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Acute Toxicity And Phytochemical Screening Of Aqueous Extract Of *Tetrapleura Tetraptera* Bark On *Clarias Gariepinus* Juveniles

Fidel Uchenna Mbata*, Gabriel Robert Okayi, Lateef .O. Tiamiyu, Lucy Ukighil

Department of Fisheries and Aquaculture, University of Agriculture,P.M.B. 2373, Makurdi, Nigeria

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Email: fidelmbata@gmail.com

ABSTRACT

The study was carried out to investigate the acute toxicity of *Tetrapleura tetraptera* aqueous bark extract on *Clarias gariepinus* juvenile following static bioassay procedures. A preliminary test was carried out and five concentrations was ascertained as 0mg/L, 120mg/L, 140mg/L, 160mg/L,180mg/L, 200mg/L. The 96hour LC50 was 154.76mg/L with lower and upper confidence limit of 147.08mg/L and 162.83mg/L respectively. Fish exposed to aqueous bark extract exhibited some abnormal behavioural changes including eventual death but was not observed in the control fish. Water quality parameters showed significant difference with exception in pH and temperature, but were within tolerable limits for fish culture. Phytochemical analysis of aqueous bark extract of T*etrapleura tetraptera* revealed the presence of saponin strongly than other constituents. Results investigated revealed aqueous bark extract exert piscicidal property and can be used as biological control to disinfect ponds.

INTRODUCTION

Tetrapleura tetraptera (Fabaceae Family) is a medium sized deciduous tree with fern-like foliage and highly characteristic fruits. It is a perennial tree which grows along the Western Coast of Africa. The plant's common names are: Aidon (English), Tiv (Kyoho), "Abogolo" among the Igala people of north central Nigeria, and "Dorowa" among the Hausa people of Northern(Sunday et al.,2014), Oshoho (Igbo), Aridan(Yoruba), Ighimiaka (Bini) and Uyayak (Efik).The plant is popularly known as Prekese in the Twi language of Ghana (Osei-tutu and Nhancale, 2010).

The aquatic environment is a sink of toxic contaminants which find their way to the water bodies through industrial, domestic and agricultural activities (Ololade and Oginni,2010). The use of plant piscicides such as *Tephrosia candida, Tephrosia purpurea, Mundule asericea, Acacia pennata* (Weiss 1973),*Tetrapleura tetraptera, Parkia filicoides, Tephrosia vogelii* is common among fish farmers in controlling pests and predators. It is therefore of considerable interest to investigate plants derived piscicides which are not only available but also cheap, effective and biodegradable. These qualities are possessed by some local plants in Nigeria thus they have a role to play in the eradication of wild, resident species of fish from aquacultural ponds prior to stocking because many of these species prey on or compete with fry and fingerlings of desired fish species.

The favour of some plants over the use of others to crop fish may be inferred on the basis of active ingredients it contains (Fafioye and Adebisi, 2001). However, these active ingredients such as alkaloids, resin, tannin, saponin, nicotine, disogenin etc. are toxic to fish at high concentrations and wear off within a short time (Adewumi, 1991).

Indiscriminate use of piscicides poses a great risk to aquatic organisms, especially food fishes and consequently to humans. Therefore, a good control measure that will be effective in killing the target organism at minimum doses which is not hazardous to people and animals and the environment but easily available and economical should be sought

This study is therefore to evaluate the potential of aqueous bark extract of *Tetrapleura tetraptera* as a piscicide, to determine the mortality rates of the different concentrations of the extract as well as observe behavioural changes in *Clarias gariepinus* fingerlings subjected to these concentrations and also provide baseline information on the active constituents of aqueous bark extracts of *Tetrapleura tetraptera*.

MATERIALS AND METHODS

Study site

This study was carried out in the indoor Fish Hatchery at the Department of fisheries and aquaculture, Makurdi, Benue State, using plastic bowls which were covered with net to prevent fish from jumping out.

Sample collection

The experimental fish (Juveniles) was obtained from Tidoo Fish Farm, Wadata, Makurdi and used for the study. The experimental fish had a mean weight of 21.5 ± 2.2g. The fish was acclimatized for one week (7days) during which the fish was fed twice daily (0800morning and 1600evening) with a commercial feed (Coppens).

The bark of *Teterapleura tetraptera* were collected from a farm site at Vandeikya L.G.A, Mbaduku Council Ward of Benue state. The bark was air dried for seven days to a constant weight. The dried bark was pulverized using mortar and pestle and sieved through a 0.25mm mesh size sieve to get a fine powder and it was stored in an air tight bottle as described by (Omoniyi et al., 2002).

Preparation of aqueous bark extract of *Tetrapleura tetraptera*

A quantity of 100g of the resultant fine powder of *Tetrapleura tetraptera* bark was weighed using Melter weighing scale and dissolved in 500ml of water at a room temperature (25±5°C) in 1litre sample bottle, the mixture was shaken and allowed to stand for 24 hours after which it was decanted and filtered using muslin cloth. The filtrate was used immediately in the experiments (Saravanan, et al.,2010).

Phytochemical analysis of *Tetrapleura tetraptera*

The aqueous bark extract of *Tetrapleura tetraptera* was oven dried at a temperature of 55°C for 3 days using hot air oven (ISO Temp Oven, MODEL 301) for phytochemical screening of the aqueous extract to test for the presence of flavonoids, tannins, saponin, alkaloids, phenol, steroids, cardiac glycoside, anthraquinone and terpenes. This analysis was carried out in the Biochemistry Laboratory, Toxicology Section, National Veterinary Research Institute, Vom, Jos. The analysis was carried out by following procedures described by Sofowora, 1993; Trease and Evans, 1989.

Preliminary test

After the acclimatization period of 7 days, trail experiments was carried out to determine the suitable concentration of the prepared *Tetrapleura tetraptera* aqueous extract bark that was used for the experiment and these five concentrations were determined;120mg/L, 140mg/L, 160mg/L, 180mg/L, 200mg/L of stock solution was obtained for aqueous bark extract.

Experimental design and acute toxicity test

A total number of one hundred and eighty (180) juveniles was used in the acute toxicity experiment and completely randomized design (CRD) was used in the experiment, the various concentrations served as treatment which were replicated and there was a control for all treatments and replicates. A total number of eighteen (18) plastic tanks each with a capacity of 50L was used for the static bioassay experiment, each containing 20L of water (Bore-hole water). Each of the tanks was stocked with Ten (10) healthy juveniles of C. *gariepinus* selected randomly and weighed for the experiment and juveniles were not fed for 24hours prior to the start of the experiment. The lethal concentrations used were 0mg/L(control), 120mg/L, 140mg/L, 160mg/L, 180mg/L and 200mg/L.

The 96-hour LC50(lethal concentration that cause 50% mortality) was determined using a probit analysis method for acute toxicity test as recommended by USEPA (2000).The water quality parameters was monitored daily and examined using digital water parameter checker (Hanna waterproof digital tester H198129 and Lutron DO meter DO5509 for measuring dissolved oxygen). Mortality was observed and recorded 12h, 24h, 48h, 72h and 96h and behavioural changes of the fish was observed. During exposure period, dead fish observed was immediately removed from each test tank and recorded in order to avoid polluting the tanks.

Data analysis

The statistical analysis of the data was subjected to one analysis of variance (ANOVA) statistical test at p-value <0.05 to determine the differences between the control and levels of treatment and calculated using SPSS 17.0. Data obtained from the 96 hour acute toxicity test was subjected to regression analysis.

RESULTS AND DISCUSSION

Table (i) shows the mortality record of *Clarias gariepinus* juveniles exposed to different concentrations of *Tetrapleura tetraptera* aqueous bark extract for 96 h. Fish mortality increased with increasing concentration, but later decreased with time. With 24hours having the highest record of mortality and 96hours having the least record of mortality in the different concentration, there was no mortality observed in 12hours. This shows that mortality is dose-dependent test. The experimental fish exhibited some abnormal behavioral changes at the time of exposure to the toxicant which include upright suspension, excessive air gulping, frequent surface to bottom movement, bottom settlement, erratic swimming behavior and Aggressive behavior before death occurred. All these behaviours revealed an indication of physiological stress in fish. The behavioural changes observed in this study is in agreement with the findings of Jegede (2013), Keremah et al.,(2010) and Ayoola (2011). Kori-Siakpere and Oviroh (2011) reported similar behavioural changes in *Clarias gariepinus* subjected to Nicotiana tobaccum leaf dust toxicity. Also works by Ayuba et al., (2012) on the acute toxicity of Datura innoxia leaf extract on *Clarias gariepinus* fingerlings showed that test fish during the exposure period exhibited various behavioral patterns which were similar to the ones of the present study. The erratic swimming behaviour prior to death in this study can be conveniently associated with the impact of toxicants on fish.
Death or mortality resulted from the inability of the gill surface to actively carry out gaseous exchange which is also due to an impairment of the respiratory activity. This finding was in agreement with works of Alkahem et al., 1998, who reported similar obsevation on Oreochromis niloticus exposed to trichloroform.

Table 1: Mortality record of *Clarias gariepinus* juveniles exposed to different concentrations of *Tetrapleura tetraptera* aqueous bark extract for 96 hours.

Concentration (mg/L)	12 Hrs Mortality	24 Hrs Mortality	48 Hrs Mortality	72 Hrs Mortality	96 Hrs Mortality	Total Mortality
0.0mg/L	0	0	0	0	0	0
120mg/L	0	1	2	1	0	4
140mg/L	0	4	3	1	2	8
160mg/L	0	5	3	2	0	10
180mg/L	0	21	4	0	1	26
200mg/L	0	21	5	1	1	28

Table 2: Mortality rate of *Clarias gariepinus* juveniles exposed to different concentrations of *Tetrapleura tetraptera* aqueous bark extract for 96 hours.

Concentration (mg/L)	Log_{10}Conc	Total Number of Test Fish	Total Mortality after 96 Hours	Percentage Mortality rate (%)	Probit Mortality Value
0.0mg/L		30	0	0	
120mg/L	2.0792	30	4	13.33	3.87
140mg/L	2.1461	30	10	33.33	4.39
160mg/L	2.2041	30	16	53.33	5.08
180mg/L	2.2553	30	26	86.67	6.13
200mg/L	2.3010	30	28	93.33	6.48

Table 3: Mean water quality parameters obtained in the acute toxicity of *Tetrapleura tetraptera* aqueous bark extract on *Clarias gariepinus* juvenile for 96 hours.

Concentration (mg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Total Dissolved Solid (ppm)	Electrical Conductivity (µS)
0.0mg/L	4.80 ± 0.00^{a}	8.05 ± 0.00^{f}	28.10 ± 0.04^{a}	300.60 ± 0.40^{c}	601.00 ± 0.32^{e}
120mg/L	4.81 ± 0.01^{a}	8.07 ± 0.01^{e}	28.10 ± 0.06^{a}	304.80 ± 0.58^{b}	608.00 ± 0.55^{c}
140mg/L	4.64 ± 0.01^{b}	8.17 ± 0.00^{d}	27.78 ± 0.04^{b}	305.80 ± 0.37^{b}	615.00 ± 0.32^{b}
160mg/L	4.43 ± 0.00^{c}	8.22 ± 0.00^{c}	27.30 ± 0.03^{c}	310.00 ± 0.32^{a}	622.00 ± 0.32^{a}
180mg/L	4.21 ± 0.00^{d}	8.40 ± 0.00^{a}	27.80 ± 0.03^{b}	298.40 ± 0.40^{d}	$598.00\pm.032^{f}$
200mg/L	4.12 ± 0.00^{e}	8.34 ± 0.00^{b}	27.70 ± 0.03^{b}	301.00 ± 0.32^{c}	$604.00\pm.032^{d}$

*means in the same column with different superscripts differ significantly (p<0.05)

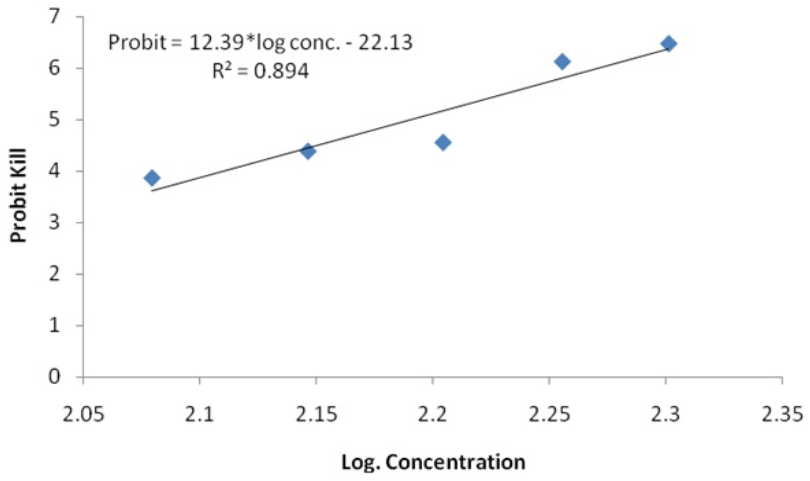

Figure 1: Linear relationship between Log. Concentration of *Tetrapleura tetraptera* aqueous bark extract and the probit kill (Mortality) of *Clarias gariepinus* juveniles.

Table 4: Phytochemical screening of Tetrapleura tetraptera aqueous bark extract

Chemical Constituent	Inference
	Bark
Alkaloid	-
Flavonoid	-
Saponin	++
Tannin	+
Glycoside	+
Steroid	+
Athraquinone	-
Terpenes	+

Key: (+) = Present, (++) = strongly present, (-) = Absent

Table (ii) shows mortality rate of *Clarias gariepinus* juveniles exposed to different concentrations of *Tetrapleura tetraptera* aqueous bark Extract for 96hours. This result reveals that after 96hours, 200mg/l had the highest mortality rate of 28 juveniles with a percentage mortality rate of 93.33% and 120mg/l had the least mortality rate of 4 juveniles with percentage mortality rate of 13.33%. Fish mortality increased with increasing concentration. Mortality increased sharply and then decreased gradually as the number of hours increased. This implies that the toxicity of *Tetrapleura tetraptera* aqueous extracts disappeared with increase in time (hours). This is due to its biodegradable properties. This corresponded with the findings of Edet and Ikpi (2008) indicating that the higher the concentration of water extracts of fruit of Tetrapleura tetraptera the higher the mortality of fingerlings of Clarias gariepinus. This study corroborates with the study by Jegede and Olanrewaju (2012) where the piscicidal effect of Nicotiana tobaccum leaf dust on African giant catfish fingerling was investigated.

Table (iii) shows the mean water quality parameters obtained in the acute toxicity of *Tetrapleura tetraptera* aqueous bark extract on *Clarias gariepinus* Juvenile for 96hours. This result reveals that there was a significant difference ($p<0.05$) of the tested water parameters among the different concentrations. The water quality parameters of these test solutions, though fluctuated during the bioassay and were significantly different from those of the control tanks, were within suggested tolerance range and so could not have affected the mortality of the test fish.

Figure (i) shows the linear relationship between Log. Concentration of *Tetrapleura tetraptera* aqueous barks extract and the Probit kill (mortality) of *Clarias gariepinus* Juveniles. This reveals that the LC50was 154.76mg/L with a lower limit of 147.08mg/L and an upper limit of 162.83mg/L, therefore the 95% confidence limit of the LC50 ranges from 147.08mg/L to 162.83mg/L. The LC50 of 154.76mg/l and 476.85mg/l for aqueous bark extract is markedly lower than the LC50 value observed by Winkaler et al., 2005 who reported a higher LC50 of 2740mg/L at 96hours for Nile tilapia when treated with aqueous extract of Neem (Azadirachta indica). Similarly, Odioko et al.,2016 reported a 96hours LC50 value of 14125.38mg/L for Clarias gariepinus juveniles exposed to fruit extract of sponge plant (Luffa cylindrica) . Keremah et al.,2010 on the contrary had an LC50 after 96hours of 0.65mg/l for Clarias gariepinus and 0.59mg/l for *Heterobranchus bidorsalis* reported on acute toxicity of L.alopecuroides to Clarias gariepinus and *Heterobranchus bidorsalis* fingerling which were far lesser than the LC50 of the present study.

The variations in LC50 observed in these studies in comparison with the present study can be attributed to the type of plants and part of the plants used, size of fish or other aquatic organism, environmental factors, water parameters, selective action of toxicants and the active phytochemical constituent present in the plant.

The regression equation of the relationship was calculated to be Probit =12.39 ×Log Conc. – 22.13 and had an R square value (r2) of 0.894. These expressions, the regression equation r2 value showed a positive correlation. This result indicates that the mortality of Clarias gariepinus juveniles and the concentrations of the bark and fruit extracts are correlated positively, further revealing that mortality is dose – dependent meaning the mortality rate of the test fish increased with increase of the concentration of the extract. This observation is similar to reported works by Kori-siakpere and Oviroh (2011) on the acute toxicity of tobacco leaf dust on *Clarias gariepinus* juveniles and Ayuba et al., 2012, who worked on the acute toxicity of Datura innoxia leaf extract on *Clarias gariepinus*.

Table (iv) reveals the phytochemical screening of aqueous bark extract of *Tetrapleura tetraptera* plant. This result shows the presence (+ve) of saponin, tannin, steriod, glycoside and terpenes with saponin strongly present in aqueous bark extract. Saponin was responsible for damaging the respiratory epithelia, RBC, hemoglobin and haematocrit levels and increased oxygen consumption (Francis et al.,2002a). Thus causing impairment on the respiration of Clarias gariepinus juveniles used in this study. This further explains in this study that fish assimilate saponin directly into their bloodstream via their gills as also reported by Elpel, 2000. Similarly Kritzon 2003 reported the presence of saponins acts on respiratory organs of the fish and attributed the mortality of fish to impaired respiratory activities.

It also showed the absence (- ve) of anthraquione and alkaloid in aqueous bark extract. Presence of tannin and phenolic compounds was recorded in aqueous bark extract of *Tetrapleura tetraptera* in this present study and it is similar with findings of Mishra and Gupta (2013). These phytochemicals are known to suffocate the fish by destroying the respiratory organs or interfering with the biochemical respiratory pathway thereby causing death or forcing the fish to the surface to gulp for air(Andel,2000).

CONCLUSION

The present study reavealed aqueous bark extract to contain active phytochemical constituents and thus posses toxic and piscicidal effect on juveniles of *Clarias gariepinus*. Although can be used as biological disinfectant against undesireable fish species in nursey ponds since its biodegradeable and its effect diminish with time but it is advised that the use of aqueous bark extract in any water body should be done with caution in order to conserve the biodiversity of fish and other aquatic organisms

Disclosure statement

No potential conflict of interest was reported by the author.

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