Growth Pattern and *in vitro* Antibacterial Activity of Some Cultivated Herbs against Fish Pathogenic and Food Poisoning Bacteria

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ABSTRACT

The global concerns on the deleterious effect of synthetic antimicrobials in the production of food animals call for suitable and sustainable natural alternatives, one of which is the use of herbal products. This study aimed to investigate the growth pattern of some herbs of medicinal values and their antibacterial potentials against some fish pathogenic and food poisoning bacteria, compared with Aquamedics (a synthetic antibiotic) using agar well diffusion assay. The results showed that the number of leaves produced in *Euphorbia hirta* was more than the leaves produced in *E. hererophylla*; notwithstanding, *E. heterophylla* was higher than *E. hirta*. *Ocimum gratissimum* showed similar pattern of growth with *E heterophylla* during the first 8 weeks while *Phyllanthus amarus* is characterized with production of higher number leaflets at the early growth stages. All the herbal extracts investigated showed presence of flavonoids and tannins. The ethanol *E. hirta* extract (EHE) and *Jatropha gossypiifolia* extract (JGE) exhibited significantly higher ($P < 0.05$) zone of inhibition against *Pseudomonas aeruginosa* and *P. fluorescens*, compared to other extracts and Aquamedics. The JGE also had higher zone of inhibition against *Aeromonas hydrophila* (26.67mm), and *Streptococcus agalactiae* (25.67mm) compared to Aquamedics (22.33mm) and other extracts while EHE, *E. heterophylla* and *Moringa oleifera* exhibited similar zones of inhibition, 23.67mm, 23.00mm, 21.67mm, respectively, compared with 22.33mm obtained for Aquamedics against *A. hydrophila* . The methanol extracts of JGE, EHE and *P. amarus* extract also exhibited higher zone of inhibition against *P aeruginosa*, compared to other extracts and the synthetic antibiotic. However, the synthetic antibiotic demonstrated

higher zone of inhibition than the aqueous extracts of the herbs, except EHE which had similar inhibitory activity with Aquamedics against *P. fluorescens*. On the over all, the ethanol extracts seemed to have more potent antibacterial activity than other extracts. The present study has demonstrated the moderately inhibitory activities of the ethanol and methanol extracts of *Euphorbia* species, *J. gossypiifolia* and *P. amarus* against the food poisoning *Pseudomonas* species and pathogenic *A. hydrophila* and *S. agalactiae*. These extracts are therefore recommended for utilization and further *in vivo* study as herbal antibacterial agents for fish health management and fish preservation.

Keywords: Growth pattern of herbs, phytochemicals, fish pathogens, zone of inhibition

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INTRODUCTION

The continuous rise in the cost of feed ingredients in the production of farmed terrestrial and aquatic animals is a major concern in animal husbandry as well as losses arising from diseases, most especially bacterial diseases of aquaculture species (Hardi *et al*., 2019). Chemotherapeutic agents are often used as additives for prophylactic, therapeutic measures and to promote production. However, the continuous utilization of these synthetic drugs makes the residues to remain in the tissues of food fish and other animal products, with the resultant deleterious effects such as development of resistant pathogen strains which can be transferred from fish and other animal products to humans resulting in immune suppression, destabilization of helpful bacterial populations as well as the environmental pollution (Sajid *et al*., 2011; Gent *et al*., 2012; Aly and Albutti, 2014; Reverter *et al*., 2017; Santos and Ramus, 2018). Hence research into suitable organic alternatives to the synthetic drugs for sustainable fish production is crucial.

In the recent decades the global scientific communities have focused on the utilization of natural alternatives to the synthetic drugs in the production of fish or livestock meant for consumption; including prebiotics, probiotics, organic acid and phytobiotics (herbal products). Out of these alternatives, herbal products seem to be the best because they are naturally and readily available, accessible, less expensive, easily produced and tend to be more biodegradable compared to synthetic drugs (Olusola *et al*., 2013; Reverter *et al*., 2014). The utilization herbal-based extracts for prophylactic or chemotherapeutic for various fish diseases has been considered to be best alternative for controlling the spread of bacterial infection by increasing the fish immunity and the innate behavior of fish (Reverter *et al*., 2014, Adeniyi et al., 2017; Abdel-Tawwab *et al*., 2018). Earlier studies on the utilization of medicinal plants such as caraway (Ahmad and Abdel-Tawwab, 2011), walnut and onion (Bello *et al*., 2012) *Aloe vera* (Mahdavi *et al*., 2013), Roselle (Adeyemo, 2014), fluted pumpkin (Dada, 2015), cotton leaf (Adeniyi and Lawal, 2017), tamarind (Adeniyi *et al*., 2018a, 2018b), *Garcinia kola* (Nyadjeu *et al*., 2019), lemon (Adeniyi, 2020) have shown that this alternative has growth-promoting and/or immune-stimulating potentials. The availability and accessibility of these plants herbal products at commercial level is crucial for sustainability of organic farming of fishes and terrestrial animals. Although several attempts have been made to investigate both *in vitro* and *in*

vivo antimicrobial potential of herbs on both human and fish pathogens (Oluwafemi and Debiri, 2008; Najiah *et al*., 2011; Panda and Ray, 2012; Pedge and Ahirrao, 2012; Bello *et al*., 2013; Kalsom *et al*., 2013; Adeniyi *et al*., 2017; Adeshina *et al*, 2018; Adeniyi, 2020). *Euphorbia hirta*, *E. heterophylla*, *Jatropha gossypiifolia*, *Ocimum gratissimum*, *Moringa oleifera*, *Phyllanthus amarus*, *Senna alata*, *Tithonia diversifolia*, and *Vernonia amygdalina* are herbal plants belonging to the families Euphorbiaceae, Lamiaceae, Moringaceae, Phyllanthaceae, Fabaceae, and Asteraceae that are widely utilized owing their significance in ethnomedicine, but with paucity of scientific information on their cultivation and antibacterial effects on aquatic pathogenic microbes. Therefore this study aimed to investigate the *in vitro* antimicrobial potential of *E. hirta*, *E. heterophylla*, *J. gossypiifolia*, *O. gratissimum*, *M. oleifera*, *P. amarus*, *S. alata*, *T. diversifolia*, and *V. amygdalina* against *Aeromonas hydrophila*, *A. salmonicida*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli.*

MATERIALS AND METHODS

Cultivation of herbal plants

The seed / stem of the herbs used for planting were locally sourced around the villages and towns in Kwara State and cultivated on an herbal garden section of an integrated homestead farm in Ilorin, in the Southern Guinea Savannah agro-ecological zone of Nigeria. The land was cleared, beds were manually prepared and hoe weeding was done as at when due. Each plot size measured 5.0 m × 5.0 m with 0.5 m in-between plots and 1.0 m in-between blocks. Poultry manure was applied during land preparation by incorporation into the soil at the rate of 5 tons/ha at two weeks before planting. Herbal plants were planted at their respective plant spacing in a randomized complete block and replicated three times. All the cultivated herbal plants, except *Vernonia amygdalina* were propagated by seed. The *E. hirta*, *E. heterophyla* and *P. amarus* were planted by drilling between rows at 20 cm for *E. hirta* and 30 cm for the other 2 herbs. Details of the planting are presented in Table 1.

Table 1: Details of planting of herbal plants

Herbal plants	Method of propagation	Seed rate	Spacing	Plant population/ha
Euphorbia hirta	Seed	5kg	20 cm	NA
Euphorbia heterophylla	Seed	5kg	30 cm	NA
Ocimum gratissimum	Seed	NA	60 cm × 30 cm++	55,555
Phyllanthus amarus	seed	NA	30 cm	NA
Senna alata	Seed	NA	60 cm × 60 cm+	27,777
Vernonia amygdalina	Stem	NA	60 cm × 60 cm+	27,777

+ = One plant per stand, ++= Two plants per stand, NA = Not applicable

Soil analysis

Composite soil sample at the experimental site at 0-20 cm depth were collected in a zig-zag pattern before planting and taking to the laboratory to determine the physical and chemical properties. Soil particles were analyzed using the hydrometer method (Bouyoucous, 1962 modified by Gee and Bauder, 1986). The soil sample was air-dried in the laboratory and sieved with 2 mm diameter mesh. Soil pH was determined using glass electron pH meter in 1:2 soil / water ratio, hence ten grams (10g) of the soil sample was weighed into 100mL beakers (in duplicate) and 20mL distilled water was added; Each beaker was stirred several times for about 30 minutes. The pH of the samples was measured by immersing the glass electrode pH into the clear solution on to suspension. Care was taken not to stir the suspension before taking the pH measurement. The soil total nitrogen was determined using the Kjeldahl digestion method as described by Bremner and Mulvaney (1982), which involved digestion, distillation and titration: 10g of the air-dried and sieved soil sample was weighed into 250 mL digestion flask and gently mixed with 100 mL HCl was added; the mixture digested at for 1 hour at 420°C using an automated digestor (8 Holes, Foss Tecator digestor, Denmark), allowed to cool for 20 minutes and then Kjeltec auto distillation unit (Kjeltec 8200, Denmark). The distillate was then titrated with standardized hydrochloric acid (HCl) using titrator dispenser (Solarus (R) Hischmann Laborgerate GmbH and Co. KG., Germany). Titre value was also determined for Blank (B) of each batch. The total nitrogen (%) was estimated using the formula below:

Total nitrogen (%) = 100 x [(T – B) x N x 14.007] / W (mg)

Where, T=Titre value of sample, B=Titre value of blank, N=0.1(Normality of acid), and W=Weight of sample

Available P was determined by Bray 1 method (Anderson and Ingram, 1998): 2g of the air-dried and sieved soil sample was weighed into 250 conical flask. 30 mL of $HClO_4$ was added and digested on a hot plate in the fume cardboard at 130°C until solution became clear. As digested was completed, fume $HClO_4$ appeared and soil residue become white, the flask was removed from the hot plate and allowed to cool to room temperature. 50 mL of distilled water was added and filtered into 100 mL standard flask and phosphorus was determined colometrically. Total soil organic carbon was determined using the acid dichromate wet-oxidation procedure described by Walkley and Black (1934), in which 1g of the sample was put into 250mm conical flask, followed by addition of 100mL 0.167M Potassium dichromate (vi) and 20mL concentrated H_2SO_4; the flask was then swirled gently until the soil and reagent were thoroughly mixed and vigorously swirled for one minute and allowed to stand on a sheet of asbestos for about three minutes; 10mL distilled water was then added, followed by three drops of ferroin indicator and titrated with 0.5M Iron (vi) ammonium sulphate. The titre value was recorded when colour changes from green through blue to red moron; the titre value was multiplied by 1.33 to give percentage organic carbon. Exchange bases were extracted with neutral ammonium acetate solution buffered at pH 7.0, using the method of Anderson and Ingram (1998) as described by Adeoye and Agboola (1985): briefly, 10g of the air dried and sieved soil sample was weighed into conical flask, and 100mL ammonia acetate was added, the mixture was put on mechanical shaker for one hour, filtered and made up to 100 ml with ammonium acetate. Exchangeable bases: calcium, magnesium, potassium and sodium were determined from the filtrate by atomic absorption spectrophotometer. The cation exchange capacity of the soil was determined with 1M NH_4OAc (1M ammonium acetate), buffered at pH 7.0 (Rhoades, 1982).

Plants and extraction

The shoots of *E. hirta*, *E. heterophylla, and P. amarus* were harvested at 8 weeks to obtain the leaves and seeds while the leaves of *J. gossypiifolia*, *O.*

gratissimum, *M. oleifera*, *S. alata*, *T. diversifolia*, and *V. amygdalina* were harvested at 12 weeks; rinsed with clean water, drained and air-dried under shade in the laboratory for 14 days. Each of the dried herbal materials was blend into uniform powder using a kitchen blender machine and labeled. A known weight of each herbal material was added to the solvents at a ratio of 1:10 (weight/volume), using distilled water, ethanol and methanol (Ghassan *et al*., 2012; Adeniyi *et al*., 2017) to obtain three different extracts for each herb. Each of the blend herbal materials was folded in filter paper, immersed in the solvent and extracted using Soxhlet apparatus for 6 hours. After 6 hours of extraction, each crude extracts were concentrated, using rotary evaporator (IKA® RV10 digital, Artisan Technology Group, Champaign, USA). The resulting concentrated crude extracts of each herb were labeled accordingly and kept in freezer until they are used for the antimicrobial and phytochemical screening.

Qualitative phytochemical screening

The extracts was screened for phytoconstituents such as alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids qualitatively, following the description of Trease and Evans (1996) and Sofowora (1993). Concisely, each of the concentrated extracts of the herbs was tested for the following phytochemical as follows:

Test for alkaloids

Two (2) mL of 1% HCl was used to dissolve 0.5g of each extracts and heated gently; Wagner's reagent was added to the mixture. Turbidity of the mixture with reddish brown precipitate revealed presence of alkaloids.

Test for cardiac glycosides

Keller-Kiliani test was used: To 0.5g of each of the extracts diluted to 5 mL in water was added 2 mL of glacial acetic acid, containing one drop of 2% $FeCl_3$ solution: this was underlayed with 1 mL of concentrated H_2SO_4. A brown ring at the interface indicated the presence of cardiac glycosides.

Test for flavonoids

Two (2) mL of 2% of sodium hydroxide (NaOH) was mixed with 0.5g of each extract; thereafter 1 mL of concentrated tetraoxosulphate (VI) acid (H_2SO_4) was added. A yellow colouration indicated the presence of

flavonoids.

Test for saponins

Five (5) mL of distilled water was use to dissolve 0.5g of each extracts in a test tube and mixed vigorously. Three drops of olive oil was added to the frothing and another vigorous mixing was done. The appearance of foam showed the presence of saponins.

Test for steroids

Ten (10) mL anhydrous chloroform was used to dissolve 0.5g of each extracts and filtered. The filtrate was mixed with 1mL of acetic anhydride followed by the addition of 1mL concentrated concentrated tetraoxosulphate (VI) acid (H_2SO_4) down the side of the test tube to form a layer underneath. The test tube was observed for green colouration which indicates presence of steroids.

Test for tannins

0.5g of each extracts was boiled in 10 mL of distilled water in a test tube. One (1) mL of 10% alcoholic iron (III) chloride ($FeCl_3$) was added and observed for brownish green colouration.

Test for terpenoids

Two (2) mL of chloroform was added to 0.5g of each of the concentrated extracts; thereafter c3 mL of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration at the interface indicated the presence of terpenoids.

***In vitro* antibacterial screening**

Organisms

The preserved stocks of pure isolates of *Aeromonas hydrophila*, *A. salmonicida*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Escherichia coli* obtained from the Microbiology Unit of University of Ibadan Teaching Hospital were used for the study

Preparation of isolates

The bacteria were sub-cultured on nutrient agar (Oxoid, Hampshire, England) from the preserved slants and then grown in peptone water

(Oxoid, Hampshire, England) for 24 hour before use. The 24-hour old of each of the test organisms was standardized to 0.5 McFarland standards (1 x 10^6 CFU/ml) following the description of Clinical and Laboratory Standard Institute (CLSI, 2012).

Preparation of extracts

Each of the concentrated extracts of the herbs was dissolved in the solvents (distilled water, ethanol or methanol) used for extraction to produce the desired concentration of 10mg/mL (extract/solvent) (CLSI, 2012; Adeniyi *et al*., 2017).

Agar well diffusion assay

The zone of inhibition was determined using the description of CLSI (2012) using agar well diffusion assay. Concisely, Mueller-Hinton agar (Oxoid, Hampshire, England) was prepared according to the manufacturer's description, sterilized, allowed to cool to room temperature and poured into plates to about 4mm depth under an aseptic condition and allowed to solidify. Thereafter, about 100µL of each standardized isolate suspensions were spread on the Mueller-Hinton agar plates in triplicates. Three wells were bored on each plate with a sterile 6mm diameter core borer; 100 µL of each of the crude extracts of the herbs at 10mg/mL of the three solvents were introduced into the wells in three replicates, allowed to stand at room temperature for about 30 minutes. Controls were also set up in parallel using the solvent used for extraction as well as synthetic antibiotic (Aquamedics) containing oxytetracycline and erythromycin. The plates were incubated at 37°C for 24 hour and observed for zones of inhibition diameter. The zones of inhibition (mm) of the each of the aqueous, ethanol and methanol extracts of the nine herbs were measured with plastic transparent ruler and recorded.

Statistical analysis

Data obtained on growth pattern were analyzed using descriptive statistics, while the data on antibacterial activity were subjected to one way analysis of variance using SPSS (Statistical package for social sciences IBM SPSS Statistics for Windows, IBM Corp., Version 23, Armonk, NY) software while Duncan multiple range test was used to compare differences among means at 5% probability.

RESULTS

Physicochemical analysis of soil of the experimental sites and poultry manure

The physicochemical components of soil sample from the experimental site and poultry manure is presented in Table 2. The soil reaction is slightly acidic, sandy loam in texture, the percentage nitrogen and phosphorous are low. Poultry manure was on the other hand very strongly alkaline in nature with very high organic matter content and available phosphorus. The soil and poultry manure are low in exchangeable calcium with very low exchangeable magnesium. The values of the physicochemical parameters of the poultry manual were generally higher than that of the experimental soil sample.

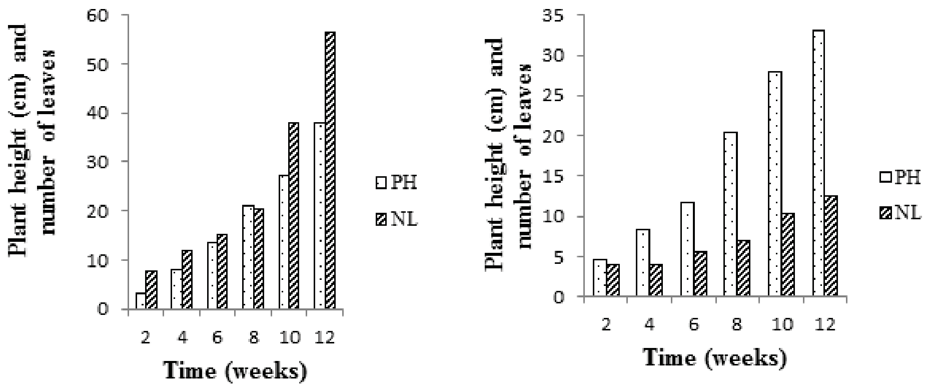
Meteorological conditions during the experimental period

The meteorological information between April and December at the experiment sites is presented in Table 3. The rainfall during the experimental period (June – September) ranged from 40.7-150.2mm. The rainfall was very high in May, but fall drastically between June and August, while the highest was in the month of September and ceased in October. The average air temperature and relative humidity were 24.54 and 76. 03, respectively.

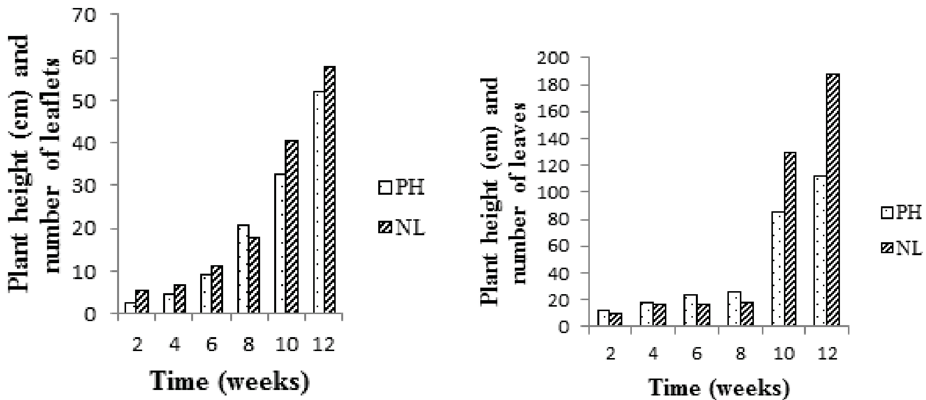
Table 1: Physicochemical analysis of soil and poultry manure of the experimental site

Parameters	Soil	Broiler manure
pH (H_2O)	6.15	10.47
Organic Carbon (%)	1.63	3.48
Organic Matter (%)	2.81	6.01
Textural Class	Sandy loam	-
Nitrogen (%)	0.19	1.26
Available Phosphorus (ppm)	4.85	9.41
Exchangeable Calcium (cmol/Kg)	0.285	0.855
Exchangeable Magnesium (cmol/Kg)	0.085	0.090
Exchangeable Sodium (cmol/Kg)	0.028	0.557
Exchangeable Potassium (cmol/Kg)	0.001	0.026
Exchangeable Acidity (cmol/Kg)	0.152	0.136
CEC (cmol/Kg)	0.551	1.664
Base Saturation (%)	72.41	91.83

Table 2: Meteorological data for the experimental site



Source: Lower Niger River Basin Development Authority (Hydrological section), Ilorin, Nigeria.



O. gratissimum (Fig. 2b). African basil, *O. gratissimum*, showed slow growth rate at the early growth stages between 2 and 6 weeks after planting, followed by a very sharp growth between 8 and 10 weeks (Fig. 2b). Senna *alata* (Fig. 3a) is characterized with short height but followed normal growth curve, with branching at 6 weeks and numerous broad paripinnate leaves at the older stage. On the other hand, *Vernonia amygdalina* exhibited slow growth rate at 2-8 weeks (Fig. 3b) with considerably higher height but with fewer leaves than *S. alata*. The *S. alata* did not flower while no branching

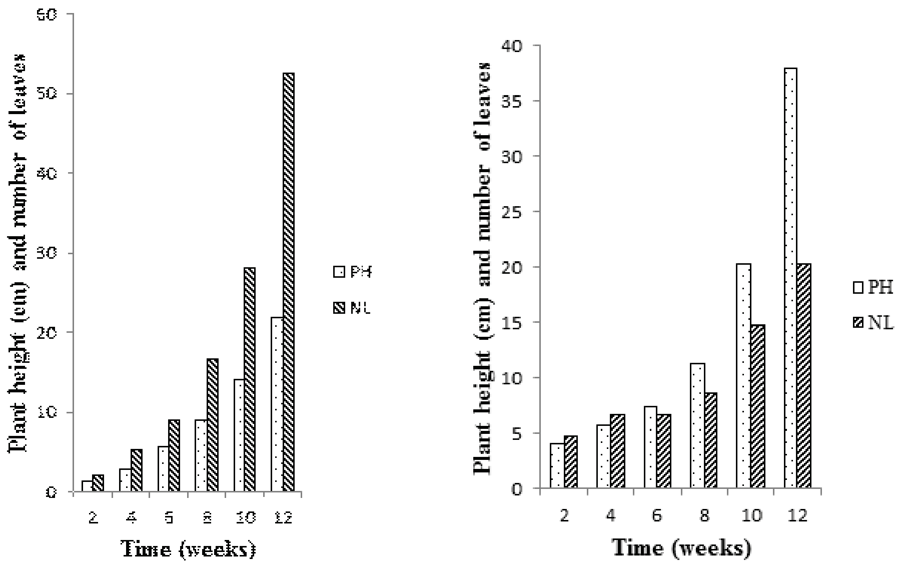


Fig. 3: Plant height (PH) and number of leaves (NL) of *Senna alata* (3a) and *Vernonia amygdalina* (3b) at 2 week's interval after planting

Phytochemical components of the herbs

The phytochemical components of ethanol, methanol and aqueous extracts of the 9 herbs screened in the current study are shown on Table 4. The result showed that saponins, tanins, terpenoids, alkaloids and flavonoids were present in most of various extracts of the herbs. However steroids were absent in *O. gratissimum* and *S. alata* extracts as well as in the ethanol and methanol extracts of *E. hirta*, *E. heterophylla*, *J. gossypiifolia* and *P. amarus*. Alkaloids were also absent in *J. gossypiifolia* extracts and in the aqueous extract of the *Euphorbia* species, while on the other hand, all the extracts contain cardiac glycosides, flavonoids and tannins.

Table 3: Phytochemical components of some herbs using different solvents

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Herbal plants	Alkaloids			CG			Flavonoids			Saponins			Steroids			Tannins			Terpenoids		
	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A	E	M	A
Euphorbia hirta	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-
E. heterophylla	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+
Jatropha gossypiifolia	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+
Moringa oleifera	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ocimum gratissimum	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
Phyllanthus amarus	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
Senna alata	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
Tithonia diversifolia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vernonia amygdalina	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+

+ = Present - = Absent CG = Cardiac glycosides E = Ethanol extracts M = Methanol extract A = Aqueous extract

Antibacterial activity of the selected plant extracts

The result of the antibacterial activities of the herbal extracts showing their zones of inhibition against the bacteria in the present study is presented in Tables 5, 6 and 7. Table 5 shows that ethanol *Euphorbia hirta* extract (EHE) and *Jatropha gossypifolia* extract (JGE) exhibited significantly higher ($P < 0.05$) zones of inhibition against *P. aeruginosa*, compared to the synthetic antibiotic. The ethanol extract of JGE also had higher ($P < 0.05$) zone of inhibition against *Aeromonas hydrophila* (26.67mm) and *Streptococcus agalactiae* (25.67mm) compared to 22.33mm obtained for the synthetic antibiotic against both organisms, while EHE, *E. heterophylla* and *M. oleifera* exhibited similar ($P > 0.05$) activities with the synthetic antibiotic against *A. hydrophila*. Also the ethanol extract of *S. alata* seemed to be effective against *A. salmonicida* while JGE, *P. amarus* extract (PAE) and *Vernonia mygdalina* ethanol extracts possess similar ($P > 0.05$) inhibitory effect against *P. fluorescens*, compared to the synthetic antibiotic. On the other hand, higher ($P < 0.05$) zones of inhibitions, 28.67mm and 26.00mm, were obtained from the synthetic antibiotics against *S. aureus* and *E. coli*, respectively, compared to the herbal extracts. The methanol extracts of JGE, EHE and PAE exhibited higher ($P < 0.05$) zone of inhibition against *P aeruginosa*, compared to other extracts and the synthetic antibiotic while on the other hand, EHE and PAE exhibited similar zones of inhibition with the antibiotic against *P. florescens* (Table 6). However, the zones of inhibition of methanol EHE and JGE seemed to be lower than the values exhibited by ethanol extract against the microbes. The aqueous extracts of the herbs exhibited lower ($P < 0.05$) zones of inhibition, except EHE that had similar zone of inhibition with the synthetic antibiotic against *P. fluorescens* (Table 7). On the over all, the ethanol and aqueous extracts showed highest and lowest antimicrobial activity, respectively, against all the microbes with EHE, JGE and PAE as the best 3 extracts with higher antibacterial potential.

Herbal extracts	PA	PF	Strep	SA	EC	AS	AH
Euphorbia hirta	26.33^{a}	25.00^{a}	22.00^{b}	22.33^{bcd}	21.33^{b}	18.33^{d}	23.67^{b}
E. heterophylla	21.00^{cd}	20.00^{c}	15.67^{de}	18.33^{d}	19.33^{b}	19.33^{d}	21.67^{b}
Jatropha gossypiifolia	25.67^{ab}	24.33^{a}	25.67^{a}	25.00^{b}	23.33^{b}	24.67^{ab}	26.67^{a}
Moringa oleifera	20.00^{cd}	19.67^{c}	18.67^{c}	21.00^{bcd}	19.67^{b}	20.67^{c}	23.00^{b}
Ocimum gratissimum	23.33^{abc}	21.00^{bc}	16.00^{d}	21.33^{bcd}	22.67^{b}	21.00^{c}	12.67^{d}
Phyllanthus amarus	22.33^{bcd}	23.33^{ab}	10.69^{f}	24.00^{bc}	21.67^{b}	19.67^{cd}	13.33^{d}
Senna alata	20.00^{cd}	20.00^{c}	16.00^{d}	19.00^{d}	19.33^{b}	26.67^{a}	15.33^{d}
Tithonia diversifolia	19.33^{d}	19.00^{c}	15.33^{de}	24.00^{bc}	21.67^{b}	20.33^{c}	18.33^{c}
Vernonia mygdalina	22.67^{bcd}	24.67^{a}	13.00^{e}	24.00^{bc}	21.67^{b}	20.00^{cd}	15.00^{d}
Antibiotic	19.33^{d}	23.67^{ab}	22.33^{b}	28.67^{a}	26.00^{a}	25.00^{ab}	22.33^{b}

^{a,b,c} Means within a column with different superscript are significantly different (P < 0.05).

PA = *Pseudomonas aeruginosa* PF = *Pseudomonas fluorescens* Strep = *Streptococcus agalactiae* SA = *Staphylococcus aureus* EC = *Escherichia coli* AS = *Aeromonas salmonicida* AH = *Aeromonas hydrophila*

Table 6: Zone of inhibition (mm) of herbal methanol extracts (mg/mL) against some fish pathogenic and food poisoning bacteria

Herbal extracts	PA	PF	Strep	SA	EC	AS	AH
Euphorbia hirta	20.67^{a}	21.00^{ab}	20.67^{a}	23.67^{b}	19.67^{b}	19.67^{b}	22.00^{b}
E. heterophylla	14.67^{c}	18.00^{c}	16.67^{bc}	21.33^{b}	14.33^{b}	17.00^{b}	15.67^{de}
Jatropha gossypiifolia	23.00^{a}	19.33^{bc}	23.00^{a}	24.00^{b}	22.67^{a}	23.00^{b}	25.67^{a}
Moringa oleifera	14.67^{c}	17.00^{c}	20.33^{ab}	15.67^{c}	13.00^{b}	13.67^{cd}	18.67^{c}
Ocimum gratissimum	12.67^{c}	13.00^{d}	12.67^{de}	14.33^{cd}	11.33^{b}	16.33^{c}	14.67^{de}
Phyllanthus amarus	21.00^{a}	21.33^{ab}	21.00^{a}	22.33^{b}	19.67^{b}	19.67^{b}	10.67^{f}
Senna alata	14.00^{c}	14.00^{d}	12.67^{c}	14.00^{cd}	12.00^{b}	14.33^{def}	16.67^{d}
Tithonia diversifolia	12.33^{c}	13.00^{d}	11.67^{de}	12.33^{d}	11.00^{b}	14.00^{ef}	13.33^{de}
Vernonia amygdalina	12.00^{c}	12.67^{d}	12.00^{c}	13.33^{cd}	13.33	11.67^{d}	13.67^{ef}
Antibiotic	19.33^{b}	23.67^{a}	22.33^{a}	28.67^{a}	26.00^{a}	22.67^{a}	22.33^{b}

^{a,b,c} Means within a column with different superscript are significantly different (P 0.05).

PA = *Pseudomonas aeruginosa* PF = *Pseudomonas fluorescens* Strep = *Streptococcus agalactiae* SA = *Staphylococcus aureus* EC = *Escherichia coli* AS = *Aeromonas salmonicida* AH = *Aeromonas hydrophila*

Table 7: Zone of inhibition (mm) of herbal aqueous extracts (mg/mL) against some fish pathogenic and food poisoning bacteria

Herbal extracts	PA	PF	Strep	SA	EC	AS	AH
Euphorbia hirta	16.00^{cd}	25.00^{a}	13.33^{b}	14.67^{b}	17.00^{b}	12.33^{c}	13.00^{c}
E. heterophylla	18.00^{bcd}	14.00^{c}	12.33^{bcd}	15.00^{b}	14.00^{c}	10.33^{e}	12.00^{cd}
Jatropha gossypiifolia	18.33^{bc}	17.00^{b}	13.33^{b}	15.33^{b}	15.00^{bc}	14.67^{b}	15.67^{b}
Moringa oleifera	20.33^{ab}	12.00^{d}	11.33^{cde}	13.33^{bc}	13.67^{cd}	10.67^{de}	10.33^{ef}
Ocimum gratissimum	12.67^{e}	10.67^{e}	10.33^{e}	12.33^{c}	10.67^{e}	10.33^{e}	10.00^{f}
Phyllanthus amarus	18.33^{b}	16.00^{b}	12.67^{bc}	16.33^{b}	14.00^{c}	13.33^{bc}	11.67^{cde}
Senna alata	12.00^{e}	10.33^{e}	10.67^{e}	10.33^{c}	10.67^{e}	10.33^{e}	10.00^{f}
Tithonia diversifolia	15.33^{d}	10.33^{e}	11.33^{cde}	12.33^{c}	11.33^{d}	10.33^{e}	10.33^{ef}
Vernonia mygdalina	13.00^{e}	10.67^{e}	13.33^{b}	10.33^{c}	10.33^{e}	10.33^{e}	10.33^{ef}
Antibiotic	19.33^{b}	23.67^{a}	22.33^{a}	28.67^{a}	26.00^{a}	22.67^{a}	22.33^{a}

^{a,b,c} Means within a column with different superscript are significantly different (P 0.05).

PA = *Pseudomonas aeruginosa* PF = *Pseudomonas fluorescens* Strep = *Streptococcus agalactiae* SA = *Staphylococcus aureus* EC = *Escherichia coli* AS = *Aeromonas salmonicida* AH = *Aeromonas hydrophila*

DISCUSSION

The higher percentage nitrogen, alkalinity and the available phosphorus contents observed in the current study was similar to the previous studies (Enwezor *et al*., 1989; Olowoake and Ojo, 2014; Alabi et al (2017); Fasakin et al., 2019). The soil is also very low in exchangeable sodium, potassium and cation exchangeable capacity (USDA–SCS, 1974) but the quantity is moderate in poultry manure. Our observation on low soil exchangeable sodium and potassium, when compared to poultry manure is similar to the

report of Ogunleye *et al*. (2019). The low nitrogen contents of the soils could be attributed to the characteristic nature of tropical soils and nutrient uptake from the previous crop. Medium to low content of organic matter content may be attributed to the nature of vegetative cover. The relatively low to very low content of exchangeable bases may be due to the nature of parent materials from which soils are formed or due to climatic conditions location, the more the erosion, the more weathering and the more loss of these exchangeable bases. Low rainfalls were experienced from June to August with peak in September during the study. The highest rainfall observed in September during the study coincided with the observation in a previous study (Ogunleye et al., 2019) in which the peak of rainfall was September, even in the Rainforest region of Nigeria while the cessation of rainfall in October is similar to previous report (Fasakin *et al*., 2019) at the experimental site. Although the scientific information on the growth pattern of herbal plants is very scarce, the current study has shown that the plants cultivated in this study could be grown on moderately fertile soil within three months for utilization as organic antibacterial agents and hence it is possible to have 3 cycles per year, especially with irrigation in off rain seasons and could be much more sustainable when integrated with organic aquaculture.

Aeromonas and Pseudomonas species are opportunistic pathogenic bacteria, which are naturally present in aquatic habitat with devastating effects on cultured freshwater fishes, owning to the stressful condition of the semi-intensive or intensive culture systems with acute to chronic clinical signs causing significant morbidity, mortality and enormous economic losses (Noga 2012; Hardi *et al*., 2016). Streptococcosis is a zoonotic disease of global concern caused *Streptococcus* species, including *Streptococus agalatiae*, *S. dysgalactiae* and *S. iniae*, affecting man, freshwater and saltwater fish species worldwide (Gent *et al*., 2012; Abdelsalam *et al*., 2013; Li *et al*., 2014; Li *et al*., 2015; Assis *et al*., 2016; Iregui *et al*., 2016; Delannoy *et al*., 2016; Mengmeng *et al*. 2019. The *E. coli* and *S. aureus* have also been identified as resistant food-borne bacterial pathogens associated with aquatic products and microbial food poisoning which threatens public health (Onmaz *et al*., 2015; Saharan *et al*, 2019).The rise in global concern on the adverse effects of uncontrolled utilization of synthetic antibiotics to control infectious diseases in the production of food fish necessitates search for alternative natural products, including herbal additives in aquaculture.

The present study has demonstrated the antibacterial activities of *E. hirta*, *E. heterophylla*, *J. gossypiifolia*, *O. gratissimum*, *M. oleifera*, *P. amarus*, *S. alata*, *T. diversifolia*, and *V. amygdalina* extracts, in which the ethanol extract showed the highest inhibitory effect. The antibacterial activity shows that the herbal products could be utilized as natural/organic antimicrobial and food preservative agents against the selected pathogens. The findings in the present study affirm the earlier reports on the antibacterial activities of *Euphorbia* species (Ogbulie *et al.* 2007; Saravanan *et al.,* 2012; Oyedun, 2017), *M. oleifera* (Onsare *et al*, 2013; Abubakar and Usman, 2016; Isitua *et al*., 2016; Abubakar and Usman, 2016; Ervianingsih *et al*., 2019), *V. mygdalina* (Adetunji *et al*., 2013), *T. diversifolia* and *J. gossypiifolia* (Okiti and Osuntokun, 2020). The observed antimicrobial effect could be associated with the presence of bioactive compounds like alkaloids, tannins, terpenoids and flavonoid in the herbal extracts. The phytochemicals observed in the current study coincided with previous observations on *Phyllanthus* species (Samali *et al*., 2012; Sen and Batra, 2013), *E. hirta* (Essiett and Okoko, 2013), *M. oleifera* (Saini *et al*., 2016), and *T. diversifolia* (Okiti and Osuntokun, 2020). The antibacterial activities of plant-based antimicrobials have been ascribed to the presence of bioactive phytochemicals, including secondary metabolites such as alkaloid, saponin, tannin, terpenoids and phenolic compounds which have been associated with antimicrobial activities of herbal extracts and thus formed the basis for the ethnomedical uses of these plants as organic therapeutics (Zablotowicz *et al*., 1996; Lewis and Ausubel, 2006; Falodun *et al*., 2012; Olushola *et al*., 2013; Apiamu *et al*., 2013; Ahmad *et al.,* 2017) for the culture of aquatic animals and livestock meant for human consumption in other to mitigate the aforementioned deleterious effects of synthetic antibiotics.

In conclusion, the present study has shown the antibacterial potentials of some cultivated herbs against varieties of pathogenic (*A. hydrophila*, *A. salmonicida*, *P. aeruginosa*, *P. fluorescens*, *S. agalactiae*) and food poisoning bacteria (*Pseudomonas* species, *S. aureus* and *Escherichia coli*) of aquaculture species and products. Moderate inhibitory activity was exhibited by ethanol extracts of *Euphorbia* species, *Jatropha gossypiifolia* and *Phyllanthus amarus* against all the microbes.

Further investigations on the *in vivo* antibacterial of these herbal extracts to determine their efficacy as prophylactic, therapeutic and preservative

agents in fish health management and preservation is recommended.

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REFERENCES

Abdelsalam, M., A. Ashe and A. E. Eissa. (2013). *Streptococcus dysgalactiae*: An emerging pathogen of fishes and mammals. *International Journal of Veterinary Science Medicine*, 1: 1-6.

Abdel-Tawwab, M., I. Adesina., A. Jenyo-Oni., E. K Ajani and B. O. Emikpe. (2018). Growth, physiology, antioxidant and immune response of African catfish, *Clarias gariepinus* (B), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection. *Fish and Shellfish Immunology*, 78: 346-354.

Abubakar, I. and A. Usman. (2016). Phytochemical and antibacterial investigations of moringa (*Moringa oleifera*) leaf extract on selected bacterial pathogens. *Journal of Microbiology and Antimicrobials*, 8 (5): 28-33.

Adeniyi, O. V. (2020). Growth performance of *Clarias gariepinus* (Burchell 1822) fed diets fortified with lemongrass (*Cymbopogon citratus*). *Acta Veterinian Eurasia*, 46: 21-29.

Adeniyi, O.V. and A. O. Lawal. (2017). Growth performance of African catfish fed diet supplemented with *Gossypium herbacium* powder as dietary feed additive. *Journal of Agriculture and Ecological Research International*, 11(2): 1-7.

Adeniyi, O. V., F. E. Olaifa., B. O. Emikpe and S. T. Ogunbanwo. (2017). Phytochemical components and antibacterial activity of *Tamarindus indica* Linn. extracts against some pathogens. *Biotechnology Journal International,* 17 (2): 1-9.

Adeniyi, O. V., F. E. Olaifa., B. O. Emikpe and A. A. Oyagbemi. (2018a).

Experimental evaluation of the wound-healing and antioxidant activities of tamarind *Tamarindus indica* pulp and leaf meal in the African catfish (*Clarias gariepinus*). *Acta Veterinaria Eurasia*, 44: 63-72.

Adeniyi, O. V., F. E. Olaifa and B. O. Emikpe. (2018b). Growth performance and nutrient digestibility of *Clarias gariepinus* (Burchell 1822) fed diets fortified with *Tamarindus indica* pulp and leaf meal. *Asian Fisheries Science*, 31: 17-31.

Adeoye, G. O. and A. A. Agboola. (1985). Critical levels for soil pH, available P, K, Zn and Mn and maize ear leaf content of P, Cu and Mn on sedimentary soils of southwestern Nigeria. *Fertilizer Research,* 6(1): 65-67.

Adeshina, I., A. Jenyo-oni., B. O. Emikpe and E. K. Ajani. (2018). Effects of solvents on phytoconstitunets and antimicrobial activities of *Ocimum gratissisum* and *Euglena caryophyllata* extracts on Listeria monocytogenes. *Acta Veterinaria Eurasia*, 44: 31-38.

Adetunji, C. O., O. O. Olaniyi and A. T. J. Ogunkunle. (2013). Bacterial activity of crude extracts of *Vernonia amygdalina* on clinical isolates. *Journal of Microbiology and Antimicrobial*s, 5(6): 60-64.

Adeyemo, M. A. (2014). Effects of roselle as dietary additive on growth performance and production economy of *Clarias gariepinus*. *Journal of Emerging Trends in Engineering and Applied Sciences*, 5(7): 1-8.

Ahmad, A., O. M. Faja, A. A. Sharad and G. Usup. (2017). Isolation, identification and distribution of potentially pathogenic bacteria associated with selected water bodies and fish organs in different locations in Peninsular Malaysia. International Journal of *Advanced Scientific and Technical Research.* 1(7), http://www.rspublication.com/ijst/index.html ISSN 2249-9954

Ahmad, M. H. and M. Abdel-Tawwab. (2011). The use of caraway seed meal as a feed additive in fish diets: Growth performance, feed utilization and whole body composition of Nile tilapia, *Oreochromis niloticus* (L) fingerlings. *Aquaculture*, 314:110-114.

Alabi, K. O., A. A. Wahab., O. O. Lawal., I. A. Aderolu and B. A. Senjobi. (2017). Characterization and classification of soils of Kwara state University Teaching and Research farm, Malete, Nigeria. *Journal of*

Science, Technology, Mathematics and Education 13(2): 100 – 106.

Aly, S. M. and A. Albutti. (2014). Antimicrobials use in aquaculture and their public health impact. *Journal of Aquaculture Research and Development*, 5: 247. doi: 10.4172/2155- 9546.1000247

Anderson, J. and J. Ingram. (1998). Tropical soil biology and fertility, a handbook of methods. Second Edition, Centre for Biosciences and Agriculture International (CABI), Wallington, 221.

Apiamu A., U. F. Evuen and U. I. Ajaja. (2013). Biochemical Assessment of the effect of Aqueous Leaf Extract of *Euphorbia heterophylla* Linn on Hepatocytes of Rats. IOSR *Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTET), 37-41.

Bagalkotkar, G., S. R. Sagineedu., M. S. Saad and J. Stanslas. (2006). Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: a review. *Journal of Pharmacy and Pharmacology*, 58: 1559–1570.

Bello, O. S., F. E. Olaifa and B. O. Emikpe. (2012). The effect of walnut (*Tetracarpidium conophorum*) leaf and onion bulb residues on the growth performance and nutrient utilization of *Clarias gariepinus* juveniles. *Journal of Agricultural Science* 4(12): 205- 213.

Bello, O. S., F. E. Olaifa., B. O. Emikpe and S. T. Ogunbanwo. (2013). Potential of walnut (*Tetracarpidium conophorum* Mull. Arg) leaf and onion (*Allium cepa* Linn) bulb extracts as antimicrobial agents for fish. *African Journal of Microbiology Research*, 19: 2027- 2033.

Bremner, J. M. and C. Mulvaney. (1982). Nitrogen total: In Methods of soil analysis Part 2, A. L. Page and R.H. Miller (eds.), vol. 9 of Agronomy Monograph, pp. 595–624, ASA, SSSA, Madison, Wis, USA.

Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal*, 54(5): 464-465.

CLSI (2012). Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute's Twenty-Second Information Supplement, 32.3: 44-160.

Dada, A. A. (2015). Use of fluted pumpkin (*Telfairia occidentalis*) leaf powder as feed additive in African catfish (*Clarias gariepinus*) fingerlings.

International Journal of Biological and Chemical Sciences, 9(1): 301-307.

Delannoy, C., R., Zadoks, M. Crumlish, D. Rodgers, F. Lainson, H. Ferguson, J.Turnbull and M. Fontaine. (2016). Genomic comparison of virulent and non-virulent *Streptococcus agalactiae* in fish. *Journal of Fish Diseases*, 39:13–29.

Enwezor, W. O., E. J. Udo., N. J. Usoro., K. A. Ayotade., J. A. Adepetu., V. O. Chude and C. C. Udegbe. (1989). Fertilizer use and management practices for crop in Nigeria. Series 2, *Federal Minister of Agriculture, Water Resources and Rural Development Nigeria,* Pp.52-56.

Ervianingsih, M., R. Mursyid., N. Annisa., I. Zahran., J. Langkong and I. Kamaruddin. (2019). Antimicrobial activity of moringa leaf (*Moringa oleifera* L.) extract against the growth of *Staphylococcus epidermidis*. IOP Conf. Series: Earth and Environmental Science 343, *The 1st International Conference of Interdisciplinary Research on Green Environmental Approach for Sustainable (ICROEST)* 2019 3–4 August 2019, University of Muhammadiyah Buton, Indonesia. IOP Publishing. doi:10.1088/1755-1315/343/1/012145

Essiett, U. A. and A. I. Okoko. (2013). Comparative nutritional and phytochemical screening of the leaves and stems of *Acalyphafirm briata* Schum. Thonn. and *Euphorbia hirta* Linn. *Bulletin of Environment, Pharmacology and Life Sciences*, 2 (4): 38- 44.

Falodun, A., N. O. Engel-Lutz., O. Erharuyi and E. C. Ekokotu. (2012). Preliminary antioxidant and antimicrobial activities ofEuphorbia heterophylla(whole plant) (Euphorbiaceae). *Journal of Pharmaceutical and Allied Sciences, 3.* 365 – 369.

Fasakin, K., A. I. Afe and N. A. Saka. (2019). Growth and yield response of okra types and times of application in the Southern Guinea Savanna Agro-Ecozone of Nigeria. *Journal of Agricultural Sciences*, 64 (4): 353-366

Gee, G. and W. Bauder. (1986). Particle size analysis. In: Dane, J.H, and Topp, G.C. (editions). Soil Science Society of America Book Series No. 5. ASA and SSSA, Madison, WI. pp 255-293.

Gent, Y., K. Y. Wang., X. L. Huang., D. F. Chen., C. W. Li., S. Y. Ren., Y. T. Liao., C. Z. Y. Zhou., Q. F. Liu., Z. J. Du and W. M. Lai. (2012).

Streptococcus agalactiae, an emerging pathogen for cultured Ya-fish, *Schizothorax prenanti* in China. *Transboundary and Emerging Diseases*, 59: 369-375.

Ghassan, A., S. Harbant and S. Muhammad. (2012). Evaluating eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides. *Annals of Agricultural and Environmental Medicine*, 19(4): 673-676.

Hardi, E. H., W. K. Irawan., W. Suwinarti and R. A. Nugroho. (2016). Antibacterial activity of *Boesenbergia pandurata, Zingiber zerumbet* and *Solanum ferox* extracts against *Aeromonas hydrophila* and *Pseudomonas* sp. *Nusantara Bioscience*, 8(1): 18-21.

Hardi, E. H., R. A. Nugroho., I. W. Kusuma., W. Suwinarti., A. Sudaryono and R. Rostika. (2019). Borneo herbal plant extracts as a natural medication for prophylaxix and treatment of *Aeromonas hydrophila* and *Pseudomonas fluorescens* infections in tilapia (*Orechromis niloticus*). *F1000 Research*, 7: 1847

Isitua, C. C., I. N. Ibeh and J. N. Olayinka. (2016). Antibacterial activity of *Moringa oleifera* Lam leaves on enteric human pathogens. *Indian Journal of Applied Research*, 6 (8): 553- 557.

Kalsom, U., S. Siddique., N. Shahzad., T. Ghous and S. Andleeb. (2013). *In vitro* screening of herbal extracts and antibiotics against bacteria isolated from fish products at retail outlets. *British Microbiology Journal*, 3(1): 19-31.

Li, L., R. Wang., W. Liang., T. Huang., Y. Huang., F. Luo., A. Lei., M. Chen and X. Gan. (2015). Development of live attenuated *Streptococcus agalactiae* vaccine for tilapia via continuous passage *in vitro*. *Fish and Shellfish Immunology*, 45:955–963.

Lewis, K. and F. M. Ausubel. (2006). Prospects of plant derived antibacterial. *National Biotechnology*, 24: 1504-1507.

Mahdavi, M., A. Hajimoradloo and R. Ghorbani. (2013). Effect of *Aloe vera* extract on growth parameters of common carp (*Cyprinus carpio*). *World Journal of Medical Sciences* 9(1): 55-60.

Najiah, M., M. Nadirah., Z. Arief., S. Zahrol., L. W. Tee., A. D. Ranzi., A. S.

Amar., A. R. Laith., R. Maham., S. Suzanz and R. J. Aida. (2011). Antibacterial activity of Malaysian edible herbs extracts on fish pathogenic bacteria. *Research Journal of Medicinal Plant*, 5: 772- 778.

Noga, E.J. (2012). Fish diseases diagnosis and treatment. Second Edition. Singapore: Wiley – Blackwell. 519pp.

Nyadjeu, P., J. Angoun., N. P. Ndasi and M. E. Tabi-Tomedi. (2019). Effect of *Garcinia kola* seeds supplemented diet on growth performance and gonadal development of *Oreochromis niloticus* juveniles breed in ponds. *Fisheries and Aquatic Sciences* 22:20 https://doi.org/10.1186/s41240-019-0136-1

Ogbulie, J. N., C. C. Ogueke., I. C. Okoli and B. N. Anyanwu. (2007). Antibacterial activities and toxicological potentials of crude ethanol extracts of *Euphorbia hirta*. *African Journal of Biotechnology*, 6: 1544-1548.

Ogunleye, M. T., J. G. Bodunde., E. A. Makinde., O. P. Sobukola and B. A. Shobo. (2019).Comparative growth and yield of Roma vf tomato fruits with organic and inorganic fertilizer. *International Organic Agriculture Research and Development*, 16: 3-12.

Okiti, A. F. and O. T. Osuntokun. (2020). Antimicrobial, phytochemical analysis and molecular docking (*In-silico* Approach) of *Tithonia diversifolia (Hemsl.) A. Gray* and *Jatropha gossypiifolia* L on selected clinical and multi-drug resistant isolates. *Journal of Advances in Microbiology* 20(6): 1-18.

Olowoake, A. A. and J. A. Ojo. (2014). Effect of Fertilizer Types on the Growth and Yield of *Amaranthus caudatus* in Ilorin, Southern Guinea, Savanna Zone of Nigeria. *Advances in Agriculture*. Article ID 947062, 5 pages http://dx.doi.org/10.1155/2014/947062

Olusola, S. E., B. O. Emikpe and F. E. Olaifa. (2013). The potentials of medicinal plant extracts as bio-antimicrobials in aquaculture. *International Journal of Medicinal and Aromatic Plants*, 3: 404-412.

Oluwafemi, F. and F. Debiri. (2008). Antimicrobial effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi*. *African Journal of Biomedical Research*, 11: 215-219.

Onsare J. G., H. Kaur and D. S. Arora. (2013). Antimicrobial activity of

Moringa oleifera from different locations against some human pathogens. *Academia Journal of Medicinal Plans,* 1(5): 80-91.

Panda N. P. and Ray P. (2012). A study of some indigenous plant extracts against two human pathogens. *Asian Journal of Experimental Biological Sciences*, 3(1): 175-179.

Pedge, S. S, and S. D. Ahirrao. (2012). Antimicrobial activities of turmeric and ginger root used in the treatment of fish diseases. *International Journal of Biosciences*, 2(2): 81-84.

Reverter, M., N. Bontemps., D. Lecchini., B. Banaigs and P. Sasal. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture*, 433: 50–61.

Reverter, M., N. Tapissier-Bontemps., P. Sasal and D. Saulnier. (2017). Use of medicinal plants in aquaculture. 9, 223-261. In: *Diagnosis and control of diseases of fish and shellfish*, B. Austin, B., & Newaj-Fyzul, A. (eds). Hoboken, New Jersey: John Wiley and Sons Ltd.

Rhoades, J. D. (1982). Cation exchange capacity. In: Methods of soil analysis, Part 2 Chemical and microbiological properties, 2nd Edition, A.L. Page (Ed.). *Agronomy* 9: 149 – 157.

Saini, R. K., I. Sivanesan and Y. Keum. (2016). Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. *Biotech* 6, 203. https://doi.org/10.1007/s13205-016-0526-3

Sajid, M., S. Prabjeet., H. S. Munir and M. Khusheeba. (2011). Emerging role of immunostimulants in combating the disease outbreak in aquaculture. *International Aquatic Resources*, 3: 147-163.

Samali, A., D. T. Florence., O. A. Odeniran and O. N. Cordelia. (2012). Evaluation of chemical constituents of *Phyllanthus niruri*. *African Journal of Pharmacy and Pharmacology*, 6 (3): 125-128.

Santos, L. and F. Ramos. (2018). Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. *International Journal of Antimicrobial Agents*, 52(2): 135-143.

Saravanan, R., D. Dhachinamoorthi., K. Senthilkumar., M. Srilakshmi and T. Divya-Sri. (2012). Antibacterial activity of *Euphorbia hirta* extracts. *IJRAP* 3(3), May-June 2012. www.ijrap.net

Sen, A. and A. Batra. (2013). The study of *in vitro* and *in vivo* antioxidant activity and total phenolic content of *Phyllanthus amarus* Schum Thonn: A medicinally important plant. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5: 947.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria. 150pp.

Trease, G. E. and W. C. Evans. (1989). *Pharmacognosy*, 11th Edition. Brailliar Tiridel Canada,Macmillian publishers. 176-180pp.

USDA-SCS (US Department of Agriculture, Soil Conservation Service), 1974. National Engineering Handbook. Section 15. Chapter 4: Border Irrigation. National Technical Information Service, Washington, DC.

Walkley, A. and I. A. Black. (1934). An examination of the digestion method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci*ence, 37: 29-38.