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Acute Toxicity of Sponge Plant (*Luffa Cylindrica*) Fruit Extract on African Catfish (*Clarias Gariepinus*, Buchell 1822) Juveniles

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Abstract: **The study examined the acute toxicity of Sponge plant (Luffa cylindrica) fruit extract (a piscicide) on Clarias gariepinus juveniles under laboratory conditions using static non renewable bioassays over a period of 96 hrs. The fish (mean weight and length 14.72±3.63g and 13.12±1.01cm) were exposed to concentrations of 13500mg/L, 15000mg/L, 16500mg/L and 18000mg/L L. cylindrica extract. The physicochemical parameters of test media were relatively stable except the TDS and conductivity which increased with increase in concentration and exposure time. The LC_{50} of the fruit extract was 14125.38m/L while the LT_{50} was found to be 28.18hrs, 38.02hrs, 70.80hrs and 151.36hrs for 18000mg/L, 16500mg/L, 15000mg/L and 13500mg/L respectively. The ANOVA revealed significant variation between treatments and control for fish mortality (F=129.83 at P<0.05). The physiological changes analyzed revealed that TBF decreased while the OBF increased with increase in concentration and exposure time. The fish exposed to the extract displayed some behavioural changes like prolonged vertical movement, rapid movement and jumping, changes in skin colour with heavy secretion of mucus. Pathological changes were observed on the gills (loss of lamellae, necrosis, and serious lamellae distortion) and liver (alterations in the tissues with steatosis, vacuolations and necrosis) of the test organism. The result of this study shows that the extract of L. cylindrica fruit is toxic to fish which implies that stringent measures should be taken to ensure the restraint of its usage by the local fishers to reduce the potential risk of poisonous fish consumption and pollution of aquatic ecosystem.**

Keywords: **Acute toxicity, *Luffa cylindrica*, *Claria sgarepinus*, Extract, Piscicides.**

1. INTRODUCTION

Extracts from more than 60,000 species of plants are used for different purposes in the world [1] approximately 1,190 pure chemical substances extracted from higher plants are used in medicine throughout the world [2]. The record numbers of flowering plants in Nigeria in 1995 range between 150,000 and 200,000 species in some 300 families and 10,500 genera [3].Some extract from flowers, bark, pulp, seed, fruit, root, leaves and even the entire plant are found to be poisonous [2]. Some of the plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties [4]. Plants of different families have been applied for killing fish all over the world [4, 5, 6]. The active ingredients in the plant part used have differing capacities, and ways of action depending on the method of application: it can be applied directly in the form of extract, aqueous or dissolved in alcohol [6]. Plant extracts are called botanicals and when toxic to fish are called piscicides [2].

Piscicidal plants contain different active ingredients known as alkaloids such as nicotine, pyrethrum, ryania, rotenone, coumerin, resin, akuammine, tannins, saponins and diosgenin [7, 8]. When fishes are exposed to botanicals, they may

suffer from stress without necessarily killing them [2]. Stress response is characterized by the biochemical and physiological changes which may be manifested in acute and chronic toxicity tests [4].

Vimal and Manohar[9] investigated the toxicity of *Euphorbia antiquorum* latex extract to fresh water fish *Poecilia reticulate*. In their investigation, they noted that the mortality of fish occurred steadily with increase in time and concentration of the latex extract. In another study on the acute toxicity of lyophilized aqueous extract of *Psychotria microphylla* leaf on *Clarias gariepinus* by Orji *et al.* [10], they found out that the fish exhibited hyperactivity characterized by surfacing and jumping outside of water, loss of balance, erratic swimming, respiratory distress, rapid opercula movement, incessant gulping of air, spiral movement, discolouration of the whole body and excessive mucus secretion within 15 min of exposure. In acute concentrations prior to death, fish aggregated at the air-water interface gasping for air with their mouth permanently opened.

A widely used piscicide in Nigeria is *Luffa* [6]. Luffa (*Luffa cylindrica* or *Luffa aegyptiaca*) is commonly called vegetable sponge, sponge gourd, loofa, sponge plant, bath sponge or dish cloth gourd and is a member of cucurbitaceous family [11]. The plant is widely distributed within the savannah belts of West Africa including Nigeria where it is reported to have domestic and medicinal values [12, 13]. The local farmers in Nigeria use the fruits of this plant to harvest fish from water bodies for human consumption. However, there is no available scientific work done on the piscicdal effects of *L. cylindrica*. Thus, this necessitated the need to investigate the toxic effects of this plant on the aquatic organisms.

2. MATERIALS AND METHODS

Collection of Experimental Organisms and Plant:

A total of two hundred (200) juveniles of *Clarias gariepinus* were obtained from a private fish farm at Aluu, Port Harcourt, Nigeria. The juveniles were of a mean weight range of 14.72 ± 3.63g and length of $13.12 \pm$ 1.01cm.The juveniles were carefully transported to the laboratory in the evening in an open plastic bucket covered with nylon net. In the laboratory, they were immediately transferred into holding tanks. The fish were acclimatized for two weeks (14 days) prior to the commencement of the experiment. The holding tanks were cleaned and the water renewed once in three days [14].The test fish were fed twice daily during the period of acclimatization, using Coppens procured from Animal Affairs, Rumuodumaya, Port Harcourt.

The test plant was collected in Choba Campus University of Port Harcourt, close to the Faculty of Agriculture demonstration farms and was taken to the Department of Plant Science and Biotechnology for proper identification using appropriate keys [15].

Preparation of the Test media for Acute Bioassay:

Phytochemical screening was carried out on the fruit of *L. cylindrica* to detect the chemical components present. Standardized chemical tests which were modified by Harbornes and Baxter [16] were employed for the assessment.

The fresh fruit of *Luffa cylindrica* was sliced into smaller sizes using a knife and homogenized using Binatone Electric Blinder [17]. Aqueous content was filtered using a dry Whatman filter paper into a graduated 1L measuring cylinder to obtain the pure extract without adding water. The aqueous content was collected into a bottle, covered and put in a refrigerator at 4^0C to keep it fresh until the time of administration Adewole [18]. Five different concentrations were prepared, 0.00 mg/L, 13500 mg/L, 15000 mg/L 16500 mg/L and 18000 mg/L). The test media were allowed to stand for 30 min [19] for proper mixing before introducing the fish. The fish were randomly placed in 15 plastic containers (three replicates) at the rate of ten (10) juveniles per container.

Acute Toxicity Test:

Standard static bioassay procedures were employed based on Fish mortalities were observed and recorded at 24, 48, 72 and 96 hours. Fish behaviour were observed and a fish is considered affected by the plant toxicant when it manifests erratic swimming behaviour, hyperactivity, hyperventilation and pronounced ataxia coinciding with decreased capacity to respond to visual stimuli. A fish is considered dead when it does not respond to mechanical prodding and is removed immediately. Physiological changes were observed using operculum beat frequency (OBF) and tail beat frequency (TBF) of the fish once every day for the four days the acute test lasted. The physicochemical parameters of test media were determined using standard methods [20]. At the end of 96 hrs, the surviving fish were scarified to determine histological changes in their gills and livers.

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The susceptibility of the organisms to the extract was determining using the LC_{50} and LT_{50} which is the concentration that will kill 50% of the population in 96hrs and the time it takes 50% of the population to die in each concentration. The LC_{50} and LT_{50} were determined using probit analysis as described by Vincent [21].

3. RESULT

Phytochemical Components of *Luffa cylindrica* Fruit:

The chemical components of the fruit of *Luffa cylindrica* are given in Fig. 1. The result of the phytochemistry analysis showed that the fruit of *Luffacylindrica* contain 11major constituents which are; Phenols, Tanins, Alkaloids, Flavonoids, Saponins, Oxalate, Cyanogenic glycoside, Anthraquionols, Steroids, Terpenoids and Phytate. The analysis also showed that alkaloids were 0.65%, followed by saponins with 0.55% while steroids were least with 0.012%.

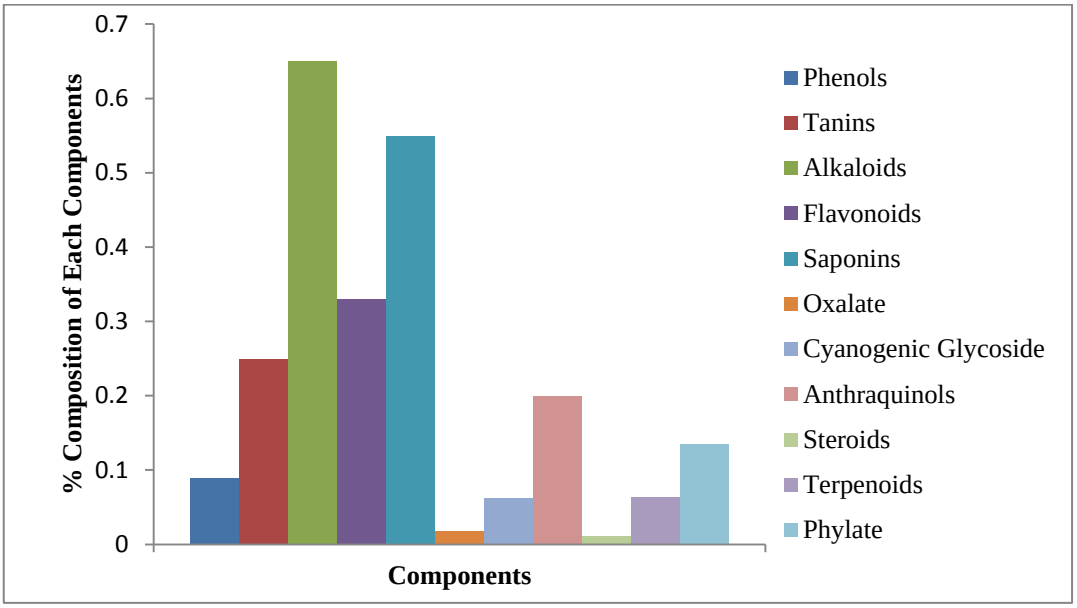

Fig. 1: Percentages of Phytochemical components of *Luffacylinrdrica* Fruit.

Physico-Chemical Parameters:

The result of physicochemical parameters of test media is presented in table 1. The pH, DO, TDS and temperature of the test media were within WHO [22] standard. The result for the conductivity showed that the two highest concentration of 18000 mg/L and 16500 mg/L had the highest concentration and the value obtained 165.47±7.91 mg/L and 102.23±23 mg/L was higher than WHO [22] standard of 100 mg/L. The values obtained for the other concentrations were within WHO/FMEVN standard. The differences in conductivity values was statistically significant ($F=86.80$, $P<0.05$).

Acute Toxicity Test Result:

The mortality of *Clarias gariepinus* juveniles exposed to water contaminated with the fruit extract of *Luffa cylindrica* is shown in table 2. The control group had no mortality while mortality increases with increase in concentration and time among the treated groups. The lowest concentration which is 13500 mg/L recorded 43.3% mortality while the highest concentration had 96.7% mortality rate. The ANOVA revealed a statistical significant difference between treatments for fish mortality ($F=78.81$ at $P<0.05$). The 96 hours LC_{50} which is the lethal concentration that kills 50% of the test organism in 96hrs, was observed at 14125.38 mg/L concentration, as shown in Fig 2.The LT_{50} for 18000 mg/L, 16500 mg/L, 15000 mg/L and 13500 mg/L concentrations were found to be 28.18 hrs, 38.02 hrs, 70.80 hrs and 151.36 hrs respectively, as shown in Fig 3.

Table 1: Physico-Chemical Parameters of the different concentrations for Acute Toxicity Test

Parameters	Control 0.00 mg/L	Treatment1 13500 mg/L	Treatment2 15000 mg/L	Treatment3 16500 mg/L	Treatment4 18000 mg/L	WHO Standard (2003)
pH	6.70 ±0.20[a]	6.20±0.46[a]	5.97±0.55[a]	5.90±0.63[a]	5.83±0.50[a]	6.5 – 8.5

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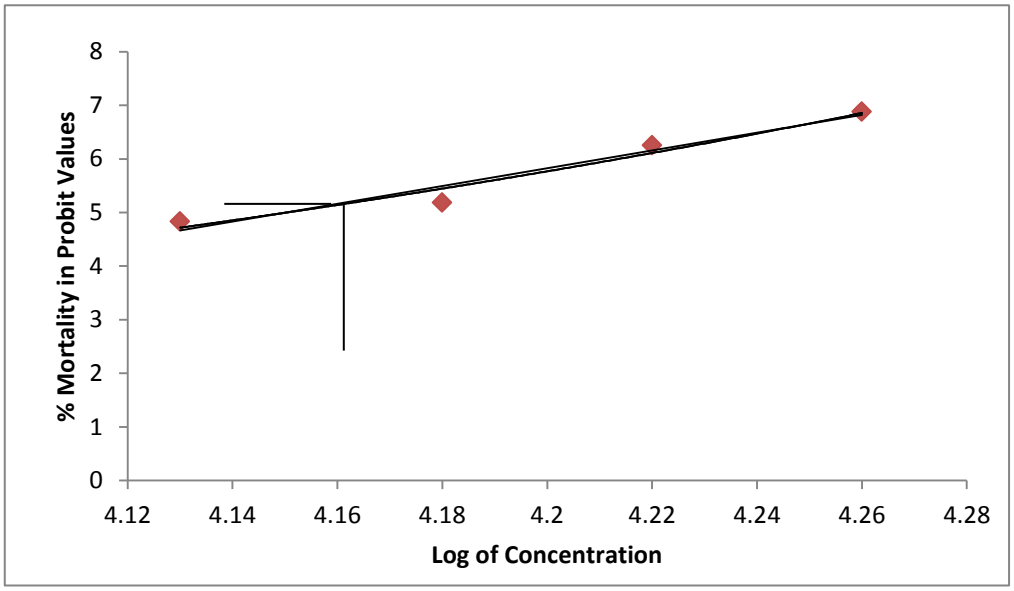
DO (mg/L)	6.10 ±0.20^{a}	5.80±0.27^{a}	5.30±0.30^{b}	4.53±0.21^{c}	4.40±0.10^{c}	> 4.0
Temperature (^{0}C)	27.07±0.31^{b}	27.53±0.31^{a}	27.77±0.31^{a}	27.57±0.06^{a}	27.57±0.21^{a}	20 – 30
TDS (mg/L)						
Conductivity (uS/cm)	3.37±1.85^{e}	45.40±2.33^{d}	58.13±2.24^{c}	68.83±4.47^{b}	107.20±6.56^{a}	250
	23.1±5.20^{e}	68.83±4.47^{d}	88.83±3.50^{c}	102.23±6.53^{b}	165.47±7.91^{a}	100

Note: Values in each row with the same superscript are not significantly different at P >0.05

Table 2: Mortality of *Clariasgariepinus* exposed to acute concentrations of *L. cylindrica* fruit extract

Treatments (Conc. in mg/L)	Number of Fish/Tank	Total Mortality			Mean Mortality	Percentage Mortality
		Replicate 1	Replicate 2	Replicate 3		
0.00	10	0	0	0	0	0
13500	10	4	5	4	4.33	43.3
15000	10	6	6	5	5.67	56.7
16500	10	9	9	9	9	90
18000	10	10	9	10	9.67	96.7



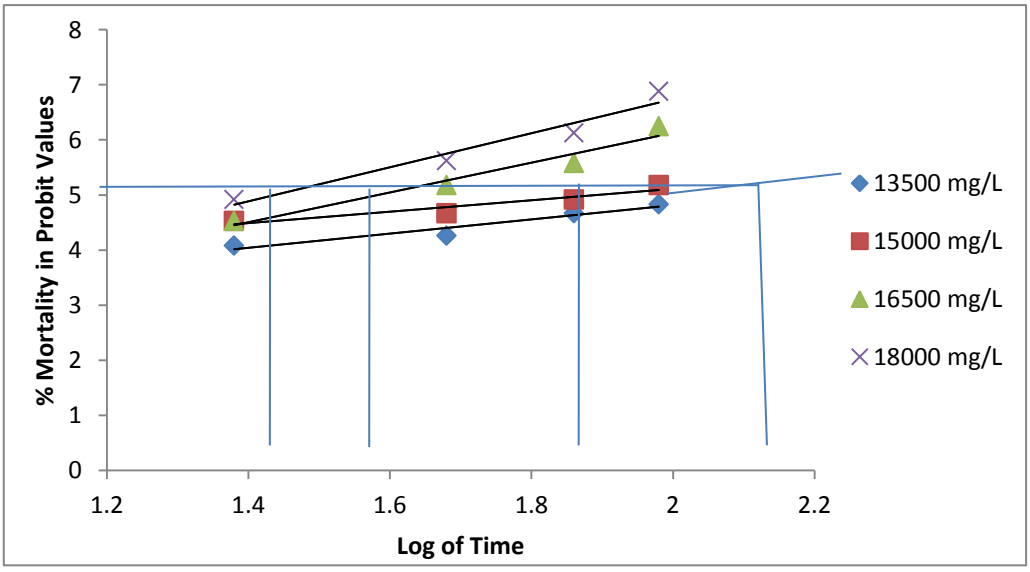


Fig 2, 3: Graph of Percent Mortality against Log of Concentrations of Fruit extract of *Luffa cylindirca* (LC_{50} Graph), Graph of Percentage Mortality in Probit Value against Log of Time (LT_{50} Graph)

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Behaviour and Physiological Responses:

Rapid erratic movement was observed in the different concentrations. There were some forms of body vibration, followed by rapid mouth movement. At the initial stage, the fishes showed prolonged vertical movement to the surface of the water, with their mouth opened at regular intervals, gasping for air. Changes in skin colouration were observed after several hours of exposure and excess mucus secretion, also the lips and eyes became swollen and reddish with weaker ones (fishes) at the bottom of the test media. These behavioural changes were more obvious with increase in concentration. The dead fish floated on the surface of the plastic aquaria immediately after death increased operculum beat frequency and reduced tail beat frequency prior to death (Table 4 and 5). The OBF values increased from 51.33±7.76 for the control to 174.67±5.51 for 18000 mg/L which is the highest concentration while the TBF values decreased from 13.67±1.53 for the control to 4.67±2.08 for the highest concentration (18000 mg/L) Table 4 and 5 respectively. The differences in Opecular beat frequency and tail beat frequencies across the groups were statistically significant (P <0.05).

Histological Observation:

The sections of the gills of the juveniles of *Clarias gariepinus* exposed to the various concentrations of the fruit extract of *Luffa cylindrica* viewed at x400 magnification in the Olympus light microscope showed that there were various pathological changes. These pathological changes ranged from mild epithelial lifting, swollen lamellae to slight hyperplasia especially in the lower concentrations of 13500 mg/L and 15000 mg/L (Plates 1a and b), the effect on the gills were more severe with increase in concentration. There was loss of lamellae and mass degeneration, necrosis and serious lamellae distortion at 16500 mg/L and 18000 mg/L; there was hyperplasia and general fusion.

Likewise the microscopic examination of the controlled experiment for *C. gariepinus* juveniles showed that there were no abnormalities in the liver appearance. The liver of the fish exposed to the various concentrations of *Luffa cylindrica* fruit extract show pathological alterations in the tissues with steatosis, vacuolations and necrosis

Table 4: Mean Opecular Beat Frequency (OBF) of *Clarias gariepinus* Juveniles at Different concentrations of *Luffa cylindrica* Fruit extract.

	Time (hrs)			
Concentration (mg/L)	**24**	**48**	**72**	**96**
0.00	50.33 ± 7.02^{b}	50.33 ± 3.06^{d}	51.00 ± 11.53^{d}	51.33 ± 7.76^{e}
13500	58.73 ± 3.06^{b}	64.00 ± 4.58^{c}	66.33 ± 4.16^{d}	75.00 ± 7.00^{d}
15000	71.67 ± 4.51^{a}	91.33 ± 3.22^{b}	104.00 ± 6.56^{c}	119.67 ± 16.44^{c}
16500	76.00 ± 6.56^{a}	95.00 ± 8.54^{ab}	125.00 ± 8.72^{b}	139.33 ± 3.06^{b}
18000	83.00 ± 9.17^{a}	106.67 ± 10.26^{a}	161.33 ± 14.01^{a}	174.67 ± 5.51^{a}

Note: Values in each column with the same superscript are not significantly different at P >0.05

Table 5: Mean Tail Beat Frequency (TBF) of *Clarias gariepinus* Juveniles at Different concentrations of *Luffa cylindrica* Fruit extract

	Time (hrs)			
Concentration (mg/L)	**24**	**48**	**72**	**96**
0.00	13.67 ± 0.56^{d}	13.67 ± 1.53^{a}	13.67 ± 1.53^{a}	13.67 ± 1.53^{a}
13500	16.00 ± 100^{d}	14.33 ± 1.16^{a}	12.00 ± 1.00^{ab}	10.33 ± 1.53^{b}
15000	18.67 ± 2.08^{c}	13.33 ± 1.53^{a}	9.33 ± 1.53^{bc}	7.00 ± 1.00^{c}
16500	22.67 ± 1.53^{b}	14.00 ± 10.26^{a}	9.00 ± 2.00^{bc}	6.00 ± 1.00^{c}
18000	26.67 ± 1.53^{a}	12.67 ± 1.73^{a}	7.33 ± 1.53^{c}	4.67 ± 2.08^{c}

Note: Values in each column with the same superscript are not significantly different at P >0.05

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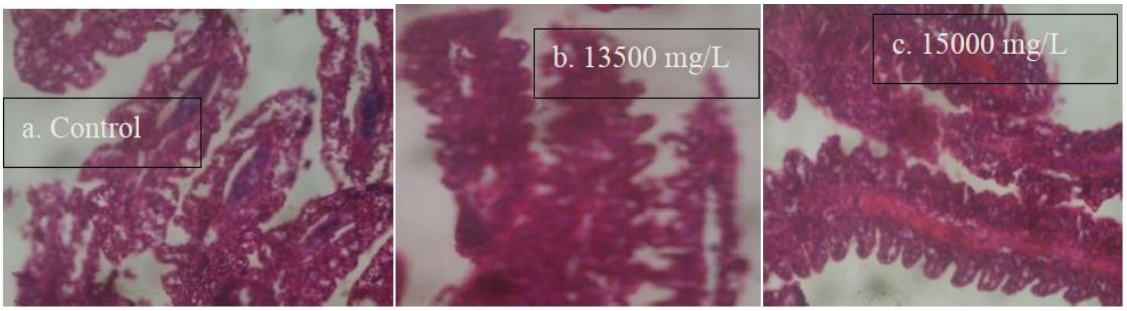
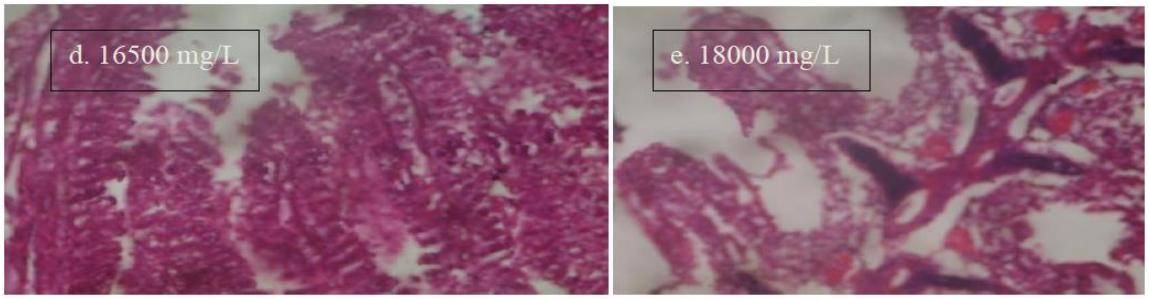
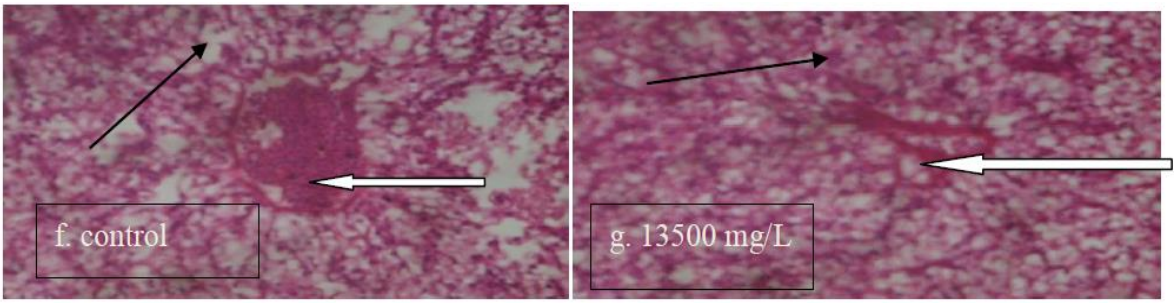


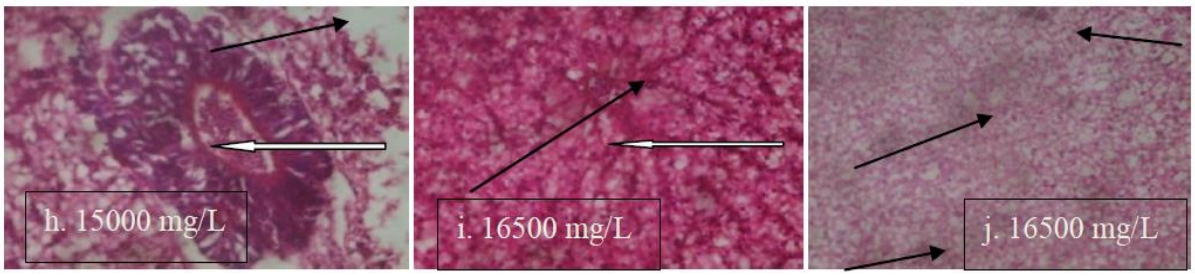
Plate 1 a: Photomicrograph of gills of control group of *C. gariepinus* shows normal appearance of gill filaments, lamellar and no visible lesion. b: Photomicrograph of gills of *C. gariepinus* exposed to13500 mg/L of *L. cylindrica* extract shows epithelium lifting, showing swollen lamellae and slight hyperplasia. c: Photomicrograph of gills of *C. gariepinus* exposed to15000 mg/L of *L. cylindrica* extract showing hypertrophy and epithelium proliferation (x 400)



d: Photomicrograph of gills of *C. gariepinus* exposed to16500 mg/L of *L. cylindrica* extract (x 400) showing a seriously distorted lamellae and necrosis. e: Photomicrograph of gills of *C. gariepinus* exposed to18000 mg/L of *L. cylindrica* extract (x 400) showing hyperplasia, massive lamellae necrosis and fusion of lamellae.



f: Photomicrograph of liver of control group of *C. gariepinus* (x 400)shows normal liver cells as indicated by the arrows. b: Photomicrograph of liver of *C. gariepinus* exposed to13500 mg/L of *L. cylindrica* extract (x400) showing congestion of the central vein, infiltration of hepatocytes and vacoulation.



h: Photomicrograph of liver of *C. gariepinus* exposed to15000 mg/L of *L. cylindrica* extract (x400) showed that there was mononuclear cellular infiltration and vacuolation of the hepatocytes within the liver acinus. i: Photomicrograph of liver of *C. gariepinus* exposed to16500 mg/L of *L. cylindrica* extract (x 400) shows necrosis of the hepatocytes and infiltration were noticed with wide spread pancreatic necrosis, fatty degeneration as indicated by the arrows. j: Photomicrograph of liver of *C. gariepinus* exposed to13500 mg/L of *L. cylindrica* extract (x 400) showing hepatocyte necrosis with degenerated hepatocytes, hepatocyte vacuolationsand haemorrhage within the centrolubular area of the liver and fatty degeneration as indicated by arrows.

4. DISCUSSION

Phytochemistry of *L. cylindrica* and Physico-Chemical Variables:

Hazardous organic chemical tend to have adverse effect on the aquatic environment and the life they support. Results from this study show that exposure of *Clarias gariepinus* to various concentrations of *Luffa cylindrica* ranging from 13500 mg/L, 15000 mg/L, 16500 mg/L and 18000 mg/L has adverse effects on the juveniles of *Clarias gariepinus*. These effects include; behavioural changes, morphological changes physiological changes, haematological changes and death.

Fish botanical piscicides contain some biologically active compounds such as alkaloids, resin, tannin, saponin, nicotine, diosgenin that are toxic to fish at high concentration [12]. The chemical constituents of *L. cylindrica* mainly alkaloids, flavoniods and saponins which are commonly used as fish poison [23] could be responsible for the high mortality in *Clarias gariepinus*. These constituents such as phenols, tannins, alkaloids, flavonoids, saponins, oxalate, cyanogenic glycoside, anthraquinols, steroids, terpenoids and phylate, have also been reported by Partap *et al*.[13] when they studied the medicinal use of *Luffa cylindrica* and that of Absalom *et al*. [24]for *Balanites aegyptiaca*.

During this study, some ecological parameters were investigated; results revealed that *Luffa cylindrica* did not affect the water temperature in the different concentrations. Physico-chemical parameters such as temperature, pH, dissolved oxygen, pH, electric conductivity and total dissolved solids are important factors which affect fish health, growth and reproduction [1]. In this study, the monitored parameters were noted to be significantly different from the control except for temperature and pH. The pH of the water samples varied from concentration to concentration and the values obtained for the different treatments where lower than the standard given by WHO [22] for fish survival. WHO [22] recommended pH range of 6.5 to 8.5 for fresh water fishes, the decline in pH with time may be due to the production of acidic products of metabolism [25] by the plant material in water. Electrical conductivity and TDS also increased across the different treatments, this may be due to the chemical composition of *L. cylindrica* [26]. DO is one of the most important factor for all living organisms especially fish survive [27]. As revealed from this study, DO of the water samples were decreased by increasing concentration of *L. cylindrica*. Prasad *et al*. [28] reported that the reduction in dissolved oxygen content in a bioassay media as toxicant concentration increased may be due to antioxidant property of the toxicant. The physic-chemical parameters monitored in this study tend to have contributed little or none to the toxicity of *L. Cylindrica* fruit extract.

Behavioural and Physiological Responses:

The results showed that *Luffa cylindrica* affects the behaviour of *Clarias gariepinus*, this was evident in the erratic swimming on exposure to *L. cylindrica* extract. Also morphological changes were observed in *Clarias gariepinus* exposed to higher concentration of *Luffa cylindrica* extract during this study, this was observed in colour changes of the skin with swollen and reddish eyes and lips, heavy secretion of mucus and this was due to the toxic property of the extract. Orji *et al*. [11] observed similar behaviour when exposed *Clarias gariepinus* juveniles were exposed to lyophilized aqueous extract of *Psychotria microphylla* leaf. The result of this study agrees with Yeeken and Fawole, [29]; Adeboyejo *et al*. [30] and Abalaka *et al*. [31] when they exposed fish to acute concentrations of different plant extracts. Similarly, Ubahe *et al*. [32] reported irrational behaviour in *Clarias gariepinus* exposed to *Hypoestes forskalei* extract, and these include vigorous movement, fast back stroke movement, restlessness, increased opercular movement and jumping. The erratic behaviour prior to death in the present and past studies can convincingly associated with the impact of toxicants on fish. The excessive mucus secretions observed in the exposed fish agrees with the report of Jothivel and Paul [33]; Abalaka and Auta[34]and Orji *et al*., [10]. Excessive mucus secretions are natural defense mechanisms by exposed fish to coat their body surfaces in order to prevent and/or reduce the absorption of the offending toxicant [35]. However, such excessive mucus secretions are reported to reduce respiratory activity in fishes [36]

Again, the display of the irrational reaction by the fish may be due to the fact that *L. cylindrical* contained a stimulant that affect the nervous system of the fish there by causing excitation, restlessness and jumping exhibiting rapid respiratory movement with increased opercula beat which could be attributed to respiratory distress [37].The piscicidal abilities and phytotoxic properties of plant extracts have been reported by several researchers such as Adewole [18]; Akinwande *et al*. [38]; Ayoola *et al*. [39]and Fafioye [40].

Adverse physiological effects of *L. cylindrica* on *Clarias gariepinus* were evident in the central nervous system (CNS) depression, resulting in narcosis which is followed by death. The effects also include interference with respiration of

Clarias gariepinus, this is evident in increased rate of operculum movement which may have resulted from increased in oxygen demand to metabolize the toxicant, and primarily; oxygen depletion in test media which was evident in *Clariasgariepinus* gulping for air. This is true because analysis of dissolved oxygen in the test media, showed that dissolved oxygen decreased with increase in concentration of *L. cylindrica* leaf extract, the decreased in dissolved oxygen resulted in high mortality in the higher concentration of the extract, the result agrees with Dede [41]whose studies showed that oxygen stress encounter by the fish is responsible for the respiratory distress, and death was due to their inability to withstand oxygen depletion of the water induced by organic compound of the water soluble fraction of crude oil. Similar oxygen stress imparted by water soluble fraction of crude oil has been studied in shrimp-*Palaemona dspersus* [42] and *Clarias gariepinus* [43].

Mortality (LC_{50} and LT_{50}):

The observed dose dependent increase in mortality rate is indicative of the possible effect and danger of the pollutant on fish species and highlights its negative impact on the natural environment. This observation agrees with other reports on fish [17; 44; 45; 46] and macrobenthic invertebrates [47] exposed to contaminants. The ecological implication is that the indiscriminate use of the substance could lead to fish body burden, bioaccumulation, and fish kill and biodiversity loss in the natural environment [9].

High mortalities due to exposure to piscicides have also been reported by several authors [9; 10; 17; 48]. The 96 hrs LC_{50} was 14125.38 mg/L which is well higher than that reported by other authors for different ichthyotoxic plants. Fafioye *et al.* [49] recorded 3.4 and 3.2ppm for extract of *Parkia biglobosa* and *Raphia vinifera* respectively using *Clarias gariepinus* juveniles. A higher LC_{50} have been recorded by Cagauan *et al*. [35] who worked on ten local botanical pisicicides on *Oreochromis niloticus* and *Gambusia affinis*. A median lethal concentration (LC_{50}) of 0.35 mg/L has been reported for *Clarias gariepinus* exposed to *Psychotria microphylla* leaf extract [10], Ubong *et al*. [50] who recorded LC_{50} of 20ml/L in their work. The toxicological actions of *L. cylindrica* may be due to the presence of flavonoid, saponins, tannins, alkaloids and triterpenoid [51].

Pathological Effects of *L. cylindrica* Fruit Extract on *Clarias gariepinus* Juveniles:

Toxicants introduced into aquatic systems can cause changes in tissues and organs of organisms leading to alterations of physiological functions. The observations in this study show that following the exposure of *C. gariepinus* to *Luffa cylindrica* fruit extract, the expression of toxicity differed considerably across the organs studied. This concurs with studies which proposed that toxicants are most times organ specific in reaction [52; 53].

The effects on the gills and liver were very pronounced and strongly implicate *Luffa cylindrica* extracts as a toxicant because these organs are important for diffusion of oxygen and detoxification of xenobiotics. Significant pathological changes like erosion of gill filament, rarefaction of cartilage, presence of fibrin thrombi (vascular changes resulting in blockage/clotting in blood vessels) in capillaries and lymphatics, oedema and clubbing of gill filament tips will lead to an overall reduction in the efficiency of gill filaments to aid in diffusion of oxygen across the gill lamellae resulting in the development of a hypoxic condition within the fish [53]. This is as a result of loss of epithelial cells in the gill hence, normal gaseous exchange across these cells would be compromised which resulted in hypoxia. Some authors have indicated that gill lesions do not only indicate possibilities of impaired respiratory functions but impaired osmo-regulatory functions too [53, 54]. The gill being a very simple structure can only manifest a limited number of reactions in adverse environmental conditions [53]. Mallat [55] reviewed 133 published studies on fish gill pathology and concluded that the structural alterations in fish gill are a stereotyped physiological reaction to environmental stressor. Degrees of the pathological changes in the each tissue were directly concentration-dependent

Pathological changes observed in liver tissues indicate that the extract is both directly hepatotoxic (vacuolar degeneration of hepatocytes around the periportal region) and indirectly hepatotoxic (degeneration around the centrilobular region) [56]. Witthawaskul *et al.* [57] posited that saponin mixtures directly impact liver and kidney functions and similar responses due to saponin contained in *L. cylindrica* fruit extract were observed in this study. Studies have shown that vacuoles in the cytoplasm of the hepatocytes contain lipids and glycogen, which is related to the normal metabolic function of the liver thus vacuolar degeneration, will result in a depletion of the glycogen reserves in the hepatocytes [53; 58]. Vacuolar degeneration will result in stress to fish because glycogen acts as a reserve of glucose to supply the higher energetic demand occurring in such situations [59]. Adeogun *et al*., [53] observed similar pathological changes in

fingerlings of *C. gariepinus* exposed to sub-lethal concentrations of the different parts of the fruit of *R. hookeri*aqueous extract. The liver is considered the most important target organ from a toxicological point of view because of its role in detoxification, modifications and excretion of xenobiotics [14].

5. CONCLUSION

The study has shown that the fruit extract of *Luffa cylindrica*is toxic to *Clarias gariepinus* with 96-hr LC_{50} value of 14125.28 mg/L. This indicates that, its effect on lower biota and non-target organisms could even be far more devastating. The phytochemical screening of *Luffa cylindrical* fruit revealed that it contains phenols, tanins, alkaloids, flavonoids, saponins, oxalate, cyanogenic glycoside, anthraquionols, steroids, terpenoids and phytate as the active ingredients that give the plant its potency on the fish organs. Water physico-chemical parameters were also affected by the fruit extract and led to stress factors that were reflected in behaviours distress like restlessness, increased respiratory rate, gulping of air and loss of balance of the test fish. Therefore the use of the fruit extract of *L. cylindrica* could lead to contamination of an ecological system, thus posing great threat to fish and other non-target organisms. Therefore, monitoring the behavioural, physiological responses and pathological changes in the African sharp-tooth catfish (*Clarias gariepinus*) will assist in monitoring, evaluating aquatic pollution and fish health in rivers and other water bodies were piscicides and other obnoxious means are used for fishing.

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