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Efficacy of Aqueous Crude Fruit Extract of Desert Date (*Balanites aegyptiaca*) in Anaesthetization of African Catfish (*Clarias gariepinus*) Fingerlings

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ABSTRACT

Chemicals have been used to anaesthetize fish but due to their hazardous effects on the environment, fish and humans environmentally friendly plant anaesthetics are being sought. Fruit of Desert date (*Balanites aegyptiaca*) is nontoxic to humans but has been reported to have anaesthetic potentials on fish. This study investigates anaesthetic effect of aqueous crude fruit extract of *B. aegyptiaca* on African catfish (*Clarias gariepinus*) fingerlings. Phytochemical and proximate compositions of the fruit were screened. Exactly 120 *C. gariepinus* fingerlings (mean weight 32.13±2.43g and mean total length 23.88±2.11cm) were used for the experiment. A total of 10 fingerlings were exposed to each of 2.00, 2.50, 3.00, 3.50 and 4.00g/L concentrations of aqueous crude fruit extract of *B. aegyptiaca* and a control in 6 plastic tanks (45x28x25cm) filled with 10L of tap water. Setup was arranged in randomized block design and replicated. Temperature, dissolved oxygen, pH, free carbon dioxide and total alkalinity were monitored. Results revealed long mean induction (25.05±3.35 min) and recovery (108.35±2.45 min) times with resultant mortalities (40%) of *C. gariepinus* fingerlings. significant correlation (P<0.01) exists between concentration of the fruit extract and induction time as well as between fruit extract concentrations and survival of fingerlings. Aqueous crude fruit extract of *B. aegyptiaca* is, therefore, not effective for anaesthetization of *C. gariepinus* fingerlings hence should be avoided.

Keywords: Anaesthetic, *Balanites aegyptiaca*, *Clarias gariepinus*

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INTRODUCTION

Aquaculture manipulations and transportation cause stress in fish which result into suppression of immunity, physical injury, or even death (Neiffer & Stamper, 2009) [1]. The stress caused by aquaculture practices could be avoided through anaesthesia. According to Javahery & Moradlu (2012)[2], anaesthesia is a biological state with complete loss of sensation or loss of voluntary neuromotor control caused by chemical or non chemical means. Anaesthetics are therefore used to immobilize fish for easy handling during aquaculture practices such as harvesting or capturing, sorting, tagging, sampling, artificial reproduction procedures and surgery (Matin, Hossain & Hashim, 2000[3]; Javahery, Nechoubin & Moradlu, 2012[4]).

Stuart (1985)[5] reported that the widely used chemical anaesthetics for fish anaesthesia have gradually changed so much so that many of the first generation anaesthetics are now out of use due to health hazards, lack of adequate efficacy and adverse physiological effects on fish (Ramanayaka & Atapattu, 2006) [6]. In aquaculture chemical anaesthetics are gradually replaced with organic ones such Tricane Methanesulphate (MS-222), benzocaine, etomidate, metomidate, 2-phenoxyethanol, quinaldine, quinaldine sulphate, ketamine, and eugenol (Hajek, Kłyszejko, Dziaman, 2006[7] and Neiffer & Stamper, 2009)[1]. Out of these anaesthetics only MS-222 has been permitted for use in food fish anaesthesia mainly due to environmental and health risks (Marking & Mayer, 1985[8]; Ramanayaka & Atapattu, 2006 [6]) hence the search for environmentally friendly plant anaesthetics (Marking & Mayer, 1985[8]; Kamble, Saini, & Ojha, 2014[9]) becomes necessary. This study therefore seeks to find out the efficacy of aqueous fruit extract of *Balanites aegyptiaca* in anaesthetization of *Clarias gariepinus* fingerlings.

The Experimental Plant (*B. aegyptiaca*)

Balanites aegyptiaca commonly called Desert date, Soapberry tree, Egyptian balsam or thorn tree (Chothani & Vaghasiya, 2011) [10] is a member of the family

Zygophyllaceae. In northern Nigeria it is commonly referred to as 'Aduwa' by the Hausa tribe while the Fulani tribe calls it 'Tanni' (Onyema, Chinedu & Ahmad, 2017) [11]. It is called 'mchunju' by Swahilis, 'Hingot' by Indians and 'Heglig' by Arabs while Amharics in Ethiopia call it 'Bedena' (Tesfaye, 2015) [12]. It is a deep-rooted, evergreen or semi-deciduous, multi-branched, spiny tree which grows up to 12m in height. The fruit of *B. aegyptiaca* is an ellipsoid drupe, about 2.5-4.0cm long and 1.2.0cm in diameter (Tesfaye, 2015) [12]. The ripe fruit is brown or pale brown to yellow and resembles a small date. In humans the edible bitter-sweet pulp is used as food or confectionary, and laxative as well as for the treatment of constipation (Nkunya, Weenen, & Bray, 1990)[13].

Phytochemical Constituents of *B. aegyptiaca* and other Plants

Salihu, Nwozo & Oloyede (2013)[14] screened the fruit mesocarp of *B. aegyptiaca* collected from Ilorin, Kwara State, Nigeria and reported presence of alkanoids, saponin, steroid, flavonoids, and cardiac glycosides. Absalom, Nwadiaro & Wophill (2013)[15] also worked on the fruit mesocarp collected from Jos, Nigeria and reported presence of alkaloids, tannins, saponins, anthraquinones, steroids, flavonoids and cardiac glycosides as active ingredients. Similarly, Vijay, Nivedita, & Bellundagi (2013)[16] collected *B. aegyptiaca* fruit from Rajasthan Jaipur, India and reported the presence of alkanoids, flavonoids, glycosides, saponins, and terpenoids. In their phytochemical screening, Kumar, Sangeetha, Suchitra, Ravishinkar, & Yashovarma (2016)[17] also reported the presence of amino acids, carbohydrates, steroids, saponins coumarins and triterpenoids while alkanoids, tannins, flavonoids and phenols were absent in the fruit of *B. aegyptiaca* from Udupi, India.

Proximate Composition of *B. aegyptiaca* and other Plants

Proximate composition of plant parts have been documented by many authors. Umar, Abubakar, Alhassan, Yahaya, Hassan, & Sani (2014)[18] reported analyzed the flower of *B. aegyptiaca* and reported 10.8% crude protein, 43.3% moisture and 4.50% crude lipid. Ekpete, Edori & Fubara (2013[19]) reported 1.28% crude protein, 0.53% crude lipid, 80.53% moisture content and 0.21% crude fibre for fruit of *Psidium guajava* while Marcel & Bievenu (2012)[20] reported 24.18 and 25.37% crude protein for *Hippocratea myriantha* and *Urera Trinervis* leaves respectively. Percentage crude protein of *Brassica oleracea* reported by Emebu & Anyika (2011)[21], was 11.67% while moisture content and crude fibre were 81.38 and 3.00% respectively. Raimi, Oyekanmi & Farombi (2014)[22] reported 21.16% crude protein for *Manihot esculentus* and 18.80% for *Ceiba pentandra* leaves while moisture content were 4.85% for *Manihot esculentus* and 5.30% for *Ceiba pentandra* leaves. Marcel & Bievenu (2012)[20] earlier reported 24.18% crude protein for *Urera trinervis* leaf. Achi, Onyeabo, Ekelem-Egedigwe, & Onyeanula (2017) [23] proximately analyzed leaves of *Ficus capensis* and reported 104% moisture, 6.65% ash, 6.31% crude protein and 73.77 carbohydrate.

The Experimental Fish (*C. gariepinus)*

The African catfish (*C. gariepinus*) is a large, eel-like fish, usually of dark gray or black coloration on the back, fading to a white belly. *C. gariepinus* has an average adult length of 1–1.5m. African catfish can weigh up to 60kg (Froese, Rainer & Pauly, Daniel, 2014) [24] These fish have slender bodies, flat bony heads and broad, terminal mouths with four pairs of barbels. They also have large accessory breathing organs composed of modified gill arches (Froese, *et al.*, 2014) [24]. Though *C. gariepinus* is hardy and tolerates poor water quality conditions, it is susceptible to stress during transportation and aquaculture practices such as sorting, tagging, artificial reproduction procedures, sampling and harvesting (Javahery *et al.*, 2012) [4]. *C. gariepinus* is found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems (Clay, 1989) [25].

MATERIALS AND METHODS

Place of Collection of *Balanites aegyptiaca* Fruits

The fruits of the experimental plant (*Balanites* aegyptiaca) were collected from Gashua, Bade Local Government Area of Yobe State; North-eastern Nigeria. The State borders the Nigerian states of Bauchi, Borno, Gombe, and Jigawa. It also borders Diffa and Zinder Regions of the Republic of Niger to the North. The climatic conditions of the state are hot and dry for most of the year, except in the southern part of the state which has a milder climate due to its location in the dry Savannah belt (Hamidu, 2010) [26].

Bade Local Government Area, one of the seventeen local government areas of Yobe State, has its Headquarters at Gashua. It is located in the north-western part of the state at 12°52"5°N 11°2"47°E. It is bordered in the North by Karasuwa Local Government area, in the south by Jakusko Local Government area, in the east by Bursari Local Government area and in the west by Jigawa state, Nigeria (Hamidu, 2010) [26].

Obtaining Fruits of *B. aegyptiaca* and *C. gariepinus* Fingerlings

. The fruit of the plant was collected in Gashua, Bade local Government area of Yobe state. A quantity of 1kg of ripe fruits was collected from five (5) *B. aegyptiaca* plants located 3km north of Gashua town. The fruits were collected by deliberately striking the branches of the plant with a long stick and the dropped ripe fruits were handpicked.

A quantity of 120 freshly caught *C. gariepinus* fingerlings was obtained from artisanal fishermen along River Yobe and transported in two oxygenated cellophane bags (60 fingerlings per bag) to Biology laboratory of Umar Suleiman College of Education, Gashua for acclimation.

Preparation of Fruit of *B. aegyptiaca*

The obtained fruits of the experimental plant were washed with clean water several times to remove soil, dust or dirt. A quantity of 1kg of the fruit was shade dried to prevent the loss of active components. The dried samples were pulverized with pestle and mortar and sieved with 0.5mm sieve into fine powder and stored in an airtight plastic container.

Acclimation of *C. gariepinus* Fingerlings

The experimental fish (*C. gariepinus* Fingerlings) were acclimated at Biology Laboratory of Umar Suleiman College of Education, Gashua, Yobe State, Nigeria in six 20L capacity round plastic tanks (20 fingerlings per tank) for 14 days. Fingerlings were fed to satiation with commercial feed (Vital®) twice daily. Water was changed every other day while uneaten feed and faecal matter were siphoned out daily. Fish were starved 24 hours prior to the experiment.

Phytochemical Screening of Pulverized Crude Fruit of *B. aegyptiaca*

Standard phytochemical test was carried out on the pulverized crude fruit using methods of Trease & Evans

(1984)[27] and Soforowa (1982) [28] to determine the presence of Alkaloids, flavonins, saponins, tannins, cardiac glycosides, and terpens & steroids. Others are balsams, phenols, and resins.

Determination of Proximate Composition of Pulverized Crude Fruit of *B. aegyptiaca*

The proximate composition of the pulverized crude fruit of *B. aegyptiaca* was determined using the standard methods of Association of Official Analytical Chemist (AOAC) (2003)[29]. The following parameters were examined: moisture content, crude protein, crude fat, and fibre as well as ash contents.

Preparation of Definitive Sedative Concentrations of Pulverized Crude Fruit of *B. aegyptiaca*

Prior to the administration of the crude fruit extract to the experimental fish, preliminary experimental trials (Audu, Omirinde, Gosomji & Wazhi, 2017) [30] were carried out to determine suitable concentrations for the experiment. Subsequent to series of experimental trials 5 concentrations (2.00, 2.50, 3.00, 3.50 and 4.00g/L) of crude fruit extract of *B. aegyptiaca* were determined as definitive sedative concentrations.

Experimental Design

The experiment consists of 12 rectangular plastic tanks (45x28x25 cm) and 120 *O. niloticus* fingerlings (mean weight 32.13±2.43g and mean total length 23.88±2.11cm) arranged in randomized block design (Rezende, Pascoal, Vianna & Lanna, 2017) [31]. Each of the six (6) tanks were filled with 10L of tap water, with 5 of the filled tanks inoculated with graded levels of aqueous crude fruit extract of *B. aegyptiaca* and ten (10) *O. niloticus* fingerlings were introduced and labeled A1, B1, C1, D1 and E1 while the sixth tank (F1) that was not inoculated with the test material served as the control. Setup was replicated in Tanks A2, B2, C2, D2, E2 and F2.

Procedure for Anaesthesia of *C. gariepinus* fingerlings

Anaesthetic stages were determined as suggested by Summerfelt & Smith (1990)[32] and Javahery & Moraldu (2012)[2]. The fingerlings were simultaneously exposed to the concentrations of the plant material and observed for behavioural changes. Behavioural responses of exposed fingerlings were compared with those in the control tanks. The behavioural changes at each stage were noted and any fingerling that attained state of anaesthesia (Total loss of reflex) in any tank was removed and placed in a corresponding recovery tank containing untainted water. The times of removal and recovery of each fish were noted.

Exposure of *C. gariepinus* fingerlings to Treatment Concentrations

To each of the concentrations of the fruit extract, 10 *C. gariepinus* fingerlings were exposed by immersion method (Neifer & Stamper, 2009) [25] in tanks A1, B1, C1 D1 and E1. The remaining batch of 10 fingerlings was exposed to the control tank (F1). The setup was replicated in tanks A2, B2, C2, D2, and E2. Tank F2 served as the replicate control.

Monitoring of Water Quality Parameters

The standard methods of American Public Health Association (APHA) (1985)[33] was used to monitor water quality parameters such as temperature, dissolved oxygen (DO), free carbon dioxide (CO_2), total alkalinity (TA), and hydrogen ion concentration (pH) of the experimental tanks.

Data Analyses

SPSS statistical package (version 20) was used to analyze the data. Analysis of Variance (ANOVA) was used to determine the difference in treatment means while separation of treatment means was by Tukey's multiple comparisons test. Pearson's correlation coefficient was used to determine relationship between treatment concentrations and time of anaesthesia.

RESULTS

Phytochemical Screening of Crude Fruit of *B. aegyptiaca*

The results of the screened crude fruit of *B. aegyptiaca* using water solvent are presented in Table 1. Screening of the crude fruit extract using different tests confirmed the presence of alkanoids, flavonoids, tannins, saponins and terpenoids. Others are balsam, carbohydrates, phenols and resins. Cardiac glycosides were not confirmed in the crude fruit of *B. aegyptiaca*.

Table 1: Phytochemical Screening of Crude Fruit of *B. aegyptiaca* using Water as Solvent

PHYTOCHEMICAL	FRUIT	COLOUR	TEST
Alkanoids	+	Orange	Dragendoff's
Flavonoids	+	Cream light yellow	Lead acetate
Tannins	+	Deep blue	Ferric chloride
Saponins	+ +	Honeycomb froth	Foam test
Terpens & Steroids	+	Reddish brown ring	Burchard's
Cardiac glycosides	–	–	Salkowki's
Balsam	+	Dark green	General test
Carbohydrates	+	Blue-black	Benedict's
Phenols	+	Deep bluish green	-
Resins	+	Violet	-

++ High presence + Moderate presence – Absence

Proximate Analyses of Crude Fruit of *B. aegyptiaca*

Proximate analyses of Fruit of *B, aegyptiaca* revealed 16.40% crude protein, 11.60% crude fibre, and 10.90% crude lipid. Moisture content was 3.56% and Nitrogen free extract (NFE) constitutes 50.03% of the total nutrients (Table 2).

Table 2: Proximate Composition of Crude Fruit of *B. aegyptiaca* obtained from Gashua, Yobe State, Nigeria

PARAMETER	PERCENTAGE COMPOSITION (%)
Crude protein	16.40
Crude fibre	11.60
Crude lipid	10.90
Ash content	8.00
Moisture content	3.56
Nitrogen Free Extract (NFE)	49.54

Water Quality Analyses of Crude Fruit of *B. aegyptiaca*

Water quality parameters of experimental tanks during anaesthesia of *C. gariepinus* fingerlings with concentrations of aqueous crude fruit extract of *B. aegyptiaca* are presented in Table 3. Mean DO decreased with increase in fruit extract concentration. Control recorded mean DO of 5.55±0.20mg/L but declined to 2.05±0.65mg/L in the lowest concentration (2.00g/L) of the extract. The mean DO concentration steadily decreased from 1.50±0.50mg/L in treatment concentration 2.50g/L to 0.8±0.30mg/L in 4.00g/L. Free CO_2, however, increased with increase in concentration of the plant extract. Lowest free CO_2 (2.60±0.10mg/L) was recorded in fruit extract concentration 2.00g/L while the highest value (16.15±0.25mg/L) was recorded in the highest concentration (4.00g/L) of the fruit extract. TA also increased with increase in plant extract concentration. Lowest TA (33.50±3.50mg/L) was noted in the control followed by the least extract concentration (2.00g/L) which recorded 73.00±9.00mg/L. TA steadily increased to its peak (150.00±15.00mg/L) in the highest extract concentration (4.00g/L). There are no significant variations (P>0.05) in pH and temperature of treatment tanks compared with the control. There is, however, significant correlation (P<0.01) between fruit extract concentrations and DO, free CO_2 and TA concentrations. Increase in fruit extract concentration decreases DO while increase in the extract concentration increases free CO_2 and TA concentrations.

Table 3: Mean Water Quality Parameters of Experimental Tanks during Anaesthesia of *C. gariepinus* Fingerlings with Concentrations of Aqueous Crude Fruit Extract of *B. aegyptiaca*

Parameter	Concentrations (g/L)					
	0.00	2.00	2.50	3.00	3.50	4.00
DO (mg/L)	5.55±0.20	2.05±0.65*	1.50±0.50*	1.10±0.40*	1.05±0.45*	0.80±0.30*
Temperature (^{0}C)	29.00±1.00	29.00±1.00	29.00±1.00	30.00±2.00	30.00±2.00	30.00±2.00
pH	7.40±0.20	7.15±0.15	6.85±0.05	6.70±0.20	6.60±0.20	6.50±0.10
Free CO_2 (mg/L)	2.60±0.10*	13.1±0.10*	13.15±1.45*	15.00±3.00*	15.45±1.75*	16.15±0.25*
TA (mg/L)	33.50±3.50	73.00±9.00	91.50±2.50*	93.00±3.00*	115.50±2.50*	150.00±15.00*

Values with Asterisks (*) in the same row are significantly different compared with the control

Anaesthetic Effect of Crude Fruit Extract of *B. aegyptiaca* on *C. gariepinus* fingerlings

Mean effect of aqueous fruit extract of *B. aegyptiaca* on *C. gariepinus* fingerlings is presented in Figure 1. Behavioural responses of *C. gariepinus* fingerlings depend on the concentration of the extract and time of exposure. Induction time decreased with increase in concentration of the extract while recovery time increased with increase in concentration of the fruit extract. Statistics reveal significant correlation (P<0.01) between concentration of the fruit extract and induction time as well as between fruit extract concentrations and survival of fingerlings. After exposure to the lowest concentration (2.00g/L) of the fruit extract the fingerlings loss their normal behaviour (NM) after 10.35±0.15 min. The mean time taken to loss normal behaviour decreased to 9.35±0.45 min in 2.50g/L concentration of the fruit extract. The mean time of loss of normal behaviour continued to decrease progressively from 6.45±0.05 to 5.30±0.20 and 4.35±0.15 min in 3.00, 3.50 and 4.00g/L of the fruit extract respectively. In the light sedation stage (LS) *C. gariepinus* fingerlings show slight loss of reactivity to external stimuli and slight decrease in opercula ventilation rate in the lowest concentration (2.00g/L) of the fruit extract at mean time of 15.15±1.05 min. Duration of this behaviour progressively decreased to 14.30±0.50, 12.00±0.40 and 9.30±1.15 min in concentrations 2.50, 3.00, and 3.50g/L respectively. The shortest time (6.35±0.45 min) in the light sedation stage was recorded in the highest concentration (4.00g/L) of the fruit extract.

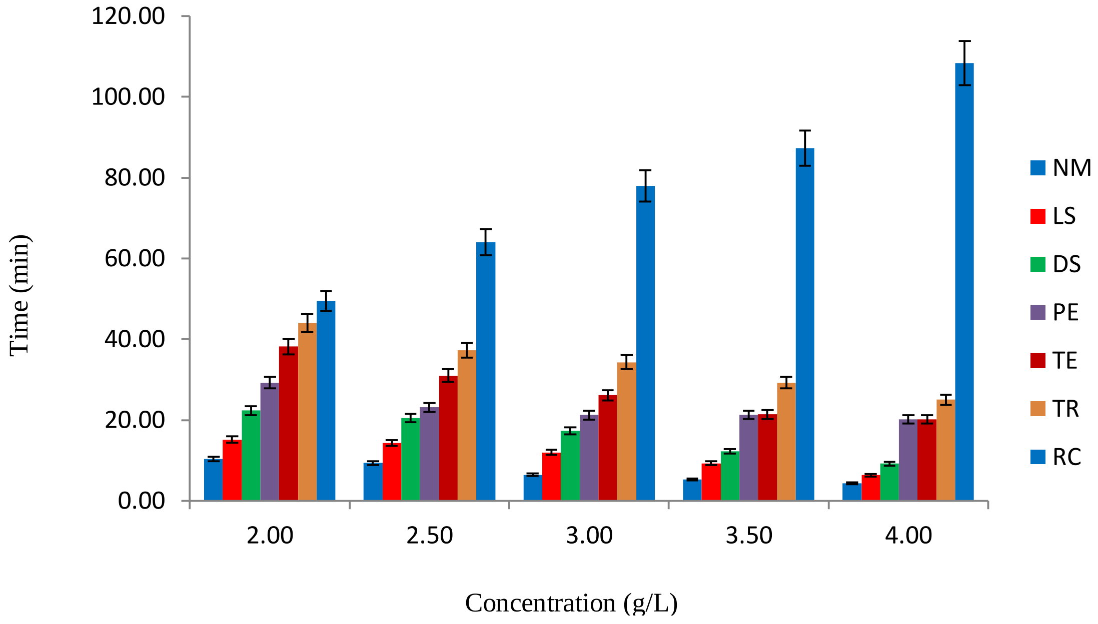

Figure 1: Mean Induction Times of *C. gariepinus* Fingerlings during Anaesthesia with Aqueous Crude Fruit Extract of *B. aegyptiaca*

NM=Normal behaviour: Normal swimming and regular opercula ventilation rate, reactive to external stimuli

LS=Light Sedation: Normal swimming and regular opercula ventilation rate, reactive to external stimuli

DS=Deep Sedation: Deep fall in opercula ventilation rate, slow swimming and partial loss of reaction to external stimuli.

PE=Partial loss of equilibrium: Partial loss of muscular tone, erratic swimming, increased opercula ventilation rate and reaction only to strong tactile or vibration stimuli.

TE=Total loss of equilibrium: Total loss of muscular tone and equilibrium, slow and regular opercula movements

TR=Total loss of reflex: Opercula movement is slow and irregular, total loss of reflex and reaction to external stimuli

RC=Recovery: Restart of opercula movement, erratic movement of the body

The mean time taken for *C. gariepinus* fingerlings to reach deep sedation stage (DS) also decreased with increase in concentration of the fruit extract. The longest mean deep sedation time (22.35±0.10 min) was recorded in the lowest fruit extract concentration (2.00g/L) while the shortest time (9.20±0.20 min) was recorded in the highest fruit extract concentration (4.00g/L). The longest mean time (29.25±1.45 min) of Partial loss of equilibrium (PE) in *C. gariepinus* fingerlings was recorded in the lowest concentration (2.00g/L) of the fruit extract while the shortest mean sedation time (20.15±0.25) was recorded in concentration 4.00g/L of the fruit extract. Fingerlings in the lowest Concentration (2.00g/L) of the extract attained the state of total loss of equilibrium (TE) at mean time of 38.15±2.35 min and progressively declined to 20.15±2.35 min in the highest concentration (4.00g/L) of the extract. In the total loss of reflex (anaesthetic) stage *C. gariepinus* fingerlings attained anaesthesia at a mean time of 44.00±1.35 min followed by fingerling in the 2.50g/L fruit extract concentration which were anaesthetized at 64.00±2.00 min. the mean anaesthetization time progressively decreased with increase in fruit extract concentration and the shortest mean anaesthetic time of 25.05±3.35 min was recorded in the highest extract concentration (4.00g/L). Statistical analyses revealed significant correlation between fruit extract concentration and recovery time. Mean recovery time of exposed fingerlings increased with increase in fruit extract concentration. The shortest mean recovery time (49.45±1.35 min) was recorded in the lowest plant extract concentration (2.00g/L) while the longest mean recovery time of 108.35±2.45 min was recorded in the highest plant extract concentration (4.00g/L).

Survival of *C. gariepinus* fingerlings (Figure 2) in the two lower extract concentrations (2.00 and 2.50g/L) was 100% while concentrations 3.00 and 3.50g/L recorded 90 and 80% survivals respectively. The lowest percentage survival (60%) was recorded in the highest extract concentration (4.00g/L). The 10, 20 and 40% recorded mortalities in fruit extract concentrations 3.00, 3.50 and 4.00g/L respectively were within 6 to 24 hours after the experiment.

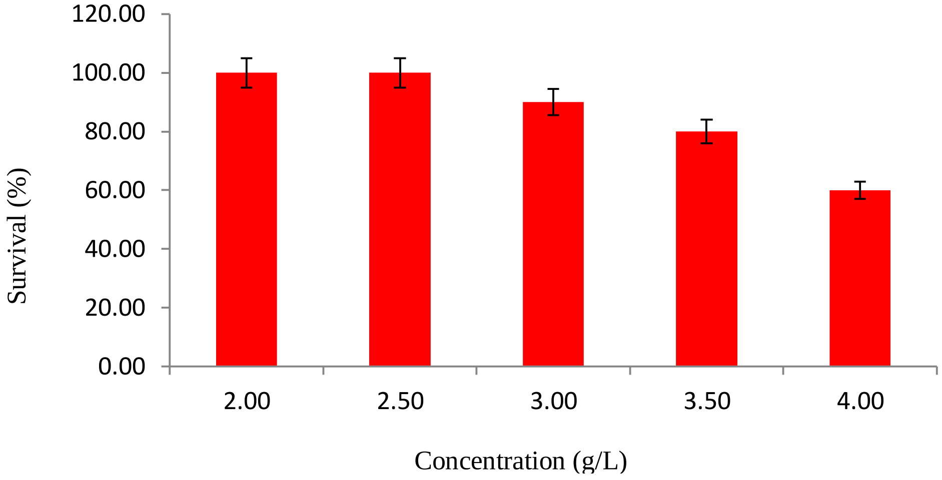

Figure 2: Mean Percentage Survival of *C. gariepinus* Fingerlings during Anaesthesia with Aqueous Crude Fruit Extract of *B. aegyptiaca*

Opercula Ventilation Rate (OVR) per Minute of *C. gariepinus* Fingerlings during Anaesthesia with Aqueous Crude Fruit Extract of *B. aegyptiaca*

Mean opercula ventilation rate (OVR) per minute of *C. gariepinus* fingerlings during anaesthesia with graded concentrations of aqueous crude fruit extract of *B. aegyptiaca* is presented in Table 4. Statistical analyses showed significant difference (P<0.05) between OVR of the fingerlings exposed to the treatments compared to those in the control. There was decrease in OVR/min as the concentration of the fruit extract increases. The mean OVR/min of *C. gariepinus* fingerlings exposed to lowest concentration (2.00g/L) of the fruit extract was 73.15±0.35 but progressively decreased to 40.15±1.00 in the highest treatment concentration (4.00g/L) while the mean OVR/min of fingerlings in the control was 95.30±1.00. Statistically, there is correlation between OVR/min of *C. gariepinus* fingerlings and the concentrations of aqueous fruit extract of *B. aegyptiaca.*

Table 4: Mean Opercula Ventilation Rate (OVR) per min of *C. gariepinus* Fingerlings during Anaesthesia with Graded Concentrations of Aqueous Crude Fruit Extract of *B. aegyptiaca*

CONC.	**OVR/MIN OF FINGERLINGS**										**MEAN**
(mg/L)	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	**OVR/MIN**
2.00	63.50 ±0.50	81.00± 3.00	65.50 ±0.50	65.50± 1.50	66.50 ±0.50	78.00 ±1.00	80.00 ±1.00	83.00 ±1.00	82.00 ±0.00	66.50± 1.50	73.15±0.35
2.50	61.00 ±1.00	60.50± 1.50	67.50 ±7.50	67.00± 4.00	74.00 ±4.00	63.00 ±0.50	62.50 ±0.50	59.00 ±1.00	61.50 ±0.50	60.00± 2.00	63.60±1.00
3.00	56.00 ±4.00	57.50± 3.50	55.00 ±1.00	55.50± 4.50	59.50 ±5.50	61.50 ±1.50	56.50 ±1.50	57.50 ±3.50	54.00 ±3.00	55.00± 2.00	56.80±1.60
3.50	50.50 ±1.50	53.00± 1.50	48.50 ±0.50	52.00± 1.00	47.00 ±0.00	47.00 ±1.00	48.00 ±0.00	50.50 ±1.50	50.50 ±0.50	50.00± 0.00	49.95±0.35
4.00	43.00 ±1.00	44.50± 3.50	44.00 ±2.00	39.00± 1.00	41.50 ±0.50	41.50 ±3.50	36.50 ±2.50	37.00 ±0.00	37.00 ±1.00	37.50± 3.50	40.15±0.15
0.00	99.00 ±4.00	100.00 ±8.0	96.00 ±2.00	108.00 ±9.0	91.50 ±0.50	99.00 ±3.00	93.50 ±1.50	94.50 ±2.50	78.00 ±0.00	98.50± 3.50	95.30±1.00

DISCUSSION

Phytochemicals are screened to determine the anaesthetic potential of the plant material. Phytochemicals with anaesthetic properties include alkanoids, flavonoids, terpenoids, cardiac glycosides and anthraquinones (Tsuchiya, 2017) [34]. Phytochemicals of crude fruit of *B. aegyptiaca* obtained in Gashua Yobe State, Nigeria include alkanoids, flavonoids, terpenoids, saponins, and cardiac glycosides. Others are tannins, phenols and resins. These show that fruit extract of *B. aegyptiaca* in the present study has anaesthetic potential. The phytochemical constituents of fruit of *B. aegyptiaca* obtained from Gashua is similar to the findings of Salihu et al. (2013)[14] who screened and reported the presence of alkanoids, saponin, steroid, flavonoids, and cardiac glycosides in fruit mesocarp of *B. aegyptiaca* collected from Ilorin, Kwara State, Nigeria. The current result also corroborates the work of Vijay et al. (2013)[16] who reported the presence of alkanoids, flavonoids, glycosides, saponins, and terpenoids in *B. aegyptiaca* fruit collected from Rajasthan Jaipur, India but not in line with the findings of Kumar et al. (2016)[17] who reported the absence of alkanoids, tannins, flavonoids and phenols in fruit of *B. aegyptiaca* collected from Udupi, India.

Proximate compositions of plant parts used in fish study are analyzed to investigate the nutrient levels and determine whether the plant part can be incorporated in diets of fish.

Proximate analyses of crude fruit extract of *B. aegyptiaca* in the present study recorded moderate percentage crude protein (16.40%) but higher than 10.8% crude protein of flower of the same plant (Umar *et al.*, 2014)[18] and 1.28% crude protein of fruit of *Psidium guajava* (Ekpete *et al.,* 2013) [19] as well as 1.25% crude protein of fruit of *Musa paradisiaca* (Ekpete *et al.,* 2013) [19] and 11.67% for *Brassica oleracea* (Emebu & Anyika, 2011) [21]. The crude protein content of *B. aegyptiaca* in the present study is however lower than 21.16% for *Manihot esculentus* leaves and 18.80% for *Ceiba pentandra* (Raimi et al., 2014) [22]. It is also higher than 24.18% crude protein for *Urera trinervis* leaf (Marcel & Bievenu, 2012) [20]. Protein is vital for various body functions such as body development, maintenance of fluid balance, formation of hormones, enzymes and sustaining strong immune function (Emebu and Anyika*,* 2011) [21]. The moderate protein value recorded in this study suggests that fruit of *B. aegyptiaca* could be incorporated in animals other than fish diet as a protein supplement owing to the plant's potential to anaesthetize fish. Results of the present study revealed low moisture content (3.56%) compared to 87.30% for *Carica papaya* and 89.60% for *malus domestica* fruits reported by Ekpete *et al.* (2013)[19]. Moisture content of the present study is also much lower than that of *Ficus capensis* leaves (104.53%) reported by Achi *et al*, (2017)[23]. Moisture in food determines the shelf life, rate of digestion, and absorption/assimilation of food within the body system (Olumiyiwa, Babafemi & Johnson, 2004) [35]. The low moisture contents of the parts of *B. aegyptiaca* are, therefore, indicative of long shelf life, implying that the parts could be dried under ordinary conditions and kept for longer period of time (Okia, Agea, Kwetegyeka, Okiror, Kimondo &Teklehaimanot*,* 2013)[36]. Crude fibre (11.60%) of *B. aegyptiaca* in the present study is comparatively similar to 11.80% crude fibre of fruit of the same plant reported by Wakawa, Audu & Sulaiman (2018)[37] but higher than 0.75% for *Musa paradisiaca,* and 0.11% for *citrus lanatus* (Ekpete *et al.,* 2013) [19]. The high crude fibre content of fruit of *B. aegyptiaca* indicates that it may aid digestion and softens stool hence ease bowel movement (Ayoola and Adeyeye, 2009) [38]. The high ash content (8.00%) of *B. aegyptiaca* in this study is comparably similar with that of fresh leaves (8.89%) reported by Okia *et al.* (2013)[36] and fruit (6.00%) reported by Wakawa *et al.* (2018)[37] of same plant. Its value, however, is higher than those reported for fruits of *Citrus lanatus* (2.50%) and *Irvingia gabonensis* (2.50%) reported by Ekpete *et al.* (2013)[19]. The high ash content of *B. aegyptiaca* shows that it may have appreciable amount of mineral elements. The crude lipid content (10.90%) recorded in this study is similar to 10.90% for fruit reported by Wakawa *et al.* (2018)[37] but higher than 2.02% for leaves reported by Okia *et al.* (2013)[36] and 4.50% for flower (Umar *et al.,* 2014)[18] of same plant.

Efficacy of an anaesthetic depends on certain water quality parameters. Increase in temperature, according to Stehly and Gingerich (1999)[39], may increase the efficacy of certain anaesthetic. In this study temperature is not correlated with concentration of the plant extract hence couldn't have affected the efficacy of *B. aegyptiaca* on anaesthesia of *C. gariepinus* fingerlings. DO in the present study is inversely correlated with the plant extract concentration and also showed significant variation with the control. This result is in agreement with the earlier findings of Audu, Adamu & Ufodike (2013)[40] who reported decrease in DO as concentration of *Cannabis sativa* increases but contradicts the findings of Anju, Solomon & Cheikyula (2015)[41] that DO concentration remained constant in all concentrations of *Tephropsia vogelii* aqueous crude leaf extract during tranquilization of *Heterobranchus longifilis*. The low DO in the higher concentrations of the extract in this study could have been responsible for the recorded mortalities due to hypoxia (Environmental Protection Agency (EPA), 2006[42]; Mallya, 2007) [43].

The present study shows that aqueous fruit extract of *B. aegyptiaca* on *C. gariepinus* fingerlings followed the patterns of fish anaesthetic suggested by Summerfelt & Smith (1990)[32] and Javahery & Moraldu (2012)[2]. Behavioural responses display by the fingerlings in the treatment tanks in the present study include loss of equilibrium, erratic swimming, jerky movement, air gulping, jumping above the water surface and vertical standing close to the surface of the water. Similar behavioural responses have been reported by Adebola & Ayo (2014)[44] and Audu *et al.* (2013)[40]. These behavioural signs exhibited by experimental fish may be due to disruption of activities of the nervous system and biochemical derangements (Fafioye, Fagade & Adebisi, 2005)[45]. The increase in mortality rate with increase in concentration of the extract in this study is also similar to the report of Audu, et al. (2017)[30]. The results of the current study however revealed longer induction and recovery times of *C. gariepinus* fingerlings. The mean induction time of 25.05±3.35 min and recovery time of 108.35±2.45 min recorded in the highest concentration in this study is much longer than the mean induction (5.18±0.07 min) and recovery (30.19±1.23 min) times of *Oreochromis niloticus* exposed to aqueous leaf extract of the same plant reported by Wakawa, Audu & Sulaiman (2019)[46] as well as induction (7.00±0.20 min) and recovery times (68.00±1.05 min) of *Heterobranchus bidorsalis* juveniles reported by Adebayo and Olufayo (2017)[47] during anaesthesia with *Datura stramonium* leaf extract. The longer recovery time recorded in this study may be due to increased duration of exposure to the plant material which prolonged the anaesthetic effect (Sladky, Swanson, Stockpot, Loomis & Lewbart, 2001)[48]. The variations in the induction and recovery times of the fingerlings in this study compared with the results of other workers could be due to variation in fish species, anaesthetic compound or induction time.

CONCLUSION

Conclusively, aqueous fruit extract of *B. aegyptiaca* is not an effective anaesthetic for *C. gariepinus* fingerlings owing to long induction and recovery times and the high mortality rate of the fingerlings therefore should not be used as anaesthetic for *C. gariepinus* fingerlings.

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