Comparative Acute Toxicity of Aqueous Extract of *Aloe vera* (*Aloe barbadensis)* Leaves and Roots on Fingerlings of African Catfish, *Clarias gariepinus* (Siluriformes: Clariidae)

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Abstract

The comparative acute toxicity of the aqueous extract of *Aloe vera* leaves and roots on fingerlings of the African Catfish (*Clarias gariepinus)* was conducted under static bioassay in the laboratory for 96h to examine and compare the toxic effects of the plant leaves and roots on the fish. Range finding bioassays were conducted to get the range of concentrations for the definitive bioassays. The range of concentrations of test media for the leaves was 0 - 650 mg L^{-1} while that of the roots was 0 - 980 mg L^{-1}. The median lethal concentrations (LC_{50}) were determined using probit analysis. Ten active experimental organisms of about the same size were randomly placed with scoop net in each of the test medium, each concentration having replicates including untreated media. The 96h LC_{50} of the leaves against exposed fingerlings was 380.6 mg L^{-1} with lower and upper confidence limits of 324.3 and 426.1 mg L^{-1} respectively while that of the roots was 554.7 mg L^{-1} with lower and upper confidence limits of 509.5 and 606.7 mg L^{-1} respectively. Paired t-test showed that there was no significant difference (P>0.05) between the test *A. vera* leaves and roots on the test species. The water quality parameters showed that the leaves caused increased temperature, conductivity, dissolve oxygen, pH, alkalinity, hardness and ammonia while the roots caused an increase in temperature, conductivity, alkalinity, hardness and ammonia and there was a decrease in pH while dissolved oxygen remained the same Based on toxicity ranking, the LC50 of the test plant materials on fingerlings of *C. gariepinus* indicated that the leaves were found to be moderately more toxic than the roots

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Introduction

The use of plants for healing purpose is getting increasingly popular as they are believed to be beneficial and free of side effects (Leonardo *et al*., 2000). Plants are used for different purposes because some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties (Cagauan, 1995).

However, the occurrences of these fish poison plants are varied based on location. Different parts of plants which contain toxic substances used in poisoning fish include the roots, seeds, fruits, barks or leaves (Gabriel and Okey, 2009). Gabriel and Okey (2009) posited that ichthyotoxic plants used for baiting and stupefying of fish are often crushed and cast into stagnant, slow moving water or spread on mud flats to poison fish. Ichthyotoxic plants have been used as fish poisons or narcosing chemicals by the artisanal fishermen for decades in the harvesting of fish in slow flowing waters (Oribhabor *et al*., 2014). Studies of Neuwinger (2004) and Fafioye *et al.* (2004) indicated that the use of fish poison plants and other plant products is one of the methods in traditional methods of fish capture. Plant extracts used as piscicides in capture fisheries and aquaculture are considered advantageous when compared to the back drop of using persistent and synthetic chemicals (Gabriel and Okey, 2009). The use of plants from different families in catching fish globally due to availability and low cost implication is a traditional practice. Phytochemical evaluation indicates that piscicidal or ichthyotoxic plants contain different active ingredients such as alkaloids, flavonoids, saponins, tannins, phythates, glycosids, oxylates etc. These active ingredients are known to be toxic to fish and other aquatic organisms even at low concentration (Tiwari and Singh, 2003; Goktepe et al., 2004). Ichthyotoxins present in these fish poisonous plants act by stunning fish when it passes

through the gills or when ingested, making fish to float in the water surface for easy capture (Kritzon, 2003).

There are several publications on effects of piscicidal plants on different species of fish, but much has not been done using *A. vera.* Taiwo *et al.* (2005) studied the effect of consumption of aqueous extract of raw *A. Vera* leaves on the histopathology and biochemistry of rat and Nile tilapia (*Oreochromis niloticus*). For fish, results obtained showed mortality, skin depigmentation, shriveled gills and sunken eyes. For rats, no mortality was recorded but diarrhea, catarrhal enteritis, villous atrophy, liver, kidney and heart damage were the hallmarks of the intoxication of rats with *A. vera* leaves extract, and these were more severe as the concentration of the extract increased. This result shows that consumption of water containing extracts of raw *A. vera* is very toxic to fish and rats. Methanolic extract of *A. vera* toxicity has also been tested on rats and it was found that the methanol extract of the plant does not produce significant toxic effect in rats during acute and sub-acute treatments (Saritha and Anilakumar, 2010). A study on analgesic efficacy and adverse effects of *A. vera* in wister rats have also been carried out and the result revealed that the aqueous extract of the plant gel showed significant analgesia (Ghosh *et al*., 2011). Ekanade *et al.* (2015) assessed the safety of aqueous extract of *A. vera* in haematology of Wister rats. It was observed in the results that rats administered with the aqueous extract of *A. vera* had increased value of the packed cell volume (PCV), red blood cell counts and other red cell indices. A significant ($P<0.05$) increase in PCV was observed in rats administered with the extract for the period of 24 hours. White blood cells also showed significant ($p<0.05$) increase, particularly in rats administered with the extract for 24 hours. *A. vera* has also been used to study dietary effects on growth performance, skin and gastro-intestine morphology in rainbow Trout (*Oncorhynchus mykiss*) and the results suggested that the plant especially at 0.1% and 1% feed administration may enhance the growth performance, gastrointestinal and skin morphology in the test species (Heidarieh *et al.,* 2013). Mahdavi *et al*. (2013) also studied the effect of *A. vera* extract on growth performance of common carp (*Cyprinus carpio*). The results obtained showed that the growth performance of common carp after 8 weeks feeding on the diets containing different levels (0.1, 0.5 and 2.0%) of *A. vera* ethanolic extract tended to have better growth performance as compared with the control diet.

Although the plant, *A. vera* has been used in several toxicological studies, little or no information have been documented on its toxic effects on *C. gariepinus*. Also, most of the documented works on *A. vera* were only on the leaves of the plant. This paper therefore determined the comparative acute toxicity of *A. vera* leaves and roots aqueous extracts on the fingerlings of the African catfish, *C. gariepinus*

Materials and Methods

The 96h LC_{50} values of *A. vera* leaves and roots aqueous extracts were determined in static bioassays on *C. gariepinus* fingerlings between April and June 2017 at the Department of Fisheries and Aquatic Environmental Management laboratory, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The study area is geographically located at latitude $5^{o}2^{'}26^{"}$N and longitude $7^{o}55'19^{"}$ E. *C. gariepinus* fingerlings reared under controlled condition free of pollutants were procured from Safe Foods Multipurpose Cooperative Society located at No.6 Phenson Street off Esuene Street, Uyo Local Government Area, Akwa Ibom State, Nigera and transported to the laboratory in a plastic container (30l volume, 52cm diameter 50cm depth) with water from the site of collection. In the laboratory, the fish fingerlings were kept in holding plastic containers (30l volume, 52cm surface diameter, 34cm width and 20cm depth) half filled with dechlorinated bore hold water which was continuously aerated with silver lake (SL-2800) aerator. The fingerlings were kept in the containers for at least two weeks, to allow them acclimate to laboratory conditions ($29^{o}C \pm 1^{o}C$) before using them in bioassays. About 100 individuals were kept in each container. During this period of acclimation, the fishes were fed twice daily (mornings and evenings) with coppens feed at 5% of their body weight and the water in the containers were changed every 48hr to avoid accumulation of toxic waste metabolites from the specimens and remnants of food particles. Also, dead and weak individuals were immediately removed and the total mortality recorded during the acclimation period was less than 5% (Adeyemo, 2005). Acclimation of test organisms to laboratory conditions and experimental procedures were in accordance with guidelines for bioassay techniques (APHA *et al*., 1985).

The plant materials, *A. vera* were purchased at a flower garden along IBB Avenue beside Idongesit Nkanga Secretariat in Uyo Local Government Area, Akwa Ibom State, Nigeria. The leaves and roots of *Aloe barbadensis* were washed with clean water to free them from sand and debris. The leaves and roots were cut into tiny pieces and air dried in the laboratory to constant weight. The dried samples were pulverized with a clean mechanized grinding machine to a fine powder which was then sieved through 0.25mm sieve. The stock solution was

prepared by dissolving 50g of each of the specimen in 500ml of dechlorinated borehole water for 24 hours. The mixture was kept at room temperature for 24 hours. Thereafter, the mixture was filtered through Whatman's filter paper (No.1). The prepared aqueous extract of both specimens were refrigerated and used for the static bioassay tests following standard procedures (Reish and Oshida, 1987).

The extracts of A. vera leaves and roots were screened to identify their constituents of bioactive compounds (Tannins, saponins, flavonoids, alkaloids and carbohydrates) through preliminary phytochemical screening as described by Sofowora, 1993; Harborne, 1998; and Ogbuewu, 2008.

Clean plastic containers (20l volume, 31cm surface diameter, 31cm width and 19cm depth) were employed in all bioassays. A predetermined volume of each test compound was pipetted into a measuring cylinder and made up to 1l by adding appropriate units of dechlorinated borehole water as diluents, to achieve the desired concentration of the test compound. Active specimens of about the same size (mean weight 2.33 ± 0.81g; mean length 0.72 ± 0.9cm) were randomly assigned to bioassay containers, already containing the test media prepared. In all bioassays, a total of 10 active animals were placed in each container. Test were run at several concentrations and untreated controls. In each treatment, there were two replicates. Test animals were exposed to several concentrations of each test compound after range-finding bioassays were conducted (Table 1).

Table 1: Toxicant concentrations to which *C. gariepinus* fingerlings were exposed.

Test *A. vera* part	Concentrations (mg L^{1})
Leaves	300, 350, 500, 550 and 650
Roots	350, 400, 450, 500, 550, 650, 950 and 980

Mortality assessments were made by examining each animal separately every 24 hours over a 96hour experimental period. *C. gariepinus* fingerling was considered dead when respiratory and tail movements stopped, and no response to gentle prodding with a rod.

Water temperature, conductivity, dissolved oxygen, pH, ammonia, alkalinity and hardness were determined in the acclimation media, untreated control and each test-compound-treated medium at the beginning (0 hr) and end (96 hr) of each bioassay.

Temperature was determined by using mercury in-glass thermometer, conductivity by HANNA conductivity meter (Model H19812 – 5), dissolved oxygen by using HANNA dissolved oxygen meter model H19146, pH by pH meter (HANNA product model HA989), hardness by the EDTA titrimetric method, alkalinity by titrimetric method, and ammonia colorimetrically using ammonia test kits. The physical and chemical parameters of acclimation media were maintained optimally and are summarized in Table 2.

Table 2: Summary of the physical and chemical parameters of the acclimation media

Physical and chemical parameters	Mean ± S. E.
Temperature (^{o}C)	25.9 ± 0.5
Conductivity (µg/cm)	98.6 ± 0.4
Dissolved oxygen (mg l^{-1})	5.8 ± 0.1
pH	6.3 ± 0.1
Hardness (mg l^{-1} $CaCO_3$)	22.4 ± 0.3
Alkalinity (mg $l^{-1}CaCO_3$)	213.0 ± 10.4
Ammonia (mg l^{-1})	7.6± 0.6

*SE = Standard error

The toxicity data based on quantal response (mortality) was analysed by probit analysis (Finney, 1971). The analysis, including the equation for probit line, and paired t-test use to test for significance between the toxicity of the leaves and roots of *A. vera* was achieved via computer programme using IBM SPSS Statistics 20. Indices of toxicity/susceptibility level were based on the 96h LC_{50} values.

Results

Some phytochemical constituents of the test *A. vera* plant

The results of phytoconstituents analysis conducted on *Aloe vera* leaves and roots aqueous extracts revealed the presence of some bioactive components such as alkaloids, tannins, flavonoids, terpenoids and carbohydrates (Table 3). In the leaves, alkaloids, flavonoids and carbohydrates were all copiously present while tannins and terpenoids were moderately present and saponins was absent.

In the root extract of the test plant, tannins and carbohydrates were found to be moderately present while alkaloids, flavonoids and terpenoids were slightly present with saponins being absent

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Table 3: Phytochemical components of *A. vera* leaves and Roots Aqueous Extract

Constituents	Leaves	Roots
Alkaloids	+++	+
Tannins	++	++
Flavonoids	+++	+
Saponins	-	-
Terpenoids	++	+
Carbohydrates	+++	++

+++ =Copiously Present; ++ = Moderately Present; + =Slightly Present; - = Absent

Physical and chemical parameters of the test media

The physical and chemical parameters of the test media are summarized in Table 4 below. The parameters indicated that when the test A. vera plant parts were tested against C. gariepinus, over 96h periods, the leaves caused increase in temperature, conductivity, dissolved oxygen, pH, alkalinity, hardness and ammonia; but in the case of the root, there was increase in temperature, conductivity, alkalinity, hardness and ammonia while there was a decrease in pH and dissolved oxygen remained the same

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Table 4: The physical and chemical parameters of test media.

Parameters	Mean ± SE			
	Leaves		Roots	
	0hrs	96hrs	0hrs	96hrs
Temperatures	27.4 ± 0.1	28.5 ± 0.2	25.4 ± 0.2	28.8 ± 0.5
Dissolved Oxygen (mg L^{-1})	6.3 ± 0.1	7.7 ± 0.3	5.9 ± 0.1	5.9 ± 0.1
pH	5.8 ± 0.4	6.5 ± 0.3	6.2 ± 0.1	5.9 ± 0.1
Conductivity (µS/cm)	100.9 ± 0.7	199.9 ± 7.3	100.4 ± 7.4	190. 1 ± 9.8
Ammonia (mg L^{-1})	2.5 ± 0.3	51.7 ± 3.7	5.6 ± 0.2	28.6 ± 1.5
Hardness (mg L^{-1} $CaCO_3$)	8.1 ± 0.3	14.6 ± 1.3	10.0 ± 0.5	22.7 ± 0.4
Alkalinity (mg $L^{-1}CaCO_3$)	44.6 ± 0.9	156.3 ± 12.1	53.0 ± 0.9	130.5 ± 2.1

Acute toxicity of *A. vera* leaves and roots under static bioassay procedure against *C. gariepinus* fingerlings

Based on 96h LC_{50}, *A. vera* leaves were more toxic against *C. gariepinus* than the roots. The computed 96h LC_{50} values for leaves and roots being 380.6mg/L and 554.7mg/L respectively. Computed toxicity factor based on 96h LC_{50} values showed that the root was 0.7 times less toxic than the leaves (Table 5). Paired t-test showed that there was no significant difference (P>0.05) between the test *A. vera* leaves and roots tested against the test species (Table 6). The log-dose probit graph depicting the relative toxicity of *A. vera* leaves and roots against *C. gariepinus* based on the 96h values were non-parallel (Fig. 1). Based on toxicity ranking, the LC50 of the test plant materials on fingerlings of *C. gariepinus* indicated that the leaves were found to be moderately more toxic than the roots.

Table 5: Comparative toxicities of the *A. vera* plant parts against *C. gariepinus* fingerlings

A. vera plant parts	96hLC$_{50}$ (95% CL) mg L^{-1}	Slope ± S.E	D.F	Regression Equation (Probit Response)	T.F
Leaves	380.6 (324.3 – 426.1)	5.6 ± 1.2	4	Y= -14.4 + 5.6X	1
Roots	554.7 (509.5 - 606.7)	6.2 ± 0.9	7	Y= -17.0+6.2X	0.7

L.C = lethal concentration, C.L = 95% confident limit, T.F = toxicity factor

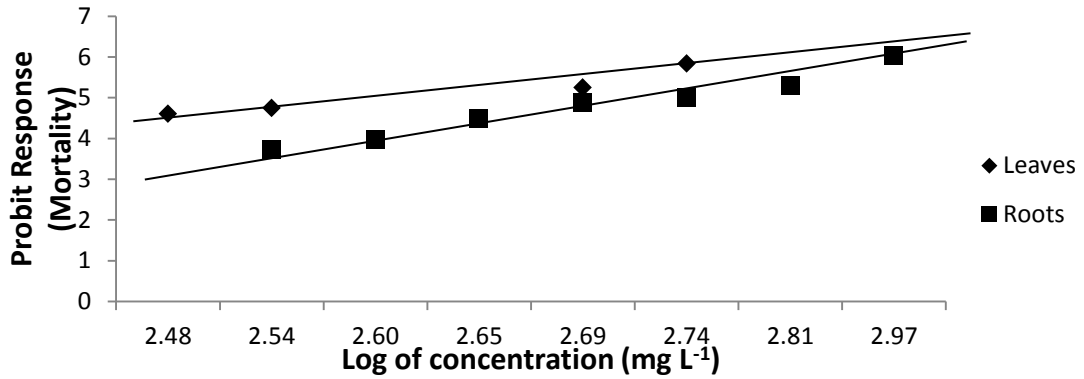

Fig.1: Log-dose probit graph depicting the relative toxicity of A. vera leaves androots against *C. gariepinus* based on the 96h LC$_{50}$ values.

Table 6. Test of significance for the 96hLC$_{50}$ value between the *A. vera* leaves and roots to the test species

Paired t-test				
Variables	Standard Error mean	t	Sig.	t-test probability
Leaves-Roots	30.0	0.667	0.541	P>0.05

Discussion

The acute test showed that all the test species in the treatment media had increased mortality with increasing concentrations of the toxicants while there was no death recorded in the control media. Results obtained for water quality parameters for the 96h static bioassay using *A. vera* leaves on *C. gariepinus* showed that mean water temperature, dissolved oxygen, pH, conductivity, ammonia, hardness and alkalinity increased significantly while for the roots, temperature, conductivity, ammonia, hardness and alkalinity increased significantly. There was a decrease in pH and dissolved oxygen remained the same. For example, the pH of the various concentrations of the water with A. vera extract was in the acidic range in this study. This agrees with findings of Taiwo et al. (2005) who had similar observation when they studied the consumption of aqueous extract of raw A. vera leaves on the histopathology and biochemistry of rat and tilapia. Similar observation of pH being in the acidic range was recorded by Fafioye (2012) when he worked on the acute and sub-acute toxicities of five plant extracts on white tilapia, *Oreochromis niloticus*. Fafioye (2012) also recorded increase in conductivity, hardness and alkalinity as observed in this study. Slight increase in temperature was recorded by Ayuba *et al*. (2012) in the study of acute toxicity of *C. gariepinus* exposed to *Datura innoxia* leaf extract. This is in agreement with the result of this study where there was a slight increase in temperature value after the experiment compared to the control. In this study, one of the physical observations in the test media was mucus production and accumulation in the gills. This might be as a result of increase in the activity of mucus cells due to subsequent exposure to pollutants. This agrees with the report of Oti (2002) who made similar observations when he exposed *C. gariepinus* fingerlings to cassava mill effluent.

In this study, ichthyotoxins were observed to be present in different parts of the test plant used (leaves and roots). This agrees with findings of Gabriel and Okey (2009); Tyler (1986) that different parts of plants including roots, seeds, fruits, barks, leaves, tuber, flowers etc. contain toxic substances used in

poisoning fish. Phytochemical analysis revealed that both leaves and roots had the same levels of tannins (moderately present), saponins absent, alkaloids and flavonoids were copiously present in the leaves and slightly present in the roots. Terpenoids was moderately present in the leaves and slightly present in the roots while carbohydrates were found to be copiously present in the leaves and moderately present in the roots. Tyler (1994) reported that *A. vera* latex contains the anthraquinone glycosides – aloin A and B which are potent laxatives. This result is similar to findings of Ukwubile *et al.* (2013) who reported slight differences in the phytochemicals of different ichthyotoxic plants.

Comparing the toxicity of the test plant parts in this study with findings of earlier studies, it was observed that there were variations in the toxicities of different ichthyotoxic plants used in catching fish. Eyo *et al.* (2013) reported the 96h LC_{50} of 163.02 mg L^{-1} of *Carica papaya* seed aqueous extract tested against *C. gariepinus* juveniles. The results showed that the 96h LC_{50} value of aqueous extracts of pawpaw seed powder to *C. gariepinus* juveniles was higher than the value obtained while tilapia fingerlings were exposed to similar concentrations of pawpaw seed aqueous extract (Ayotunde and Offem, 2008). Ayuba *et al*. (2012) reported LC_{50} of 120.23 mg L^{-1} for *C. gariepinus* fingerlings exposed to aqueous extract of *Datura innoxia* leaf. Ayuba and Ofojekwu (2002) also reported 96h LC_{50} for *Datura innoxia* root extract against *C. gariepinus* to be 204.17 mg L^{-1}.

The 96h LC_{50} of 380.6 mg L^{-1} recorded against *C. gariepinus* in the test of leaves of *A. vera* for this study is less toxic than the 96h LC_{50} of 120.23 mg L^{-1} for leaves of *D. innoxia* against *C. gariepinus* reported by Ayuba *et al*. (2012). Ijioma *et al*. (2015) who worked on the comparative acute toxicity and hypoglycaemic studies of five Nigerian indigenous medicinal plants in experimentally induced hypoglycaemic rats reported LD_{50} values of 3300mg/kg for *Acalypha wilkesiana* leaf extract and 3750mg/kg for *Moringa oleifera* leaf extract. Abalaka and Auta (2010) also recorded 296.14 and 225.48 mg L^{-1} for aqueous and ethanol extracts of *Parkia biglobosa* pod respectively against *C. gariepinus*. The 96h LC_{50} of 554.7 mg L^{-1} recorded in the test of roots of *A. vera* against *C. gariepinus* of this study is less toxic than the 96h LC_{50} of 204.17 mg L^{-1} of the root of *D. innoxia* against *C. gariepinus* reported by Ayuba and Ofojekwu (2002). Much work has not been done using the roots of plants. The findings of Ayuba *et al*. (2012) for *D. innoxia* leaves and roots against *C. gariepinus* implied that the leaves of the plant was more toxic than the roots based on 96h LC_{50} values. This agrees with findings of this study where the leaves of *A. vera* are more toxic than the roots based on 96h LC_{50} values. The difference in the results of the present study and those of earlier researchers may be due to the differences in toxicants, their concentrations, of the test species (Ayuba *et al.*, 2012).

The behavioural alterations that occurred before death in this study may be as a result of nervous impairment due to blockage of nervous transmission along the nervous system and various affector sites, failing organs and retarded physiological processes in fish body functions (Shah, 2002). This may have resulted from enzymes dysfunction and paralysis of respiratory muscles or the depression of respiratory centers and disturbance in energy pathways leading to the depletion of energy (Gabriel *et al*., 2010).

Conclusion

This study revealed that *A. vera* leaves and roots cause a regular trend in mortality of *C. gariepinus* fingerlings which increased with increased concentrations. Based on toxicity ranking, the LC_{50} of the test plant materials on fingerlings of *C. gariepinus* indicated that the leaves were found to be moderately more toxic than the roots. Therefore, the use of *A. vera* in aquatic environment should be done with caution. Further study could be conducted to exploit the possibility of using the plant for biological control and eradication of predators and unwanted organisms in the ponds by farmers instead of using agrochemicals.

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