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Antibacterial Activity of Ethanolic Extract of *Acalypha wilkesiana and Peperomia pellucida* on Fish Pathogens

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Abstract: The study was conducted to investigate the antibacterial activity of ethanolic extract of *Acalypha wilkesiana and Peperomia pellucida* leaves against Catfish infected with bacterial fin-rot. The menace of fin-rot in fingerlings of *Clarias gariepinus* is becoming high and this can impair on fish productivity. Agar well diffusion assay was used and Whatman filter paper soaked in plant extracts was placed on inoculated Mueller-Hinton agar plate to determine inhibitory action and zones of inhibition of plant extracts on the isolated bacteria recorded.The identified bacterial pathogen includes *Streptococcus* sp, *Bacillus cereus, Klebsiella sp., Enterobacter aerogenes and Escherichia coli*. The plant extracts inhibited the organisms using disc agar diffusion test. The highest zone of inhibition was 0.75mm by *Acalypha* on *Streptococcus sp*. While the least was 0.50mm *Acalypha on klebsiella sp., Enterobacter aerogenes and E. coli.* Also *Peperomia* had 0.50mm on *E. aerogenes. Acalypha* extract inhibited all the bacterial isolates tested. Gentamycin a positive control inhibited all the isolates except *Bacillus cereus* while water, a negative control inhibited no bacteria. Preliminary photochemical testing revealed the presence of tannins, flavonoids, saponin, phylobatanins, alkaloids, glycosides and cardiac glycosides from slightly present to highly present on *Acalypha* while glycoside and phylobatanins was absent in *Peperomia;* saponins, flavonoids, alkaloids and cardiac glycosides varied from slightly to highly present. The leaves of these plants has inhibitory action on isolated bacteria and can be used to treat bacterial fin-rot of infected catfish *Clarias gariepinus.*

Keywords: *Acalypha wilkesiana, peperomia pellucida, ethanolic extract, antibacterial activity, Abeokuta Ogun State.*

I. INTRODUCTION

Aquaculture is the fastest-growing animal food producing sector with an average annual increase of 6% per year in the period 1990–2010 (FAO, 2012). Despite the popularity of farming in Nigeria, the fish farming industry can best be described as being at the infant stage when compared to the large market potential for its production and marketing (Nwiro, 2012). The Nigerian fishing industry comprises of three major sub sectors namely the artisanal, industrial and aquaculture of which awareness on the potential of aquaculture to contribute to domestic fish production has continued to increase in the country (Adewuyi, Phillip, Ayinde and Akerele, 2010). A right step towards arresting the demand-supply deficit for fish is aquaculture, which involves raising fish under controlled environment where their feeding, growth, reproduction and health can be closely monitored (Ejiola and Yinka, 2012).

Aquaculture has been highly adapted as an alternative to natural fishing from open waters and can greatly increase the protein intake of the people but the problem of disease is hampering its productivity.

This is evidenced by a host of diseases. Among the various diseases, those caused by pathogenic bacteria represent the gravest threat to aquaculture (Davis and Hayasaka, 1983).

These fishes are susceptible to various diseases that are hampering its growth from infectious and non-infectious diseases and can impair greatly on its production capability. (Jeney and Jeney, 1995). Antimicrobials and other veterinary drugs are administered regularly as additives in fish food or sometimes in baths and injections and are used as prophylactics (prevent diseases before they occur), therapeutics (treat sick animals) or growth promoters (Rico *et al*., 2013).

In fish disease Management Synthetic chemicals and antibiotics have been used but due to the side effects and emergence of antibiotic resistant strains of Micro-organisms (Davies, 1994). However, the bacteria develop resistance, either by mutations or by acquiring new genes from other bacteria to these molecular targets of macro molecular biosynthesis target. There is the need for alternative to combat the problem and search is on-going, hence antimicrobials from plant origin would be beneficial in this direction. As a result, there is a continuous search for antimicrobials from plant sources. These plants are used either alone or in combination with known antibiotics for treating bacterial infections (Collin and Pareicia, 1970). The renewed interest in the use of medicinal plants may be attributed to cheapness, availability, fast rate of decomposition and are eco-friendly with little side effects. Plants with medicinal properties have been used by man to treat and control human ailments and are still relevant in modern times. (Nair *et al*, 2004, Stiffness and Douros, 1982). Some of the proposed solutions are the use of natural products (plant extracts) or probiotics (beneficial microbial strains) in the culture of fish and shrimp (Citarasu, 2010).

Phytochemical research based on ethnopharmacologial information is generally considered an effective approach in the discovery of new anti infective agents from higher plants (Kloucek *et al*., 2005). The genus 'Acalypha' comprises about 570 species (Riley, 1963) *A. wilkesaina* belongs to the Euphorbicaceae family.This plant have been used to treat skin infections and other ailments (Akinde and Odeyemi, 1987) its antimicrobial property (Adesina *et al*, 1980; 2000, Kabir *et al.*, 2005, Oladunmoye, 2006, Erute and Oyibo, 2008). *Peperomia Pellucida* belongs to the family Piperaceae. It is an annual, shallow-rooted herb (Ghani, 1998). Khan and Omoloso (2002) reported the anti-microbial activity of *P. pellucida* extract against numerous species of bateria including *Bacillus subtilis, Escherichia coli,pseudomonas aeruginosa and Staphylococcus*

aureus. Xu *et al* (2006) have Studied the bioactive compound from *P.pellucida* and have reported that the crude extract of the plants cause cytotoxicity against the cancer cell lines HL-60, MCF-7 and HeLa. This study was done using *Acalypha wilkesaina and Peperomia pellucida* against some bacteria isolated from fin-rot infected Catfish, *Clarias gariepinus.*

II. MATERIAL AND METHODS

Plants preparation and extraction

Acalypha wilkesiana and Peperomia pellucida were collected from Federal University of Agriculture, Abeokuta (FUNAAB) Arboretum. Extraction was done using soxhlet apparatus.40g of fresh leaves was placed in a soxhlet and extracted with 150ml 95% ethanol. The extract was done until the solvent in the soxhlet turned colourless. The extract was concentrated by recovering the solvent using the soxhlet apparatus until the extract become just pourable. It was poured into a beaker and put in the oven at 40^{o}C to dry. The volume is reduced to concentrated form of 25mls yielding a 6:1 ratio to give 100% concentration.

Phytochemical testing

Testing for the present of Phytochemical was carried out using the methods in Sofowora (1982) for testing the organic constituents.

Test for alkaloids

About 0.5g of extract was stirred with 3 ml of 1% aqueous Hydrochloric acid on a steam bath and filtered, 1 ml of the filtrate was treated with few drops of these reagents:

1. Mayer's reagent

2. Picric acid solution

3. Dragendor ff's reagent. precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids.

Test for saponins

The 0.5g of the extract was shaken with water in a test tube. Frothing which persists on warming was taken as a preliminary evidence for presence of saponins.

Test for tannins

A 0.5g of the extract was stirred with 1ml of distilled water and filtered. Ferric chloride solution was added to the filtrate. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins.

Test for cardiac glycosides

(Keller killiani test)

Using 0.1g of the extract and was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. A 1ml of concentrated sulphuric acid was added gently by the side of the test tube. A brown ring obtained at the interphase indicates the presence of de oxy sugar characteristic of cardenolides.

Test for flavonoids

A 2g of powered sample was detanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the detanned sample. The mixture was filtered while hot. The filtrate was cooled and 5ml of 20% sodium hydroxide was added to equal volume of the filtrate. A yellow solution indicates the presence of flavonoids.

Test for Phylobatanins

About 2ml of aqueous extract was added to 2ml of 1% HCL and the mixture was boiled. Deposition of a\ red precipitate was taken as an evidence for the presence of Phylobatanins.

Test for reducing Sugar (in glycosides)

The residue was re-dissolved in water in the water bath. Two milliliter of the solution in a test tube was added 1ml each of Fehling's solution A and B. The mixture was shaken and heated in a water bath for 10min, a brick-red precipitate indicates a reducing sugar.

Concentration used

The 100% concentration of the plant extracts was used on the test organisms isolated from infected Catfish, *Clarias gariepinus.*

Test organisms

The test organisms were bacterial isolates from infected fin-rot Catfish, *Clarias gariepinus*. The following organisms were identified and used for the experiment: *Bacillus cereus, Enterobacter aerogenes, Escherichia coli, Streptococcus sp, Klebsiella sp.*

Identification of organisms

The isolates were identified after the characterization of the organisms using bio-chemical tests

Bacterial susceptibility testing

Agar Diffusion test

Disk diffusion assay was conducted according to Alderman and Smith (2001). The surface of an agar plate was inoculated with a bacterial suspension on Muller-Hinton agar. Sterile 6mm paper filters disks were soaked in 22 microlitre of the aqueous plant extract in triplicate and paper disc embedded with sterile water was used as the negative control, placed on the inoculated agar plate, and incubated at 37^{o} C for 24 hrs. Zones of inhibition produced after incubation were measured in millimetres (Abayo, 1982; Okwori *et al*., 2007). Results are the average of the three measurements.

III. RESULTS

The Phytochemical testing of the extract of *Acalypha wilkesiana* showed positive reactions for saponins, tannins, flavonoids, phylobatanins, alkaloids glycosides and cardiac glycosides from slightly present to highly present (table 1). In addition, the plant extract of *Peperomia pellucida* gave positive reactions for saponins, tannins, flavonoids, alkaloids, glycosides and cardiac glycosides from slightly present to highly present and negatives reactions to phylobatanins and glycoside from glycosides (table 2).

Acalypha extract inhibited the growth of all the organisms tested as indicated by their zones of inhibition in (table 3). However, *Peperomia pellucida* extract inhibited only the growth of *Enterobacter aerogenes. Acalypha* extract with zone of inhibition of (0.75mm) was the highest on *Streptococcus sp*, followed by

(0.60mm) *Bacillus cereus*, others were (0.50) on the remaining bacteria. The positive control Gentamycin had average zone of inhibition of 0.90mm while the negative control did not show any zone of inhibition. The zone of inhibition of 0.75mm and 0.70mm were similar on S*treptococcus sp* (table 3).

IV. DISCUSSION

Extracts of 2 plants were screened for antibacterial activity against pathogenic bacterial in fish. The disc diffusion assay was used. Extracts from these plants species inhibited growth of at least one of the tested bacterial, while *Acalypha wilkesaina* inhibited all the bacterial isolates. The presence of zones of inhibition on the agar plates showed that the tested organisms which included both Gram positive and Gram negative organisms. Although the zones of inhibition were lower than that exhibited by the standard drug Gentamycin, this could be due to the fact that the plant extract is crude and contains other constituents that do not posses antibacterial property. Though the plant extract of *Acalypha* extract and standard drug gentamycin were similar on *Streptococcus sp*. Generally, the antibacterial activity of the *Acalypha* extract against *E.coli*, *Streptococcus* and other bacterial agrees with earlier works by Akinde and Odeyemi, 1987), Adesina *et al*, 1980, Kabir *et al.*, 2005) and Oladunmoye, 2006). In addition, the antibacterial activity of *Peperomia* extract against *Enterobacter aerogenes* agrees with study by Xu *et al* (2006), Khan and Omoloso (2002) on the antimicrobial activity against numerous species of bacterial including *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, andSstaphylococcus aureus*.

The Phytochemical testing of the extract showed that the *Acalypha* extract contains saponins, tannins, flavonoids, phylobatanins, alkaloids and cardiac glycosides From slightly present to highly present. This result is similar to what Oladunmoye, 2006) report on the presence of organic constituents of afformentioned. Basu *et al* (2007) reported on the Phytochemical present on *Acalypha* and include alkaloids, tannins, flavonoids which were moderately present while carotenoid, saponin was highly present. The saponin of this work is highly present their's was moderately present. The difference could be due to environmental and edaphic factors in the geographical areas in which the plants were located. *Peperomia* plant extract showed the presence of saponin, tannins, flavonoids, alkaloids cardiac glycoside from slightly present to highly present except phylobatanins and glycoside that is absent. Tannins and saponins are responsible for the antibacterial activity of the plant extracts (Gloor, 1997). Four compounds were reportedly found in *Peperomia* plant extract by Lee Seong Wei *et al*., 2011 in which none of the identified compound was reported by Xu *et al.*2006. Phytol, being the major compound in *P. pellucida*, is one of the most important diterpenes and possessed both antimicrobial and anticancer activities Kumar *et al*., 2010.

Ammara *et al*., (2009) reported the anti-microbial efficacy of the dried leaf extracts of *Peperomia pellucida* on *P. mirabilis* and *P. aeruginosa with* highest inhibitory activity using ethanol as the medium of extraction. They opined that the stronger extraction capacity of ethanol could have produced greater active constituents responsible for anti-microbial activity. This suggests that the active components of this plant may be highly polar compound, and the active principle dissolved completely in the alcohol after soaking the leaves for 24 hrs. This showed ethanol to be better than water as solvent for extraction of *P. pellucida* when used on some pathogenic bacteria.

Table1: Qualitative Phytochemical analysis of Ethanolic extractof *Acalypha wilkesiana* leaf

Phytochemicals	Result
Saponin	Moderately Present
Tannins	Highly Present
Flavonoids	Moderately Present
Phylobatanins	Moderately Present
Alkaloids	Slightly Present
Glycosides Reducing Sugar	Moderately Present
Glycoside	Highly Present
Cardiac Glycosides	Slightly Present

Table 2: Qualitative Phytochemical analysis of ethanolic extract of *Peperomia pellucida*

Phytochemicals	Result
Saponin	Slightly Present
Tannins	Moderately Present
Flavonoids	Moderately Present
Phylobatanins	Absent
Alkaloids	Slightly Present
Glycosides Reducing Sugar	Moderately Present
Glycoside	Absent
Cardiac Glycosides	Slightly Present

Table 3: antibacterial activity of Ethanolic extract of *Acalypha wilkesiana and Peperomia pellucida* leaves showing zones of inhibition at 100% concentration.

Isolates	Mean zone of Inhibition Diameter (mm)			
	Acalypha	Peperomia	Water (control)	Gentamycin 10µg
Streptococcus	0.75	0	0	0.7
Bacillus cereus	0.6	0	0	0
Klebsiella	0.5	0	0	0.9
Enterobacter aerogenes0.50	0.5	0.5	0	0.9
Escherichia coli	0.5	0	0	0.9

CONCLUSION

Ethanolic extract of *A. wilkesaina* has antibacterial activity on all bacterial organisms isolated from infected Catfish, *Clarias gariepinus*, while *Peperomia* extract has antibacterial activity on *Enterobacter aerogenes* only. The results have shown these extracts possess antibacterial properties and suggest that these extracts may be useful in the treatment of bacterial infections and other related fish diseases and to enhance immune responses. The toxicity level of the plant extracts new to be worked upon to achieve a safe regimen.

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