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# Research Article
# Assessment of Ichthyotoxicity of *Lonchocarpus cyanescens* on the African catfish, *Clarias gariepinus* Fingerlings and Anuran Tadpoles

Blessing Julius Oribhabor and Nsikak Nse Akanse

Department of Fisheries and Aquatic Environmental Management, University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria

## Abstract

**Background and Objectives:** *Lonchocarpus cyanescensis* is a common tropical plant used traditionally by fisher folks to stun and catch fish in Akwa Ibom State, Nigeria. *Lonchocarpus cyanescens* is a shrub of twining habitat, belonging to the tribe Dalbergiae of the natural order Leguminosae. This study was conducted to determine the bioactive components and assess the acute toxicity of the aqueous extract of the *L. cynescens* on *C. gariepinus* fingerlings and anuran tadpoles. **Materials and Methods:** The 96 h $LC_{50}$ values of *L. cyanescens* bark aqueous extracts were determined in the laboratory under static bioassay conditions against *C. gariepinus* fingerlings and anuran tadpoles. Range finding bioassays were conducted to get the range of concentrations for the definitive bioassays. The range of concentrations of test media for *C. gariepinus* fingerlings was 0.6-5 mg $L^{-1}$ while that of anuran tadpoles was 0.6-2 mg $L^{-1}$. The median lethal concentrations ($LC_{50}$) were determined using probit analysis. **Results:** The 96 h $LC_{50}$ values indicated that *L. cynescens* was more toxic against *C. gariepinus* fingerlings than the anuran tadpoles. Unpaired t-test also showed that the test plant was significantly ($p<0.05$) more toxic against the fingerlings than tadpoles. The mean water quality parameters were within the optimal range requirement for the test species. **Conclusion:** The study indicated that *L. cyanescens* exerted piscicidal effects on *C. gariepinus* fingerlings and anuran tadpoles. The indiscriminate use of this plant to catch fish by local fishermen should be discouraged.

Key words: *Lonchocarpus cyanescens*, *Clarias gariepinus*, anuran tadpoles, ichthyotoxicity, static bioassay

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Corresponding Author: Blessing Julius Oribhabor, Department of Fisheries and Aquatic Environmental Management, University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria Tel: +2348033558270



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The eradication of predators and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially grown fry and fingerlings of desired species is a common management practice in fish farming operations. Undesirable fish that enter fish culture ponds through water supplies, birds or fish seeds brought into fish farms could account for up to about 40% losses in commercial fish and shrimp harvest[1]. Some fish farmers overcome this problem by using cyanide or other similar poisons that could have adverse impact on other organisms in the food chain and humans. Some apply tea seed cake in the control of predators and trash fishes, while others adopt pond draining which is usually not feasible and ineffective in control and eradication of undesirable fishes at commercial level. Inlets are sometimes screened to avert the entry of eggs or larvae of wild fish canal water sources. This technique is also not as effective as usually thought[2]. Ponds are usually sundried and the pond bottom cracked to eliminate undesirable fish. However, this practice is usually impossible especially during the wet season. Application of fish piscicides is the best way of eradicating undesirable fishes in pond water[3]. Fish piscicides could be herbal or synthetic. Synthetic piscicides are undegradable, pose the problem of environmental resistance, pest resurgence and could have harmful effects on non-target organisms[4]. 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[5]. Plant piscicides are unanimously used by fisher folks to stun or catch fish because they are readily available and almost collected at no cost, highly toxic to fish and very easy to apply. They leave no residues in the environment and are easily reversed in fish subjected to chronic concentration[6]. Secondly, plants are virtually an inexhaustible source of structurally diverse and biologically active substances[7]. Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties[8]. This can be extracted from flowers, bark, pulp, seeds, roots, leaves and even the entire plant[9]. The rotenones, saponins and cyanide account for nearly all varieties of fish poison, others are ichthyothereol, triterpene and other ichthyotoxins[10].

Plants which are widely used as piscicides are tea seed cake and derris root and their toxic effects have been well documented[11]. However, conventional plant piscicides are either not within the reach of the fish farmers or their use may not be cost effective, especially for farmers in developing countries such as Nigeria. There is therefore, the need for more information reported on the piscicidal useful plants that have been reported to be of great biodiversity in countries like Nigeria where aquaculture is presently recording good growth[12].

*Lonchocarpus cyanescens* is a common tropical plant used traditionally by fisher folks to stun and catch fish in Akwa Ibom State, Nigeria. *Lonchocarpus cyanescens* is a shrub of twining habitat, belonging to the tribe Dalbergiae of the natural order Leguminosae. It is grouped among the medicinal plants of Akwa Ibom State[13]. The plant has alternate leaves, flat fruits which are 1-5 seeded, oblong pod pointed at both end[14,15]. The popularity of *L. cyanescens* is attributed to its usage in dye production. The indican-containing leaves and young sprouts are used after fermentation to obtain the blue indigo dye, which is used for colouring textile and other materials[13].

The African catfish, *Clarias gariepinus* belonging to the family Clariidae, is the most cultivated species in Nigeria[16]. It is ecologically important and commercially valued fish for the Nigerian fishery and aquaculture industry[17].It possesses vital characteristics such as the ability to tolerate handling stress, rapid growth rate, great yield potential, high fecundity, palatability and consumers' preference. Tadpoles are young frogs or toads found in tropical countries like Nigeria and are mostly herbivores, feeding on algae, detritus and some plants, although they will also eat other animals in small amounts[18].

Although the piscicidal potentials of *L. cynescens* has been generally known among local fishermen, there are no scientific work reported on its piscicidal activities on *C. gariepinus* and tadpoles. Therefore, this study was conducted to determine the bioactive components and assess the acute toxicity of the aqueous extract of *L. cynescens* on *C. gariepinus* fingerlings and anuran tadpoles under static bioassay conditions.

## MATERIALS AND METHODS

**Study area and source of experimental fish and tadpoles:** The 96 h $LC_{50}$ values of *L. cyanescens* bark aqueous extracts were determined in static bioassays against *C. gariepinus* fingerlings and anuran tadpoles between August and November, 2015 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°2'26"N and longitude 7°55'19"E. *Clarias gariepinus* fingerlings reared under controlled condition free of pollutants were obtained from Akanse's Fish Farm, Edem Idim Ibesit, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria and transported to the laboratory in a plastic container (30 L volume,

52 cm diameter and 50 cm depth) with water from the site of collection. The experimental tadpoles were obtained with a long handled scoop net from a pond in the same environment and transported to the laboratory under same condition.

**Acclimation:** In the laboratory, the fish fingerlings and tadpoles were kept in holding plastic containers (30 L volume, 52 cm surface diameter, 34 cm width and 20 cm depth) half filled with dechlorinated borehole water. The fingerlings and tadpoles were kept in the containers for at least 1 week, to allow them acclimate to laboratory conditions (29±1°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 while the tadpoles were fed with algae throughout the period. During this period of acclimation, the water in the aquaria was changed every 48 h to ensure good health of the experimental organisms while uneaten feed were removed by siphoning to avoid fouling. Also, dead and weak individuals were immediately removed and the total mortality recorded during the acclimation period was less than 5%[19]. Acclimation of test organisms to laboratory conditions and experimental procedures were in accordance with guidelines for bioassay techniques[20].

**Test plant material and preparation of the stock solution:** The fresh bark of *L. cyanescens* was procured from Edem Idim Ibesit, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria. The samples were washed with clean water to free them from sand and debris. They were sundried to constant weight, cut into tiny pieces and air dried in the laboratory to constant weight and the dried samples were pulverized with a sterile manual grinding machine and then sieved with 100 micron sieve to obtain the fine powder[21]. The samples produced were stored in air tight containers. Subsequently, 50 g of the test plant was dissolved in 500 mL of dechlorinated water for 24 h. Thereafter, the mixture was filtered through Whatman's filter paper (No. 1) and that served as the stock solution for the experiment. The prepared aqueous extract of the test plant was refrigerated and used for the static bioassay tests following standard procedures[22].

**General bioassay procedure:** Clean plastic containers (20 L volume, 31 cm surface diameter, 31 cm width and 19 cm depth) were employed in all bioassays. A predetermined volume of the 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 3.04±2.05 g, mean length 2.92±1.97cm) for *C. gariepinus* fingerling sand (mean weight 0.01±0.008 g, mean length 0.38±0.041cm) for anuran tadpoles 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. Tests 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).

Mortality assessments were made by examining each animal separately every 24 h over a 96 h experimental period. *Clarias gariepinus* fingerling was considered dead when respiratory and tail movements stopped and no response to gentle prodding with a rod. Anuran tadpole was considered dead if it sunk to the bottom of the test medium and no response to gentle prodding with a rod.

**Physical and chemical parameters of acclimation and test media:** 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 h) and end (96 h) of each bioassay. Temperature was determined by using mercury in-glass thermometer, conductivity by conductivity meter (Hanna product 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 1: Toxicant concentrations exposed to *C. gariepinus* fingerlings and anuran tadpoles

| Test animals | Concentrations (mg $L^{-1}$) |
|---|---|
| *C. gariepinus* | 0.6, 1, 1.4, 1.8, 2, 2.6, 3, 4, 5 |
| Anuran tadpoles | 0.6, 0.7, 0.8, 1, 1.2, 1.4, 2 |

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

| Physical and chemical parameters | *C. gariepinus* (Mean±SE) | Tadpoles (Mean±SE) |
|---|---|---|
| pH | 6.80±0.09 | 6.49±0.112 |
| Dissolved oxygen (mg $L^{-1}$) | 5.02±0.25 | 8.37±0.70 |
| Temperature (°C) | 25.37±0.20 | 26.01±0.34 |
| Conductivity (µS $cm^{-1}$) | 101.57±2.03 | 147.29±9.16 |
| Ammonia (mg $L^{-1}$) | 0.63±0.08 | 3.49±0.05 |
| Alkalinity (mg $L^{-1}$ $CaCO_3$) | 65.43±1.94 | 22.86±1.01 |
| Hardness (mg $L^{-1}$ $CaCO_3$) | 4.14±0.21 | 9.40±0.15 |

SE: Standard error

**Statistical analysis:** The toxicity data based on quantal response (mortality) was analysed by probit analysis[23]. The analysis, including the equation for probit line and unpaired t-test used to test for significance on toxicity of the test plant between *C. gariepinus* and tadpoles was achieved via computer programme using IBM SPSS Statistics 20. Indices of toxicity/susceptibility level were based on the 96 h $LC_{50}$ values. Measure of central tendency and dispersion were used to characterize the physical and chemical parameters of the acclimation and test media.

**Phytochemical screening of plant material:** The extract of *L. cyanescens* bark was screened to identify its constituents of bioactive compounds (Alkaloids, flavonoids, saponins, tannins, phytate, glycosides and oxylate) through preliminary phytochemical screening as described in literature[24-27].

## RESULTS

**Physical and chemical parameters of the test media:** The physical and chemical parameters of the test media are summarized in Table 3.

When *L. cyanescens* was tested against *C. gariepinus*, the physical and chemical parameter data over 96 h period, showed that *L. cyanescens* caused increase in temperature, pH, ammonia and conductivity, while it reduced dissolved oxygen, alkalinity and hardness of the test media (Table 3). A test of *L. cyanescens* against tadpoles indicated no significant change in temperature, dissolved oxygen and ammonia. But there was an increase in pH, alkalinity and hardness, with decrease in conductivity.

**Acute toxicity of *L. cyanescens* bark against *C. gariepinus* fingerlings and anuran tadpoles:** Based on 96 h $LC_{50}$, *L. cyanescens* bark was more toxic against *C. gariepinus* fingerlings than the anuran tadpoles. The computed 96 h $LC_{50}$ values for fingerlings and tadpoles being 0.278 mg $L^{-1}$ and 1.061 mg $L^{-1}$, respectively. Computed toxicity factor based on 96 h $LC_{50}$ values showed that *L. cyanescens* was 0.26 times less toxic against tadpoles than *C. gariepinus* (Table 4). Unpaired t-test showed that the test plant was significantly ($p<0.05$) more toxic against *C. gariepinus* than tadpoles (Table 5). The log-dose probit graph depicting the relative toxicity of *L. cyanescens* against *C. gariepinus* and tadpoles based on the 96 h values were non-parallel (Fig. 1).

**Some phytochemical constituents of the test *L. cyanescens* plant bark:** The results of phytoconstituents analysis conducted on *L. cyanescens* bark aqueous extracts revealed the presence of some bioactive components such as alkaloids, flavonoids, saponins, phytates, glycosides and oxalates (Table 6). Alkaloids, flavonoids and glycosides were slightly present, saponins and phytates were moderately present, while oxalates were copiously present.

Table 3: The physical and chemical parameters of test media for *C. gariepinus* fingerlings and anuran tadpoles

| | Mean±SE | | | |
|---|---|---|---|---|
| | *C. gariepinus* fingerlings | | Anuran tadpoles | |
| Parameters | 0 h | 96 h | 0 h | 96 h |
| Temperature (°C) | 25.50±0.005 (27.2-27.7) | 27.20±0.24 (26.0-28.3) | 26.10±0.17 (26.1-27.7) | 26.03±0.05 (26.0-26.4) |
| pH | 8.85±0.25 (7.6-9.8) | 9.31±0.11 (8.3-9.10) | 7.23±0.18 (6.33-7.83) | 8.35±0.35 (7.34-9.70) |
| Dissolved oxygen (mg $L^{-1}$) | 9.25±0.19 (8.1-9.9) | 7.53±0.53 (5.7-10.0) | 9.40±0.23 (8.64-10.4) | 9.53±0.34 (7.51-10.70) |
| Conductivity (μS $cm^{-1}$) | 115.80±1.23 (108.0-120.0) | 143.70±8.73 (114.0-183.0) | 148.80±9.4 (105.0-184.0) | 135.50±7.31 (102.0-178.0) |
| Ammonia (mg $L^{-1}$) | 0.31±0.02 (0.2-0.7) | 0.63±0.05 (0.23-0.9) | 0.48±0.04 (0.23-0.7) | 0.54±0.07 (0.33-0.89) |
| Alkalinity (mg $L^{-1}$ $CaCO_3$) | 91.39±15.9 (48.0-170.0) | 66.50±10.3 (18.0-120.0) | 90.60±11.7 (29.3-130.0) | 97.30±8.07 (60.0-130.0) |
| Hardness (mg $L^{-1}$ $CaCO_3$) | 18.41±7.85 (2.8-70.0) | 5.80±0.44 (3.6-7.20) | 4.98±0.81 (2.3-8.20) | 5.58±0.47 (3.80-8.04) |

Minimum and maximum values are represented in parentheses

Table 4: Comparative toxicities of the *L. cyanescens* bark against *C. gariepinus* fingerlings and anuran tadpoles

| *L. cyanescens* plant bark | 96 h $LC_{50}$ (95% CL) (mg $L^{-1}$) | Slope±SE | DF | Regression equation (probit response) | TF |
|---|---|---|---|---|---|
| *C. gariepinus* fingerlings | 0.278 (0.223-0.333) | 3.913±0.48 | 8 | Y = -1.087+3.913X | 1.00 |
| Anuran tadpoles | 1.061 (0.980-1.157) | 7.857±1.12 | 5 | Y = -0.201+7.851X | 0.26 |

LC: Lethal concentration, CL: 95% confident limit, TF: Toxicity factor, SE: Standard error, DF: Degree of freedom

Table 5: Test of significance for the 96 h $LC_{50}$ values of *L. cyanescens* between *C. gariepinus* fingerlings and anuran tadpoles

| | Unpaired t-test | | | |
|---|---|---|---|---|
| Variables | Standard error mean | T | Significance (2-tailed) | t-test probability |
| *C. gariepinus*-tadpoles | 0.16 | 4.321 | 0.005 | $p<0.05$ |

$\alpha = 0.05$

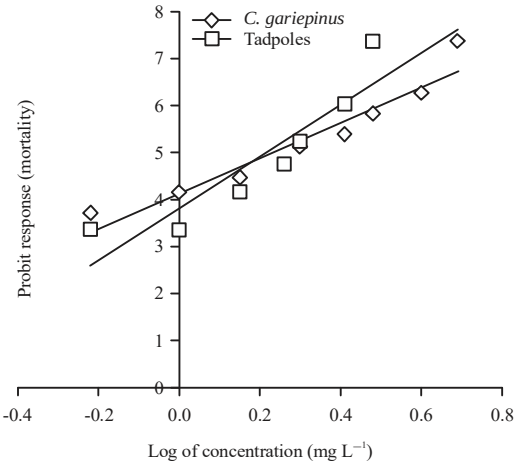


Fig. 1: Log-dose probit graph depicting the relative toxicity of *L. cyanescens* against *C. gariepinus* and anuran tadpoles based on 96 h values under static bioassay

Table 6: Phytochemical components of *L. cyanescens* bark aqueous extract

| Bioactive constituents | *L. cyanescens* bark aqueous extract |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Saponins | ++ |
| Phytates | ++ |
| Glycosides | + |
| Oxalates | +++ |

+++: Copiously present, ++: Moderately present, +: Slightly present

## DISCUSSION

The 96 h $LC_{50}$ values of this study indicated that *L. cyanescens* exerted piscicidal effects on *C. gariepinus* fingerlings and anuran tadpoles. The use of different methods to capture fish and other aquatic organisms from water such as synthetic chemicals and the use of fish poison plants and other plant products is a traditional practice[21,28]. However, the use of plant extracts as piscicides in capture fisheries and aquaculture are considered advantageous when compared to the back drop of using persistent and synthetic chemicals[29]. This study have revealed that the ichthyotoxic plant, *L. cyanescens* subjected to phytochemical screening contain some bioactive components such as alkaloids, flavonoids, saponins, phytates, glycosides and oxalates. These active ingredients are known to be toxic to fish and other aquatic organisms even at low concentrations[30-32]. Except for phytates and oxalates, earlier workers have reported other secondary metabolites for *L. cyanescens*. Seven important classes of secondary metabolites, namely: Saponin, tannin, steroid, terpenoid, cardiac glucoside, phlobatannins and flavonoids have been obtained from the phytochemical screening of leaf, stem and roots extracts of *L. cyanescens*[33]. Secondary metabolites such as alkaloids, anthraquinones, cardiac glucosides, tannins, saponins, steroids and flavonoids have been recorded from phytochemical screening of the leaves[34].

The observed incessant jumping and gulping for air, restlessness, loss of equilibrium, surface to bottom movement, sudden quick movement, resting at the bottom and death of the test species were similar to those earlier observed[35,36]. This abnormal behaviour could be due to the effects of flavonoids, alkaloids, saponins, phytates, glycosides and oxylates present in the extracts of the bark of *L. cyanescens* bark. This observation is similar to earlier findings which reported restlessness and mortalities of the *C. gariepinus* due to the effects of flavonoids, alkaloids and saponins[37]. The ichthyotoxins, saponins which destroy erythrocytes are assimilated directly through the gills[38]. Plant toxins even at low concentrations are known to elicit mucus production on the body and gills of fish[39,40]. Increased mucus secretion in fish exposed to toxicants is a defense response by which fish attempts to reduce entrance of the toxicant through the skin and the gills surfaces[41].

The mean water quality parameters for 96 h static bioassay using *L. cyanescens* for the two test species, *C. gariepinus* fingerlings and anuran tadpoles at various concentrations did not vary significantly ($p<0.005$) from values obtained from the control. The pH and ammonia were found to increase with a slight increase in temperature of the test media. However, the mean water quality parameters were within the range earlier reported as optimal requirement for African catfishes[42-44]. This indicates that the parameters did not influence the toxicity of *L. cyanescens*, hence, it might not have contributed to the mortality of the test species. This is in agreement with earlier work which exposed *C. gariepinus* to Akee apple and sausage plant extracts and indicated no significant different ($p<0.05$) in the water quality parameters[6]. The acute toxicity test showed that the 2 test species in the treatment media had increased mortality with increasing concentrations of the toxicants and showed a dose-response relationship which has earlier been reported[45].

The value of 96 h $LC_{50}$ of 0.278 mg $L^{-1}$ for *C. gariepinus* fingerlings is within the extremely toxic range of 0.35 mg $L^{-1}$ earlier reported for acute toxicity of lyophilized aqueous extract of *Psychotria microphylla* on *C. gariepinus*[46] and another study that reported 0.36 mg $L^{-1}$ for acute toxicity of *Tetrapleura tetraptera* on the tilapia, *Sarotherodon galilaeus*[47]. It is however significantly and extremely more toxic

than those earlier reported for other highly toxic ichthyotoxic plants tested on *Clariid* species[40,48-50]. The observed differences in the present study and those of earlier workers could be attributed to the type and part of the plants used, size and type of fish, environmental factors, water parameters and selective action of the plant toxicants.

The value of 96 h $LC_{50}$ of 1.061 mg $L^{-1}$ for anuran tadpoles is greatly highly toxic condition. Although it has been reported that ichthyotoxic plants besides fish have effects on other living things like water snakes and frogs in water[51,52], no literature exists on the toxicity of ichthyotoxic plants on tadpoles. However, little literature existing on the toxicity of pesticides on tadpoles reported $LC_{50}$ values as follows: 7.5 mg $L^{-1}$ for malathion acute toxicity on tadpoles of *Duttaphrynus melanostictus*[53], range of 1.25 and 5.9 mg $L^{-1}$ for tadpoles of *Rana*, *Bufo* and *Hyla speires*[54] and 2.137 mg $L^{-1}$ for tadpoles of *Rana boylii*[55].

The difference in the susceptibility of organisms to chemicals has been investigated widely by earlier workers and shown to depend on factors like cuticular disposition to penetration by the toxicant in question, the rate of enzymatic break down excretion of the compound and availability of physiological storage mechanism in the organism[56]. However, during the study it was observed that *C. gariepinus* fingerlings were extremely susceptible to the toxic effect of the *L. cyanescens*, while anuran tadpoles were highly susceptible, hence, the low concentrations used for the definitive bioassay tests. The increased mortality rate recorded in the study was due to the impairment of normal metabolism by inhibitory components in the toxicants. Tadpoles and adult amphibians are major competitors and predators in fish ponds[57]. Hence, it can be used for their control in the earthen nursery and production pond preparation for stocking.

## CONCLUSION

The study indicated that *L. cyanescens* exerted piscicidal effects on *C. gariepinus* fingerlings and anuran tadpoles. Further research on its toxicity and extract of its bioactive constituents could make it applicable in ponds for eradication of predators, competitors and unwanted fish population. The use of the plant extracts to clear ponds may be preferable to agrochemicals since they have the tendency of degrading faster without bioaccumulation in the flesh of the cultured organism and the environment. However indiscriminate use of this plant to catch fish by local fishermen should be discouraged.

## SIGNIFICANT STATEMENT

The ichthyotoxic plant, *L. cyanescens* is used traditionally to kill fish. Its toxicity to the catfish, *C. gariepinus* fingerlings and anuran tadpoles in acute toxicity tests could be attributed to observed phytochemical constituents such as alkaloids, flavonoids, saponins, phytates, glycosides and oxalates. Further research on *L. cyanescens* and extract of its active ingredients could make it useful in aquaculture for eradication of tadpoles and other predators during pond preparation for fish culture.

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