



## Toxicological evaluation of methanol extract of *Khaya senegalensis* leaves in African catfish *Clarias gariepinus*

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**ABSTRACT:** The toxicity of methanol extract of *Khaya senegalensis* leaves was evaluated in *Clarias gariepinus* over 96 h exposure. The fish were exposed to 150 mg/L, 170 mg/L, 190mg/L, 210 mg/L and 230 mg/L of the extract and a control in acute static bioassay after a trial finding to determine the median lethal concentration (LC<sub>50</sub>) of the extract. Exposed fish showed clinical signs, such as distress opercula ventilation, tail fin beats and respiratory alteration. The frequency of opercula ventilation and tail fin beats were concentration dependent and showed significant association (P<0.05). The LC<sub>50</sub> value of 199,69mg/L was established for the extract in the exposed fish where mean mortality was significantly (P<0.05) concentration and exposure dependent. This plant is to be regarded as a piscine toxicant and should be discarded from the bank of rivers and dams. © JASEM

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**KEYWORDS:** Acute effect, Histopathology, *Khaya senegalensis*, *Clarias gariepinus*

### Introduction

*Khaya senegalensis* is a tree belonging to the Meliaceae family, it has plethoric usages in Africa. The plant is intensively used as a shading tree in this part of the world. In Cotonou capital of the Republic of Benin, the government has implemented its usage for reforestation of recreational areas (Adanhounso, 2012). The tree is commonly known in Africa for its medicinal values with its capacity to cure several diseases such as malaria, helminth infection and bacteria infections (Iwu, 1993).

However, aqueous extract of *K. senegalensis* stems back, roots and the leaves are commonly used for treating jaundice, dermatoses, malaria, fever, mucous diarrhea and venereal diseases (Iwu, 1993). In addition, the ethanolic extract of *K. senegalensis* has been reported to have toxicological effect on the biochemistry, haematology and histology of rats and rabbits (Abbel-Wareth *et al.* (2014) and Onu *et al.* (2013). Abdulahi *et al.* (2012) also reported the toxic effect of *K. senegalensis* on working termites (Isoptera: Rhinothermididae).

Despite the intensive research on the aqueous extract and ethanolic extract of stem bark and leaves of *K. senegalensis* with prominent effects on terrestrial animal species, a dearth of information about the median lethal concentration and the toxicological response of the methanolic extract of the leaves on aquatic fauna including *C. gariepinus* still exist. The

study therefore aimed to evaluate the toxicity of methanol extract of *K. senegalensis* leaves in African catfish *C. gariepinus*.

### MATERIALS AND METHODS

**Extraction:** *K. senegalensis* leaves were collected from the Ahmadu Bello University (ABU), Zaria, Nigeria at the bank of the natural dam and identified and authenticated by a Chief technologist at the Herbarium of the Department of Biological Sciences, ABU Zaria, Nigeria. The leave were dried in an open airy laboratory for 2weeks and later pounded into powder using ceramic mortar and pestle and sieved through 100 mm sieve to obtain 500g of fine powder.

200g of the powder was packed into a soxhlet extractor with 5 L of 25% v/v methanol (98% vol. Sigma-Aldrich<sup>R</sup> Inc., St.Louis, MO 63178, USA) as the extracting solvent. The extract dried by placing in a water bath (40- 45 °C) for 3 to 4 h.

**Acute fish toxicity bioassay:** Juvenile *C. gariepinus* were identified at the Fishery Section, Department of Biological Sciences, ABU Zaria, Nigeria after purchase at the Bagiwa farm Funtua Katsina State. 500 fish of mean weight  $18.47 \pm 3.06$ g and standard length  $10.9 \pm 2.7$ cm Fish were acclimatized in 500L plastic tank pond for 28 days under natural day and night photoperiods (12/12h) prior to commencement of the toxicity bioassay. Pond water was changed once every three (3) days. Fish were fed twice daily

with standard Vital feed for aquaculture (Vital Fish Feed Limited, Jos, Nigeria). Fish were observed for diseases, mortality and infection. Feeding was stopped 24h prior to the 96h exposure period so as to prevent mixture in the stomach with waste from the acclimatization tank.

One hundred and twenty (360) healthy acclimatized fishes were randomly selected and distributed into 12 glass aquaria with dimension 30.50 x 30.50 x 44.25cm each containing 20 litres of dechlorinated water and 10 fish per aquaria, three of the aquaria served as control for acute toxicity bioassay.

20g of the methanolic extract of *K. senegalensis* was obtained and dissolved in distilled water (1L) to form stock solutions of 250mg/L. 0 (control), 150, 170, 190, 210 and 230mg/L were dispersed into the experimental aquaria. The mixture was allowed to stand for about five minutes to evenly distribute via diffusion before introducing the fish. The exposure of the fish into the aquaria was carried out in triplicate, and the static bioassay ceased at the end of the 96hr exposure periods. This is after a performance of a range finding test to determine the five extract concentration as described by Fafioye, *et al.* (2004)

and the control with no extract. Clinical signs such as opercula gills ventilation and tail fin beats were observed in three fish selected from each aquarium per minute. Death was recorded after 96h.

Median lethal (LC50) concentration of the extract after 96 h exposure period was determined using the statistical package XLSTAT 15.5

The temperature, P<sup>H</sup>, TDS, and Conductivity of fish culture water was ascertained using a Hana portable hand instrument HI 98129 and the dissolved oxygen contents were measured using Winkler-Azide method.

**Statistical analyses:** Data were expressed as mean (±SD) were subjected to ANOVA and to Chi-square for statistical significance (P< 0.05). XSTAT version15.5 software and graph-Pad Prism 6.0 were used.

**RESULTS AND DISCUSSION**

The physicochemical parameters of water showed that EC was 79.5µs/cm, TDS 56.79 mg/L, P<sup>H</sup> 6.92 and Temperature 24.14 °C (Table 1).

**Table 1** Physicochemical parameters of fish culture water of *C. gariepinus* exposed o methanol extract of leaves *K. senegalensis*.

Physicochemical parameters	Value
Conductivity (5µs/cm)	79.5±3.48
Total dissolved solid (TDS) mg/L	56.79±1.81
p <sup>H</sup>	6.92±0.12
Dissolved oxygen (DO) (mgO <sub>2</sub> /L)	6.66±0.14
Temperature (°C)	24.14±0.18

The physical and chemical parameters analyzed in this study are very relevant for the status of water quality tolerable by *C. gariepinus*. The result of the physical chemical in this study, conductivity 79.5 µs.cm, TDS 56.79mg/L, Temperature 24.14°C PH 6.92 and DO 6.66mgO<sub>2</sub> 132mg/L corroborate with the standard of freshwater fish water quality. The physicochemical parameters of fish culture water in this study were all within acceptable limit for the survival of *C. gariepinus*. The standard as stated by

Kalawole *et al.* (2011) reported a conductivity less than 135-100µs/cm, DO greater than 4 mg/L; PH between 6.5 to 9 and Temperature between 20-30 °C. The effect of *K. senegalensis* leaves on *C. gariepinus* showed a high mortality of 60 and 70 at concentration 210 and 230 mg/l respectively. The mortality increased significantly with the increased in concentration (P= 0.002). The median lethal concentration (LC50) of leaves extract was therefore 199.69 mg/L (Table 2).

**Table 2** Mortality in *C. gariepinus* exposed to methanol extract of *K. senegalensis* leaves over 96h period

Extract concentration (mg.L)	Log concentration	Total mortality	Percentage total mortality (%)	Probit value
0.0 (control)	0.000	0.0	0.0*	2.5*
150mg.L	2.176	1	10	3.72
170mg.L	2.230	3	30	4.16
190mg.L	2.279	4	40	4.75
210 mg.L	2.322	6	60	5.25
230 mg.L	2.362	7	70	5.52

Y= 9.33x-21.47, LC<sub>50</sub>= 199.69, \* =corrected value

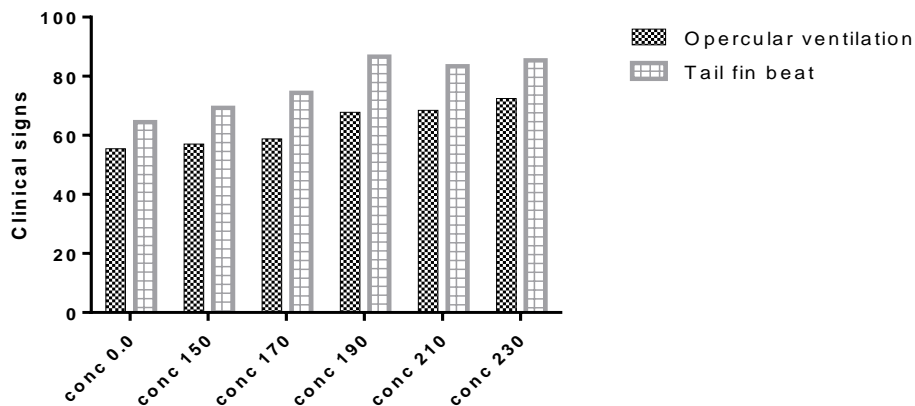
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In this study the mortality increased with the increment of concentration, this increment was shown to be significant ( $P < 0.05$ ). This showed that methanolic extract of *K. senegalensis* was 138 toxic to the exposed fish. The dependence of plants extract to mortality of *C. gariepinus* was similar Abalaka *et al.* (2015). The LC<sub>50</sub> value of 199.69 in this study was high compare to Abalaka *et al.* (2015) who had a LC<sub>50</sub> of 7.15 mg/L while analyzing the gills and skin evaluation in African sharptooth catfish, *clarias gariepinus* exposed to ethanol extract of *Adenium obesum* stem bark. Therefore theses results proved *C. gariepinus* to be resistant to methanolic extract of *K. senegalensis*. The fish seem not to be susceptible to

the toxicity of these extracts except at high doses while compare to results of Abalaka *et al.* (2015) and Ayoola (2011) on the ethanol extract of *Adebiium obesum* and *Ipomoea* aquatic. They authors reported low LC<sub>50</sub> of 7.15 and 2.6 respectively.

The opercula ventilation of gills and tail fin beats with exposure concentration are presented in Fig 1.

The opercula ventilation of gills and tail fin beats exposed to methanol extract of *K. senegalensis*, were significantly higher than in the control fish species ( $P < 0.05$ ). The opercula ventilation of gills and tail fin beats increased with toxic concentration.



**Fig 1** Means of opercula gills ventilation and tail fin beats of *Clarias gariepinus* exposed to different concentrations of methanol extracts of *K. senegalensis* for 96h with ( $P < 0.05$ ) Note: conc = concentration.

The respiratory distress caused by the increasing concentration of *K. senegalensis* extract led to increasing frequency of opercula and tails fin beats. These was attempts to increase ventilation rate to compensate for low oxygen uptake (Fernandes and Mazon, 2003) by passing large volume of water over gills at faster rates (Reebs, 2009). This study agreed with Emere and Balogun (2014) who reported an initial increased of opercula rates though the rate of opercula ventilation decreased with increased of concentrations and days of exposure. The respiratory distress might due to gill epithelia damage or excessive mucous coating of gill epithelia surfaces (Abalaka *et al.*, 2010).

**Conclusion:** Methanol extract of *K. senegalensis* leaves was deleterious to the exposed *C. gariepinus* by causing death and altering the opercula ventilation and tail fin beats of *C. ganepinus*' gills. Therefore this plant is to be regarded as a piscine plant that should be planted far a distance from the bank of

rivers and dams because its bioaccumulation in fish organs can affect food safety and may be the depletion of aquatic fauna.

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