eze A, Amadi B. Phytochemical, vitamin and proximate composition *of Dacryodes edulis* fruit at different stages of maturation. Asian Journal of Plant Science and Research. 2012;2(4):437-441.
25. Agomuo EN, Amadi BA, Duru MKC. Some biochemical studies on the leaves and fruits of *Persea americana.* International Journal of Research and Reviews in Applied Sciences. 2011;11(3):565-569.
26. Amadi BA, Duru MKC, Agomuo EN. The chemical profiles of leaf, stem, and flower *of Ageratum conyzoides*. Asian Journal of Plant Sciences and Research. 2012;2(4): 428-43.
27. Duru M, Eboagwu I, Kalu W, Odika P. Nutritional, anti-nutritional and biochemical studies on the oyster mushroom, *Pleurotus ostreatus*. EC Nutrition. 2019*;*14(1):36-59.
28. Duru M, Nwadike C, Ezekwe A, Nwaogwugwu C, Eboagwu I, Odika P, Njoku S, Chukwudoruo C. Evaluation of nutritional, anti-nutritional and some biochemical studies on *Pleurotus squarrosulus* (Mont.) singer using rats. African Journal of Biochemistry Research. 2018;12(2):7-27.
29. Duru MKC, Amadi BA, Amadi CT, Lele KC, Anudike JC, Chima-Ezika OR, Osuocha K. Toxic effect *of carica papaya* bark on body weight, haematology, and some biochemical parameters. Biokemistri. 2012; 24(2):67-71.
30. Duru M, Agomuo E, Amadi B, Iheanacho A, Nutritional evaluation and some biochemical Studies *on Ageratum conyzoides* using its different par*ts.* Proceedings of the 35th Annual International Conference, Workshop & Exhibition of Chemical Society of Nigeria*.* 2012;1:372-379.
31. Agomuo EN, Duru MKC, Amadi BA. Some bioactive constituents of *Asmina triloba* (paw paw) leaf variety. International Science Research Journal. 2013;4(2):18-22.
32. Ugbogu AE, Okezie E, Uche-Ikonne C, Duru M, Atasie OC. Toxicity evaluation of the aqueous stem extracts of *Senna alata* in wistar rats. American Journal of Biomedical Research, 2016;4(4):80-86.
33. Ho WY, Liang WS, Yeap SK, Beh BK, Yousr AHN. *In vitro* and *in vivo* antioxidant activity of *Vernonia amygdalina* water extracts, Afric J Biotech. 2012;11(17): 4090–4094.
34. Igile GO, Oleszek W, Jurzysta M, Burda S, Fanfunso M, Fasanmade AA. Flavonoids from *Vernonia amygdalina* and their antioxidant activities. J Agric Food Chem. 1994; 42: 2445–2448.
35. Farombi EO, Owoeye O. Antioxidant and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. Int J of Environ Res Pub Health. 2011;8:2533–2555.

36. Hamzah RU, Jigam AA, Makun HA, Egwin EC.2013. Antioxidant properties of selected African vegetables, fruits and mushrooms: A Review. Intech 2013;9: 203-250.
37. Huffman MA, Seifu M. Observation on the illness and consumption of a possibly medicinal plant *Vernonia amygdalina* (Del.), by a wild chimpanzee in the Mahale Mountains National Park, Tanzania. Primate. 1989;30:51-63.
38. Arit E, Olorunfemi AE, Amaku OI, Edoho JE. Studies on some biochemical effects of *Vernonia amygdalina* in Rats. Asian Journal of Biochemistry 2007;2:193-197.
39. Edim, EH, Egomi, UG, Uwem EF, Archibong OE. Review on *Gongronema latifolium (*Utasi): A novel antibiotic against Staphylococcus *aureus* related infections. International Journal of Biochemistry and Biotechnology. 2012;1(8):204-208.
40. Ugochukwu NH, Babady NE. Antioxidant effects of *Gongronema latifolium* in hepatocytes insulin dependent diabetes mellitus Filoterapia. 2002;73(7-8):612-618.
41. Anaso HU, Onochie CC. A comparative study of nutrients in *Gongronema latifolium, Piper Guidneense* and *Piper nigrum*, to testify high acceptability in local dishes. J. Sci. Eng. Technol. 1999;6(2): 1321–1832.
42. Iwu MM. Handbook of African medicinal plants, 1st edition, CRC press Inc., Florida. 1993;239-239.
43. Eleyinmi AF. Chemical composition and antibacterial activity of *Gongronema latifolium*. Journal of Zhejiang University Science B. 2007;8(5):352-358.
44. Ross IA. Medicinal plants of the world, Vol. 2: Chemical Constituents, Traditional and Modern Uses. Humane Press, Totowa NJ 07512. 2001;487.
45. Schneider C, Rotscheidt K, Breitmaier E. 4 new pregnane glycosides from *Gongronema latifolium* (Asckepiadaceae). Liebigs Annalen Der Chemie. 1993;10: 1057-1062.
46. Nicholas MS, Susan S, Nicholas W, Richard JF, Nitin VK. Gentamicin-associated acute kidney injury. QJM: An International Journal of Medicine. 2009; 102:12–880.
47. Bethesda. Gentamicin In: Liver Tox: Clinical and research information on drug induced liver injury. National Institute of Diabetes and Digestive and Kidney Diseases; 2012. Available:https://www .ncbi. nlm. nih. gov /books/NBK548706/ (Accessed on 25th 06/2020).
48. Matzke GR, Lucarotti RL, Shapiro HS. Controlled comparison of gentamicin and tobramycin nephrotoxicity. Am J Nephrol. 1983;3:11–7.
49. Raveh D, Kopyt M, Hite Y, Rudensky B, Sonnenblick M, Yinnon AM. Risk factors for nephrotoxicity in elderly patients receiving once-daily aminoglycosides. QJM. 2002; 95:291–7.
50. Barza M, Ioannidis JP, Cappelleri JC, Lau J. Single or multiple daily doses of aminoglycosides: A meta-analysis. BMJ 1996;312:338–45.
51. Schentag JJ, Plaut ME, Cerra FB. Comparative nephrotoxicity of gentamicin and tobramycin: Pharmacokinetic and clinical studies in 201 patients. Antimicrob Agents Chemother 1981;19:859–66.
52. National Institute of Health. Guide for the care and use of laboratory animals. U.S. Department of health education and welfare. Washington, D.C.: NIH Publication; 1985.
53. Searcy RL, Reardon JE, Foreman JA A new photometric method for serum urea nitrogen determination. American Journal of Medical Technology. 1976;33(1):15-20.
54. Larsen KCreatinine assay by a reaction-kinetic principle. Clinica Chimica Acta. 1971;41:209-217.
55. Forrester RL, Watafe LJ, Silverman DA, Pierre KJ. Enzymatic method for the determination of CO2 in serum. Clinical Chemistry.1979; 22(2):243–245.
56. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 1957;28(1):56-63.
57. Babson LA, Greeley SJ, Coleman CM, Philips GD. Serum alkaline phosphatase determination. Clinical Chemistry. 1966;1: 482-490.
58. Doumas BT, Perry BW, Sasse EA, Straumfjord JV. Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. Clinical Chemistry. 1973;19(9):984-93.
59. Onu M, Igwemma AA. Applied statistical techniques for business and basic sciences, Skillmark Media, 2nd Edition; 2000.

60. Wough E, Anne G, Allison B.Anatomy and physiology in health and illness. 10th edition Churchill living stone. Elsevier. 2007; pp.22.

61. Moss DW, Henderson AR. Enzymes In: Tietz fundermental of clinical chemistry, 4th Eds. Tietz NW (Ed) W.B. Saunders Company, Philadalphia. 1996;283-335.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59205