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Phytochemistryand Acute Toxicity Study of *Bridelia ferruginea* Extracts

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Abstract: Phytochemical and acute toxicity study of *Bridelia ferruginea* extracts were done to determine the secondary metabolites and toxicity level of the plant.Qualitative and quantitative analyses of *Bridelia ferruginea* stem bark indicated the presence of alkaloids, flavonoids, steroids, cardiac glycosides, tannins, saponins and phenols. The GC-MS analysis of *Bridelia ferruginea* stem bark showed thirteen (13) bioactive ingredients in ethanol extract and twenty one (21) bioactive ingredients in hexane extract. This confirms high level of its medicinal value. Though, a number of these analysed phytochemicals could be totally detrimental to man as well as farm animals. The mortality rate of *C. Gariepinus* exposed to aqueous extract of *Bridelia ferruginea* stem bark and butachlor during acute toxicity test followed concentration and time dependent pattern throughout the exposure of the test toxicant to fish.

Key words: Acute Toxicity · Phytochemicals · Butachlor and *Bridelia ferruginea*

INTRODUCTION

Bridelia ferruginea is a homegrown medicinal plant species in Nigeria. Although present in the forest vegetation, but it is frequently found in the savannah. Its common names are: Kizin (Hausa), Mareni and Iralodan (Fulani and Yoruba), Ola, Agar (Igbo) and kensangeAbia (Boki) [1-3]. *Bridelia ferruginea* is usually a crooked messy plant with 15 m high and crooked bole up to 1.80 m in girth, the commonest *Bridelia* species of the savannah woodland occurring from Guinea and Mali to Nigeria and throughout the wooded Savannah regions of Africa [4-7].

All plants produced phytochemicals as part of their usual metabolic activities. There are primary metabolites and secondary metabolites present in a few range of plants, few important ones are obtained barely in a particular genus or species [8-10]. Pigments absorbing light offer protections to the host from radiation and exhibit colours to attract pollinators.

However, the roles of secondary metabolites are numerous. For instance, some secondary metabolites are toxins used to avoid predatory animal; pheromones to attract insect for pollination, phytoalexins offer protection in opposition to bacterial and fungal attack while allelochemicals inactivate competiting plants which contend for soil and light.

Furthermore, secondary metabolites and pigments are responsible for therapeutic actions in human that could be developed to produce drugs. These secondary metabolites include glycosides, saponins, alkaloids, tannins, flavonoids, phenol, steroids and among others [10, 11, 13].

MATERIALS AND METHODS

Biological Materials and Methods

Collection of Biological Materials

Plant Material: The stem bark of *Brideliaferruginea*plant was collected fromNtsuruakpaEzzamgbo, Ohaukwu Local Government Area Ebonyi State, Nigeria. The stem bark was identified and authenticated by Prof. S.C. Onyekwelu of the Department of Applied Biology, Ebonyi State University Abakaliki.

Fig. 1: Bridelia ferruginea Stem bark

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Preparation of Plant Extract: The *Brideliaferruginea* stem bark was cut into pieces and dried on air at temperature of 25°C for two (2) weeks and pulverised into fine powder using grinding machine. Fifty grams (50g) of ground stem bark of *Brideliaferruginea*was weighed into a conical flask and 100mls of de-ionised water, mixed and shaken prior to filtration by means of a dried Whatman sift paper into a graduated 1litre measuring cylinder to obtain cold water extract. Thereafter, the extract was stored at -5°C.

Experimental Fish and Treatment: A total of two hundred and thirty four (234) juvenile African catfish (*Clarias gariepinus*) of mean weight 240 g + 25.5–30.8 cm were purchased from Chiboy's Fish Farm Abakaliki Ebonyi State. The fish were acclimatized for two weeks in a plastic container of 80 litre capacity in a laboratory condition where temperature was kept constant at 25 ± 1°C and lightening plan at 12 hours of daylight alternating with 12 hours of darkness (LD: 12:). The water in plastic container was changed daily with bore-hole water that was free from chlorine

Estimation of Flavonoids: One gram (1g) of plant extract was added together with 10 mls of diluted hydrochloric acid solution and boil for 35 minutes. After boiling, the extract was allowed to cool and filtered. Thereafter, 2 mls of the filtrate was mixed with 10 mls solution of ethylacetate followed by 10 mls of one percentage (1 %) ammoniasolution. The absorbances were read spectrophotometrically at 420 nm – 520 nm.

Determination of Saponins: One gram of the plant extract was mixed with 60 mls ammonium chloride solution, heated in a boiling tube for 4 hrs. After boiling, it was cooled and filtered. Then, 50 mls of petroleum ether was mixed with filtrate and evaporated to dryness. Later, 5 mls of acetone-ethanol solution was mixed with the residue. Thereafter, 0.4 mls each of the samples was put into 3 different test tubes and 9 mls of ferrous sulphate solution were poured into test tube followed by addition of 2mls of conc. H_2SO_4 solution. The mixture was well shaken. 10 min. later, absorbances were read at 490 nm.

Determination of Tannins: Two gram of the powdered sample was vigorously shaken for 1 minute with 3 mls of methanol into a test tube and then dispensed in a Buchner funnel while the suction was turned on. The test tube was rinsed with an addition of 3mls of methanol and the content discharge at once into the funnel. The filtrate was mixed with 40 mls of water and study within one hour. The absorbances were read at 720 nm.

Determination of Cardiac Glycosides: Two grams (2g) of the fine powdered of plant sample was waterlogged with 10 mls 70% alcohol in 2 hours and then separated with filter paper. The extract collected was then subjected to purification using lead acetate paper and Na_2HP0_4 solution prior to the addition of newly prepared Buljet's solution (containing 95 ml aqueous picric acid + 10% aqueous. The distinction linking the colour intensity of the test sample and the blank (de-ionized water and Bulyet's solution) sample gives the absorbance, which is proportional to the concentration of cardiac glycosides.

Determination of Phenols: The total phenolic concentration in plant extract was determined with the FolinCiocalten's solution. In this method, various concentrations of the test sample were put together with 0.6 mlsFolinciocalten solution in 1:10v/v dilution. 5 minutes later, 5 mls of Na_2CO_3 solution was dispensed and the new volume of test tube was made up to 10 mls with de-ionized water and stand for 90 mins at 25 °C while the absorbance of sample against blank was taken spectrophotometrically at 760 nm.

Gas Chromatography –Mass Spectrometry Analysis: Gas chromatography-mass spectrometric study of the ethanol and hexane extracts of stem bark of *Brideliaferruginea* were analyzed using a Perkin-Elmer Gas chromatography clarus 500 system and two phase between gas chromatography-mass spectrometry designed with an Elite-2, fused silica capillary column of 30 mmX0.02 mm ID X 1 one micromolardf and 100% Dimethyl poly siloxane. For gas chromatography-mass spectrometry examination, an electron-ionized method with ionization energy of 75eV was employed while helium gas of 99.999% was used as the carrier gas at steady flow rate of 1ml/min and 2il volume was injected in a 10:1 split ratio. An injection temp. 250 °C, oven temp.1000 °C (Isothermal 20r 2min.) with increase of 10 °C/minute to 200 °C and 5 °C/minute to 280 °C and stoping with a 9 minutes isothermal at 280 °C. The mass spectrum was recorded at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total gas chromatographic running time was 36 minutes.

Thereafter, the comparative percentage quantity of each constituent was determined by comparing its average peak area to the total areas, by means of software designed to measure mass spectrum and chromatogram. The analysis on mass spectra of gas chromatography-mass spectrometry was done using the database of National Institute Standard and Technology with more than 60,000 patterns. Finally, the spectra of the unidentified constituent were compared with the spectrum of the identified constituent stored in the national institute standard and technology library. The name, molecular weight and structure of the constituent of the test materials were determined.

Acute Toxicity Test: Acute toxicity test to determine the 24, 48, 72 and 96hrs LC_{50} values of aqueous extract of *B. ferruginea*stem barkand Butachlor (herbicide) on *Clarias garriepinus* was done in a semi-static bowl in the laboratory using OECD guideline NO 23 (1992). The water with butachlor concentrations and that with *Bridelia ferruginea* were changed every 24 hrs by adding fresh butachlor concentrations and aqueous extract of stem bark of *Bridelia ferruginea* in order to keep concentration constant.

Exactly 150 fish were selected and divided into two groups for acute toxicity test. Each group was sub-divided into seven groups, each containing ten (10) fish in each aquarium. The first group was exposed to 870, 750, 625, 500, 375, 250 and 125 mgL^{-1} of the aqueous extract of the stem bark of *B. ferruginea* while the second group was respectively exposed to 0.90, 0.75, 0.45, 0.30, 0.15, 0.075 and 0.038 mgL^{-1} of butachlor. A control was set up with ten fish in an aquarium. The experimentation was done in aquaria containing 40 litres of aerated tap water. The 96 h LC_{50} was determined using the probit study techniques.

Sub-acute Toxicity Test: The 96 h LC_{50} values of plant extract and butachlor on *Clarias gariepinus* obtained were 139.090 and 50.438 mgL^{-1} respectively. These 96 h LC_{50} values were used for the sub-acute toxicity test. For the biochemical studies, a total of 84 fish acclimatized for 14 days were shared into two groups, A and B. Each group was sub-divided into three with twelve (12) fish in each aquarium. The group A for aqueous extract of stem bark of *Bridelia ferruginea* were exposed to 34.77, 17.39 and 13.91 mgL^{-1} and group B for butachlor exposed to 12.61, 6.30 and 5.04 mgL^{-1} corresponding to their ¼, $^{1}/_{8}$ and $^{1}/_{10}$ 96 h LC_{50}, for sixteen days. A set of 12 fish was simultaneously maintained in an aquarium to serve as the control (0.00 mgL^{-1}).

At the end of every 4, 8, 12 and 16 days, three (3) fish were taken from each aquarium, sacrificed and peripheral blood of the fish was collected by caudal vein puncture and in some cases by heart puncture. The blood sample collected (5ml) was spinned at 3500 g revolution per minute for 30 mins while the serum was collected for biochemical assays.

RESULTS

Phytochemical Component of Stem Bark Extract of the *Brideliaferruginea*: In the present investigation, the phytochemical evaluation of aqueous extract of *Brideliaferruginea*stem bark revealed the presence of the cardiac glycosides (92.853mg/100g), phenols (44.365mg/100g), flavonoids (43.145mg/100g), tannins (19.480mg/100g) and saponins (6.220mg/100g). Cardiac glycosides have the highest concentration while the least concentration was found to be saponins.

Phytoconstituents Identified in Both Ethanol and Hexane Extract of *Brideliaferruginea* Stem Bark via Gas Chromatography-mass Spectrometric Study: The phytoconstituents in the ethanol extract of stem bark of *Brideliaferruginea*were identified through gas chromatography- mass spectrometry study. The active constituents with their molecular weight (MW), retention time (RT), molecular formulae and percentage (%) contents in the ethanol extract of stem bark of the *Brideliaferruginea*are recorded in Table 1. Thirteen (13) constituents were investigated in ethanol extract of stem bark of *B. ferruginea.* Results shown that 9,12-octadecadienoyl chloride (32.15 %), 1-fluorodecane (12.90 %), 9-octadecenoic acid (19.57 %), trans-3-Dodecane (8.27 %) and Tetradecyl 2, 2, 3, 3, 3-Pentafluoro propanoate (6.81 %) were seen as the main constituents in the ethanol extract of the stem bark of the *Brideliaferruginea* while hexane extract of the stem bark of *Brideliaferruginea* (Table 2) twenty-one (21) compounds were detected. The results revealed that 1,2,4-Trimethylbenzene (14.92 %), n-Nonane (14.67 %), 9,12-octadecadienoyl chloride (11.59 %), 1,2,-Dimethylbenzene (7.34 %), 4,5-Dimethyl -2-undecene (7.26 %), Ethylcyclohexane (6.80 %), and 1,2,4,-Trimethylbenzene (5.21 %) were also detected as the main constituents in the hexane extract of the stem bark of the *Brideliaferruginea*.

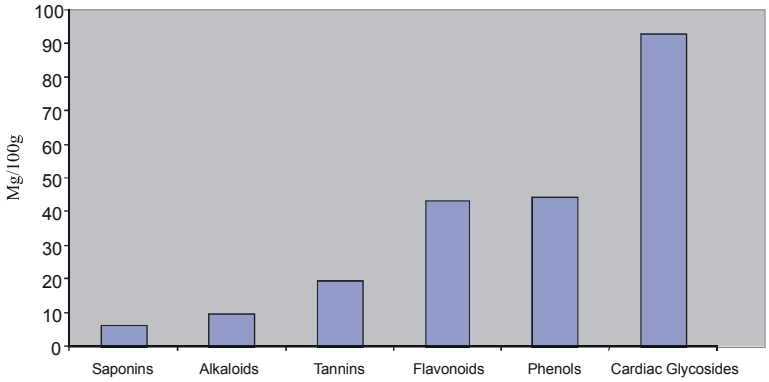

Fig. 2: Quantitative estimation of phytoconstituents of *Brideliaferruginea* stem bark

Table 1: Phytocomponents obtained in the ethanol extract of the stem bark of *Brideliaferruginea*through gas chromatography-mass spectrometry

S/no	Compounds	Molecular formular	m.wt	RT	Base peak	Peak area in %
1.	2-Ethoxypropane	$C_5H_{12}O$	88	3.100	45	8.27
2.	2-Ethoxy-1-propanol	$C_5H_{12}O_2$	104	3.100	45	8.27
3.	trans-3-Dodecene	$C_{12}H_{24}$	168	11.314	41	0.32
4.	n-Dodec-1-ene	$C_{12}H_{24}$	168	14.125	41	0.43
5.	Ethyl n-octadecanoate	$C_{20}H_{40}O_2$	312	20.717	88	1.41
6.	trans-13-Docosenoic acid	$C_{22}H_{42}O_2$	338	22.308	41	2.95
7.	Methyl 11-octadecenoate	$C_{19}H_{36}O_2$	296	22.558	55	3.54
8.	Ethyl 9-octadecenoate	$C_{20}H_{38}O_2$	310	23.458	55	3.54
9.	9-octadecenoic acid	$C_{18}H_{34}O_2$	282	23.783	41	19.57
10.	1-fluorodecane	$C_{10}H_{21}F$	160	25.167	43	12.90
11.	9,12-octadecadienoyl chloride	$C_{18}H_{31}O_{10}$	298	27.008	55	32.15
12.	Tetradecyl 2,2,3,3,3, penalfluoropropanoate	$C_{17}H_{29}F_2O_2$	360	27.192	43	6.81
13.	7- Tetradecenal	$C_{14}H_{26}O$	210	29.442	55	6.09

KEYS: RT= Retention Time in minutes, M.wt = Molecular weight in gram

Table 2: Phytocomponents identified in the hexane extract of the stem bark of *Brideliaferruginea*using GC-MS analysis

S/n	Compounds	Molecular Formular	m.wt	RT	Base peak	Peak area in %
1.	Ethylcyclohexane	C_8H_{16}	3.133	112	83	6.80
2.	4-Ethyl-5-methyl nonane	$C_{12}H_{26}$	3.300	170	43	4.98
3.	1,2-Dimethylbenzene	C_8H_{10}	3.558	106	91	7.34
4.	n-Nonane	C_9H_{20}	3.692	128	43	14.67
5.	n-Dodec-1-ene	$C_{12}H_{24}$	4.21	168	41	7.26
6.	4,5-Dimethyl-2-undecene	$C_{13}H_{26}$	4.625	182	41	4.49
7.	n-Decane	$C_{10}H_{22}$	5.067	142	43	14.92
8.	1,2,4-Trimethylbenzene	C_9H_{12}	5.275	120	105	5.21
9.	n-Tridecane	$C_{13}H_{28}$	11.400	184	57	0.45
10.	4-methyldecane	$C_{11}H_{24}$	5.392	156	43	4.37
11.	4,6,8-Trimethyl-1-nonane	$C_{12}H_{24}$	14.192	168	43	0.76
12.	n-pentadecane	$C_{15}H_{32}$	20.667	212	57	1.40
13.	2-Butyl-1-octanol	$C_{12}H_{26}O$	22.333	186	57	1.10
14.	n-Hexane	$C_{16}H_{34}$	24.925	226	57	1.45
15.	2,3-Dihydroxypropyl palmitate	$C_{19}H_{38}O_4$	25.167	330	43	2.75
16.	2-Methyloctadecane	$C_{19}H_{40}$	25.983	258	43	2.22
17.	9,12 octadecadienoyl chloride	$C_{18}H_{31}ClO$	27.017	298	55	11.59
18.	1-florodecane	$C_{10}H_{21}F$	27.192	160	43	2.75
19.	2 methylnonadecane	$C_{20}H_{42}$	27.833	282	57	2.63
20.	2-methyloctadecane	$C_{19}H_{40}$	28.792	268	43	1.23
21.	n-octadecane	$C_{18}H_{38}$	29.892	254	57	1.67

RT= Retention Time in minutes, M.wt = Molecular weight in gram

Table 3: Data on fish survival at different test concentrations and sampling time intervals in *C. gariepinus*exposed to aqueous extract of *Brideliaferrugineas*tem bark

Exposed Concentration (mgl^{-1})	Number exposed	Number of fish alive at different time intervals (hours) 24	48	72	96	%mortality
0.00	18	18	18	18	18	0.00
125	18	18	18	18	18	0.00
250	18	18	15	11	09	50
375	18	13	07	07	06	67
625	18	00	00	00	00	100

Table 4: Data on fish survival at different test concentrations and sampling time intervals in *Clariasgariepinus* exposed to butachlor.

Exposed Concentration (mgL^{-1})	Number exposed	Number of fish alive at different time intervals (hours) 24	48	72	96	%mortality Rate
0.000	18	18	18	18	18	0.00
0.038	18	18	18	18	18	0.00
0.075	18	18	18	13	12	33
0.150	18	15	00	00	00	100
0.300	18	15	00	00	00	100
0.450	18	6	00	00	00	100
0.750	18	00	00	00	00	100
0.900	18	00	00	00	00	100

Acute Toxicity Studies: Fish exposed to aqueous extract of the stem bark of *Brideliaferruginea* and butachlor showed behavioural abnormalities in response to test toxicants. Following the exposure of the test toxicants; fish were attentive, fail to swim and remained motionless in reaction to the unexpected changes within the environment. Later, fish in the test groups wanted to keep away from the test water by swimming rapidly, jumping as well as showing other irregular movements. In tank with higher concentration of the toxicants, erratic movements along with hyper excitation, faster opercula movements, surfacing and gulping of air were observed. Body pigmentation reduces while much mucus, which covered the buccal cavity body and gills were secreted. Later, the fish lost their body equilibrium, perception and noticeable rolling movement and became fatigued as well as sluggish owing to respiratory impairment. Shortly, they remained at the base of the water containers with the operculum widen open and eventually died.

The 96 h LC_{50} values of various concentrations of aqueous stem bark extract of *Bridelia ferruginea* in *Clarias gariepinus* were found to be 199.72, 165.76, 149.58 and 139.09 mg/l in that order for 24, 48, 72 and 96 hour of exposure while for butachlor, the LC_{50} values of different concentrations were found to be 64.407, 59.040, 52.485 and 50.438 mg/l in that order for 24, 48, 72 and 96 hour of introduction. A concentration-time increase in mortality rate was observed, thus, as the days of sample collection increased from 24 to 96 h the median lethal concentration necessary to kill the fish decreases.

Table 3 and 4 showed fish survival at different test concentration and sampling time intervals in *C. gariepinus* exposed to aqueous extract of the stem bark of *Bridelia ferruginea* and Butachlor. Percentage mean mortalities were recorded in Tables 3 and 4. The fish exposed to aqueous extract of the stem bark of *Bridelia ferruginea* in Table 3, mortality was highest (100 %) at 625 mg/L and lowest (50 %) in 250 mg/L after 96 hour of exposure while for butachlor- exposed fish, highest mortality (100 %) at 0.150 mg/L and lowest mortality (33 %) at 0.07 mg/L after exposure. Fish mortality increased with increasing concentration and time dependent pattern. No mortality was recorded in the control experiment during the toxicity test

DISCUSSION

In this research, qualitative and quantitative analysis of *Bridelia ferruginea* stem bark were carried out. Alkaloids, flavonoids, steroids, cardiac glycosides, tannins, saponin, as well as phenols were investigated in *Bridelia ferruginea* stem bark as shown in Figure 2. Phytochemicals and other compounds of plants are accountable for their medicinal values. The cardiac glycoside in nature is a cardioactive drug exploit for treating heart failure (congestive) as well as cardiac arrhythmia while alkaloids are used frequently in pharmacology for medications, or entheogenic rituals [5, 6]. While alkaloids act in a variety of biochemical processes in human being in addition to other animal and

equally caused bitter taste [7]. A number of these analyzed constituents could be totally detrimental to man as well as livestocks animals while several pyhtochemicals are specie specific as noted in the case of tannins [3]. Many of these compounds have confirmed to have antinutritional effect, subsequent to their capability to reduce palatability and digestibility of feed- stuffs.

GC-MS Analysis: In this study, thirteen (13) constituents were obtained from ethanol extract (Table I) and 21 compounds from hexane extract (Table II) of the stem bark of *B.ferruginea*by GC-MS investigation. Amongst the obtained constituents; tetradecanoic acid and n-hexadecanoic acid have antioxidant activity while 9,12,15-octa-decotrienoic acid, methyl ester has anti-inflammatory activity as well as treating arthritis [12].

Omega-3-fatty acids were examined to be vital for normal growth in addition to development and could play central role in preventing and treating coronary artery disease, hypertension, diabetes and arthritis, inflammatory problems, autoimmune disorder and cancer [13].

The 96h LC_{50} of 50.438 and 139.09 mg L^{-1} for butachlor and aqueous stem bark extract of *Brideliaferruginea* repesctivly, obtained indicated that both test toxicants are toxic to the *Clariasgariepinus.* The toxicity follows concentration-time dependent trend. Therefore, attributed the variation in the value obtained at different concentration and days of sample collection [10].

REFERENCES

1. Abdul–farah, M., B. Ateeg, M.N. Ali and W. Ahmad, 2004. Studies on lethal concentration and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere, 55: 257-265.
2. Adams, J., A. Garcia and C.S. Foote, 1978. Some chemical constituents of the secretion from the temporal gland of the African elephant (*Loxodonta africana*). Journal of Chemical Ecology, 4: 17.
3. Adebayo, E.A. and R.O. Ishola, 2009. Phytochemical and antimicrobial screening of thecrude extracts from the root, stem bark and leaves of *Bridelia ferruginea*. AfricanJournal Biotechnical, 8: 650-653.
4. Adewole, A.M., 2002. Evaluation of chemical components of some fish toxic plants in Ibadan. Mphil Dissertation University of Ibadan, Nigeria, pp: 241.
5. Ugwu Okechukwu, P.C., F.C. Nwodo Okwesili, E. Joshua Parker, Bawa Aburbakar, C. Ossai Emmanuel and E. Odo Christian, 2013. Phytochemical and Acute Toxicity Study of *Moringa oleifera* Ethanol Leaf Extract. International Journal of Life Sciences Biotechnology and Pharma Research, 2(2): 66-7.
6. Odo Christian, E., F.C. Nwodo Okwesili, Joshua, E. Parker, Ugwu, P.C. Okechukwu and C.C. Okonkwo, 2013. Acute Toxicity Investigation and Anti-diarrhoeal Effect of the Chloroform-Methanol Extract of the Seeds of *Persea americana*. Journal of Pharmacy Research, 6(2): 331-335.
7. Adonu Cyril, C., P.C. Ugwu Okechukwu, C.O. Esimone, Ossai, C. Emmanuel, Bawa, Abubakar and Okorie, U. Chris, 2013. Phytochemical Analyses of the Menthanol, Hot water and n-hexane extracts of the Aerial Parts of *Cassythafiliformis* (linn) and Leaves of *Cleistopholis patens* (benth). Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4(2): 1143-1149.
8. Abdul–farah, M., B. Ateeg, M.N. Ali and W. Ahmad, 2004. Studies on lethal concentration and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere, 55: 257-265.
9. Bate-Smith, S., 1962. Flavonoid compounds. In M. Florkin and H.S. Mason, Comparative Biochemistry III. New York: Academic Press, pp: 75-809.
10. Chang, J., S. Lin, Zhous, M. Wang and G. Zhu, 2011. Effects of butachlor on reproduction and hormone levels in adult zebrafish (*Daniorerio*). Journal of Experimental Toxicology.
11. Fafioye, O.O., A.A. Adebisi and S.O. Fagade, 2004. Toxicity of Parkia Biglobos and *Raphiavinifera* extracts on *Clarias gariepinus* juveniles. African Journal of Biotechnology 3(10). Available on line at http://www. Academic Journals. Org/AJB.
12. Simopoulos, A.P., 1991. Omega-3-fatty acids in health and disease and growth anddevelopment. American Journal of Clinical Nutrition, 54: 438-463.
13. Simopoulos, A.P., 1997. Omega-3-fatty acids in the prevention management of cardiovascular disease. Canadian Journal of Physiological Pharmacology, 75: 234-239.