



ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: <http://baures.bau.edu.bd/jbau>

Phyto-quantitative assessment and influence of *Erythrophleum suaveolens* extracts on *Clarias gariepinus* (Burchell, 1822)

Adekunle Adedoyin Idowu¹✉, Olamilekan Johnson Egunjobi¹, Adeleke Taofik Towolawi², Wilfred Olusegun Alegbeleye¹, Omotola Abiola Jaiyeola¹, Andrew Osereme Agbon³

¹Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, PMB 2240, Ogun State, Nigeria.

²Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta, PMB 2240, Ogun State, Nigeria.

³Animal and Environmental Biology, Federal University, Oye, Ekiti State, Nigeria.

ARTICLE INFO

Article history:

Received: 14 December 2019

Accepted: 03 April 2020

Published: 30 June 2020

Keywords:

Phytochemicals, organic compounds, piscicides, histopathology, fish toxicology

Correspondence:

Adekunle Adedoyin Idowu

✉: idowudoyin@yahoo.com



ABSTRACT

The study investigated phytotoxic effects of aqueous *Erythrophleum suaveolens* stem bark (AES) at 3.00, 2.25, 1.50 and 0.75 g/ L while leaf (AEL) at 3.50, 2.63, 1.75 and 0.88 g/ L extracts as treatments on 300 samples of *Clarias gariepinus*. Static non-renewal bio-assay test was used to determine the 96-hr acute toxicity while exposed the fish to 1/ 10th of LC₅₀ for 28 days in sub-lethal tests. Results showed that tannins, saponins and oxalate were higher in AES while flavonoids, alkaloids, cyanogenic glycosides and phenols were higher in AEL. Levels of DO reduced in the treatments unlike in control, pH varied without changing the media slightly alkaline condition, Temperature was significantly different across the treatments, EC rose as levels of treatments increased more than in control. The TDS increased except at 2.63 g/ L and higher in the treatments than control. Occurrences of air gulping, discolouration, erratic swimming and resting at the bottom of the containers were alike as the concentrations of the extracts increased. The fish gained weight across the treatments and more in the AEL than AES; their 87.52 % and 96.41 %, respectively influenced LC₅₀ of the fish. In conclusion, the AEL exhibited higher phytotoxic effect on the fish than aqueous *E. suaveolens* stem bark.

Copyright ©2020 by authors and BAURES. This work is licensed under the Creative Commons Attribution International License (CC By 4.0).

Introduction

Flora with toxic chemicals have different effects on fish and aquatic ecosystem (Fafioye, 2005). Fisher folks use plants and products to capture fish (Fafioye *et al.* 2004). Parts of plants with the ethnobotanical origin and application in catching fish had been used by Singh and Singh (2002). Oshimagye *et al.* (2014) reported uses of plant poisons to obtain fish from small water bodies. Afolayan *et al.* (2014) reported that fishing in freshwater with piscicides can elicit adverse effects on aquatic biodiversity. Agbebi *et al.* (2012) reported use of phytotoxic plant to control fish diseases. Plants are source of bioactive substances (Batabyal *et al.*, 2007), with potential adverse effects on aquatic organisms health, especially non-target species (Sun *et al.*, 2009). Natural processes like decomposition of plant parts and the entire plant contribute to phenol accumulations in aquatic environment (Ali *et al.*, 2011). If levels of phenolic substances are higher than what the aquatic organism can tolerate, organ damages or death can then result (Dahunsi *et al.*, 2011).

Erythrophleum suaveolens is a poisonous plant that belongs to the Fabaceae family with distinct foliage. The plant bears traditional names as *Obo* and *Erun* (Yoruba), *Inyi* (Igbo), *Baska* (Hausa), *Aba* (Akan-Asante, Ghana), *Digpende* (Bassari-ogo), *tali* (Koranko-Sierra leone) while in English as sassy, salswood, red water tree, and ordeal tree. The bark of the tree is the source of its common name and contains a poisonous red sap erythrophleine that was used by natives in Africa as an ordeal in trials. Phytotoxic mechanisms of *E. suaveolens* were extensively studied such as anaesthetics potentials on clarrid fish, acute toxicity on albino mice, anti-fungal properties, anti-bacterial properties, phytochemical and toxicological properties, toxicity and mutagenic activity, subchronic toxicity on rabbits, fungal events on wood, anti-oxidant and anti-bacteria of its saponin fractions, and wound healing action (Akinpelu *et al.*, 2014; Akanji and Sonibare, 2015). There is limited work on the phytotoxicity of *E. suaveolens* on fish because of its widespread use by artisanal fisherfolks

Cite this article

Idowu, A.A., Egunjobi, O.J., Towolawi, A.T., Alegbeleye, W.O., Jaiyeola, O.A., Agbon, A.O. 2020. Phyto-quantitative assessment and influence of *Erythrophleum suaveolens* extracts on *Clarias gariepinus* (Burchell, 1822). *Journal of Bangladesh Agricultural University*, 18(2): 529–536. <https://doi.org/10.5455/JBAU.78025>

(Sowunmi and Adeogun, 2002). Most studies carried out about *E. suaveolens* were on terrestrial animals with little or no research on the toxicity of its different parts on aquatic organisms, especially fish.

Fishes exposed to toxicants experience changes in their biochemistry and physiology as an adaptive mechanism to cope with stressors (Barton, 2002). Symbols of toxicity and sub-lethal effects precede changes at various levels in an organism (Lohiya *et al.*, 2002). The toxicity effects of plant extraction on fish resulted in fish mortality (Tiwari and Singh, 2004). African catfish (*Clarias gariepinus*), an omnivore freshwater fish, is a popular delicacy relished in tropical Africa (Nguyen and Janssen, 2002) with fast growth rate, high stocking-density capacities and consumer acceptability (Ayotunde *et al.*, 2011), high resistance to poor water quality and oxygen depletion (Karami *et al.*, 2010). So, it is used in many researches as a prominent culture species (Adeyemo, 2008). Thus, the main objective was to investigate the phytotoxicity of *E. suaveolens* stem bark and leaf extracts on *C. gariepinus*.

Materials and Methods

Experimental site and plant preparation

Hatchery section in the Fish farm of the Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria, provided the experiment venue. The test plant: *E. suaveolens* was from the Forestry Herbarium of FUNAAB, partitioned to stem bark and leaf. Each part was air and oven-dried at 27 - 35 °C and pulverised. Finally, the ground products were sieved to obtain fine particles using a 0.2 mm mesh. Ten (10) samples of fish in 30 plastic containers (25 Litres capacity each) and ten places were the experimental setups. Four treatments with stem bark extractions had a set of control; the leaf extractions also had the same setups. These two sets of procedures were replicated three times to make 12 treatments of stem bark extractions with three controls; leaf extractions also had the identical setups to make 30 treatments.

Quantitative phytochemical screening of E. suaveolens and water quality test

Chemical tests were carried out on the extractions to quantify total phenols according to the method described by AOAC (2002); tannins according to the Swain (1979) practices; saponins and alkaloids based on the previous described method (Obadomi and Ochuko, 2001); flavonoids following the previous process (Boham and Kocipai, 1994); cyanogenic glycosides as described by Bradbury *et al.* (1999); while oxalates and steroids according to the AOAC (2002) method. A potable water quality test kit (APHA, 2005) measured water quality parameters for the 96-hr acute toxicity test in-situ. A digital meter (Model: Amtast-AMT08) determined temperature values *in situ*. The reading was in replicates, and the average value was used to ascertain the

temperature of the experimental medium. The pH values of the medium were determined using a pH meter (Model: pH-0093). Standardisation of the pH meter was through a 4.0 buffer solution before usage. After dipping the probe part of the meter into the experimental medium in a slanting position, the recorded readings showed after a few minutes with a precision value of 0.01. The dissolved oxygen (DO) of the water in the experimental tanks was determined with a DO meter (Model: Amtast-AMT08), which was calibrated using distilled water by un-screwing the probe end of the meter, adding 2-3 drops of the distilled water into the loose end and screwing it to the meter to take the readings after the values became stable and no longer adjusted by itself. The electrical conductivity of the experimental tanks was determined using a digital meter (Model: HM-EC-3M). The meter has been factory calibrated with a 1413 µS potassium chloride solution (KCl). The probe end is inserted in the tanks to take the readings with 0.01 precision. The total dissolved solids (TDS) were determined using a digital meter (Model: HM-TDS-3). The meter has been factory calibrated with a 342 ppm sodium chloride (NaCl) solution. The probe end placed in the tanks and readings were taken with 0.01 precision after a few minutes.

Sub-lethal, range-finding and definitive tests

From the 96-hr acute toxicity test, the median lethal concentration (LC₅₀) was determined, and 1/10th of the value used for the experiment. The fish were fed *ad-libitum* with 4 mm commercial feed (Skretting) twice daily (morning at 7.00 AM and 6.00 PM) for 28 days. The test medium and water was renewed every 2 days for 28 days (Agbon *et al.*, 2002). There were three treatments and three replicates. The bio-assay test to determine the 96-hr acute toxicity of aqueous extracts of stem bark and leaf of *E. suaveolens* on sub-adult *C. gariepinus* followed the bioassay procedures described by Solbe (1995). Serial dilution was made from the stock solution at the varying concentrations of 0.50, 0.75, 1.50, 2.50, and 3.00 g/ L for stem bark extraction while 0.50, 0.75, 1.50, 2.50, 3.00, and 3.50 g/ L for the leaf extraction. Distilled water served as the control; each of the test solutions was in a rectangular 25-litre plastic tank and the water-filled to a 15-litre mark. Seven (7) sub-adult *C. gariepinus* were introduced directly into the transparent rectangular plastic. There was an observation of the concentration that had the highest mortality after 24 hours. Based on the results from the range-finding test, a 96-hr static non-renewal bioassay procedure, as described by American Society for Testing and Materials [ASTM] (1992), was carried out using seven (7) sub-adult *C. gariepinus* each at different concentration. For the stem bark, concentrations used were 3.0, 2.25, 1.50, 0.75 and 0.00 g/ L, while for the leaf; concentrations were 3.50, 2.63, 1.75, 0.88 and 0.00 g/ L. Each treatment was replicated three times. The fish under study were not given feed 48 hours before the commencement of the experiment to avoid pollution due to food regurgitation. The response patterns and mortality of the test organisms in each tank were

monitored and recorded every 24-hr until the 96-hr. Fish mortality was noticed by touching with a rod and removed when there was no movement immediately with a scoop net to avoid contamination due to rotting.

Weight measurement

Weighing of test fish in both treated and control test media commenced and terminated (after 28 days) the sub-lethal test. Weight changes in the fishes were carried out at seven-day intervals of the experiments to reduce the introduction of handling stress in the test animals. Percentage weight gain of the fishes was calculated using the formula:

% weight change of fish =

$$\frac{\text{Average final weight} - \text{Average initial weight}}{\text{Average initial weight}} \times 100$$

Statistical analysis

Data from acute toxicity were analyzed using percentage mortalities and transformed into respective probits (Finney, 1952). Logs of the concentrations were plotted against the probits to determine the LC₅₀. Descriptive and inferential statistical tool at $p < 0.05$ level of significance analyzed both water parameters and weight changes data.

Results and Discussion

Quantitative phytochemical analysis of *E. suaveolens*

The quantitative phytochemical screening (Table 1) of *E. suaveolens* extraction showed that there was higher percentage of tannins (5.00 and 4.6 %) and saponins (4.00 and 3.00 %) in both stem bark and leaf plant parts respectively, this was relatively followed by alkaloids (0.50 and 1.00 %) and flavanoids (0.10 and 0.20 %). The aqueous extractions of stem bark and the leaf of *E. suaveolens* contained varieties of other bioactive compounds through quantitative screening at relatively low concentrations. Human had explored steroids in plants as compounds for sex hormones (Okwu, 2001). Pranoothi *et al.* (2014) observed that the presence of alkaloids, phenols, flavonoids, steroids, and saponins makes a plant beneficial for antimicrobial and antioxidant purposes. Presence of alkaloids in both stem bark and leaf of *E. suaveolens* extract in one way plays a metabolic role in the living systems and protective function in animals while flavonoids in the other hand make the plant suitable against the cancer progression. When phenols mixed with flavonoid compounds in plants, there would be different activities like antioxidant, anticarcinogenic, and anti-inflammatory (Asha *et al.*, 2011). Tannins and saponins in aqueous extracts of the plant showed the potential of the plant for antimicrobial activity and reserved of pathogenic fungi just as saponins cause the leakage of proteins and degradation of cell wall enzymes from the cell. Presence of the chemical constituents of *E. suaveolens* made it poisonous to fish in this study. Ibiam

et al. (2015) stated that alkaloids created neurologic effects that could damage liver cells while alkaloids have stimulating effects on the gills and in turn, increased opercular beat and may impair respiration and osmoregulation. Saponin in-plant had been observed to cause asphyxiation and for hemolytic problems in nature and are highly toxic to fish because of their damaging effect on respiratory epithelia. Presence of flavonoids in plant interrupts respiration by inhibiting the protective mechanisms of Glutathione S-transferase (GST) in reducing oxygen radical toxic potential (Ibiam *et al.*, 2015). Flavonoids also inhibit NADH – dependent dehydrogenase (mitochondrial respiration) (Laszlo, 2015). Cyanogenic glycoside, which can adversely disturb the central nervous system and nerve mechanisms of the heart, was detected in the aqueous extract of *E. suaveolens* leaf, and this contradicted the report of Hassan *et al.* (2007) who did not find it in the foliage of *E. africanum*.

Table 1. Phytochemical screening of *E. suaveolens* for various phenolic compounds

Parameters	Stem bark	Leaf
Tannins (%)	5.00	4.60
Saponins (%)	4.00	3.00
Flavanoids (%)	0.10	0.20
Alkaloids (%)	0.50	1.00
Oxalate (%)	0.03	0.02
Steroids (%)	0.01	0.01
Cyanogenic glycosides (%)	0.02	0.03
Phenols (%)	0.05	0.32

Effects of *E. suaveolens* extracts on water physicochemical parameters

Dissolved oxygen (DO), electrical conductivity (EC, $\mu\text{S}/\text{cm}$) and total dissolved solids (TDS, mg/L) of the cultured water varied significantly ($p < 0.05$) within each extract treatment. Values of the DO unlike EC and TDS in the treatments were lower than in the control treatments. Both EC and TDS increased as the stem bark extract concentrations increased. The temperature values were not altered and pH of the water remained neutral with no significant ($p > 0.05$) differences when compared to the control treatment (Table 2). The fluctuation of these physicochemical parameters in the aquatic environment could influence levels of phytotoxins (Idodo-Umeh, 2002). The water parameters (DO, EC and TDS) variations showed that both aqueous stem bark and leaf extracts had effects on water quality (Ayotunde *et al.*, 2011). Reduction in DO as the concentration of the extract increased may be due to antioxidant property of the test plant. The low level of DO might be due to increase in the EC to corroborate the observation of Alhou *et al.* (2016). Values for both temperature and pH were within the range for culture, growth, and survival of *C. gariepinus* as previously reported (Agbon *et al.*, 2013). Levels of toxicity increased at low oxygen because of increased respiratory rate, thus growing poison affected the test fish (Jaishankar *et al.*, 2014), and deficiency of O₂ causes hypoxic condition with increased breathing rate and fish were gulping air by frequent surfacing to survive.

Ezemonye and Ogbomida (2010) reported that introduction of a toxicant into an aquatic system might decrease the DO levels and impair respiration leading to asphyxiation.

Responses of *C. gariepinus* to *E. suaveolens* extracts

Fish in control treatments displayed healthy swimming within the water medium, while the time spent by fish to move along the water column at the bottom was equal to that at the surface. The test fish had increased air gulping from both sub-lethal concentrations of extractions. The barbel deformation was not present throughout the

incremental levels of stem bark extractions, but progressively as the leaf extracts concentrations increased; the same showed in 2.63 and 3.50 g/ L. The test fish discolouration increased as the extractions of both *E. suaveolens* plant parts increased. Both erratic swimming and scratching on containers were observed and might be from the increased sub-lethal concentrations of both extractions (Table 3). The test fish rested longer at the bottom of the treated water as the treatment concentrations increased. The fish had mild fin deformation at the highest amount of both extracts: 2.25 and 3.00 g/ L of stem bark, and 2.63 and 3.50 g/ L of leaf.

Table 2. Water quality parameters during 96-hr exposure of *C. gariepinus*

Conc. (g/L)	DO (mg/L)	pH	Temperature (°C)	EC (µS/cm)	TDS (mg/L)
Control	3.93 ± 0.17	7.38 ± 0.06	27.03 ± 0.57	527.50 ± 38.40	264.00 ± 18.99
Stem bark extract					
0.75	2.80 ± 0.28 ^c	7.32 ± 0.03 ^a	27.10 ± 0.56 ^a	568.75 ± 51.59 ^a	284.75 ± 24.63 ^a
1.50	1.05 ± 0.36 ^a	7.38 ± 0.05 ^a	26.93 ± 0.66 ^a	669.25 ± 74.64 ^b	335.25 ± 37.41 ^b
2.25	1.43 ± 0.15 ^b	7.35 ± 0.05 ^a	27.05 ± 0.61 ^a	706.00 ± 84.89 ^c	352.25 ± 40.38 ^{bc}
3.00	1.35 ± 0.41 ^b	7.45 ± 0.10 ^b	26.95 ± 0.57 ^a	901.50 ± 102.10 ^d	443.00 ± 48.53 ^d
Leaf extract					
0.88	3.11 ± 0.53 ^c	7.43 ± 0.05 ^a	27.00 ± 0.56 ^a	545.50 ± 41.21 ^a	273.00 ± 20.15 ^a
1.75	1.60 ± 0.18 ^b	7.32 ± 0.03 ^{ab}	27.23 ± 0.56 ^a	750.50 ± 104.78 ^c	372.25 ± 50.56 ^c
2.63	1.06 ± 0.35 ^a	7.30 ± 0.04 ^{ab}	26.98 ± 0.59 ^a	601.00 ± 44.83 ^b	301.00 ± 21.89 ^b
3.50	1.53 ± 0.17 ^b	7.20 ± 0.07 ^b	27.20 ± 0.57 ^a	816.00 ± 91.71 ^d	412.00 ± 50.27 ^d

Means with the same superscripts down the column were not significantly ($p > 0.05$) different. DO- Dissolved oxygen; pH- Hydrogen Ion concentration; EC- Electrical conductivity; TDS- Total dissolved solids

Table 3. Responses of *C. gariepinus* in *E. suaveolens* extracts during 96-hour exposure

Behaviour	Control	Stem bark extract				Aqueous leaf extract			
		0.75	1.50	2.25	3.00	0.88	1.75	2.63	3.50
Air gulping	-	-	+	++	+++	-	+	++	+++
Barbel deformation	-	-	-	-	-	-	+	++	++
Discolouration	-	-	+	++	++	-	+	++	++
Erratic swimming	-	-	+	++	+++	-	+	++	+++
Scratching on the plastic tank	-	-	-	+++	+++	-	+	+++	+++
Resting at bottom	-	+	++	++	+++	+	++	++	+++
Hanging vertically in the water column	-	+	++	++	++	+	++	++	++
Fin deformation	-	-	+	++	++	-	-	++	++

(-): Not present, (+): Low, (++) : Mild, (+++) : High

Test fish in the media with a sub-lethal concentration of least stem bark: 0.75 g/ L and leaf: 0.88 g/ L extractions showed healthy swimming during the period of 96-hr exposure but more time at the bottom of the experimental tank. There was no skin discolouration, barbel deformation nor restlessness. Fish in the higher treatment concentration containers displayed air gulping at the surface of the exposure medium, erratic swimming, scratching by using their pelvic fins on the sides of the container from the time of exposure to death. They also showed restlessness, fish in the stem bark extracted treatment showed brownish colour while those in the leaf extracted treatments displayed green colouration when died. Barbel deformation showed in the fish at a higher concentration of the aqueous leaf extracts: 2.63 and 3.50

g/ L. The dead fish from the higher levels of aqueous stem bark and leaf extracts exhibited gill and mouth opening to the highest point. The fish without opening display had swollen mouth at the lower jaw, within the first 24 hours of the exposure time.

Mahnaz and Sadegh (2018) observed that variability in acute toxicity depends on the size, age, and condition of the test species along with experimental factors, even in a single species and single toxicant. The observation may be responsible for the abnormal behavioural responses observed in the fish exposed to the varying *E. suaveolens* extractions in this study. Tiwari *et al.* (2008) reported that animal behaviour is a neuro-tropically regulated phenomenon mediated by neurotransmitter substances.

Various abnormal responses of *C. gariepinus* to the tested aqueous extracts in this study correlated proportionally with their concentrations as observed previously (Omoniyi et al., 2002; Rahman et al., 2002). Hanging vertically in the water medium and skin discolouration suggested a respiratory impairment due to the phytotoxic effect of *E. suaveolens* extracts on the gills (Oshode et al., 2008) while Schreck et al. (2001) attributed the skin discolouration to dysfunction of the endocrine (pituitary) gland under toxic stress. The behaviour might also be from sensory organ systems impairment; predominantly the mechanoreceptor and chemoreceptor systems as reiterated by Mishra et al. (2011). Al-Otaibi et al. (2019) related the burdens to enzymatic and ionic disturbances in blood and tissues of the fish. Such behavioural changes were also said to be an indication of stress imposed by the extracts (Adesina, 2008). The loss of equilibrium might not only be from the inactivation of the central nervous system but also the failure of the lateral-acoustic system due to the inactivation of acetylcholine esterase activity (Bhat et al., 2012). Fafioye (2012) reported that unusual nervous behaviours resulted from the impacts of the phytotoxins on the test fish, due to nervous system involvement/ failure or biochemical body imbalance including hepatic compromise. Some of the observed responses in this study were as previously reported (Ajani and Ayoola, 2010; Muhammad et al., 2010) when exposed fish to acute levels of varying plant extractions.

Weight changes and lethal-toxicity of the exposed fish

The Table 4 depicted weekly and percentage weight gain of *C. gariepinus* exposed to sub-lethal (LC_{50}) concentrations of aqueous stem bark and leaf extractions of *E. suaveolens* while Fig. 1 showed weight gain of the fish and exposure days. The fish exposed to aqueous leaf extract of 0.26 g/ L had weekly higher weight gain: 162.25 ± 8.96 g and percentage weight gain: 30.00 ± 7.03 % significantly ($p < 0.05$) than fish exposed to both aqueous stem bark extract of 0.23 g/ L and control. The initial weight of the fish was not significantly ($p > 0.05$) different. The lethal toxicity of *E. suaveolens* aqueous stem bark extraction on the test fish (Table 5) showed the highest percentage (78.50 %) mortality recorded at the highest extracted treatments of 3.00 g/ L stem bark extraction and 3.50 g/ L leaf extraction but no mortality at the least concentrations of both treatments: 0.75 g/ L stem bark extraction and 0.88 g/ L leaf extraction.

Comparing Fig. 2, the linear relationship between the probit and logarithm of the median lethal concentration (LC_{50}) indicated that aqueous leaf extract ($Y = 9.3766X + 1.171$, $R^2 = 0.8752$ at 2.56 g/ L) was more toxic than the stem bark extract ($Y = 9.5718X + 1.5305$, $R^2 = 0.9641$ at 2.30 g/ L); showing that 87.52 % of 2.56 g/ L leaf extract and 96.41 % at 2.30 g/ L stem bark extract influenced 50 % of the fish mortality; lower % concentration, more toxic the extract. The fish exposed to both aqueous extracts in different quantities had higher weight gain than the fish in control treatment. The observation could be that the fish had utilised growth-promoting components of the plant; more in leaf extraction than stem bark extraction. The observed increased weight gain agreed with the previous report of Omoniyi et al. (2002). The hyperactivity, exhaustion and stimulation of peripheral nervous system with increasing metabolic activities and desiring oxygen utilisation could have been from inactivation of acetyl-cholinesterase leading to acetyl-choline accumulation at the synaptic junctions in fish (Tiwari et al., 2005).

Table 4. Weight gain of the test fish exposed to LC_{50} of *E. suaveolens* extracts

	Initial weight (g)	Final weight (g)	Percentage weight gain (%)
Control	124.61 ± 0.75^a	155.27 ± 2.07^a	24.60 ± 1.26^a
Stem bark extract (0.23 g/L)	124.82 ± 0.96^a	158.90 ± 2.78^b	27.32 ± 2.25^b
Leaf extract (0.26 g/L)	124.81 ± 1.07^a	162.25 ± 8.96^c	30.00 ± 7.03^c

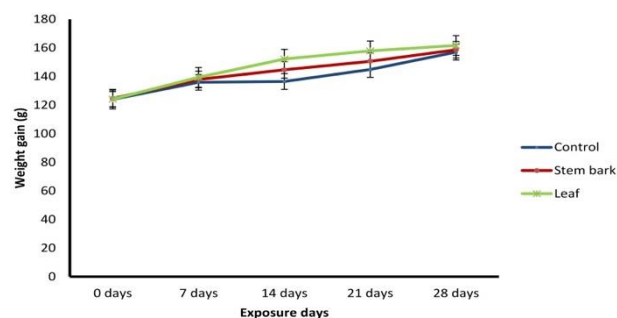


Fig. 1. Weight gain at the sub-lethal (LC_{50}) concentrations of *E. suaveolens* extracts

Table 5. Mortality of sub-adult *C. gariepinus* exposed to varying levels of aqueous extracted *E. suaveolens* over the 96-hr period

Treatment	Control	Stem bark extract				Aqueous leaf extract			
Concentration (g/ L)		0.75	1.50	2.25	3.00	0.88	1.75	2.63	3.50
Mortality (%)	0.00	0.00	14.28	42.85	78.50	0.00	42.85	42.85	78.50
Log ₁₀ Dose (conc.)	0	-0.125	0.176	0.352	0.477	-0.058	0.243	0.419	0.544
Probits	0	0	3.92	4.82	5.81	0	4.82	4.82	5.81

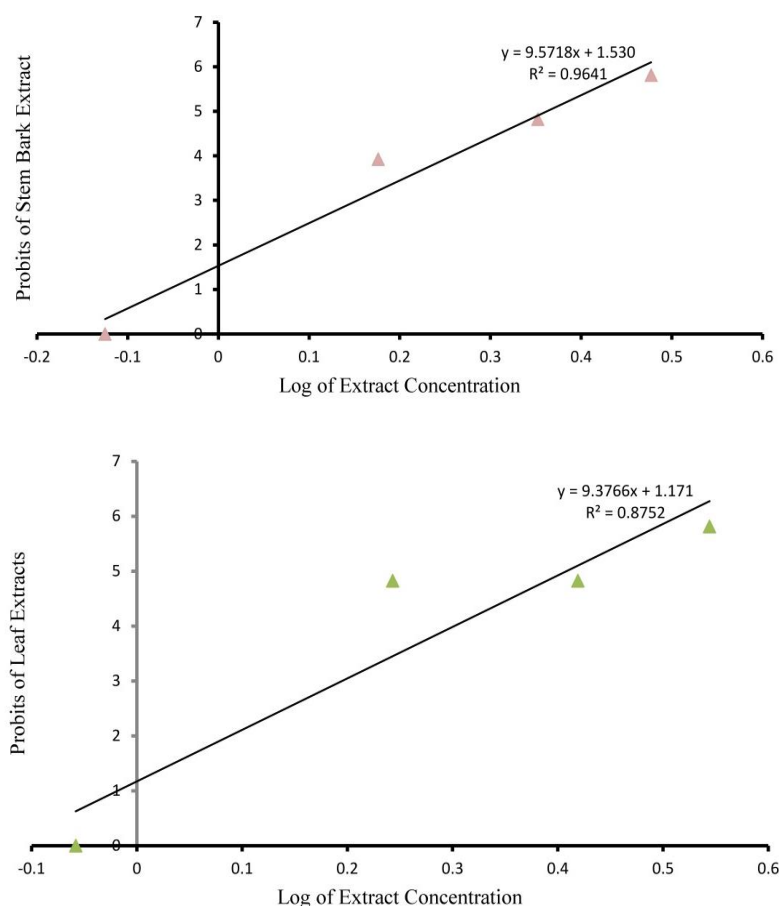


Fig. 2. Aqueous extracts of *E. suaveolens* on the test fish, 96-hr LC₅₀

The response and the subsequent death of the test fish affirmed that phytotoxic effect might be facilitated by the nervous system disturbances, which involved control of all the vital organ activities (Tiwari *et al.*, 2005). Death of the test fish increased with increase in the plant extract concentrations to denote relationship between sub-lethal (LC₅₀) concentration and mortality to indicate that phytotoxicity is dose-dependent varying the time of exposure of aquatic organisms to the plant toxins (Akinwande *et al.*, 2007; Ayoola *et al.*, 2011; Fafioye, 2012). The estimated 96-hour LC₅₀ values for both extracts were 2.30 g/ L stem bark and 2.56 g/ L leaf. The results obtained indicated that 87.52 % leaf extract was more toxic to *C. gariepinus* than 96.41 % stem bark extract and could be due to the presence of higher phytotoxins in the leaf than in the stem bark. Both quantities were lower than the values reported by Absalom *et al.* (2013) who reported LC₅₀ at 12.59 g/ L and Ayoola *et al.* (2011) who reported LC₅₀ at 7.13 g/ L while higher than the quantity observed by Musa *et al.* (2013) who reported LC₅₀ of 1.56 g/ L, which was more toxic than the quantities obtained in this study. Sub-lethal concentrations of phytotoxins in an aquatic habitat may not result in mortality of marine organism, but the chronic bioaccumulation may constitute potential health hazards not only to aquatic lives but a higher trophic life also.

Ndome *et al.* (2013) reiterated that fish exposed to a low concentration of toxicants might not reach exhausted stage by getting adapted to the stressor.

Conclusion

The presence of both extracts caused a significant reduction in dissolve oxygen levels which led to hypoxic condition at higher doses. Occurrences of air gulping, discolouration, erratic swimming and resting at the bottom of the containers were the same as the stem bark, and leaf extractions concentrations increased gradually. There was a significant difference in the weight gain of *C. gariepinus* exposed to both extracts; though weight gain was higher in fish exposed to the aqueous leaf extract (which influenced more lethal toxicity) to deduce that levels of deleterious phytotoxins were more in leaf with higher weight gain than stem bark extractions. Thus, indiscriminate disposal of *E. suaveolens* on aquatic bodies requires proper monitoring and discouragement because they are lethal at low concentrations. Lower concentrations of both plant extractions could be useful to minimise the anaemic problem in fish. Further studies should be carried out for the precise phytotoxins that influenced LC₅₀ toxicity.

Acknowledgements

The authors sincerely appreciated the Department of Aquaculture and Fisheries Management for approval of the hatchery facility and the Department of Environmental Management and Toxicology for the stepwise monitoring and completion of the study. The authors also appreciated one another for the tremendous and untiring knowledgeable contribution to the success of the study.

References

- Association of Official Analytical Chemists. 2002. Official Method of the Analysis of the Association of Official Analytical Chemistry, 17th Edn, Ch. 4 (968): 40-41.
- Adesina, B.T., 2008. Toxicity of *Moringa oleifera* (Lam.) Extracts to *Oreochromis niloticus* (Doctoral thesis), University of Ibadan, Nigeria.
- Adeyemo, O.K., 2008. Histological alterations observed in the gills and ovaries of *Clarias Gariepinus* exposed to environmentally relevant lead concentrations. *Journal of Environmental Health*, 70(9): 48-51.
- Afolayan, A.O., Borokini, T.I. and Afolayan, G.O., 2014. Sublethal effects of methanolic extract of *Raphia hookeri* on the reproductive capacity of *Clarias gariepinus*. *Hindawi Publishing Corporation, Advances in Zoology*, Article ID 615908: <https://doi.org/10.1155/2014/615908>
- Agbon, A.O., Omoniyi, I.T. and Teko, A.A., 2002. Acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on *Oreochromis niloticus* and haematological changes resulting from sublethal exposure. *Journal of Aquatic Sciences*, 17(1): 5-8.
- Agbon, A.O., Omoniyi, I.T., Akinyemi, A.A., Abdul, W.O., Adeosun, F.I. and Odulate, D.O., 2013. Effect of ecotype on haematology of *Clarias gariepinus* (Burchell, 1822). *Journal of Aquatic Sciences*, 28(1): 83-92. <https://doi.org/10.4314/jas.v17i1.19901>
- Ajani, E.K. and Ayoola, S.O. 2010. Acute toxicity of piscicidal plant extracts (*Adenia cissampeloides*) on tilapia (*Sarotherodon galidæus*) juveniles. *Iranica Journal of Energy and Environment*, 1(3): 246-254.
- Akanji, O.L. and Sonibare, M.A., 2015. Evaluation of wound healing activity of *Erythrophleum suaveolens* (Guill. and Perr.) brenan and *Moringa oleifera* (Lam) on infected albino Rats. *European Journal of Medicinal Plants*, 7(2): 67-76. <https://doi.org/10.9734/EJMP/2015/15222>
- Akinpelu, B.A., Igbeneghu, O.A., Awotunde A.I., Iwalewa, E.O. and Oyedapo, O.O., 2014. Antioxidant and antibacterial activities of saponin fractions of *Erythrophleum suaveolens* (Guill. and Perri.) stem bark extract. *Academic Journals*, 9(18): 826-833. <https://doi.org/10.5897/SRE2014.5844>
- Akinwande, A.A., Sogbesan, A.O., Moody, F.O. and Ugwumba, A.A., 2007. Piscicidal potential of mesocarp of neem plant (*Azadirachta indica* L.) fruit on hybrid, *Heteroclaris*. *Journal of Environmental Biology*, 28(3): 533-536.
- Alhou, B., Issiaka, Y., Adamou, M.M. and Abdou, I., 2016. Effects of *Balanites aegyptiaca* on tadpoles and *Oreochromis niloticus*. *Journal of Applied Biological Sciences*, 97: 9220-9226. <https://doi.org/10.4314/jab.v97i1.7>
- Ali, S.M., Sabae, S.Z., Fayez, M., Monib, M. and Hegazi, N.A., 2011. The influence of agro-industrial effluents on River Nile pollution. *Journal of Advanced Research*, 2: 850-895. <https://doi.org/10.1016/j.jare.2010.08.008>
- Al-Otaibi, A.M., Al-Balawi, H.F.A., Ahmad Z. and Suliman, E.M., 2019. Toxicity bioassay and sub-lethal effects of diazinon on blood profile and histology of liver, gills and kidney of catfish, *Clarias gariepinus*. *Brazilian Journal of Biology*, 79 (2): 326-336. <https://doi.org/10.1590/1519-6984.185408>
- American Public Health Association. 2005. Standard Methods for Examination of Water and Wastewater. American Public Health Association, WWA. Washington, D.C., USA.
- Asha, K., Rasika, C.T., Nirmala, R.D. and Jyoti, P.S., 2011. Antioxidant potential from stem bark of *Juglans regia* L. *Annals of Biological Research*, 2(1): 176-180.
- American Society for Testing and Materials. 1992. Annual Book Of ASTM Standards: Water And Environmental Technology, ASTM, 11(04): 1426 pp. Philadelphia, PA, USA.
- Ayoola, S.O., Kuton, M.P., Idowu, A.A. and Adelakun, A.B., 2011. Acute toxicity of Nile Tilapia (*Oreochromis niloticus*) juveniles exposed to aqueous and ethanolic extracts of *Ipomoea aquatica* leaf. *Nature and Science*, 9(3): 91-99. <https://doi.org/10.5897/IJFA11.003>
- Ayotunde, E.O., Offem, B.O. and Bekeh, A.F., 2011. Toxicity of *Carica papaya* Linn: haematological and piscicidal effect on adult catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Science*, 6: 291-308. <https://doi.org/10.3923/jfas.2011.291.308>
- Barton, B.A., 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, 42: 517-525. <https://doi.org/10.1093/icb/42.3.517>
- Batabyal, L.P., Sharma, L., Mohan, P.M. and Srivastava, C.N. 2007. Larvicidal efficiency of certain seed extracts against *Anopheles stephensi*, with reference to *Azadirachta indica*. *Journal of Asia-Pacific Entomology*, 10: 251-255. [https://doi.org/10.1016/S1226-8615\(08\)60359-3](https://doi.org/10.1016/S1226-8615(08)60359-3)
- Bhat, I.D., Bhat, B.A., Vishwakarma, S., Verma, A. and Saxena, G., 2012. Acute toxicity and behavioural responses of Labeo rohita (Hamilton) to a biopesticide "NEEM-ON". *Current World Environment*, 7: 175-178. <https://doi.org/10.12944/CWE.7.1.26>
- Boham, A. and Kocipai, A., 1994. Flavonoid and condensed tannins from leaves of Hawaiian *vaccinium vaticulum* and *vicalycinium*. *Pacific Science*, 48: 458-463.
- Bradbury, M.G., Egan, S.V., Bradbury, J.H., 1999. Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. *Journal of the Science of Food and Agriculture*, 79: 593-601. [https://doi.org/10.1002/\(SICI\)1097-0010\(19990315\)79:4%3C593::AID-JSFA222%3E3.0.CO;2-2](https://doi.org/10.1002/(SICI)1097-0010(19990315)79:4%3C593::AID-JSFA222%3E3.0.CO;2-2)
- Dahunsi, S.O., Oranusi, S.U. and Ishola, R.O., 2011. Biochemical profile of *Clarias gariepinus* exposed to sub-lethal concentrations of chemical additives effluent. *International Journal of Research in Environmental Science and Technology*, 1(4): 52-58.
- Ezemonye, L. and Ogbomida, T.E., 2010. Histopathological effects of gammalin 20 on African catfish (*Clarias gariepinus*). *Applied and Environmental Soil Science*, Article ID 138019, 8 pp. <https://doi.org/10.1155/2010/138019>
- Fafioye, O.O., 2005. Plants with piscicidal activities in Southwestern Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences*, 5: 91-97.
- Fafioye, O.O., 2012. Acute and sub-acute toxicities of five plant extracts on white tilapia *Oreochromis niloticus* (Trewavas). *International Research Journal of Agricultural Science and Soil Science*, 2(13): 525-530.
- Fafioye, O.O., Adebisi, A.A. and Fagaden, S.O., 2004. Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. *African Journal of Biotechnology*, 3(11): 627-630.
- Finney, D.J., 1952. Probit Analysis, 2nd Edn. 318pp. Cambridge University Press, New York, USA.
- Hassan, S.W., Ladan, M.J., Dogondaji, R.A., Umar, R.A., Bilbis, L.S., Hassan, L.G., Ebbo, A.A. and Matazu, I.K., 2007. Phytochemical and toxicological studies of aqueous leaves extracts of *Erythrophleum Africanum*. *Pakistan Journal of Biological Sciences*, 10(21): 3815-3821. <https://doi.org/10.3923/pjbs.2007.3815.3821>
- Ibiam, U.A., Egwu, C.O., Ezeani, N., Edwin, N., Okechukwu-Ugwu, P.C. and Alum, E., 2015. Acute toxicity and biochemical response of *Clarias batrachus* exposed to *Adenia cissampeloides* stem extract. *Journal of Medicinal and Dental Science Research*, 2(1): 6-12

- Idodo-Umeh, G., 2002. Pollution Assessment of Olomoro Water Bodies, using Physical, Chemical and Biological Indices (Doctoral thesis), University of Benin, Nigeria.
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. and Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2): 60–72. <https://doi.org/10.2478/intox-2014-0009>
- Karami, A., Christianus, A., Ishak, Z., Courtenay S.C., Syed, M.A. Noor, A.M., and Noorshinah. H., 2010. Effect of triploidization on juvenile African catfish (*Clarias gariepinus*). *Aquaculture International*, 18: 851-858. <https://doi.org/10.1007/s10499-009-9307-x>
- Laszlo, K., 2015. Flavonoids: A Paper Presentation, Department of Pharmacology, Semmelweis University. Budapest, Hungary.
- Lohiya, N.K., Manivannam, B., Mishra, P.K., Pathak, N., Sririam, S., Bhande, S.S. and Pann, S., 2002. The chloroform extract of *Carica papaya* seeds induces long-term reversible azoospermia in Langur monkey. *Asian Journal of Andrology*, 41: 17-26.
- Mahnaz, S.S. and Sadegh P., 2018. Evaluation of toxicity and lethal concentration (LC50) of silver and selenium nanoparticle in different life stages of the fish *Tenulosa ilish* (Hamilton 1822). *Oceanography and Fisheries*, 7(5): 555722. <https://doi.org/10.19080/OFOAJ.2018.07.555722>
- Mishra, A., Tripathi, C.P.M., Dwivedi, A.K. and Dubey, V.K., 2011. Acute toxicity and behavioural response of freshwater fish *Mystus vittatus* exposed to pulp mill effluent. *Journal of Environmental Chemistry and Ecotoxicology*, 3(6): 167-172.
- Muhammad, A., Muhammad, A., Tufail, S., Noor, E., Imtiaz, A. and Zulfiqar, A., 2010. Replacement of Rotenone by locally grown herbal extracts. *International Journal of Agriculture and Biology*, 12(1): 70-80.
- Musa, S.M., Aura, C.M., Ogello, E.C., Omondi, R., Charo-Karisa, H. and Mbonge-Munguti, J., 2013. Haematological response of African catfish (*Clarias gariepinus* Burchell, 1822) fingerlings exposed to different concentrations of tobacco (*Nicotiana tobaccum*) leaf dust. *ISRN Zoology*, Article ID 492613: <http://dx.doi.org/10.1155/2013/492613>
- Ndome, C.B., Mowang, D.A. and Ayibaemi, T.T., 2013. Comparative acute toxicity of local detergents (Omo and Ariel) on fingerlings of the *Clarias gariepinus* ♀ x *Heterobranchius longifilis* ♂ hybrid. *AACL BIOFLUX (Aquaculture, Aquarium, Conservation & Legislation, International Journal of the Bioflux Society)*, 6 (4): 415-420.
- Nguyen, L.T.H. and Janssen, C.R., 2002. Embryo-larval toxicity tests with the African catfish (*Clarias gariepinus*): comparative sensitivity of endpoints. *Archives of Environmental Contamination and Toxicology*, 42: 256-262. <https://doi.org/10.1007/s00244-001-0007-4>
- Obadomi, B. and Ochuko, P., 2001. Phytochemical studies comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8: 203-208. <https://doi.org/10.4314/gjpas.v8i2.16033>
- Okwu, D.E., 2001. Evaluation of the chemical composition of indigenous spices and flavouring Agents. *Global Journal of Pure and Applied Sciences*, 7(3): 455- 459. <https://doi.org/10.4314/gjpas.v7i3.16293>
- Omoniyi, I., Agbon, A.O. and Sodunke, S.A., 2002. Effect of lethal and sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and haematological changes in *Clarias gariepinus* (Burchell, 1822). *Journal of Applied Science and Environmental Management*, 6(2): 37-41. <https://doi.org/10.4314/jasem.v6i2.17174>
- Oshimagye, M.I., Ayuba, V.O. and Annune, P.A., 2014. Toxicity of aqueous extracts of *Parkia biglobosa* pods on *Clarias gariepinus* (Burchell, 1822) juveniles. *Nigerian Journal of Fisheries and Aquaculture*, 2(2): 24-29.
- Oshode, O.A., Bakare, A.A., Adeogun, A.A. and Sowunmi, A.A., 2008. Eco-toxicological assessment using *Clarias gariepinus* and microbial characterization of leachate from municipal solid landfill. *International Journal of Environmental Research*, 2(4): 391-400.
- Pranoothi, K.E., Narendra, K., Suman-Joshi, D.S.D., Swathi, J., Sowjanya, K.M., Rathnakarreddi, K.V.N., Emmanuel, S.J., Padmavathi, C.H. and Krishna-Satya, A., 2014. Studies on qualitative, quantitative, phytochemical analysis and screening of *In vitro* biological activities of *Leucas indica* (L) var. Nagalapuramiana. *International Journal of Herbal Medicine*, 2(3): 30-36.
- Rahman, M.Z., Hossain, Z., Mullah, M.F.R. and Ahmed, G.U., 2002. Effect of diazinon 60EC on *Anabus testudineus*, *Channa punctatus* and *Barbades gomonotus*. *NAGA. The ICLARM Quarterly*, 25: 8-11.
- Schreck, C.B., Contreras-Sánchez, W.M. and Fitzpatrick, M.S., 2001. Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture*, 197(1): 3-24. [https://doi.org/10.1016/S0044-8486\(01\)00580-4](https://doi.org/10.1016/S0044-8486(01)00580-4)
- Singh, D. and Singh, A., 2002. Piscicidal effect of some common plants of India commonly used in freshwater bodies against target animals. *Chemosphere*, 49(1): 45-49. [https://doi.org/10.1016/S0045-6535\(02\)00178-9](https://doi.org/10.1016/S0045-6535(02)00178-9)
- Solbe, J.F. 1995. *Freshwater In Handbook of Ecotoxicology*. Edited by Peter Collins. Black Well Science Limited, Osney Mead OX 20EL, United Kingdom.
- Sowunmi, A.A. and Adeogun, A.O., 2002. A survey of piscicidal plants in South-west Nigeria. *Journal of Applied Sciences*, 5(1): 2417-2424.
- Sun, H., Yong, X. and Yi-Ping, Y., 2009. Advances in saponin-based adjuvants. *Vaccine*, 27: 1787–1796. <https://doi.org/10.1016/j.vaccine.2009.01.091>
- Swain, T. 1979. *Tannins and Lignins In Rosenthal, G.A., Berenbaum, M.R. Herbivores: Their Interaction with Secondary Plant Metabolites. Vol. II: Ecological and Evolutionary Processes*, 2nd Edn, 1992. Academic Press, Elsevier Inc., New York, USA. <https://doi.org/10.1016/C2009-0-03210-3>
- Tiwari, S. and Singh, A., 2004. Piscicidal and anti-acetylcholinesterase activity of *Euphorbia royleana* stem bark extract against freshwater common predatory fish *Channa punctatus*. *Environmental Toxicology and Pharmacology*, 18: 47-53. <https://doi.org/10.1016/j.etap.2004.05.001>
- Tiwari, S., Pandey, R.P. and Singh A., 2008. Effect of Cycloart-24-en-3β-ol from *Euphorbia Royleana* Latex on Neuroenzyme AChE and Oxidative Metabolism of Freshwater Fish, *Channa Punctatus*. *African Journal of Traditional, Complementary and Alternative Medicine*, 5(4): 332–339. <https://doi.org/10.4314/ajtcam.v5i4.31287>