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Original Article

Phytochemical composition and toxicity of the aqueous extract of *Parkia biglobosa* pods in adult *Clarias gariepinus*

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ABSTRACT: The phytochemical composition and toxicological evaluation of the aqueous extract of *Parkia biglobosa* pods in adult *Clarias gariepinus* was investigated over a 96-h exposure period. The aqueous extract of the pods was freeze-dried and screened for its phytochemical constituents. This revealed the presence of glycosides, alkaloids, saponins, steroids and triterpenes, tannins, carbohydrates and flavonoids but no anthraquinones and fixed oils. The fish were exposed to varying concentrations of the extract in a static acute toxicity bioassay. The exposed fish showed initial signs of agitated and erratic movements followed by signs of respiratory distress and abnormal nervous compromise, including mortality in some of the exposed fish. No such obvious signs of toxicity were recorded in the unexposed control fish. The appearance and intensity of the signs were both concentrations and exposure period-dependent. Mean mortality was significantly ($p < 0.05$) related to extract concentrations and exposure period. The median lethal concentration of the extract in exposed fish over the 96-h exposure period was calculated to be 115.38 mg/l. *Parkia biglobosa* pods contained some toxicologically active constituents that can be meaningfully exploited to harvest fish from water bodies. However, this should be done with great care as the abusive use (higher concentrations) of this extract could result in extract residues in the exposed fish that might pose a risk to the consumers of such fish.

KEYWORDS: *Clarias gariepinus*, *Parkia biglobosa* pods, phytochemical constituents, toxicity.

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INTRODUCTION

Various plants are reputed for their medicinal and antimicrobial values (Nascimento *et al.*, 2000; Adamu *et al.* 2005), including their pesticidal, acaricidal and trypanocidal properties (Jhingran 1975, Mgbojikwe and Okoye 1998; Atawodi 2005). Yet, some are known potent arrow and fish poisons (Geidam *et al.*, 2007; Kamalkishor and Kulkarni 2009) depending upon the type and the concentrations of their bio-active constituents. This is because plants contain structurally diverse biological substances with varying properties (Istvan 2000).

Clarias gariepinus (Burchell, 1822), which belong to the Family *Clariidae*, is widely distributed and readily available in Nigerian waters (Fagbenro, 1992). The fish is known for its hardiness (Ogundiran *et al.*, 2009) and its ability to withstand

harsh culture conditions (Hogendoorn, 1979). *Clarias gariepinus* is an important fish species in Nigeria because of its taste and high market price (Kori-Siakpere and Ubogu, 2008) resulting in its massive killings with both conventional and unconventional means, including the use of piscicidal plants or plants that are poisonous to fish. *Parkia biglobosa*, which belongs to the Family *Mimosaceae*, is also widely distributed and readily available within the savannah belts of Nigeria (Hopkins, 1983) where it is highly reputed for its medicinal and antimicrobial importance (Ajaiyeoba, 2002; Agunu *et al.*, 2005; El-Mahmood and Ameh, 2007). This is in addition to being used traditionally to stun and/or kill fish (Jenness 1967; Fafioye, 2005) where the toxicity of plants to fish arising from their phytochemical constituents normally wears off within short time (Kulakkattolickal *et al.*, 1987; Wang and Hoffman, 1991). However, extract residues have

been reported in exposed fish (Idris, 2012) and therefore, the indiscriminate use of high concentrations of these plants could lead to either total fish kill in an area or makes the killed fish to become toxic to its consumers (Van Andel, 2000). There is therefore a need to ascertain the phytochemical constituents of piscicidal plants in relation to their LC₅₀ in exposed fish so as to be able to give an insight into the appropriate concentrations of piscicidal plants to be used by the locals to kill fish with great caution. Hence, the study aimed to evaluate the phytochemical constituents and toxicity of *P. biglobosa* pods extract in adult *C. gariepinus*.

MATERIALS AND METHODS

Plant Collection, Authentication and Preparations

The pods of *Parkia biglobosa* were collected from the open field around Ruwan Kanya village, Rano Local Government Area, Kano State, Nigeria. These were subsequently authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University (A. B. U), Zaria, Nigeria with Voucher No. 900187. The pods were dried openly in the sun before pounding it into fine powder for use.

Aqueous Extraction

Six litres of distilled water was used to soak 1.00 kg of the fine powder of *P. biglobosa* pods based on the maceration method of Bentley (1977) and Ghani (1990) to obtain the filtrate, which was freeze-dried over-night using a freeze-drying machine (Lyovac GT2, AMSCO/FINN-AQUA, CAT No. 204555B4-2, Germany).

Determination of the Phytochemical Constituents

The aqueous extract of *P. biglobosa* pods was screened for alkaloids, saponins, tannins, glycosides, carbohydrates, steroids and triterpens, anthraquinones, flavonoids and fixed oils as described by Trease and Evans (1983) and Harbone (1998).

Fish Collection, Authentication and Acclimatization

Clarias gariepinus adults of 163.70 ± 3.71 g mean weight and 27.23 ± 0.19 cm mean total lengths were purchased from Kune Integrated Farms, Katsina, Katsina State, Nigeria. Fish authentication took place at the Fishery Section, Department of Biological Science, A. B. U., Zaria, Nigeria. The experimental fish were acclimatized for 21 days under natural day and night photo-periods where pond water was changed every other day. The fish were fed to satiation twice daily with 4 mm pelletized commercial catfish feed (Multi feed, Zelmach feed mill, Israel).

Experimental Design

A static acute toxicity bioassay was conducted as described by APHA (1985) with a replicate per extract concentration after performing a range finding test as recommended by

Omitoyin *et al.* (1999) to determine five extract concentrations that were used. These involved the use of 10 fish per reconstituted extracts, including the controls in transparent glass aquaria (2 x 1 x 1 cm) that were covered with mesh and fastened with binding wires to their table support base. This was to prevent the fish from jumping out of their culture water (Fafioye *et al.* 2004). Reconstituted extracts were allowed to stand for 30 minutes for proper mixing as performed by Usman *et al.* (2005) prior to fish introduction. Feeding of fish stopped 48 hours (h) prior to and during the 96-h exposure period as performed by Adeyemo (2005). This was to prevent interference with the absorption and metabolism of the extract by wastes in reconstituted extracts as suggested by Smith *et al.* (2007) and Olufayo (2009). Fish mortality was used as a measure of the toxicity of the plant extract. The exposed and unexposed fish were observed for signs of toxicity with prompt recordings at 24-h (0-h, 3-h, 6-h and 12-h) 48-h, 72-h and 96-h exposure period.

Median Lethal Concentration (LC₅₀) Determination

The LC₅₀ of the aqueous extract of *P. biglobosa* pods in the exposed *C. gariepinus* adults over the 96-h exposure period was determined based on the Arithmetic method of Karber as adapted by Dede (1992) using the formula:

$$LC_{50} = LC_{100} - \frac{\sum \text{Probit}}{\text{No. of fish per extract conc.}}$$

Where: LC₁₀₀ is the extract concentration that caused 100 % fish mortality

Statistical Analysis

Data were presented as means (± SEM) and also subjected to one-way analysis of variance (ANOVA) for statistical significance at p<0.05 using GraphPad software programme (GraphPad Prism, version 4.0, San Diego, CA).

RESULTS

The phytochemical screening of the aqueous extract of *P. biglobosa* pods revealed the presence of glycosides, tannins, saponins, steroids and triterpenes, carbohydrates, alkaloids and flavonoids but no anthraquinones and fixed oils as shown in Tables 1 and 2, respectively.

The observed signs of toxicity were divided into agitated behaviours, respiratory distress and abnormal nervous behaviours. The agitated behaviours were characterized by aggression, frequent attempts to jump out of the reconstituted extracts and raised dorsal fin with frequent surface to bottom movements. These signs increased with increasing extract concentration but decreased with exposure period except for the raised dorsal fin, which was noticed throughout the 96-h exposure period. The respiratory distress was characterized by frequent opercula movements, air gulping, vertical positioning with exposed snouts and excessive mucous

secretion. Similarly, the appearance and intensity of the respiratory distress was directly related to extract concentration but indirectly related to the exposure period except for the excessive mucous secretion and the vertical positioning with exposed snouts that increased with exposure period. However, signs of sluggish and swirling movements, increasing states of motionlessness, sudden darts, varying postures, rolling on their axis and loss of balance were exhibited in the abnormal nervous phase of the toxicity.

Table 1: Phytochemical screening of the aqueous extract of *Parkia biglobosa* pods for glycosides, saponins, carbohydrates, steroids and triterpenes, and tannins.

Parameters	Phytochemical constituents				
	Glycosides		Saponins	Carbohydrates	
Test	Fehling's Solution	Ferric chloride	Frothing	Molisch	Fehling
Observation	Brick red precipitate	Black precipitate	Honey comb froth	Purple precipitate	Brick red precipitate
Indication	+	+	+	+	+

Parameters	Phytochemical constituents		
	Steroids and Triterpenes		Tannins
Test	Salkowskii	Lieberman-Buchard	Ferric chloride
Observation	Reddish colour	Reddish-brown precipitate	Bluish-black precipitate
Indication	+	+	+

(+): present; (-): absent

Table 2: Phytochemical screening of the aqueous extract of *Parkia biglobosa* pods for flavonoids, alkaloids, anthraquinones and fixed oils.

Parameters	Phytochemical constituents				
	Flavonoids			Alkaloids	
Test	Shinoda	Sodium hydroxide	Ferric chloride	Meyer	Wagner
Observation	Yellow colour	Bluish black colour	Greenish black colour	Reddish precipitate	Whitish precipitate
Indication	+	-	+	-	+

Parameters	Phytochemical constituents		
	Anthraquinones	Fixed oils	
Test	Borntrager	Modified borntrager	Fixed oils
Observation	Transparent	Amber colour	Amber colour
Indication	-	-	-

(+): present; (-): absent

These signs increased with increasing extract concentration and increasing exposure except for sudden darts, which decreased with exposure period. Mortality was recorded in some of the exposed fish. However, the unexposed control fish did not show any of these signs of toxicity.

Table 3: Mean mortality (\pm SEM) per extract concentration per exposure period in *Clarias gariepinus* adults exposed to the aqueous extract of *Parkia biglobosa* pods.

Parameters	Exposure period	Extract concentration (mg/l)					
		0	85	100	115	130	160
Exposure period	24-h	- ^{abc}	1.0 (\pm 0.00) ^{de}	2.0 (\pm 0.00) ^f	2.0 (\pm 1.00) ^{gh}	5.0 (\pm 1.00) ^{bd}	8.0 (\pm 1.00) ^{efg}
	48-h	-	0.5 (\pm 0.50)	1.5 (\pm 0.50)	2.5 (\pm 0.50)	1.5 (\pm 0.50)	2.0 (\pm 0.00)
	72-h	-	0.5 (\pm 0.50)	-	-	-	-
	96-h	-	-	-	-	-	-
Mean mortality	-	2.0	3.5	4.5	6.5	10.0	
Percent mean mortality (%)	-	20	35	45	65	100	

Values with the same superscript in a row are statistically significant ($P < 0.05$)

There was complete (100%) mortality in the fish exposed to the highest extract concentration of 160 mg/l while the least mortality of 20% was recorded in the fish exposed to the lowest extract concentration of 85 mg/l as shown in Table 3. However, no mortality (0%) was recorded in the unexposed control fish. Mean mortality increased significantly ($p < 0.05$) with extract concentrations but decreased with exposure period. The LC_{50} of the aqueous extract of *P. biglobosa* pods in the exposed *C. gariepinus* adults over the 96-h exposure period was calculated to be 115.38 mg/l using Table 4.

Table 4: Parameters for the determination of the median lethal concentration (LC_{50}) of the aqueous extract of *Parkia biglobosa* pods in the exposed *Clarias gariepinus* adults over the 96-h exposure period.

Parameters	Extract concentration (mg/l)						Total Sum (Σ)
	0	85	100	115	130	160	
Concentration difference (CD)	0	15	15	15	15	30	
Mean fish mortality	0	2.0	3.5	4.5	6.5	10.0	
Average mean fish mortality (AM)*	0	1.00	2.75	4.00	5.50	8.25	
Probit (CD x AM)	0	15.0	41.25	60.00	82.50	247.50	446.25

(AM)*: average of the sum of preceding and proceeding mean mortality

DISCUSSION

The aqueous extract of *P. biglobosa* pods contained some bio-active substances. Some of these substances have also been reported in the leaves and root bark of the same plant (Ajaiyeoba, 2002; Agunu et al., 2005). The observed signs of toxicity, including the ultimate mortality in some of the exposed fish might have been due to these substances. This is because of the toxic nature of some of them (Da Roch et al. 2001; Bent and Ko 2004). Tannins are reported to be hepatotoxic and nephrotoxic (Nellis, 1997; Evans, 2005) due to their ability to precipitate exogenous and endogenous proteins (Bagepallis et al., 1992; Bele et al. 2010). The observed mortality in some of the exposed fish might have been due to the saponins content of the aqueous extract of *P. biglobosa* pods. This is because of the haemolytic nature of saponins in exposed fish (Bureau et al., 1998; Desai et al., 2009). The saponins content of the extract might have also impaired oxygen consumption by the exposed fish via the lowering of the surface tension of the reconstituted extracts, including the formation of colloidal substances within them (Geidam et al., 2007, Armstrong, 2008). Alkaloids are known to inhibit oxidative phosphorylation with subsequent impairment of oxygen consumption in exposed fish (Bocek, 1994; Tiwari and Singh, 2003) and therefore, might have contributed to the observed signs of respiratory distress in the exposed fish.

The agitated behaviours were attempts to escape from the toxic aquatic environment. Similar signs were reported in *C. gariepinus* exposed to the aqueous extract of *Carica papaya* seed powder (Ayotunde et al., 2011). The excessive mucous secretion was a protective response of the exposed fish to coat absorptive surfaces and prevent the continuous entry of the toxicant. Omoniyi et al. (2002) reported similar signs in *C. gariepinus* exposed to the lethal and sub-lethal concentrations of *Nicotiana tobaccum* leaf dust extract. The respiratory distress might have also been due to gill epithelial damage as earlier reported by Fafioye et al. (2004) in *C. gariepinus* exposed to extracts of *P. biglobosa* stem bark. The observed air gulping and surfacing phenomenon was attempts by the exposed fish to cope with the increasing demand for oxygen (Schmidt et al., 2005). The irregular, erratic and darting movements coupled with the observed loss of balance and the adoption of different postures by the exposed fish might be due to acetylcholinesterase inhibitory effects of the extract. Similar signs were reported in *C. gariepinus* exposed to the aqueous extract of *N. tobaccum* leaf dust (Kori-Siakpere and Oviroh, 2011).

The concentration-dependent nature of fish mortality in this study agreed with the work of Fafioye et al. (2004) who exposed *C. gariepinus* to extracts of *P. biglobosa* bark. Oxidative bio-degradation of the extract over time as suggested by Kela et al. (1989) might be responsible for the subsequent decrease in the toxicity of the extract with

increasing exposure period. However, our findings disagreed with the work of Fafioye et al. (2004) who reported an increase in toxicity with increasing exposure period. This might have been due to the static nature of the present toxicity bioassay compared to the non-static nature of the previous work by Fafioye et al. (2004). The calculated LC₅₀ value of 115.38 mg/l over the 96-h exposure period was different from the LC₅₀ value of 2.8 mg/L reported by Fafioye et al. (2004) or the LC₅₀ value of 105.83 mg/l reported by Abalaka and Auta (2010). This might be due to differences in fish age, extraction process, the nature of the toxicity bioassay and methods of the LC₅₀ determination. This is in addition to differences in the types and concentrations of the phytochemical constituents of the plant used, which is normally a function of the age and parts of the plants used as well as a function of the differences in the genetic make-up between species of the plant, including those of the climatic condition and the soil profile upon which the plants were grown (Norton, 1975; Botes et al., 2008; Borokini and Ayodele, 2012).

It was concluded that the aqueous extract of *P. biglobosa* pods contained some toxicologically active bio-constituents. These substances can be meaningfully exploited with great care to harvest fish from water bodies but their abusive use (higher concentrations) could result in extract residues in the exposed fish with the possible risk of toxicity in the consumers of such fish.

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