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Research Article

Phytobiotics Effects of Pawpaw (*Carica papaya*) Leaves and Fluted Pumpkin (*Telferia ocidentalis*) Leaves Extracts against Certain Aquatic Pathogens

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ABSTRACT

Due to an increase in the rate at which microorganism are resistant to antibiotics, there is need to assess the antimicrobial effect of some medicinal plant extracts in the treatment of fish pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Bacillus substilis*, *Staphylococcus epidermidis*, *Salmonella typhi* and *Aeromonas hydrophila*. The antimicrobial activity and inhibition diameter of methanolic and ethanolic extracts of pawpaw and fluted pumpkin leaves were evaluated against eight (8) clinical strains of bacteria isolated from *Clarias gariepinus* using agar well diffusion method. Minimum inhibitory concentration and phytochemical screening of these plants were determined using standard methods. Data were analyzed using descriptive statistics. The results of the present study showed that the zone of inhibition varies with the bacteria and the type of extracts. Ethanolic extract has better diameter of zone of inhibition than the methanolic extracts with highest zone of inhibition recorded in *Pseudomonas aeruginosa* (16.00 ± 0.02 mm) for fluted pumpkin leaves extracts and the lowest in *Staphylococcus epidermidis* (8.00 ± 0.01 mm) and *Aeromonas hydrophilia* (8.00 ± 0.00 mm) for fluted pumpkin leaves extracts respectively. Minimum inhibitory concentration of methanolic and ethanolic extract of pawpaw and fluted pumpkin leaves on the pathogenic bacteria tested were 425 µg/mL, and 850 µg/mL respectively. Phytochemical screening of these plants revealed the presence of saponins, flavonoids, tannins, cyanogenic glucosides and amino acid and proteins. The result have shown that methanolic and ethanolic extracts of pawpaw and fluted pumpkin leaves extracts possess antimicrobial functions and serve as a source of antimicrobial agents against fish pathogens.

Keywords: Antimicrobial; Pawpaw leaves; Fluted pumpkin leaves; Fish pathogen; Phytochemical

INTRODUCTION

Fish is a member of a group of organisms that consist of all gill bearing aquatic craniates that lack limbs with digits, like any other animal, fishes suffer from diseases and parasites [1]. The problem of fish diseases may be due to the development of antibiotic resistant bacteria, due to poor water quality and the misuse of antibiotic drugs which lowers the immune system of fishes due to the presence of microbes or pathogen in the water [1]. Recently there is an increasing awareness to determine the efficacy of plants as an alternative to treat microbial diseases of aquatic animal.

A medicinal plant is any plant which in one or more of its organ contains substances that can be used for the synthesis of useful drugs. Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds which have curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants [2]. Such plants include fluted pumpkin leaves and pawpaw leaves, although both plants appear to have broad spectrum of antimicrobial activities [3]. They are various reports on the microbial properties of a number of plant extracts, but their scientific evaluation remains as nascent area

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for intensive investigations especially with reference to aquatic pathogens.

This study was therefore aimed at screening for the antimicrobial properties of methanolic and ethanolic extracts pawpaw and fluted pumpkin leaves extracts against eight isolated fish pathogenic bacteria, with a possibility of determining the minimum inhibitory concentration by which the plant extracts can be added in fish feeds.

MATERIALS AND METHODS

Plant collection

Telferia ocidentalis and *Carica papaya* were obtained in Ayeka, Okitipupa, Nigeria and was identified by Dr. DO Aworinde in the Department of Biological Sciences (Botany Programme), Ondo State University of Science and Technology, Okitipupa, Nigeria.

Preparation and extraction of plants material

Leaves extraction: The extraction of the *T. ocidentalis* and *C. papaya* was carried out as described by Ajaiyeoba and Fadare [4]. These plants were air dried for a period of 4 weeks. The air dried *T. ocidentalis* and *C. papaya* were grinded into powder, using hammer mill. 170 g of the *T. ocidentalis* and *C. papaya* were weighed respectively using a weighing balance. Eight hundred and fifty (850) mL of ethanol and methanol was measured each using a measuring cylinder and was decanted into the cup containing each plant extracts with appropriate labeling. The mixture was then homogenized and kept for 48 hours under room temperature at constant stirring at regular interval. Each labeled mixture was then filtered using a sterile muslin cloth after which the extraction was obtained, the filtrate was kept in the refrigerator at 40°C until require for further use.

Phytochemical screening

Detection of saponins: Froth test: Extracts were diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins. Foam test: Extract of 0.5 g was shaken with 2 mL of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols ferric chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

Tannin: Extract of 2 mL was taken up in 10 mL distilled water, and filtered. Then few drops of ferric chloride reagent were added to 1 mL of the filtrate. The mixture was observed for the formation of blue, blue black, green or green black coloration or precipitate.

Detection of flavonoids: Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color indicates the presence of phenol. Glucosinolates: Extracts were treated with few drops of chloroform followed by filtration as described by Adeoye and Oyedapo method [5]. Concentration tetraoxosulphate (IV) acid was carefully layered at the bottom of the tube without disturbing the solution. It was observed for the formation of a sharp brown ring at the chloroform/sulphuric acid interface.

Test for triterpenes and steroids: The Salkowski test: Extract of 3 mL was warmed in 5 mL of chloroform solution was then treated with a small volume of concentrated tetraoxosulphate (IV) acid and shaken. The red colour produced within few minutes indicates a positive reaction.

Detection of protein and amino acids: Xanthoproteic test: The residues were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of protein.

Media preparation

All media used were prepared according to manufacturer's instruction. The media were allowed to cool after sterilization to about 400°C before pouring into sterile Petri dishes.

Source of test organisms

The microorganisms isolated from *C. gariepinus* were *A. hydrophila*, *S. aureus*, *S. pyogens*, *B. subtilis*, *E. coli*, *S. typhii*, and *P. aeruginosa*. The isolation and characterization of bacteria using a biochemical test was carried out at the Microbiology Laboratory Faculty of Science at University of Ibadan. *Aspergillus niger* and *S. epidermidis* were collected from the laboratory stock of Department of Biological Sciences, Ondo State University of Science and Technology. The pure cultures collected were labeled, sub-cultured on nutrient agar slant and nutrient broth(s) and potato dextrose agar (PDA) and were kept inside refrigerator at 40°C until it is required for study.

Antimicrobial assay

A well diffusion assay as described by [6] was used. Pre poured indicator [pathogen (4 mm depth)] was overlaid with a 10 mL soft agar (0.7%) lawn of indicator culture (thus generating a potential mat for the indication of bacteria). Wells of 10 mm diameter were cut into these agar plates using cork borer and 0.1 mL of these plants extract was placed into each well [6]. Distilled water was used as negative control while oxytetracycline (10 mg and 20 mg) was used as positive control. The plates were examined for zone of inhibition which was scored positive, if the width of the clear zone was 10 mm or longer. The diameter of the inhibition zones were taken to be proportional to the logarithm of the antimicrobial of the antimicrobial compounds in *T. ocidentalis* and *C. papaya* [7].

Isolation of microorganism/total microbial count

The gills, skin, intestine and liver sample of *C. gariepinus* were separately macerated and put into sterile capped test tube containing sterilized distilled water and were homogenized [8]. Serial dilution was carried out and 1 mL each from 10-3 to 10-5 dilution factor was dispersed into petri dishes that were appropriately labeled and molten sterilized medium was poured aseptically into petri dish. The plates were swirled gently for even distribution of inoculums and allowed to set/gel and then incubated at 37°C for 24 hours. The organism grew into visible

different colonies after 24 hours. Total viable counts and *Enterobacteriacea* counts were determined and the result was expressed in log_{10}CFU/mL.

Minimum inhibitory concentration of fluted pumpkin and pawpaw leaves

Double dilution of 1700 µg/mL of these plant extract were made into 2 mL volume of broth to 3.37 µg/mL. One row of the test was inoculated with 0.02 mL of 1 in 10 dilution of the broth culture of the organism. The test was incubated at 37°C for 24 hours aerobically. The minimum inhibitory concentration was the lowest concentration that prevented the growth of bacteria after 24 hour incubation [6].

Statistical analysis

The microbial load of fish tissue (skin, gills, intestine and liver) and antimicrobial and antifungal activities (diameter of zone of inhibition, mm) of pawpaw and fluted pumpkin leaves against tested pathogens resulting from the experiment were subjected to one way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Science version 20).

RESULTS

Phytochemical analysis of fluted pumpkin and pawpaw leaves extracts

Preliminary phytochemical screening of fluted pumpkin and pawpaw leaves extracts for primary and secondary metabolites showed the presence of saponins, tannins, alkaloid, amino acid and protein while phenols were not detected in both plants, flavonoids were not detected in fluted pumpkin leaves but the presence of cyanogenic glycoside was not detected in pawpaw leaves but present in fluted pumpkin leaves shown in Table 1.

Table 1: Determination of important phytochemical of ethanolic and methanolic extracts of fluted pumpkin and pawpaw leaves. Keys: +++=present in high quantity, ++=moderately present, +=present in low quantity, -=negative or not present.

	Parameter	Methanol Value	Ethanol Value
	Alkaloid	-	-
	Saponins	++	++
	Tannin	++	++
	Flavonoids	-	-
	Amino acid and protein	++	++
	Phenols	-	-
Fluted pumpkin leaves	Cyanogenic glycoside	++	++
	Alkaloid	-	-
	Saponins	++	++
	Tannin	++	++
	Flavonoids	++	++
	Amino acid and protein	++	++
	Phenols	-	-
Pawpaw leaves	Cyanogenic glycoside	-	-

Determination of microbial load in Clarias gariepinus

The microbial load of fish tissue (skin, gills, intestine and liver) were determined and the result show that the highest *Enterobacteriacea* counts was recorded in skin and least in liver while no *Enterobacteriacea* was recorded on control. Also, the highest total viable count was recorded in skin and least in liver while no total viable counts was recorded in the control as shown in Table 2.

Table 2: Microbial load of skin gills, intestine and liver of C. *gariepinus*.

Organ	Organism	Microbial load (log10CFU/g)
Liver	Enterobacteriacea counts	6.29 ± 0.99
	Total viable counts	7.12 ± 0.23
Skin	Enterobacteriacea counts	9.82 ± 0.20
	Total viable counts	12.43 ± 0.95
Intestine	Enterobacteriacea counts	7.86 ± 0.17
	Total viable counts	8.56 ± 0.21
Gills	Enterobacteriacea counts	8.95 ± 0.46
	Total viable counts	10.36 ± 0.14
Control	Enterobacteriacea counts	-
	Total viable counts	-

Detection of antimicrobial activities of fluted pumpkin and pawpaw leaves extracts

Ethanolic and methanolic extracts of fluted pumpkin and pawpaw leaves respectively shows antibacterial and antifungal properties in the present study. The fluted pumpkin leaves shows the highest activities with all the pathogens investigated. Although both the fluted pumpkin and pawpaw leaves showed no activities against *S. pyogens* and the methanolic extract of pawpaw leaves shows no activities against *B. subtilis* while the ethanolic extract of fluted pumpkin leaves shows no activities against *S. typhii*. Antifungal activity was also recorded against *A. niger* with the highest inhibition recorded in pawpaw leaves extracts (Table 3).

Table 3: Antibacterial activities (diameter of inhibition zone, mm) of methanolic and ethanolic extracts of fluted pumpkin and pawpaw leaves. Keys: - =no diameter of zone of inhibition; oxy=Oxytetracycline.

Method of Extraction	Pathogen	Fluted pumpkin leaves	Pawpaw leaves	Control (distilled water)	Control 2 (Oxy) (10mg/mL)
METHANOL	*Pseudomonas aeruginosa*	10 ± 0.02	10 ± 0.05	-	13.00 ± 0.02
	Bacillus subtilis	10 ± 0.03	-	-	11.00 ± 0.01
	Staphylococcus aureus	10 ± 0.04	10 ± 0.02	-	9.00 ± 0.01
	Staphylococcus epidemidis	14 ± 0.01	18 ± 0.01	-	12.00 ± 0.05
	Streptococcus pyogenes	-	-	-	8.50 ± 0.03
	Salmonella typhi	8 ± 0.02	12 ± 0.01	-	12.80 ± 0.07
	Escherichia coli	18 ± 0.01	16 ± 0.04	-	14.00 ± 0.01
	Aeromonas hydrophila	14 ± 0.03	12 ± 0.03	-	15.00 ± 0.02
	Aspergillus niger	4 ± 0.01	6 ± 0.01		-
ETHANOL	*Pseudomonas aeruginosa*	16 ± 0.02	14 ± 0.01	-	13.00 ± 0.02
	Bacillus subtilis	14 ± 0.06	12 ± 0.02	-	11.00 ± 0.01

Staphylococcus aureus	12 ± 0.03	8 ± 0.02	-	-
Staphylococcus epidemidis	8 ± 0.06	14 ± 0.07	-	16.00 ± 0.05
Streptococcus pyogenes	-	-	-	16.50 ± 0.03
Salmonella typhi	-	16 ± 0.02	-	12.80 ± 0.07
Escherichia coli	14 ± 0.01	12 ± 0.01	-	17.00 ± 0.01
Aeromonas hydrophila	14 ± 0.03	8 ± 0.02	-	13.00 ± 0.02
Aspergillus niger	3 ± 0.02	4 ± 0.05	-	-

Minimum Inhibitory Concentration (MIC) of pawpaw and fluted pumpkin leaves extracts

The minimum inhibitory concentration of ethanolic and methanolic of fluted pumpkin and pawpaw leaves against eight bacteria isolated from *C. gariepinus* were examined in the present study and their potency were assessed by minimum inhibitory concentration and it was recorded that 425 µg/mL and 850 µg/mL for methanolic and ethanolic extract of pawpaw and fluted pumpkin leaves respectively against all the tested pathogens (see table 4A and 4B).

Table 4A: Minimum inhibitory concentration (µg/mL) assay of ethanolic and methanolic extracts of fluted pumpkin leaves on isolated fish pathogen (+ = no inhibition; - = inhibition).

	ETHANOL										METHANOL									
Pathogen	1700	850	425	212.5	107.5	53.8	26.9	13.45	6.73	3.37	1700	850	425	212.5	107.5	53.8	26.9	13.45	6.73	3.37
Pseudomonas *aeruginosa*	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Bacillus substillis	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Staphylococcus epidermidis	-	-	-	-	+	+	+	+	+	+	-	-	_	+	+	+	+	+	+	+
Streptococcus pyogenes	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Salmonella typhii	-	-	+	+	+	+	+	+	+	+	-	-	_	+	+	+	+	+	+	+
Escherichia coli	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
Aeromonas *hydrophila*	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4B: Minimum inhibitory concentration assay (µg/mL) of methanolic and ethanolic extracts of pawpaw leaves on isolated fish pathogen (Keys: + = no inhibition; - = inhibition).

	ETHANOL										METHANOL									
Pathogen	1700	850	425	212.5	107.5	53.8	26.9	13.45	6.73	3.37	1700	850	425	212.5	107.5	53.8	26.9	13.45	6.73	3.37
Pseudomonas aeruginosa	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
Bacillus subtillis	-	-	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
Staphylococcus epidermidis	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
Streptococcus pyogenes	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
Salmonella typhi	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
Escherichia coli	-	-	-	-	_	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
Aeromonas hydrophila	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

DISCUSSION

Many naturally occurring compounds found in plants have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens [9,10]. The concentration of metabolites in pawpaw and fluted pumpkin leaves extracts were moderately available, this study shows that phytochemical constituent's such as cyanogenic glycoside, saponins, tannin, flavonoids, alkaloids, phenols and amino acid were present in these plants and the results of the study agreed with the report of Nwanna and Pietta [3,11].

The result of this study shows that the microbial load of the liver, skin, gill and intestine of *C. gariepinus* varies with the skin and gill having the highest value of total viable count and *Enterobacteriacea* count, this is in accord with Shalaby et al. [8] that the bacteria load is greater on the skin and gills than any part of the fish as these part are ones constantly exposed to challenges and the lowest total viable count and *Enterobacteriacea* counts was recorded on the liver. This result is in accord with Bello et al. [6].

Pawpaw and fluted pumpkin leaves extracts showed antibacterial activity against the eight isolated fish pathogens from *C. gariepinus*, which may reflect the antibacterial activity of plant active ingredients that inhibit bacterial growth which is in accord with Abu Shanab et al. [12]. It was also recorded that ethanolic extracts had better effect in the inhibition compared to methanolic extract, the reason may be due to the fact that ethanol is the best solvent for the active compounds extracted from the plant. Also, the difference in antibacterial activity of a plant extracts might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), time of harvesting of plant materials and drying method used before the extraction process. The highest antibacterial activity of fluted pumpkin and pawpaw leaves was recorded on *P. aeruginosa* while the lowest antibacterial activity was recorded on *S. pyogenes*. Antifungal activity was also recorded on *A. niger* with the highest zone of inhibition recorded on ethanolic extract of pawpaw leaf.

The minimum inhibitory concentration assay carried out on pawpaw and fluted pumpkin leaves indicated that 425 µg/mL and 850 µg/mL is the minimum inhibitory concentration required for both methanolic and ethanolic extracts of the pawpaw and fluted pumpkin leaves required to inhibit the eight isolated fish pathogens (*P. aeruginosa*, *A. hydrophilia*, *B. subtilis*, *S. typhi*, *S. epidermidis*, *S. pyogenes*, *S. aureus* and *E. coli*). The result is in agreement with the report of Awe and Omajasola [13,14].

CONCLUSION

Fluted pumpkin and pawpaw leaves extracts exhibit a wide range of antimicrobial activity, with this antibacterial properties *T. ocidentalis* and *C. papaya* can play an important role in fish disease management with *T. ocidentalis* having the highest antibacterial activity against the isolated fish pathogens. Also,

inclusion of *T. ocidentalis* and *C. papaya* in fish feeds could be used for the treatment of fish diseases and enhanced fish yield in rural and urban aquaculture farms.

REFERENCES

1. Ihedioha JI, Chineme CN. Fundamentals of Systemic Veterinary Pathology. Great AP express publishers limited, Nigeria. 2004;pp: 109-129.
2. Kayode AA, Kayode OT. Some medical values of Telfairia occidentalis: A review. Am J Biochem Mol Biol. 2011;1:30-38.
3. Nwanna EE. Antioxidant and hepatoprotective properties of Telfairia occidentalis leaf (Fluted Pumpkin). Thesis and Dissertations (Biochemistry), Federal University of Technology, Akure, Nigeria. 2008.
4. Ajaiyeoba EO, Fadare DA. Antimicrobial potential of extracts and fractions of the African walnut-Tetracarpidium conophorum. Afr J Biotechnol. 2006;5:2322-2325.
5. Adeoye BA, Oyedapo OO. Toxicity of erythrophleum stem-bark: role of alkaloids fraction. Afr J Tradit Complement Altern Med. 2004;1:45-54.
6. Bello OS, Olaifa FE, Emikpe BO, Ogunbanwo ST. Potentials of walnut (Tetracarpidium conophorum Mull. Arg) leaf and onion (Allium cepa Linn) bulb extracts as antimicrobial agents for fish. Afr J Microbiol Res. 2013;7:2027-2033.
7. Maria EF, Aida AP, Derviz H, et al. Bacteriocin production by lactic acid bacteria isolate from regional chesses. J Food Prot. 1994;57:1013-1015.
8. Shalaby AM, Khattab YA, Abdel Rahman AM. Effects of garlic (Allium sativum) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia. Journal of Venomous Animal Toxins including Tropical Diseases. 2006;12:172-201.
9. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34:1589-1596.
10. Deans SG, Ritchie G. Antibacterial properties of plants essentials oils. International Journal of Food Microbiology. 1987;5:165-180.
11. Pietta PG Flavonoids as antioxidants. Journal of Natural Products. 2000;63:1035-1042.
12. Abu Shanab B, Adwan G, Abu Safiya D, Jarrar N, Adwan K. Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. Turkey Journal of Biology. 2004;28:99-102.
13. Awe S, Omojasola PF. Comparative study of the antibacterial activity of Piliostigma reticulatum barks extract with some antibiotics. Ethnobotanical Leaflets. 2009;13:1197-1204.
14. Osoba AO. The control of gonococcal infections and other sexually transmitted diseases in developing countries-with particular reference to Nigeria. Nigeria Journal of Medical Science. 1979;2:127-133.