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Phytochemical Constituents of *Cnestis ferruginea* and Its Toxicity in Fish

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ABSTRACT The study explored the potentials of *C. ferruginea* as a nutritional supplement while also testing its possible toxicity to fish. Standard proximate and mineral analysis procedures were used to estimate nutritional composition, while Lorke's and Tainter and Miller's methods were used to determine LD_{50} of *C. ferruginea*. Results were presented as means±SD, and analysis of variance was used to test for differences in means. The values obtained for each parameter measured were significantly different ($p<0.05$) across all the samples. The analyzed ash contents for the crude powder, aqueous, ethanolic extracts were 1.47±0.02, 1.06±0.01, and 0.85±0.01, respectively. A low protein concentration of less than 5% was recorded across the samples. A decreasing trend (75.05±0.25 < 52.09±0.19 < 31.21±0.61) was observed in the carbohydrate values in the crude powder, aqueous and ethanolic extracts, respectively. The mineral composition of *C. ferruginea* across the samples was significantly different ($p<0.05$). The crude powder sample had the highest values recorded for all the minerals analyzed, followed by the aqueous and then ethanolic extract except for iron and copper, where ethanolic extract had higher values than aqueous extract. The values obtained for alkaloids, flavonoids, saponin, tannin, phenolic compounds, and oxalates showed a significant increase ($p<0.05$) in the crude powder sample, aqueous extract, and ethanolic extract, respectively. However, the ethanolic extract had higher values than the aqueous extract for glycosides, terpenoids, and steroids. Of the phytochemical components analyzed, oxalate had the highest value (16.11±0.01) followed by saponin (12.31±0.19), alkaloids (8.12±0.00), and tannin (6.43±0.17). The lowest values were recorded in steroids (0.11±0.00), terpenoids (0.29±0.01), and glycosides (0.34±0.01). LD_{50} was calculated to be 223.61 mg/kg for aqueous extract of *C. ferruginea* and 170.29 mg/kg for ethanolic extract (Lorke's method), while 124.82 mg/kg was obtained for aqueous extract and 128.63 mg/kg for ethanolic extract of *C. ferruginea* (Miller and Tainter method). The behavioural responses observed include weakness, hanging within the water column, and slow response to feeding. Mortality was recorded in some doses. The results indicate that *C. ferruginea* is a potential source of energy and minerals when incorporated into the fish diet. Its phytochemical constituent is wealthy and could be used in fish health management. However, the plant is toxic and cautiously applied with an LD_{50} value lower than 2.500 mg/kg.

Keywords: Aquaculture; fish health; LD_{50}; phytochemical; toxicity

INTRODUCTION

About 80% of the world's population still depend solely on traditional or herbal medicine to treat diseases, mainly in Africa and other developing nations (Okoye *et al*., 2014). Medicinal plants are vital storehouses of bioactive compounds and nutrients, including minerals and vitamins (Adnan *et al*., 2010). When consumed by fish or livestock, they transfer their biologically active constituents to them and aid in preventing and treating diseases (Adnan *et al*., 2010; Iranloye *et al*., 2010; Achi *et al*., 2017). Recently, the interest in natural products from plants and their use has increased tremendously in aquaculture. Caruso *et al*. (2013) found that 46% of the fish farmers surveyed in West Java (Indonesia) used plants in their farms, most of which were also traditionally used in human medicine. A study conducted by Sule *et al*. (2019) in Southwestern Nigeria also confirmed the use of medicinal plants by fish farmers.

In most cases, fresh plants were directly introduced into the rearing water and used to improve water quality, reduce fish stress, increase fish resistance to pathogens and treat fish diseases. Medicinal plants may be effective in treating various diseases owing to their antioxidant and anti-inflammatory activities; however, the consumption of certain phytochemicals may cause some acute and chronic toxic effects (Ipek *et al*., 2020). The daily consumption of specific phytonutrients may easily reach high levels when high doses of related compounds containing dietary supplements are taken simultaneously (Ipek *et al*., 2020). Farmers need to know the correct phytonutrient dose(s) in fish diets. Hence, the plant's safety assessment of the whole bio-active compounds should be well established before use.

Cnestis ferruginea, also known as 'gboyin gboyin' or 'omu aja' (Yoruba), 'fura amarya' (Hausa), 'amu nkita' (Igbo), 'ukpo-ibieka' (Edo), and 'usiere ebua' (Efik), is a perennial shrub widely distributed in Africa and bears orange-red fruits with velvet hairs on the follicle (Irvin, 1961; Adisa *et al*., 2010). This plant is used in traditional medicine for a variety of purposes. The leaf decoction is used by the Yoruba tribe of South West Nigeria as a laxative, enema for dysentery and gonorrhea. It has also helped treat conjunctivitis, syphilis, gum pain, wounds, dysentery, and gonorrhea (Funsho *et al*., 2013). According to their traditional use, natural compounds are often assumed to

be safe. However, several studies have reported that many plant species used as food ingredients or in traditional medicine present mutagenic, carcinogenic, or toxic properties (Deciga-Campos *et al*., 2007; Mohd-Fuat *et al*., 2007). Furthermore, available and known literature (to the best knowledge of the authors) on the toxicity of *C. ferruginea* extracts are only known to have been tested in mice and rats. *C. ferruginea* is a potential aquafeed additive; however, there is presently no information on its toxicity or level of safety in fish diets. Hence, it is crucial to conduct a study looking into its toxicity. Findings from this study will serve as a guide on safe inclusion levels of *C. ferruginea* in future studies and its potential in fish nutrition and health management.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *C. ferruginea* were collected from a forest at Ayetoro town, Ogun State. The leaves were identified and authenticated in the Forestry Wildlife and Fisheries Department, Faculty of Agricultural Science, Olabisi Onabanjo University, Ogun State.

The collected plant leaves were rinsed in clean water and air-dried at room temperature for two weeks. The air-dried leaves were pulverized to powder using an electric blender machine. The powder obtained was weighed, and small portions of the crude powdered leaves were used to prepare the extracts and the nutritional and mineral analyses.

Preparation of extracts

A method developed by the authors was adopted for extraction. 300 gr each of the pulverized leaves was soaked separately in 3.6 litres of water at ratio 1:12 and 2.7 litres of ethanol at ratio 1:9 at room temperature for 72 hours with constant mixing within this period. The solutions (aqueous and ethanolic) were filtered using a muslin cloth and concentrated using a water bath. The final volume of the solid extracts obtained weighed 80.3 gr and 151.34 gr, respectively, for water and ethanol. These were stored in a refrigerator until further use.

Determination of the nutritional composition of C. ferruginea leaves

The dry matter, moisture, ash, crude fat, crude protein, carbohydrate, and crude fibre contents of the leaves of *C. ferruginea* were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000).

Mineral analysis of C. ferruginea leaves

Calcium, magnesium, potassium, phosphorus, zinc, manganese, copper, and iron contents of *C. ferruginea* leaves were quantitatively analyzed using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Varian 710-E.S. series, S.M.M. Instruments, Cape Town, South Africa).

Phytochemical screening of C. ferruginea leaves

Standard procedures to identify the phytochemical constituents include Dragendorff's test for alkaloids, alkaline reagent test for flavonoids, and foam test for saponin (Tiwari *et al*., 2011). Ferric chloride test, Libermann Burchard's test, and sodium hydroxide test were used for tannins, steroids, terpenoids, and coumarins, respectively (Jayapriya & Shoba, 2014). Finally, glycosides and phenolic compounds were identified using the Kellar-Kiliani test (Rajesh *et al*., 2014). The quantitative determination of the phytochemical composition such as oxalate and phytate of C. ferriginea leaves was performed using the procedures described in Unuofin *et al*. (2017) and Ifemeje *et al*. (2014).

Determination of LD_{50}

Experimental fish

African mud catfish (*Clarias gariepinus*) with sizes ranging from 300 gr to 1 kg were used for this experiment. Healthy fish were purchased from a reputable fish farm in Ogun State and transported to the Department of Forestry, Wildlife and Fisheries, College of Agricultural Science Ayetoro, Ogun State. The fish were acclimatized for two weeks in plastic tanks before toxicity tests.

Lorke's method (1983)

Aqueous and ethanolic leaf extracts of *C. ferruginea* were orally administered to the procured fishes. In the first phase, nine fishes were divided into three groups of 3 animals for each treatment (aqueous and ethanolic), making 18 fish samples. Aqueous and ethanolic extracts weighing 10 mg, 100 mg, and 1000 mg were administered to the fishes through the feed per kg body weight and were observed for 24 hours to monitor their behavioural responses and check if mortality occurred. In the second phase, three animals were divided into one fish for aqueous and ethanolic extracts (1600 mg/kg, 2900 mg/kg, and 5000 mg/kg). A total of 6 experimental fish were used in the second phase. Each group was observed for 24 hours, and the number of death was recorded. The arithmetic method of Lorke (1983) was used to determine LD_{50}. The equation is given as:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where,

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Miller and Tainter's method (1944)

C. gariepinus was divided into five groups, which contained four fishes that weighed 1 kg for each treatment. The aqueous and ethanolic extracts were administered in 10, 50, 90,130, and 170 mg/kg oral doses. The fishes were observed during the 2nd and at the 6th hour and 24th hour. Behavioural responses and mortality were recorded. The data obtained were used to calculate the probit values. These were plotted against log-doses, and then the dose corresponding to probit 5%, i.e., 50%, was calculated.

Statistical analysis

Analysis of variance (ANOVA) at a 5% significance level was used to test the data obtained for nutritional, mineral, and phytochemical compositions of the aqueous and ethanolic extracts of *C. ferruginea*.

RESULTS AND DISCUSSION

Nutritional composition of C. ferruginea leaves

The increasing world fish demand pressure has led to intensive aquaculture practices. As a result, diseases are emerging. To prevent or control the emergence of diseases, fish farmers apply antibiotics. Furthermore, many chemical substances are applied to fish feed or water to aid growth, boost reproduction or treat water (Durojaiye & Sule, 2018). However, the use of chemical substances in treating diseases and raising fish is not without their challenges,

majorly, antibiotic resistance and public health concerns from consuming residues of these substances in the flesh of treated fish. Thus, the search for alternatives to these chemical substances in fish health management has led to herbal plants. Plants are known to contain a diversity of compounds with varied beneficial functions. However, some are reported to be toxic even at low concentrations. Hence, instead of relying on trial and error, understanding the chemical composition of a plant of interest is vital as this will serve as a guide before administration to fish.

The nutritional composition of *C. ferruginea* leaves presente in Table 1. The moisture contents recorded showed an increasing trend (crude powder > aqueous > ethanolic) with values of 13.64±0.04, 42.32±0.09, and 62.84±0.06, respectively. The analyzed ash contents for the crude powder, aqueous, ethanolic extracts were 1.47±0.02, 1.06±0.01, and 0.85±0.01, respectively. The considerable ash content observed suggests that *C. ferruginea* has moderate elemental mineral composition. For crude fat, the crude powder sample had the highest value (3.27±0.06) followed by ethanolic extract (1.33±0.02), and the lowest value was recorded in the aqueous extract (0.32±0.01). Plants are generally characterized by low lipid content (Achi *et al*., 2017). This may explain the value obtained in this study-dietary fibre regulates bowel movement, proper digestion, and effective eradication of wastes from the body. Fibre also lowers serum cholesterol, constipation, and heart diseases (Viuda-Martos *et al*., 2010; Narzary & Basumatary, 2019). The values of 2.08±0.07, 1.40±0.02, and 0.98±0.01 were recorded for crude fibre in powder, aqueous and ethanolic extracts, respectively. The crude powder had the highest protein content (4.22±0.03), followed by aqueous extract (2.76±0.03) and ethanolic extract (2.14±0.03). The result showed a low protein concentration with less than 5% recorded across the samples. Hence it is not a good protein source for fish (Spinelli, 1979). A decreasing trend (75.05±0.25 < 52.09±0.19 < 31.21±0.61) was observed in the crude powder, aqueous, and ethanolic extracts; respectively, its meal can be a good source of energy. The values obtained for each parameter measured were significantly different ($p<0.05$) across all the samples.

The mineral content of C. ferruginea leaves

Minerals are important in fish nutrition as they have been credited in sustenance and improvement functions of the muscle, heart, and brain and the production and maintenance of strong bones and teeth (Jequier & Constant, 2010; Haruna *et al*., 2015). The mineral composition of *C. ferruginea* leaves across the samples were significantly different ($p<0.05$) (Table 2). The crude powder sample had the highest values recorded for all the minerals analyzed, followed by the aqueous and then ethanolic extract except for iron and copper, where ethanolic extract had higher values than aqueous extract. Manganese (1.69±0.01, 1.06±0.04, 0.74±0.06), iron (4.66±0.14, 1.44±0.16, 3.86±0.04), zinc (2.82±0.04, 2.16±0.04, 0.90±0.00) and copper (0.79±0.01, 0.37±0.03, 0.59±0.01) were present in considerable amounts while potassium (134.68±0.12, 131.47±0.13, 5.17±0.06), phosphorus (68.23±0.23, 63.11±0.11, 7.17±0.17), magnesium (23.74±0.06, 21.48±0.02, 2.87±0.00) and calcium (11.86±0.04, 9.74±0.06, 3.11±0.09) contents were relatively high in the crude powder, aqueous extract and ethanolic extract respectively. Except for the ethanolic extract, the plant can be an excellent source of minerals, especially potassium and phosphorus, which are important in maintaining physiological processes like energy generation,cell growth, and the structural framework for DNA and RNA (Gharibzahedi & Jafari, 2017).

Table 1. Proximate composition of *C. ferruginea*.

Parameters	Crude powder (g/100 g)	Aqueous extract (g/100 g)	Ethanolic extract (g/100 g)
Moisture	13.64±0.04^{c}	42.32±0.09^{b}	62.84±0.06^{a}
Dry matter	86.10±0.22^{a}	57.23±0.55^{b}	36.78±0.33^{c}
Ash	1.47±0.02^{a}	1.06±0.01^{b}	0.85±0.01^{c}
Fat	3.27±0.06^{a}	0.32±0.01^{c}	1.33±0.02^{b}
Crude fibre	2.08±0.07^{a}	1.40±0.02^{b}	0.98±0.01^{c}
Crude protein	4.22±0.03^{a}	2.76±0.03^{b}	2.14±0.03^{c}
Carbohydrate	75.05±0.25^{a}	52.09±0.19^{b}	31.21±0.61^{c}

*Means with a different superscript in the same row are significantly different ($p<0.05$).

Table 2. Mineral composition of *C. ferruginea*.

Mineral composition	Crude powder (g/100 g)	Aqueous extract (g/100 g)	Ethanolic extract (g/100 g)
Calcium	11.86±0.04^{a}	9.74±0.06^{b}	3.11±0.09^{c}
Magnesium	23.74±0.06^{a}	21.48±0.02^{b}	2.87±0.00^{c}
Potassium	134.68±0.12^{a}	131.47±0.13^{b}	5.17±0.06^{b}
Phosphorus	68.23±0.23^{a}	63.11±0.11^{b}	7.17±0.17^{c}
Iron	4.66±0.14^{a}	1.44±0.16^{c}	3.86±0.04^{b}
Zinc	2.82±0.04^{a}	2.16±0.04^{b}	0.90±0.00^{c}
Copper	0.79±0.01^{a}	0.37±0.03^{c}	0.59±0.01^{b}
Manganese	1.69±0.01^{a}	1.06±0.04^{b}	0.74±0.06^{c}

*Means with a different superscript in the same row are significantly different ($p<0.05$).

Table 3. Phytochemical composition of *C. ferruginea*.

Phytochemical composition	Crude powder (g/100g)	Aqueous extracts (g/100 g)	Ethanolic extracts (g/100 g)
Alkaloids	8.12±0.00[a]	7.62±0.05[b]	2.06±0.04[c]
Flavonoids	3.72±0.15[a]	3.02±0.01[b]	1.06±0.00[c]
Glycosides	0.93±0.01[a]	0.34±0.01[c]	0.79±0.00[b]
Saponin	12.31±0.19[a]	10.97±0.13[b]	3.47±0.02[c]
Tannins	6.43±0.17[a]	5.64±0.16[b]	1.09±0.11[c]
Phenolic compound	1.67±0.03[a]	1.03±0.07[b]	0.88±0.06[b]
Terpenoids	0.88±0.02[a]	0.29±0.01[c]	0.78±0.02[b]
Steroids	0.39±0.01[a]	0.11±0.00[c]	0.29±0.01[b]
Oxalate	16.11±0.01[a]	12.66±0.06[b]	4.89±0.01[c]
Phytate	1.37±0.03[a]	0.53±0.07[c]	1.06±0.04[b]

*Means with a different superscript in the same row are significantly different ($p<0.05$).

Table 4. Mortality of experimental fish for Lorke's method.

Dose (mg/kg)	Aqueous extract	Ethanol extract
Phase 1		
10	1/3	1/3
100	0/3	0/3
1000	0/3	0/3
Phase 2		
1600	0/1	1/1
2900	0/1	0/1
5000	0/1	1/1

Table 5. Behavioural Responses of experimental fish to Aqueous Extract (Lorke's method).

Phase 1			
Time	10 mg	100 mg	1000 mg
0 hour	No unusual reaction observed	No unusual reaction observed	No unusual reaction observed
2nd hour	Weak and responded poorly to feed	Fairly active and responded fairly well to feed	Active and responded fairly well to feed
6th hour	Continual weakness with fish hanging within the water column	Weakness sets in	Hanging of some fish within the water column
24th hour	One mortality occurred	Recovery from weakness	Recovery from weakness
Phase 2			
Time	1600 mg	2900 mg	5000 mg
0 hour	No unusual reaction observed	No unusual reaction observed	No unusual reaction observed
2nd hour	Fairly active	Fairly active and responded well to feed	Fairly active and responded well to feed
6th hour	Continual weakness in fish	Weakness occurs	Fairly active
24th hour	Recovered from weakness	Recovery from weakness	Fairly active

Phytochemical composition of C. ferruginea leaves

The crude powder recorded the highest values for all parameters measured. The values obtained for alkaloids, flavonoids, saponin, tannin, phenolic compounds, and oxalates showed a significant increase ($p<0.05$) in the crude powder sample, aqueous extract, and ethanolic extract, respectively. However, the ethanolic extract had higher values than the aqueous extract for glycosides, terpenoids, and steroids. Of the phytochemical components analyzed, oxalate had the highest value (16.11±0.01) followed by saponin (12.31±0.19), alkaloids (8.12±0.00), and tannin (6.43±0.17). The lowest values were recorded in steroids (0.11±0.00), terpenoids (0.29±0.01), and glycosides (0.34±0.01) (Table 3).

Table 6. Behavioural responses of experimental fish to ethanolic extract (Lorke's method).

Phase 1			
Time	10 mg	100 mg	1000 mg
0 hour	No unusual reaction observed	No unusual reaction observed	No unusual reaction observed
2nd hour	Respond to feed fairly and slightly slow in movement	Active and responded very well to feed	Active and responded very well to feed
6th hour	Fairly active	Active	Active.
24th hour	One mortality occurred	Consistently active	Consistently active
Phase 2			
Time	1600 mg	2900 mg	5000 mg
0 hour	No unusual reaction observed	No unusual reaction observed	No unusual reaction observed
2nd hour	Active and responded fairly well to feeding	Active and responded fairly well to feeding	Active and responded fairly well to feeding
6th hour	Fairly active and hanging within the water column	Weakness sets in, and hanging of fish is observed	Consistently active
24th hour	Mortality occurred	Increased weakness	Mortality occurred

Table 7. LD_{50} determination for aqueous and ethanolic extracts of *C. ferruginea* (Miller and Tainter method).

Aqueous extract					
Group	Doses mg/kg	Log doses	Percentage dead	Percentage corrected	Probits
1	10	1	25	25	4.33
2	50	1.69	0	6.25	3.49
3	90	1.95	0	6.25	3.49
4	130	2.11	0	6.25	3.49
5	170	2.23	50	50	5.00
Ethanol extract					
Group	Doses mg/kg	Log doses	Percentage dead	Percentage corrected	Probits
1	10	1	0	6.25	3.49
2	50	1.69	25	25	4.33
3	90	1.95	0	6.25	3.49
4	130	2.11	0	6.25	3.49
5	170	2.23	0	6.25	3.49

Table 8. Behavioural responses in *C. gariepinus* to aqueous and ethanol extracts of *C. ferruginea* (Miller and Tainter method).

Doses mg/kg	Aqueous extract	Ethanol extract
10 mg	Fairly active One mortality was recorded	Responded to feeding Active
50 mg	They did not respond to feed well and were reasonably active	Fairly responds to feeding Weak
90 mg	Some of the fish were hanging Respond to feeding fairly	One mortality occurred Responds to feeding Active
130 mg	Responds to feeding Weak	Responds to feeding Active
170 mg	Some of the fish are weak Two mortality was recorded	Responds to feeding Consistently active

Alkaloids have been reported to possess anti-cancer, antimicrobial, analgesic, anti-fungal, and anti-inflammatory (Saxena *et al*., 2013). Saponins also possess antimicrobial properties, protecting them from microbial pathogens (Sczkowski *et al*., 1988). They could also be beneficial in modulating blood lipids, inhibiting tumour growth, and

strengthening the immune system (Igidi & Edene, 2014). Terpenoids possess antibacterial, anti-viral, and anti-parasitic properties (Franklin *et al*., 2001). Hence, the leaves of *C. ferruginea* can be helpful in fish health management. Phytochemicals screening of *C. ferruginea* revealed alkaloids, flavonoids, glycosides, saponins, tannins, phenolic compounds, terpenoids, steroids, oxalate, and phytate. However, according to Akharayi *et al*. (2012), terpenoids were not found in the plant, while Olugbade *et al*. (1982) reported the absence of saponin. Enemor *et al*. (2015) reported an abundance of alkaloids, moderate presence of flavonoids, glycosides, and saponins with the calm presence of tannins in the petroleum ether extract of *C. ferruginea*. This report revealed the presence of glycosides and flavonoids in small quantities, which agrees with Essiet *et al*. (2013). The tannin levels observed in this study are high and can interfere with digestive processes by inhibiting protease. Saponin levels were also high, retard growth and damage intestinal mucosa in fish (Francis *et al*., 2001). Hence, caution must be sought before inclusion in fish feed.

In phase 1 of Lorke's method, mortality was recorded in the fish fed 10mg/kg dose of aqueous and ethanolic extracts of *C. ferruginea*. No mortality was observed in fish fed aqueous extract of *C. ferruginea* in Phase 2, while one mortality was recorded for fish fed 1.600 mg/kg and 5.000 mg/kg of ethanolic extract of *C. ferruginea* (Table 4). From this result, LD_{50} was calculated to be 223.61 mg/kg for aqueous extract of *C. ferruginea* and 170.29 mg/kg for ethanolic extract. The behavioural responses of the test animals fed *C. ferruginea* aqueous extract in the first few hours showed no abnormal reaction. Six hours into the trial, fish became weak and responded somewhat to feeding. This weakness progressed till the 12th hour, and at the 24th hour, death was recorded in fish fed 10 mg/kg of C. ferruginea aqueous extract (Table 5). A reverse trend was observed in fish fed ethanolic extract of *C. ferruginea*, with fish consistently active for the entire feeding trial. However, mortality was recorded at the end of phase 1 (10 mg/kg) and phase 2 (1.600 mg/kg and 5.000 mg/kg) (Table 6).

Toxicity is the degree to which a substance can affect an organism. Many toxicity studies are conducted to evaluate the toxic effect that could threaten consumers' lives. LD_{50} is the amount of material given all at once, which causes the death of 50% of a group of test animals. It is a way to measure the short-term poisoning potential of a material. LD_{50} less than 500 mg/kg indicates high toxicity, 500 to 1.000 mg/kg indicates moderate toxicity while 1.000 to 2.000 mg/kg indicates low toxicity. The LD_{50} obtained from this study for *C. ferruginea* range from 124.82 mg/kg to 223.61 mg/kg, indicating that the plant is toxic. However, Venkatesh *et al*. (2003) opined that 250 mg/kg dose of *C. ferruginea* had no adverse effect on the experimental animals from previous toxicity tests conducted. This variation could be a difference in the phytochemical composition of the test ingredient used.

The log doses and probits for aqueous and ethanolic extracts of *C. ferruginea* are shown in Table 7. From these values, the LD_{50} was determined. The LD^{50} value of 124.82 mg/kg was calculated for aqueous extract, while 128.63 mg/kg was recorded for ethanolic extract of *C. ferruginea*. The behavioural responses observed include weakness, hanging within the water column, slow response to feeding. Mortality was recorded in some doses (10 mg/kg aqueous, 50 mg/kg ethanolic and 170 mg/kg aqueous) (Table 8).

CONCLUSION

The present study reveals the nutritional composition, mineral content, phytochemical composition, and toxicity of *C. ferruginea* in fish. The results show the potential for the use of the plant in aquaculture, especially in nutrition and health management. The plant contains a high level of carbohydrates. Hence, it will be a good source of energy to fish. Furthermore, its rich phytochemical constituent could help treat pathogenic and parasitic diseases in farmed fish. The extract of the plant can also be used to control aquatic parasitic fauna and aquatic pests. However, the use of this plant in aquaculture should be done with caution as it is toxic with an LD_{50} value range of 124.82 mg/kg to 223.61 mg/kg. Hence, it can cause economic loss if carelessly applied.

REFERENCES

Achi, N.K., C. Onyeabo, C.A. Ekeleme-Egediwe and J.C. Onyeanula. 2017. Phytochemical, proximate analysis, vitamin and mineral composition of aqueous extract of *Ficus capensis* leaves in South-Eastern Nigeria. Journal of Applied Pharmaceutical Science. 7 (3): 117-122.

Adisa, R.A., M.I. Choudhary, E.O. Adewoye & O.O. Olorunsogo. 2010. Hypoglycaemic and Biochemical Properties of *Cnestis ferruginea* Afr. J. Trad. C.A.M. 7 (3): 185-194. https://doi.org/10.4314/ajtcam.v7i3.54774

Adnan, M., J. Hussain, T.M. Shah, Z.K. Shinwari, F. Ullah, A. Bahader, N. Khan, A.L. Khan, & T. Watanabe. 2010. Proximate and nutrient composition of medicinal plants of humid and sub-humid regions in North-west Pakistan. Journal of Medical Plants Research. 4 (4): 339-345.

Akharaiyi, F.C., B. Bolatito & F.C. Adetuyi. 2012. Antibacterial, phytochemical, and antioxidant properties of *Cnestis ferruginea* D.C. (Connaraceae) extracts. Journal of Microbiology, Biotechnology and Food Sciences. 2 (2) 592-609.

AOAC. 2000. Official Method of Analysis. 17th ed. The Association of Official Analytical Chemists International, Gaithersburg, MD, U.S.A.

Caruso, D., A.M. Lusiastuti, T. Taukhid, J. Slembrouck, O. Komarudin & M. Legendre. 2013. Traditional pharmacopoeia in small-scale freshwater fish farms in West Java, Indonesia: an ethnoveterinary approach. Aquaculture. 416-417: 334-345. https://doi.org/10.1016/j.aquaculture.2013.09.048

Deciga-campos, M., I. Rivero-couz, M. Arriaga-albam, G. Castaneda-corral, G.E. Angeles-lopez, A. Navarrete & R. Mata. 2007. Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. J. Ethnopharmacol 110: 334-342. https://doi.org/10.1016/j.jep.2006.10.001

Durojaiye, A.F & S.O. Sule. 2018. A Preliminary Assessment of Aquamedicines Used at Eriwe Fish Farm Community in Ogun State. Ogueji, E.O. (Ed). 33rd Annual Conference of Fisheries Society of Nigeria (FISON) at Lagos State Polytechnic, Ikorodu, Lagos, 29th October-2nd November, 2018. 52-56 pp.

Sule, S.O., A.F. Durojaiye & T.A. Ojetayo. 2019. Health Management Practices Adopted in Fish Production at Eriwe Fish Farming Community, Ogun State, Nigeria. Nigerian Journal of Scientific Research. https://journal.abu.edu.ng/index.php/njsr/article/view/66

Enemor, E.C., T.N. Akagha, K.G. Ngwoke, T.H. Gugu, A.N. Oli, C.O. Eze, B.C. Ugwu, P.C. Ejikeugwu & M.C. Ugwu. 2015. Phytochemical analysis and antimicrobial activity of ethanolic stem extracts of *Cnestis ferruginea* on multidrug-resistant bacteria isolated from raw retail meat sold in Awka, Nigeria). Pharm. Sci. & Res. 7 (11): 1044-1049.

Essiet, U. & U.I. Akpan. 2013. Comparative phytochemical and physicochemical properties of *Aspilia africana* (Pers) and *Tithonia diversifolia* petals as a scientific backing to their tradomedicinal potentials. International Journal of Modern Biology and Medicine. 3 (2): 88-100.

Francis, G., H.P.S. Makkar & K. Becker. 2001. Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 199:197-227.https://doi.org/10.1016/S0044-8486(01)00526-9

Franklin, L.U., G.D. Cunnington & D. Young. 2001. Terpenes-based pesticide treatments for killing terrestrial arthropoda including among others, lice, lice eggs, mites and ants https://patents.google.com/patent/EP1211938A1/en.

Funsho, O.O., R. Yinusa, A.A. Olajire & O.O. Mathew. 2013. Haematological and some biochemical profiles in male rats treated with *Cnestis ferruginea* (de Candolle) root extract and its pure fractions. African journal of pharmacy and pharmacology. 7 (20): 1231-1235

Gharibzahedi, S.M.T & S.M. Jafari. 2017. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and Nanoencapsulation. Trends in Food Science & Technology. 62: 119-132. https://doi.org/10.1016/j.tifs.2017.02.017

Haruna, S.S., O. Ahmed & J.O. Titilayo. 2015. Nutritional and anti-nutritional composition of *Lantana camara* leaf. Journal of Investigational Biochemistry. 4 58-60.

Ifemeje, J.C., C. Egbuna, J.O. Eziokwudiaso & F.C. Ezebuo. 2014. Determination of the anti-nutrient composition of *Ocimum gratissimum*, *Corchorus olitorius*, *Murraya koenigii Spreng* and *Cucurbita maxima*. International Journal of Innovation and Scientific Research. 3 (2): 127-133.

Igidi, O.J & C.E. Edene. 2014. Proximate and phytochemical compositions of *Napoleona vogelii* hook fruit. The International Journal of Engineering and Science. 3 (6): 46-5.

Ipek, S.Ö & Y. Faruk. 2020. Phytonutrients in Food. Elsevier Woodhead Publishing. 255 pp.

Iranloye, B., K. Oyeusi & A. Alada. 2010. Effect of Aqueous Extract of *Phyllanthus amarus* Leaves on Implantation and Pregnancy in Rats. Nigerian Journal of Physiological Science. 25 (1): 63-66.

Irvin, F.R. 1961. Woody plants of Ghana. Oxford University Press. 146-147 pp.

Jayapriya, G. & F.G. Shoba. 2014. Screening for the phytochemical activity of *Urechites lutea* plant. Asian Journal of Plant Science and Research. 4 (6): 20-24.

Jéquier, E & F. Constant. 2010. Water as an essential nutrient: The physiological basis of hydration. European Journal of Clinical Nutrition. 64 (2): 115-123. https://doi.org/10.1038/ejcn.2009.111

Lorke, D. 1983. A new approach to practical acute toxicity testing. Archives of Toxicology. 54: 275-287. https://doi.org/10.1007/bf01234480

Miller, L.C & M.L. Tainter. 1944. Estimation of ED_{50} and its error by means of log-probit graph paper. Proceedings of the Society for Experimental Biology and Medicine. 57 (2): 261-264. https://doi.org/10.3181/2F00379727-57-14776

Mohd-fuat, A.R., E.A. Kofi & E.G. Allan. 2007. Mutagenic and cytotoxic properties of three herbal plants from Southeast Asia. Tropical Biomedicine. 24 (2): 49-59. https://pubmed.ncbi.nlm.nih.gov/18209708/

Narzary, H & S. Basumatary. 2019. Amino acid profiles and anti-nutritional contents of traditionally consumed six wild vegetables. Current Chemistry Letters. 8 (3):137–144.

Okoye, T.C., P. Uzor, C.A. Onyeto & E.K. Okereke. 2014. Safe African medicinal plants for clinical studies. In: Toxicological survey of African medicinal plants. Elsevier, U.S.A. 742 pp.

Olugbade, T.A., J.O. Oluwadiya & W.A. Yisak. 1982. Chemical constituents of *Cnestis ferruginea* petroleum ether fraction. Journal of Ethnopharmacology. 6 (3): 365-370. https://doi.org/10.1016/0378-8741(82)90058-7

Rajesh, K.D., S. Vasantha, N.V. Rajesh & A. Panneerselvam. 2014. Qualitative and quantitative phytochemical analysis in four pteridophytes. International Journal of Pharmaceutical Sciences Review and Research. 27 (2): 408-412.

Saxena, M., J. Saxena, R. Nema, D. Singh & A. Gupta. 2013. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry Center for Microbiology and Bio-Technology Research and Training, Bhopal, India. 8192 (1): 168-182

Sczkowski, C.P., M. Kalinowska & Z. Wojciechowski. 1998. The 3-Oglucosylation of steroidal saponins and alkaloids in eggplant (*Solanum melongena*); evidence for two separate glycosyl transferences. Phytochemistry. 48: 1151-1159.

Spinelli, J. 1979. Influence of feed in finfish quality. In: Finfish nutrition and feed technology. Eds: Havler, J.E. and Tiews, K. Proceedings of a World Symposium sponsored by EIFAC/FAO/ICES/IUNS, Hamburg, 20-23 June, 1978 Schr.Bundesforschungsanst.Fisch., Hamb. (14/15) 2: 45-52

Tiwari, P., B. Kumar, M. Kaur, G. Kaur & H. Kaur. 2011. Phytochemical screening and extraction: A review. Int Pharm Sci. 1: 98-106.

Unuofin, J.O., G.A. Otunola & A.J. Afolayan. 2017. Essential oil composition, nutrient and anti-nutrient analysis of *Vernonia mespilifoli*. Research Journal Botany. 12 (2): 38-45. https://dx.doi.org/10.3923/rjb.2017.38.45

Venkatesh, S., R.G. Dayanand, R.B. Madhawa, M. Ramesh

& A.V.N. Apparao. 2003. Antihyperglycemic activity of Caralluma. Fitoterapia. 73: 274-279

Viuda-Martos, M., M.C. López-Marcos, J. Fernández-López, E. Sendra, J.H. López-Vargas & J.A. Pérez-Álvarez. 2010. Role of fiber in cardiovascular diseases: A review. Comprehensive Reviews in Food Science and Food Safety. 9 (2): 240-258. https://doi.org/10.1111/j.1541-4337.2009.00102.x