PLANT PHYTOCHEMICALS AND INHIBITORY ROLES AGAINST FISH DISEASES

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ABSTRACT

The Phytochemical potentials of different plant parts have been reported which necessitated further assessment on their inhibitory roles against fish diseases. This research investigated the phytochemicals present in the plants of Lemon grass (*Cymbopogon citratus),* Neem plant *(Azadirachta indica),* Pawpaw plant (*Carica papaya),* Scent plant (*Occimum gratissimum),* Mango plant (*Mangifera indica),* bitter plant (*Vernonia amygdalina),* Moringa plant (*Moringa oleifera)* with barks of these plants being the parts used for the study. Using qualitative and quantitative methods of phytochemical determination, zones of inhibition were assessed against Gram positive and Gram-negative bacteria using in-vivo and in-vitro with plant parts extracts. Neem, mango and Moringa bark, scent leaf, bitter leaf, pawpaw, neem and moringa leaves and whole lemon grass extracts showed best results in terms of qualitative and quantitative presence and were able to exert antibacterial properties on some tested fish diseases of *Aeromonas veronii* with values of 0.70 and 0.40mm diameter. A highest zone of inhibition was recorded in bitter leaf and the lowest in Neem leaf against *Bacillus subtilis* with values of 0.60 and 0.45mm diameter. Highest zone of inhibition was recorded in mango leaf and lowest in Neem leaf against *Staphylococcus aureus* with values of 0.90 and 0.36mm diameter. Highest zone of inhibition was recorded in bitter leaf and lowest in Neem bark against *Pseudomonas aeruginosa* with values of 0.75 and 0.20mm diameter. Neem, mango and Moringa bark, scent leaf, bitter leaf, pawpaw, and moringa leaves have phytochemicals capable of inhibiting fish diseases.

INTRODUCTION

The plants used for this research were Lemon grass (*Cymbopogon citratus),* Neem plant *(Azadirachta indica),* Pawpaw plant (*Carica papaya),* Scent plant (*Occimum gratissimum),* Mango plant (*Mangifera indica),* bitter plant (*Vernonia amygdalina),* Moringa plant (*Moringa oleifera)* and some selected parts of the plants such as leaves and barks. These plants are rich in secondary metabolites and phytochemical compounds which have an effect against bacteria diseases in fish. Medicinal plants have been used in human medicine as immune boosters for millennia. Furthermore, they are alternatives to antibiotics in aquaculture (Van Hai, 2015).

Studies on botanicals in available literature show that some of these selected plants contain some phytochemicals that have antibacteria properties in treating human diseases, hence these were explored to test their efficacies in the treatment of fish diseases. Several parts of these medicinal plants were used to extract the active substances. Use of leaves have been reported by (Abutbul *et al*., 2004; Ekanem *et al*., 2004; Rattanachaikunsopon and Phumkhachorn, 2009; Alexander *et al*., 2010; Kim *et al*. 2011;Harikrishnan *et al*.2011b) and use of bark has also been reported by (Ji *et al*., 2012; Zhou *et al.,* 2017).

Medicinal plants are rich in various secondary metabolites and phytochemical compounds such as tannins, alkaloids, and flavonoids which affect various diseases in fish (Citarasu 2010; Pandey and Madhuri 2010; Ravikumar *et al*., 2010; Pandey *et al*., 2012). These active substances are mostly extracted with ethanol (Yao *et al*., 2010; Kim *et al*., 2011; Harikrishnan *et al*., 2011b; Harikrishnan *et al*., 2011c; Hu *et al*., 2014; Thanigaivel *et al*., 2015.

Medicinal plants give good results when the bath method is used in various solutions as reported by Wu *et al*., 2011 and Thanigaivel *et al*., 2015.

The antibacterial properties of medicinal plants have been investigated with application in aquaculture (Reverter *et al*., 2014), and it is well known that these plants have antibacterial activity against Gram-positive and Gram-negative bacteria (Van Hai, 2015). Most of these are administered against *Aeromonas hydrophila Vibrio parahaemolyticus, ibrioharveyi, Vibriocarchariae, Vibriosplendidus, Streptococcus iniae, Edwardsiella tarda, Pseudomonas eruginosa*, and *Streptococcus agalactiae*. Some of these bacteria chosen are those which serve as probiotic and mostly from Gram-positive bacteria associated with the genus *Lactobacillus*. According to Fuller, R. A. (1993), most probiotics proposed in aquaculture belong to the lactic acid bacteria (*Lactobacillus, Carnobacterium*, etc.), to the genus *Vibrio* (*Vibrio alginolyticus*, etc.), to the genus *Bacillus*, or to the genus *Pseudomonas*. The choice of the other bacterial organisms was made based on predominant and most common presence in the study. Research on the use of plant extracts as a novel product to treat fish diseases is on-going with this study further looking at the aftermath effects on histopathology of the liver and the gills. Compared to previous studies which did not transcend examination of the qualitative phytochemicals present in these extracts, the quantitative analyses adopted for this study show that these phytochemicals present in the plant extracts.

MATERIALS AND METHODS

Study area

Part of this study was carried out in Odogbolu township, the headquarters of Odogbolu Local Government Area, northwest of Ogun State, with an area of 541km^2 at 6^{0}50'N 3^0 46'E, the location of private fish farms located along the Uren river where clusters of fish farmers using earthen ponds to raise *Clarias* in commercial quantities were visited and healthy catfish samples collected, placed in empty jerry cans and transported to Microbiology laboratory, Federal University of Agriculture. Abeokuta for further studies. Abeokuta is contiguous to Ogun-Oshun River Basin Development Authority located along the Abeokuta-Ibadan road, northeastern end of the city, 15 km from Abeokuta city, on Latitude 7^{0}09'20.56" N and Longitude 3^{0}20' 42.32" E. In addition, catfish were also raised from fingerlings to sub-adult and were inoculated with bacterial organisms at the Fish Ponds and Hatchery farms of Lagos State University, Ojo, in Ojo Local Government area of Lagos State, Nigeria, located on Latitude 6^{0}27'59.99" N; Longitude 3^{0}10'60" E. The fish farm is used by staff and students for training and practical purpose.

Collection of plant samples

The following plants were used: *Cymbopogon citratus* (Lemon grass), *Azadirachta indica* (Neem plant), *Carica papaya* (Pawpaw), *Occimum gratissimum* (Scent leaf), *Mangifera indica* (Mango plant), *Vernonia amygdalina* (Bitter leaf plant), *Moringa oleifera* (Moringa plant) and parts of the plant used were the leaves and barks. They were collected within Odogbolu township and taken to the Chemistry laboratory, Lagos State University for extraction while concentrated extracts for phytochemical screening were taken to Nigerian Institute of Science Laboratory Technology, Ibadan, Oyo State (NISLT) for analyses.

Plant parts extraction

Plant parts such as leaves and barks were extracted using ethanol as solvent using Cowan's (1999) method.

Ethanolic extraction

Forty gramme (40g) of the plant part was weighed using electronic sensitive analytical balance (METTLER PM 400) extracted using Soxhlet extractor. 40g of the plant part was placed inside the thimble and 150 ml of 95% ethanol was placed inside the round bottom flask and heated at 60^0C for 4 hours. The ethanol inside the flask evaporated leaving the extract behind. Thereafter, the extracts were air dried with vacuum pressure machine to reduce the volume to concentrated form. This was repeated for all the other plants. The extracts from the plants were then used for phytochemical screening and sensitivity tests on isolated and identified fish bacteria.

Phytochemical screening

Qualitative and quantitative analyses of the plant parts were carried out to determine the presence or absence of the different organic constituents in the ethanolic extracts, using method adopted by Oloyede (2005). This screening was carried out at the Nigerian Institute of Science Laboratory Technology, Ibadan, Oyo State.

Qualitative screening methods

Test for alkaloids: About 0.2 g of each of the samples were boiled with 5 ml of 2% hydrochloric acid on a steam bath for 5 minutes. The mixtures were allowed to cool and were then filtered, with the filtrates shared in equal proportion into 3 test tubes and labeled A, B, C. Portion of the filtrate which was 1ml was treated with 2 drops of Dragendroff's reagent (a red precipitate was shown) and Mayer's reagent (a creamy white colored precipitate indicated the presence of alkaloid), Oloyede (2005).

Test for flavonoids: About 0.5 g of each of the samples were introduced into 10mls of Ethyl acetate and heated in boiling water for 1 min. The mixtures were then filtered, and the filtrates used for the following test; 4 ml of the filtrates were shaken with 1 ml of 1% aluminum chloride solution and kept in beaker for further use. Formation of a yellow colour in the presence of 1 ml dilute Ammonia solution indicated the presence of flavonoids (Oloyede, 2005)

Test for steroids: Approximately 9ml of ethanol was added into the plant extract sample and refluxed for few minutes and then filtered. The filtrate was concentrated to 2.5ml in a boiling bath; then 5ml of hot distilled water was added to the concentrated solution. The mixture was allowed to stand for 1 hour and the waxy matter was filtered off and 0.5ml of chloroform in a test tube added carefully to 1ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface showed the presence of Steroids (Oloyede, 2005).

Test for saponin: About 0.1 g of each of the plant extract samples was boiled with 5ml of distilled water for 5 minutes. The obtained mixtures were filtered while still hot and the filtrates were then used for the following tests. To 1ml of the filtrates, 2 drops of olive oil was added, the mixtures were shaken and observed for the formation of emulsion. 1ml of each filtrate was diluted with 4ml of distilled water. The mixture was vigorously shaken and then observed on a stand for stable froth (Oloyede, 2005).

Test for tannins: Into 2g of each of the samples was added 5ml of 45% ethanol and boiled for 5 minutes. The mixtures were cooled and filtered. 3 drops of lead sub acetate solution were added into

1ml of each filtrate. A gelatinous precipitate were observed which indicated the presence of Tannins. 0.5 ml of bromine water was added into another 1ml each of the filtrates. A pale brown precipitate was observed indicating the presence of Tannins (Oloyede, 2005).

Test for glycosides: Two grams (2g) of each of the samples was mixed with 30ml of distilled water and heated for 5 minutes on a water bath, filtered and used as follows: 0.2ml of Fehling solution A and Fehling solution B were added into 5ml of the filtrates until they turned alkaline and heated in a water bath for 2 minutes. A light blue colouration was observed (instead of brick red precipitate) which indicated the absence of glycosides (Oloyede, 2005).

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Oloyede, 2005).

Quantitative screening method

The quantitative phytochemical analysis was carried out according to Harboune's (1973) method.

Determination of steroid: One gram (1g) each of the samples was weighed out and macerated with 20ml of ethanol. The mixtures were filtered and 2ml each of the filtrates was pipetted into test tubes. Then 2ml of Ceric sulphate was added into the test tubes and allowed to stand for 30minutes and the absorbance was measured at 550nm on spectrophotometer.

Determination of flavonoids: About 1g each of the samples were measured out and macerated with 20ml of ethyl. The mixture was filtered and then 5ml of the filtrate was measured into test tubes. To each test tube that contained the filtrate, 5ml of dilute ammonia was added and the mixture was shaken. The upper layers were collected and absorbance was measured at 490nm on spectrophotometer (Harboune, 1973).

Determination of saponin: One gram each of the samples was weighed out and macerated with 10ml of petroleum ether. The supernatant was decanted into a beaker and another 10ml of petroleum ether was added. The supernatant was decanted and mixed with the first. The mixture was evaporated to dryness and 6ml of ethanol was added. 2ml of the mixture was pipetted into a test tube. 2ml of color reagent was added and the mixture was allowed to stand for 30minutes, then the absorbance was read at 550nm on spectrophotometer (Harboune, 1973).

Alkaloid determination: About 1g of the samples was weighed out and macerated with 20ml of 20% H_2SO_4 in ethanol (1:1). The mixture was filtered and 1ml of the filtrate was pipetted into test tubes. 5ml of 60% H_2SO4 and 5ml of 0.5% formaldehyde in 60% H_2SO4 were added and the content of the test tube were mixed properly. The mixture was allowed to stand for 3hr and the absorbance was measure at 568nm on spectrophotometer (Harboune, 1973).

Determination of tannin: The tannin content of the extracts was determined by Pearson's (1976) method. One gram (1g) of the test sample was weighed out into a conical flask and 10ml of water was added. The mixture was shaken at 5 minutes interval for 30 minutes and filtered to get the extract. About 2.5ml of the supernatant was transferred into a test tube and 2.5ml of standard tannic acid

solution was transferred into a 50ml flask. Then 1ml of Folin-Denis reagent was added into the flask followed by 2.5ml of saturated Na_2CO3 solution. The absorbance was read at 720nm spectrophotometrically after 90 minutes and incubated at room temperature. The Tannin % is calculated as follows:

$$\text{Tannin}(\%) = \frac{A_n XC}{As} \times \frac{100}{W} \times \frac{V_f}{Va}$$

Where:

An = Absorbance of test sample; As = Absorbance of standard solution;

C = Concentration of standard solution; W = Weight of sample used

V_f = Total volume of extract; Va = Volume of extract analyzed

Determination of phytate: Phytate content was determined according to Pearson's (1976) method. Approximately 0.5g of sample was weighed out into a 500ml flat bottom flask, placed in a shaker and extracted with 100ml of 2.4% Hydrochloric acid for one hour at 25^0C. After this, the mixture was decanted and filtered. 5ml of the filtrate was diluted with 25ml with distilled water and 10ml of it was collected into a flask and 15ml of 0.1M sodium chloride was added to it. The mixture was passed through No. 1 Whatman filter paper to elute inorganic phosphorus and 15ml of 0.7M sodium chloride was added to elute phytate. The absorbance was read at 520nm on spectrophotometer.

Determination of oxalate: The determination of oxalates was carried out by the titration method of AOAC (2010). Two grams of the sample was suspended in a mixture of 190ml of distilled water and 10ml of 6N HCl in a 250ml volumetric flask and digested for one hour at 100^0C, cooled and made up to 200ml with distilled water. The digest was filtered through Whitman No. 1 Filter paper using a suction pump. A duplicate proportion of 125ml of the filtrate was measured into 250ml beakers and four (4) drops of methyl red indicator was added into each beaker. Concentrated NH_3 solution was added drop wise until the test solution changed from its salmon pink color to a faint yellow color (pH 4-4.5). Each portion was heated up to 90°C and 10ml of 5% $CaCl_3$ was added while being stirred constantly. After heating, it was cooled and left-over night at 5°C. The supernatant was decanted and the precipitate completely distilled in 10ml of 20% (v/v) H_2SO4 solution. At this point, the filtrate resulting from digestion of 2g of the sample was combined and made up to 300ml. Aliquots (125ml) of the filtrate was heated until near-boiling and then titrated against 0.05M standard $KMnO_4$ solution to a faint pink color. Oxalic acid content was calculated using the formula:

$$\text{Oxalate mg/100g} = \frac{T \times (V_{me})(D_f) \times 10^5}{ME \times M_r}$$

Where:

T = Titre of KMnO4; Vme = Volume-mass equivalent (i.e., 1ml of 0.05M KMnO4 solution is equivalent to 0.00225g anhydrous oxalic acid); D_f = The dilution factor

ME = The molar equivalent of KMnO4 in oxalic acid (KMnO4 redox reaction is 5)

M_f = The mass of sample used

Determination of cyanide: Cyanide content was determined according to Onwuka's (2005) method. Five grams (5g) of the sample was weighed into a conical flask and 50ml of distilled water was added to it and the solution was allowed to stand overnight and then filtered. 1ml of the sample filtrate was measured into a test tube and 4ml of alkaline picrate was added and it was allowed to stand for 5 minutes. The absorbance was read at 490nm on spectrophotometer. The reading was taken with the reagent blank at zero.

Sterilization of materials

Working tables were swabbed with ethanol to disinfect them. All the glass wares were washed and air-dried before they were sterilized in the hot air oven at 160^0C for 1 hour. Petri dishes were sterilized by putting them in petri dish cans in the hot air oven at 160^0C for 1 hour; cotton plugged and aluminium foil-covered conical flasks were sterilized in the hot air oven at 160^0C for 1 hour, and the wire loop was sterilized by flaming it red-hot using a spirit lamp (Cheesbrough, 2006)

Collection of fish samples

Thirty healthy sub-adult catfishes were used for this research with five (5) fishes taken from a population of fifty fishes (50) from the small ponds with access in all the farms. They were collected from private fish farms in Odogbolu, Odogbolu Local Government, Ogun State; the fishes were collected from the earthen pond farms using random sampling with slips of papers where the names of farmers were written out and are put into a bag, mixed thoroughly and draw one after the other without replacement, so that all the farms in the population has the same chance of being selected. Farms were categorized as big, medium and small farms based on fish population. The farms randomly selected with their corresponding fish population are as follows: Elegbeji Farms (Farm 1):100,000 ; Sanni Farms (Farm 2) :15,000; Kunle Farms (Farm 3) :16,000; Johnson Farms (Farm 4): 100,000; Adewale farm (farm 5): 45,000 and Awosanya Farms (Farm 6) : 45,000. The live fish was put in the water in a plastic container (Jerry cans) the upper part of which was cut out to allow for aeration, and taken immediately to the Laboratory of Department of Microbiology, Federal University of Agriculture, Abeokuta for microbial analyses.

Quantitative anti nutrients presence in plant extracts

Quantitative phytochemical presence of anti-nutrients in the plant parts revealed that phytic acid of 2.80% was highest in bitter leaf and lowest in Neem bark with 1.40 %, with oxalate having the highest percentage of 3.78 in mango leaf and lowest of 0.45% in Moringa bark. Tannin content was 7.29% in scent leaf, 1.84% in bitter leaf; cyanide was not present in all the plant parts (Table 9).

Table 1: Quantitative phytochemical percentage of phytic, oxalate, tannin and cyanide present in experimental plants parts

PLANT PART	PHYTIC ACID	OXALATE Mg/g	TANIN (MEAN±SE)	CYANIDE
Bitter leaf	2.80±0.10	0.81 ± 0.10^{b}	1.84 ± 0.10^{c}	ND
Pawpaw leaf	2.35±0.10	0.63 ± 0.10^{b}	4.09 ± 1.00^{bc}	ND
Scent leaf	2.68±1.00	0.90 ± 0.10^{b}	7.29 ± 1.00^{a}	ND
Mango bark	1.28±1.00	2.07 ± 1.00^{ab}	4.40 ± 1.00^{abc}	ND
Mango leaf	2.27±1.00	3.78 ± 1.00^{a}	3.52 ± 1.00^{bc}	ND
Moringa bark	1.52±1.00	0.45 ± 0.10^{b}	2.55 ± 1.00^{bc}	ND
Moringa leaf	2.57±0.05	1.51 ± 0.59^{ab}	2.77 ± 0.03^{bc}	ND
Neem leaf	2.65±0.05	1.44 ± 0.83^{ab}	5.63 ± 0.02^{ab}	ND
Neem bark	1.40±0.13	1.98 ± 1.53^{ab}	3.10 ± 1.42^{bc}	ND
Total	2.17±0.21	1.50±0.30	3.91±0.44	ND

Mean values with different superscript along the column were significantly different at $p < 0.05$.
ND= Not Detected

Quantitative and Qualitative phytochemical presence in experimental plant extracts

Mango leaf had 8.69 mg/g of alkaloids, which was the highest, followed by 0.53 mg/g in Neem bark for alkaloids; flavonoids was higher in moringa leaf and the lowest in neem leaf with values of 4.90 and 0.0 mg/g respectively; saponin was the highest in bitter leaf and the lowest in neem bark with values of 4.66 and 0.31 mg/g respectively. Tanin was the highest in scent leaf and the lowest in neem bark with values of 7.29 and 1.16 mg/g respectively; Phenol in pawpaw leaf was the highest and least in neem leaf with values of 5.51 and 1.47 mg/g respectively (Table 10 a and b).

Table 2: Quantitative phytochemical presence in experimental plant parts

	Mean±SE (mg/g)				
Plant part	Alkaloids	Flavonoids	saponin	Tannin	Phenol
Bitter leaf	8.69 ± 1.00^{ab}	2.21 ± 1.00^{bc}	4.66±1.00a	1.84 ± 1.00^{cd}	3.63 ± 1.00^{abc}
Pawpaw leaf	8.46 ± 1.00^{abc}	1.98 ± 1.00^{bc}	2.34 ± 1.00^{abc}	4.09 ± 1.00^{bcd}	5.51 ± 1.00^{a}
Scent leaf	5.61 ± 1.00^{cd}	1.40 ± 1.00^{bc}	$0.77\pm.0.10^{c}$	7.29 ± 1.00^{a}	3.21 ± 1.00^{abc}
Mango bark	5.21 ± 1.00^{d}	2.71 ± 1.00^{bc}	2.22 ± 1.00^{abc}	4.40 ± 1.00^{bc}	4.93 ± 1.00^{ab}
Mango leaf	10.49 ± 1.00^{a}	2.93 ± 1.00^{ab}	3.52 ± 1.00^{ab}	3.52 ± 1.00^{bcd}	4.10 ± 1.00^{abc}
Lemon grass whole	6.78 ± 1.00^{bcd}	3.22 ± 1.00^{ab}	0.42 ± 1.00^{c}	1.68 ± 1.00^{cd}	4.29 ± 1.00^{abc}
Moringa bark	5.90 ± 1.00^{bcd}	4.90 ± 1.00^{a}	3.49 ± 1.00^{ab}	2.55 ± 1.00^{cd}	4.70 ± 0.80^{ab}
Moringa leaf	1.85 ± 0.02^{e}	3.80 ± 0.02^{ab}	0.43 ± 0.01^{c}	2.77 ± 0.03^{bcd}	2.12 ± 0.01^{bc}
Neem leaf	1.34 ± 0.04^{e}	0.01 ± 0.00^{c}	0.85 ± 0.19^{bc}	5.63 ± 0.02^{ab}	1.47 ± 0.01^{c}
Neem bark	0.530 ± 0.40^{f}	3.17 ± 0.01^{ab}	0.31 ± 0.14^{c}	1.16 ± 0.52^{d}	2.33 ± 0.02^{bc}

Mean values with different superscript along the column were significantly different at $p < 0.05$.

Table 3: Qualitative phytochemical analysis of the experimental plant parts

Plant parts	Alkaloids	Flavonoids	Saponin	Tannin	Cardiac glycoside	Phenol	Anthraquinone	Terpenoid	Phylobatanin	Steroid
Bitter leaf	+	+	+	+	+	+	+	+	-	+
Pawpaw leaf	+	+	+	+	+	+	+	+	+	+
Scent leaf	+	+	+	+	+	+	-	+	+	+
Mango bark	+	+	+	+	+	+	+	+	+	+
Mango leaf	+	+	+	+	+	+	+	+	+	+
Lemon grass whole	+	+	+	+	-	+	-	-	-	-
Moringa bark	+	+	+	+	+	+	+	+	-	-
Moringa leaf	+	+	+	+	+	+	+	-	+	+
Neem leaf	+	+	+	+	+	+	+	+	+	+
Neem bark	+	+	+	+	+	+	+	+	+	+

+ indicates presence of the phytochemical - indicates absence of phytochemical

Zones of inhibition

Zones of inhibition of plant extracts and antibiotic compared favourably against the different bacterial isolates. The highest zones of inhibition were recorded in bitter leaf and the lowest in moringa bark and lemon grass against *Aeromonas veronii* with values of 0.70 and 0.40mm diameter. The highest zones of inhibition was recorded in bitter leaf and the lowest in neem leaf against *Bacillus subtilis* with values of 0.60 and 0.45mm diameter (Table 4).

The highest zones of inhibition were recorded in mango leaf and the lowest in neem leaf against *Staphylococcus aureus* with values of 0.90 and 0.36mm diameter. The highest zones of inhibition was recorded in bitter leaf and the lowest in neem bark against *Pseudomonas aeruginosa* with values of 0.75 and 0.20mm diameter (Table 5)

Table 4: Zones of inhibition of Aquaceryl antibiotic and extracts from plant parts against bacterial isolates cultured from *Clarias gariepinus*

Plant parts/Antibiotic	Inhibition zone (mm diameter)(Mean±SE)	
	Aeromonas veronii	*Bacillus subtilis*
Bitter leaf	0.70±0.10^{a}	0.60±0.00^{b}
Moringa bark	0.40±0.00^{b}	0.60±0.10^{b}
Neem bark	0.55±0.05^{ab}	0.55±0.05^{a}
Neem leaf	0.42±0.02^{b}	0.45±0.05^{a}
Lemon grass whole	0.40±0.00^{b}	0.55±0.05^{a}
Antibiotic	0.48±0.04^{b}	0.58±0.02^{b}
Total	0.49±0.03	0.55±0.02^{a}

Mean values with different superscript along the column were significantly different at $p < 0.05$.

Table 5: Zones of inhibition of Aquaceryl antibiotic and extracts from plant parts against bacterial isolates cultured from *Clarias gariepinus*

Plant parts/Antibiotic	Inhibition zone (mm diameter)(Mean±SE)	
	Staphylococcus aureus	*Pseudomonas aeruginosa*
Mango leaf	0.90±0.00^{a}	0.55±0.05^{ab}
Scent leaf	0.50±0.00^{b}	0.60±0.20^{ab}
Bitter leaf	0.45±0.05^{bc}	0.75±0.05^{a}
Moringa bark	0.45±0.05^{bc}	0.35±0.05^{ab}
Pawpaw leaf	0.37±0.07^{bc}	0.40±0.00^{ab}
Neem bark	0.40±0.00^{bc}	0.20±0.00^{b}
Neem leaf	0.36±0.01^{c}	0.42±0.02^{ab}
Lemon grass whole	0.36±0.04^{c}	0.50±0.30^{ab}
Aquaceryl	0.46±0.01^{bc}	0.50±0.04^{ab}
Total	0.47±0.04	0.47±0.05

Mean values with different superscript along the column were significantly different at $p < 0.05$.

DISCUSSION

The qualitative assay indicated the presence of alkaloids, flavonoids, saponin, tannin, cardiac glycoside, phenol, anthraquinone, terpenoid, phylobatanin and steroid in most of the plant extracts. The quantitative method was used to determine the quantity in mg/g of each phytochemical in the extracts.

On leaf of bitter plant, the tannin content of ethanol extract of this work was 1.84 ± 1.00 mg/g and was higher than 0.37 ± mg/g as reported by Atangwho *et al*. (2009). The result was also higher than 0.55± 0.01 mg/g squeezed washed bitter plant. 1.03 ± 0.00mg/g blanched bitter plant and less than 2.04 ± 0.05 mg/g of raw bitter leaf and less than 1.92 ± 0.02 mg/g of solar dried bitter plant in tannin as reported by Yusufu *et al*., 2015. The reasons could be due to seasonal variation in the plant physiology as of the the rainy season when it was collected. Erasto *et al*., 2006; Farombi and Owoeye, 2011; Kiplimo *et al*., 2011; Toyang and Verpoorte, 2013; Adedapo *et al*., 2014; Quasie *et al*., 2016; Luo *et al*., 2017, also reported the presence of tannin in their research work. Tannin is known to have antibacterial effects on bacteria. Amodu *et al*. (2013) and Luo *et al*. (2017) corroborated its bactericidal activity against bacteria.

For flavonoids, it was 2.21± 1.00 mg/g which was higher than 0.87 ± 0.02 mg/g as reported by Atangwho *et al*., 2009. In addition, this result was lower than what was reported by Yusufu *et al*., 2015 for raw bitter leaf of 12.43± 0.06 mg/g, blanched bitter leaf 3.22 ± 0.03 mg/g, solar dried 3.11± 0.03mg/g and 3.35 ± 0.04 for squeezed washed. The reasons could be due to planting location and seasonal variation in weather as of the time of collection.

Saponin content of leaf of bitter plant was 4.66 ± 1.00 Mg/g, higher than 2.15 ± 0.01 mg/g reported by Atangwho *et al*., 2009. It was higher than 0.72 ± 0.01 mg/g for raw bitter leaf, 0.30 ± 0.00 mg for blanched bitter leaf, 0.43 ± 0.01mg/g for solar dried and 0.25 ± 0.03 for squeezed washed leaf as reported by Yusufu *et a*l., 2015. The reasons could be due to extraction variables like temperature and time.

Alkaloids content of this work was 8.69 ± 1.00 mg/g, higher than 2.13 ± 0.04 mg/g reported by Atangwho *et al*., 2009. It was higher than 6.65 ± 0.06 mg/g for raw bitter leaf, 3.22 ± 0.03 mg/g for blanched bitter leaf, 2.12 ± 0.02 mg/g for solar dried and 4.41± 0.04 mg/g for squeeze washed reported by Yusufu *et al*., 2015.

Phenol content of this work was 3.63± 1.00 mg/g higher than 0.35± 0.00 mg/g for polyphenol reported by Atangwho *et al*., 2009. The reasons could be due to extraction variables like concentration and particle size.

On *Azadirachta indica* (Neem leaf and bark). Neem plants are rich in alkaloids, saponin, flavonoids and other phytochemicals as reported in this research. Also, SaiRam, *et al*. (2000) reported the presence of triterpenoids, phenolic compounds, carotenoids, steroids, valavinoids, ketones and tetra-triterpenoids azadirachtin in neem plant.

The flavonoids content of this work were 0.01 ± 0.00 mg/g and 3.17 ± 0.0 mg/g respectively. This result was lower than 0.39± 0.02 mg/g for neem leaf reported by Atangwho *et al*., 2009. In addition, the 0.01 ± 0.00 mg/g for flavonoid in neem leaf was lower than the results reported by Yusufu *et al*., 2015 for raw 12.43± 0.06 mg/g, Blanched leaf 3.22 ± 0.03 mg/g, solar dried leaf 3.11 ± 0.03 mg/g and squeeze washed leaf 3.35 ± 0.04 mg/g.

Saponin of *Azadirachta indica* (Neem leaf and bark) of 0.85 ± 0.19 mg/g and 0.31 ± 0.14 mg/g; Tanin 5.63 ± 0.02 mg/g and 1.16 ±0.12 mg/g and Phenol 1.47 ±0.01 mg/g and 2.33 ±0.02 mg/g of this work is higher than Neem leaf Saponin 0.56 ±0.01 mg/g, Tanin 0.63 ±0.01 mg/g and phenol of 0.35 ±0.00 mg/g reported by Atangwho *et al*., 2009. In addition, Tanin of Neem leaf of 5.63 ± 0.02 mg/g of this work was higher than what Yusuf *et al*., 2015 reported of raw Neem leaf 2.04±0.5 mg/g, blanched leaf 1.03±0.00 mg/g, solar dried leaf 1.92± 0.02 mg/g, squeezed washed leaf 0.55± 0.01 mg/g. While Saponin of Neem leaf of 0.85 ± 0.19 mg/g was higher than those reported by Yusufu *et al*., 2015 of raw 0.72 ±0.01 mg/g, blanched 0.30±0.00 mg/g, solar dried 0.43± 0.01 Mg/g, squeeze washed 0.25± 0.03 mg/g.

Alkaloid of *Azadirachta indica* (Neem leaf and bark) of 1.34 ± 0.04 mg/g and 0.53± 0.40 mg/g was lower than raw 6.65 ±0.06 mg/g, blanched 3.22 ± 0.03 mg/g, solar dried 2.12 ±0.02 mg/g, and squeeze washed 4.41 ±0.04 mg/g as reported by Yusufu *et al*., 2015.

On scent plant leaf *Ocimum gratissimum,* the phenol content using ethanol as solvent was 3.21 ±1.00 mg/g higher than 0.17 ±0.03 mg/g reported by Ladipo *et al*., 2010 on their investigation of *Ocimum gratisimum* extracts on selected enterobacteriaceae.

On scent plant leaf, the alkaloid content was 5.61 ±1.00 mg/g higher than 0.82 ±0.02 mg/g reported by Ladipo *et al*., 2010. However, Akinmoladun *et al*., 2007 reported the absence of alkaloids in the aqueous extracts of the leaf, The Tanin and Flavonoids of this work of 7.29±1.00 mg/g and 1.40±1.00 mg/g were higher than 1.95±0.02 mg/g and 0.68±0.03 mg/g respectively as reported by Ladipo *et al*., 2010. Akinmoladun *et al*., 2007 also confirmed the presence of flavonoids in the aqueous and ethanolic leaf extracts. Flavonoids were also reported to be absent in the ethanolic extract of the leaf (Nweze and Eze, 2009). While the Saponin of 0.77±0.10 mg/g of this work was lower than 1.92±0.05 as reported by Ladipo *et al*., 2010. The reasons in variation in the phytochemical content of the leaf extract could be due to seasonal variation which has direct effect on plant physiology and in turn affects the phytochemical composition.

These plant extracts contained phytochemicals that acts as antibacterial against the fish diseases. This was also in line with finding from research that plant product application in aquaculture for disease control is one of promising alternatives to antibiotic. They act as antibacterial due to their active

chemical ingredients (Reverter *et al*., 2014).

Similarly, Hakan and Arzu's (2015) report from the disc diffusion assay showed that some plants exhibited antibacterial activities against pathogenic fish bacteria and these could be considered a promising source of new drug candidates in aquaculture industry.

In addition, Bamola *et al*. (2018) reported the traditional medicinal uses of 21 plants species belonging to different families and their antibacterial properties.

Conclusion

The concentrated plant part extracts from 2.5 mg/ml to 10 mg/ml were effective against the tested pathogens.

The 2.5 mg/ml of Pawpaw leaf, Moringa bark was found to be effective in the treatment of catfish infected with *Pseudomonas aeruginosa* with improved blood parameters coupled with normal to mild vacuolar degeneration of liver and normal to atrophy of epithelial cells of secondary lamellae of gills. In addition, 2.5mg/ml of pawpaw leaf extracts was only effective in the treatment of *Staphylococcus aureus* inoculated on catfish with improved blood profile such as WBC, HGB, RBC, MCV, MCHC coupled with normal to mild, diffused vacuolar degeneration of liver and normal to atrophy of epithelial cells of secondary lamellae of the gills. Also, 2.5mg/ml of whole lemon grass, bitter leaf and neem leaf extracts were able to treat *Aeromonas veronii* inoculated against catfish with improved blood parameters such as WBC, HGB, RBC, HCT, PLT, PCT, MCV, MCHC with normal to mild diffuse vacuolar degeneration of liver and the gills exhibited normal to atrophy of epithelial cells of secondary lamelae after treatment. The 2.5mg/ml of Moringa bark and neem leaf extracts were effective in the treatment of *Bacillus subtilis* inoculated catfish with improved blood parameters and normal to mild vacuolar degeneration of liver. Also, there was atrophy of epithelial cells of secondary lamellae of the gills.

The 5.0mg/ml of whole lemon grass and neem bark extracts were effective against *Staphylococcus aureus*. However 5.0mg/ml whole lemon grass extracts were effective on *Bacillus subtilis.* The 5.0mg/ml of neem bark and Moringa bark extracts respectively were effective on *Aeromonas veronii*.

The 7.5mg/ml each of lemon grass, scent leaf, neem leaf extracts were found to be effective in the treatment of catfish inoculated with *Pseudomonas aeruginosa*. Also, 7.5mg/ml each of mango leaf, bitter leaf and neem leaf extracts were effective against *Staphylococcus aureus*. In addition, 7.5mg/ml of neem bark and Moringa bark extracts respectively were effective on *Aeromonas veronii*

Moreover, 7.5mg/ml of bitter leaf extracts and neem bark extracts respectively were effective against *Bacillus subtilis* infected fish.

The 10.0mg/ml of mango leaf, bitter leaf and neem bark extracts were effective in the treatment of *Pseudomonas aeruginosa* inoculated catfish. Also, 10.0mg/ml of Scent leaf and Moringa bark extracts were effective against *Staphylococcus aureus*

Productions of these extracts need further development into novel products to be made available to farmers.

CONCLUSION

This study concluded that neem, mango and moringa bark; scent, bitter, pawpaw, and moringa leaves and whole lemon grass extracts contained botanicals that exerted antibacterial properties on the identified fish pathogens and reduce reliance on synthetic antibiotic.

A B C

D E F

G

Legend:

Figure A: Neem (*Azadirachta indica*) plant showing its leaves

Figure B: Lemon grass (*Cymbopogon citratus*) plant

Figure C: Mango (*Mangifera indica*) plant

Figure D: Bitter leaf (*Vernonia amygdalina*) plant

Figure E: *Moringa oleifera* plant showing its leaves and flowers

Figure F: Pawpaw (*Carica papaya*) plant showing fruits and leaves

Figure G: Scent leaf (*Ocimmum gratissimum*) plant

Source: Field survey, 2016

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