



Synergistic Effects of Medicinal Plants *Carica papaya*, *Telferia occidentalis* and Oxytetracycline against some Pathogens Isolated from *Clarias gariepinus*

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Abstract

The problem of fish diseases may be due to the development of antibiotic-resistant bacteria and there is an increasing awareness to determine the efficacy of plants as an alternative to treat microbial diseases of aquatic animals. Antibacterial and antifungal synergistic evaluation of methanolic and ethanolic extracts of Fluted Pumpkin Leaves (FPL), Pawpaw Leaves (PL) and combination with oxytetracycline were investigated using the agar cup diffusion and broth diffusion techniques. The extracts were tested against 8 clinical strains of Gram-positive and Gram-negative bacteria and a fungal. Minimum Inhibitory Concentration (MIC) of methanolic and ethanolic extracts of pumpkin, pawpaw leaves and combination with oxytetracycline were determined using standard methods. Data were analyzed using descriptive statistics. The extracts displayed higher antimicrobial properties to the Gram-positive and G negative bacteria and these plants inhibited the growth of all the microorganisms used in the study when combine with oxytetracycline at 10 mg/ml and 30 mg/ml. The diameter of the zone of inhibition of methanolic and ethanolic extracts of fluted pumpkin and pawpaw leaves when combine with oxytetracycline (10 mg/ml and 30 mg/ml) were higher than single application and combination of the two plants with oxytetracycline. The combination of extracts of each plant with oxytetracycline was found to be the most effective combination in retarding microbial growth of all tested pathogenic bacteria. The results of MIC of methanolic and ethanolic extracts of pawpaw leaves, fluted pumpkin leaves and oxytetracycline at 10 mg/ml and 30 mg/ml range between 212.5 µg/ml - 850 µg/ml while the control doesn't show any antimicrobial properties in terms of zone of inhibition and minimum inhibitory concentration. The results observed in the pumpkin and pawpaw leaves and combination with oxytetracycline can provide additive or synergistic inhibitory effects making them more effective as antimicrobial agents against fish pathogens.

Keywords: Pawpaw Leaves; Pumpkin Leaves; Fish Pathogens; Antimicrobial Properties; Synergistic

Abbreviations: FPL: Fluted Pumpkin Leaves; PL: Pawpaw Leaves; MIC: Minimum Inhibitory Concentration; PDA: potato

dextrose agar; SPSS: Statistical Package for Social Science.

Introduction

Aquaculture is the fastest growing industry and is an important economic activity contributing to the world protein supply [1]. High mortality and serious economic losses have been reported in disease outbreaks in aquaculture [2]. The use of antibiotics and chemotherapeutics for treatment and prophylaxis has been broadly criticized for its negative impact, and research on interactions between growth, immunity and development of eco-friendly alternatives to antibiotics that may keep fish healthy such as probiotics, prebiotics and plant-based immunostimulants has increased. Indigenous technological knowledge for treating diseases is enjoying attention in fish health and disease management [3].

Plant-derived substances have recently become of great interest in pharmaceutical research coming to their versatile application. Medicinal plants are the richest bio-resources of drugs for traditional systems of medicine, modern medicine, nutraceuticals, food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs [4]. A good therapeutic antimicrobial should have a wide spectrum of activity, must not trigger any side/adverse reactions or be resistant to their therapeutic effects [1]. Some natural plants such as fluted pumpkin and pawpaw leaves could possess some of these ideal characteristics/properties but there is scanty documentation of the potential of fluted pumpkin leaves, pawpaw leaves and their combination with oxytetracycline in fish production as well as the mechanism of action of these plants as antimicrobial agents are yet to be adequately researched. This present study was therefore aimed to investigate the antimicrobial effects and minimum inhibiting concentration of fluted pumpkin leaves and pawpaw leaves singularly or in combination with oxytetracycline against 8 clinical strains of pathogenic bacteria.

Material and Methods

Plant Collection and Identification

Carica papaya was obtained in Teaching and Research Farm, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria while *Telferia occidentalis* was obtained in Ore, Ondo State on 5th July -10th July 2016 and was identified by a botanist, Mr S. M. Erinoso in the Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa.

Extraction of *T. occidentalis* and *C. papaya*

These plants were air-dried for 6 weeks (5th July- 19th August 2016) in the Fisheries and Aquaculture Laboratory,

Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. The air-dried *T. occidentalis* and *C. papaya* were ground into powder, using a hammer mill. The extraction of the *T. occidentalis* and *C. papaya* was carried out as described by Ajaiyeoba, et al. [5].

Media Preparation

All media used were prepared according to the manufacturer's instructions. The media were allowed to cool after sterilization to about 40°C before pouring into sterile Petri dishes.

Source of Test Organisms

The microorganisms isolated from *C. gariepinus* were *A. hydrophila*, *S. aureus* and *S. pyrogens*, *B. subtilis*, *E. coli*, *S. typhi*, and *P. aeruginosa*. The isolation and characterization of bacteria using a biochemical test were carried out at the Microbiology Laboratory, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria between 5th September 2016-10th October 2016. *Aspergillus niger* and *S. epidermidis* were collected from the laboratory stock of the Department of Biological Sciences, Olusegun Agagu University of Science and Technology. The pure cultures collected were labelled, sub-cultured on nutrient agar slant and nutrient broth(s) and potato dextrose agar (PDA) and were kept inside the refrigerator at 4 °C until it is required for the study.

Antimicrobial Assay

The Petri-dishes containing nutrient agar or potato dextrose agar were spread with inoculums with a sterile glass spreader. Wells of 10mm diameter were cut into these agar plates using a sterile stainless steel cork borer and each well was filled with 0.1ml of these plants extract and oxytetracycline to assess their synergistic or antagonistic antimicrobial effects. Distilled water was used as negative control while oxytetracycline (10 mg and 20 mg) was used as a positive control. The plates were incubated for 24 hours at 37°C in an incubator for bacterial growth and at 30°C for 48 hours for fungal growth. The diameter (mm) of the clear inhibitory zone formed around the well was measured using a graduated transparent meter rule. Microorganisms showing a clear zone of more than 10 mm were considered to be inhibited [6]. Mean values of triplicate data were recorded.

Minimum Inhibitory Concentration of Fluted Pumpkin and Pawpaw Leaves

Double dilution of 1700µg/ml of these plants extracts and oxytetracycline were made into 2 ml volume of broth to

3.37µg/ml. one row of the test was inoculated with 0.02ml of 1 in 10 dilutions 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 hours of 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 a one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Science version 15.0)

Result

Detection of Antimicrobial Activities of Fluted Pumpkin and Pawpaw Leaves Extracts

Ethanollic and methanolic extracts of fluted pumpkin and pawpaw leaf respectively show antibacterial and antifungal properties in the present study. The fluted pumpkin leaves show the highest activities with all the pathogens investigated. Although both the fluted pumpkin and pawpaw leaves showed no activities against *Streptococcus pyrogens* and the methanolic extract of pawpaw leaves shows no activities against *B. subtilis*. While the ethanollic extract of fluted pumpkin leaf shows no activities against *Salmonella typhi*. Antifungal activity was also recorded against *Aspergillus niger* with the highest inhibition recorded in pawpaw leaves extract (Table 1).

Method of extraction	Pathogen	Fluted pumpkin leaves	Pawpaw leaves	Control (distilled water)	Control 2 (Oxy) (10mg/ml)
METHANOL	<i>Pseudomonas aeruginosa</i>	10±0.02	10±0.05	-	13.00 ± 0.02
	<i>Bacillus subtilis</i>	10±0.03	-	-	11.00 ± 0.01
	<i>Staphylococcus aureus</i>	10±0.04	10±0.02	-	9.00 ± 0.01
	<i>Staphylococcus epidemidis</i>	14±0.01	18±0.01	-	12.00 ± 0.05
	<i>Streptococcus pyrogens</i>	-	-	-	8.50 ± 0.03
	<i>Salmonella typhi</i>	8±0.02	12±0.01	-	12.80 ± 0.07
	<i>Escherichia coli</i>	18±0.01	16±0.04	-	14.00 ± 0.01
	<i>Aeromonas hydrophila</i>	14±0.03	12±0.03	-	15.00 ± 0.02
	<i>Aspergillus niger</i>	4±0.01	6±0.01		-
ETHANOL	<i>Pseudomonas aeruginosa</i>	16±0.02	14±0.01	-	13.00 ± 0.02
	<i>Bacillus subtilis</i>	14±0.06	12±0.02	-	11.00 ± 0.01
	<i>Staphylococcus aureus</i>	12±0.03	8±0.02	-	-
	<i>Staphylococcus epidemidis</i>	8±0.06	14±0.07	-	16.00 ± 0.05
	<i>Streptococcus pyrogens</i>	-	-	-	16.50 ± 0.03
	<i>Salmonella typhi</i>	-	16±0.02	-	12.80 ± 0.07
	<i>Escherichia coli</i>	14±0.01	12±0.01	-	17.00 ± 0.01
	<i>Aeromonas hydrophila</i>	14±0.03	8±0.02	-	13.00 ± 0.02
	<i>Aspergillus niger</i>	3±0.02	4±0.05	-	-

Keys: - = no diameter of zone of inhibition, Oxy = Oxytetracycline.

Table 1: Antibacterial activities (diameter of inhibition zone, mm) of methanolic and ethanollic extracts of fluted pumpkin and pawpaw leaves

Determination of Antimicrobial Activities of Fluted Pumpkin, Pawpaw Leaves and Oxytetracycline

The methanolic and ethanolic extracts of fluted pumpkin leaf with oxytetracycline at 30mg/ml concentration shows the highest antimicrobial activity. The interaction of fluted pumpkin leaves and pawpaw leaves of methanolic and

ethanolic extracts with oxytetracycline shows moderate antimicrobial activity, antifungal activity was also recorded against *Aspergillus niger*. In methanolic and ethanolic extracts of pawpaw leaves, fluted pumpkin leaves and oxytetracycline but no antifungal properties were recorded in the interaction of both plants with oxytetracycline. Ethanolic extracts had a better diameter of zone of inhibition, mm than methanolic extracts (Tables 2A-2C).

Pathogen	METHANOL		ETHANOL		CONTROL
	FPL+OXY1 (10mg/ml)	FPL+OXY2 (30mg/ml)	FPL+OXY1 (10mg/ml)	FPL+OXY2 (30mg/ml)	Distilled water
<i>Pseudomonas aeruginosa</i>	24±0.02	34±0.05	22±0.04	28±0.02	-
<i>Bacillus subtilis</i>	16±0.07	20±0.03	22±0.02	36±0.07	-
<i>Aeromonas hydrophila</i>	20±0.02	24±0.02	16±0.02	24±0.02	-
<i>Staphylococcus aureus</i>	16±0.05	22±0.02	22±0.04	34±0.01	-
<i>Staphylococcus epidermidis</i>	16±0.02	22±0.06	14±0.02	24±0.02	-
<i>Escherichia coli</i>	18±0.03	22±0.05	16±0.03	20±0.06	-
<i>Streptococcus pyrogens</i>	14±0.05	18±0.02	16±0.01	18±0.02	-
<i>Salmonella typhi</i>	14±0.04	16±0.02	14±0.07	24±0.025	-
<i>Aspergillus niger</i>	6±0.02	6±0.02	8±0.03	9±0.01	-

Keys: FPL = Fluted Pumpkin Leaves, Oxy = Oxytetracycline.

Table 2A: Antimicrobial activity (diameter of zone of inhibition mm) of methanolic and ethanolic extracts of fluted pumpkin leaves with oxytetracycline

Pathogen	METHANOL		ETHANOL		CONTROL
	PPL+OXY1 (10mg/ml)	PPL+OXY2 (30mg/ml)	PPL+OXY1 (10mg/ml)	PPL+OXY2 (30mg/ml)	Distilled water
<i>Pseudomonas aeruginosa</i>	20±0.03	22±0.04	16±0.02	22±0.03	-
<i>Bacillus subtilis</i>	10±0.01	34±0.02	14±0.05	16±0.02	-
<i>Aeromonas hydrophila</i>	10±0.07	24±0.05	18±0.03	20±0.02	-
<i>Staphylococcus aureus</i>	16±0.03	28±0.02	16±0.03	20±0.02	-
<i>Staphylococcus epidermidis</i>	16±0.02	28±0.01	22±0.02	22±0.05	-
<i>Escherichia coli</i>	26±0.01	42±0.02	18±0.01	24±0.02	-
<i>Streptococcus pyogenes</i>	16±0.02	30±0.04	18±0.02	22±0.02	-
<i>Salmonella typhi</i>	16±0.06	24±0.07	12±0.07	22±0.02	-
<i>Aspergillus niger</i>	4±0.01	4±0.01	4±0.01	4±0.01	-

Keys: PPL = Pawpaw leaves, Oxy = Oxytetracycline.

Table 2B: Antimicrobial activity (diameter of zone of inhibition) of methanolic and ethanolic extracts of pawpaw leaves with oxytetracycline

Pathogen	METHANOL		ETHANOL		CONTROL
	PPL+FPL+OXY1 (10mg/ml)	PPL+FPL+OXY2 (30mg/ml)	PPL+FPL+OXY1 (10mg/ml)	PPL+FPL+OXY2 (30µg/ml)	Distilled water
<i>Pseudomonas aeruginosa</i>	10±0.05	10±0.01	12±0.03	12±0.02	-
<i>Bacillus subtilis</i>	12±0.02	16±0.04	12±0.05	14±0.08	-
<i>Staphylococcus aureus</i>	18±0.02	20±0.01	14±0.01	16±0.03	-
<i>Staphylococcus epidermidis</i>	14±0.01	24±0.03	12±0.06	14±0.02	-
<i>Streptococcus pyrogens</i>	10±0.02	14±0.03	10±0.02	14±0.02	-
<i>Salmonella typhi</i>	12±0.03	18±0.02	10±0.04	16±0.01	-
<i>Escherichia coli</i>	12±0.02	24±0.06	14±0.08	20±0.02	-
<i>Aeromonas hydrophila</i>	14±0.01	16±0.05	12±0.02	14±0.01	-
<i>Aspergillus niger</i>	-	-	-	-	-

Keys: FPL = Fluted Pumpkin Leaves, PPL= Pawpaw Leaves, Oxy = oxytetracycline, - = no inhibition.

Table 2C: Antimicrobial activity (diameter of zone of inhibition) of methanolic and ethanolic extracts of fluted pumpkin leaves, pawpaw leaves extracts and oxytetracycline

Determination of Minimum Inhibitory Concentration of Methanolic and Ethanolic Extracts of Fluted Pumpkin Leaves, Pawpaw Leaves and Oxytetracycline

The minimum inhibition concentration of ethanolic and methanolic extracts of the combination of fluted pumpkin leaves, pawpaw leaves and oxytetracycline at 10g/ml and 30g/ml against eight pathogenic bacteria isolated from *C. gariepinus* were investigated and the result showed

that 212.5 µg/ml, 425 µg/ml and 212.5 µg/ml; 425 µg/ml and 212.5 µg/ml, 850µg/ml and 425 µg/ml, 850µg/ml for methanolic extracts of fluted pumpkin leaves and oxytetracycline, ethanolic extracts of fluted pumpkin leaves and oxytetracycline, (10 and 30 µg/ml); methanolic extracts of pawpaw leaves and oxytetracycline (10 and 30 µg/ml), ethanolic extracts of pawpaw leaves and oxytetracycline (10 and 30 µg/ml) and methanolic and ethanolic extract of pawpaw, fluted pumpkin leaves and oxytetracycline (10 and 30 µg/ml) respectively (Tables 3A-3F).

Pathogen	METHANOL										ETHANOL									
	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
<i>Salmonella typhi</i>	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Streptococcus iniae</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+
<i>Aeromonas hydrophila</i>	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
<i>Staphylococcus epidermidis</i>	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Escherichia coli</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Bacillus subtilis</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: + = no inhibition - = inhibition.

Table 3A: Minimum inhibitory concentration assay of methanolic and ethanolic extracts of fluted pumpkin leaves and oxytetracycline (10 mg/ml) on isolated fish pathogens

Pathogen	METHANOL										ETHANOL									
	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
<i>Salmonella typhi</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
<i>Streptococcus iniae</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+
<i>Aeromonas hydrophila</i>	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+
<i>Staphylococcus epidermidis</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
<i>Escherichia coli</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
<i>Bacillus subtilis</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: + = no inhibition - = inhibition

Table 3B: Minimum inhibitory concentration assay of methanolic and ethanolic extracts of fluted pumpkin leaves and oxytetracycline (30 mg/ml) on isolated fish pathogens

Pathogen	METHANOL										ETHANOL									
	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
<i>Salmonella typhi</i>	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Streptococcus iniae</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Aeromonas hydrophila</i>	-	-	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Staphylococcus epidermidis</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
<i>Escherichia coli</i>	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Bacillus subtilis</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	--	-	-

Key: + = no inhibition - = inhibition

Table 3C: Minimum inhibitory concentration assay of methanolic and ethanolic extracts of pawpaw leaves and oxytetracycline (10 mg/ml) on isolated fish pathogen

Pathogen	METHANOL										ETHANOL									
	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
<i>Salmonella typhi</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Streptococcus iniae</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Aeromonas hydrophila</i>	-	-	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Staphylococcus epidermidis</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Escherichia coli</i>	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
<i>Bacillus subtilis</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: + = no inhibition - = inhibition.

Table 3D: Minimum inhibitory concentration assay of methanolic and ethanolic extracts of pawpaw leaves and oxytetracycline (30 mg/ml) on isolated fish pathogen

Pathogen	METHANOL										ETHANOL									
	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
<i>Salmonella typhi</i>	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
<i>Streptococcus iniae</i>	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Aeromonas hydrophila</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Staphylococcus epidermidis</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Escherichia coli</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Bacillus subtilis</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: + = no inhibition - = inhibition.

Table 3E: Minimum inhibitory concentration assay of methanolic and ethanolic extracts of pawpaw leaves, fluted pumpkin leaves and oxytetracycline (10 mg/ml) on isolated fish pathogens

Pathogen	METHANOLIC										ETHANOLIC									
	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
<i>Salmonella typhi</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Streptococcus iniae</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Aeromonas hydrophila</i>	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+
<i>Staphylococcus epidermidis</i>	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>Escherichia coli</i>	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
<i>Bacillus subtilis</i>	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: + = no inhibition - = inhibition.

Table 3F: Minimum inhibitory concentration assay of methanolic and ethanolic extracts of pawpaw leaves, fluted pumpkin leaves and oxytetracycline (30 mg/ml) on isolated fish pathogens

Discussion

The result of this present study showed that methanolic and ethanolic extracts of pawpaw and fluted pumpkin leaves had antibacterial activity against *Salmonella typhi*, *Streptococcus pyrogens*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Bacillus subtilis*. This result agrees with the study of Nwakanma, et al. [7]; Oboh, et al. [8]; Oyewole, et al. [9] who reported the diameter of the zone of inhibition on *E. coli*, *P. aeruginosa*, *S. typhi*, *A. hydrophila* to the extracts of *T. occidentalis*. Also, the study of Lonkala, et al. [10] was similar to this present finding who reported a better diameter of zone of inhibition of extracts of *C. papaya* on *Bacillus cereus*, *S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa*. Shanab, et al. [11] reported that plant active ingredients may reflect the antibacterial activity that inhibits bacterial growth which was similar to the present study. The highest antibacterial activity of fluted pumpkin and pawpaw leaves was recorded on *P. aeruginosa* and *E. coli* for ethanolic and methanolic extracts respectively while no antibacterial activity was recorded on *Streptococcus pyrogens* for ethanolic and methanolic extracts. Antifungal activity was also recorded on *Aspergillus niger* with the highest zone of inhibition recorded on ethanolic extract of pawpaw leaves.

The interaction between fluted pumpkin leaves, pawpaw leaves extracts and oxytetracycline at the two levels of concentrations (10 mg/ml and 30 mg/ml) showed inhibitory activity against all the tested pathogens. The bioactive phytochemicals present in fluted pumpkin and pawpaw leaves extracts might have inhibited the growth of bacteria and their joint activity with oxytetracycline led to an enhanced antibacterial effect compared to the control and the values obtained in a single plant. A significant higher inhibitory action was achieved when these plants were combined at a higher concentration of 30 mg/ml of oxytetracycline than at a lower concentration of 10 mg/ml. This result revealed the presence of effective active ingredients in the plant extracts which at higher concentrations with oxytetracycline might have penetrated the cell membrane and facilitated the influx of extracts and oxytetracycline. This result was similar to the report of Olgica, et al. [12]; Odunbaku, et al. [13] who reported that combined effects of extracts with oxytetracycline showed the inhibition zone was wider than the control to multidrug-resistant pathogens.

All bacteria strained showed reduced sensitivity or resistance to the combination of the two plant extracts and oxytetracycline at the two-level concentrations. The interaction of the two plant extracts and oxytetracycline showed a lesser zone of inhibition which might be due to

the antagonizing nature of the two plant extracts when combined, the highest antimicrobial activity was recorded on the methanolic extracts for *S. epidermidis* and *E. coli* with a diameter of zone of inhibition of 24 mm at 30 mg/ml concentration of antibiotics. Although, methanolic and ethanolic extracts of pawpaw leaves, fluted pumpkin leaves and oxytetracycline show no antifungal properties were recorded in the interaction of both plants with oxytetracycline. Ethanolic extracts had a better diameter of zone of inhibition than methanolic extracts.

The minimum inhibitory concentration assay carried out on the interaction of fluted pumpkin leaves extract with oxytetracycline indicates that 212.5 µg/ml was the minimum inhibitory concentration for methanolic and ethanolic extract required to inhibit the growth of the eight isolated fish pathogens at 10 mg/ml and 30 mg/ml concentration of oxytetracycline respectively, The result was in agreement with Oboh, et al. [8] who reported inhibitory effects of ethanolic extracts of *T. occidentalis* on *E. coli* and *P. aeruginosa* while the minimum inhibitory concentration of the methanolic extract of the interaction of pawpaw leaves was 450 µg/ml and 212.5 µg/ml for ethanolic extract of pawpaw leaf with 10 mg/ml and 30 mg/ml concentration of oxytetracycline respectively.

The minimum inhibitory concentration assay carried out on the interaction of fluted pumpkin leaves, pawpaw leaves extracts and oxytetracycline indicates that 850 µg/ml was the minimum concentration for both methanolic and ethanolic extract required to inhibit the growth of the eight isolated fish pathogens. This finding was in accord with Sarawama Kumar, et al. [14] who reported that MIC of methanolic extract of *Thespesia populnea* in combination with oxytetracycline using 12 different Gram-positive and Gram-negative bacteria had inhibitory effects with the values ranging between 62.5 µg/ml to 1000 µg/ml. Also, this result was in accord with Odunbaku, et al. [13] who reported that MIC of the ethanolic extract of *Ficus exasperata* leaf against *E. coli* and *S. albus* had inhibitory effects and that the combination of the crude plant extracts and the protein synthesis inhibitors had the highest inhibitory activity which was in agreement with the present finding.

Conclusion

This study revealed that the combination of pawpaw leaves, fluted pumpkin leaves extracts and oxytetracycline had significant potential for the development of new antimicrobial treatment of several diseases caused by a microorganism. From the results of the study, these plants act in synergy with oxytetracycline. This synergy could lead to a new option for the treatment of infectious diseases and emerging drug resistance.

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