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Sensitivity of Bacteria Isolated From Fish To Some Medicinal Plants

Awe, F. A, [1], Akinyemi A. A,[2] Ezeri G. N. O,[2] Giwa-Ajeniya, A.O,[3]
[1.] *Fisheries department, Lagos State University, Ojo, Lagos, Nigeria*
[2.] *Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.*
[3.] *Chemistry Department Lagos State University, Ojo, Lagos State, Nigeria.*

----------ABSTRACT----------
Ethanolic extracts from 7 plant species were screened for antibacterial activity against streptococus sp., Bacillus cereus, Klebsiella sp., Enterobacter aerogenes and Escherichia coli, Extract of Acalypha wilkesiana has a broad-spectrum antibacterial activity on all the tested organisms, but the extract of Leucaena leucocephala showed the highest inhibitory activity only on Klebsiella sp. These plants served as an alternative to synthetic antibiotics.

Key words: *Fish pathogens, Plant extracts, antibacterial activity*

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I. INTRODUCTION

Aquaculture is fast gaining momentum as an alternative to wild fish capture, but owing to problem associated with disease and parasites is limiting its potentials. The Continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in the aquatic environment. Continuous use of synthetic antibiotics reveals the threat to consumers and non-target organism in the environment (Munirruzzaman and chowdhury, 2004., Abutbul *et al.*, 2005). Though treatments of bacterial disease with various herbs have been safely used widely in organic Agriculture, Veterinary and human medicine (Direkbusarakom, 2004). Since ancient times, medicinal plants have been used for the treatment of common infectious disease (Rios and Recio, 2005) and treatments with plants having antibacterial activity are potentially beneficial alternative in aquaculture (Abutbul *et al.*, 2005). Medicinal plants as the alternative agents are effective to treat the infectious disease and mitigate many of side effects that are associated with synthetic antimicrobials (Punitha *et al.*, 2008). In addition, plant derived phyto medicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents in this field (Punitha *et al.*, 2008). *Aeromonas hydrophilla*, the most common bacterial pathogen in freshwater fish, has been recognised to be the aetiological agent of several distinct pathological conditions including tail/ fin-rot and haemorrhagic septicemia especially in freshwater and ornamental fish (Austin and Austin, 2007).

The ability of some herbs and seaweeds to inhibit activity of bacteria having potential interest as fish pathogens has been documented (Direkbusarakom, 2004., Muniruzzaman and Chowdhurry, 2004., Abutbul *et al*.,2005; Borisutpeth *et al* 2005; Bansemir *et al*., 2006; Duber and Harder, 2008) and these plants were reported to inhibit the pathogenic bacteria in aquaculture and referred to limited number of plant species (Direkbusarakom, 2004). In view of this efforts to get novel and desirable plants which could be used continuously as a natural means of treatment for fish pathogenic bacteria. This study seek to evaluate the antibacterial activity of ethanolic aqueous extracts of 7 medicinal plants on isolated bacteria obtained from fin-rot infected Catfish, *Clarias gariepinus* fingerlings from a private hatchery in Abeokuta, Ogun State, carried out at Federal Univeristy of Agriculture, Abeokuta, Ogun State, Nigeria.

II. MATERIALS AND METHODS

Plants materials and extraction.

Plants were collected from Federal University of Agriculture, Abeokuta, FUNAAB Arboretum. Fresh leaves were collected, 40g forty grammes of plant sample was extracted using soxhlet apparatus with 150mls of 95% ethanol at 60^0C for 4 hours and liquid portion was evaporated under vacuum. The volume was

concentrated to 25mls yielding a 6:1 ratio to give 100% concentration. The plant materials, designation of treatment and plant part used are summarized in table 1.

Antibacterial Assay

Paper disc diffusion assay was used to screen the plants extracts for antibiotic activity (Prescott *et al*, 1990). The micro-organisms isolated from fin-rot infected catfish fingerlings are: *Bacillus cereus, Streptococcus sp*. Which are gram positive bacteria others are *klebsiella sp., Enterobacter aerogenes and Escherichia coli* which are gram negative bacteria. The isolation and characterization of bacteria using bio-chemical tests was carried out at Microbiology laboratory, College of Natural Science, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.Mueller Hinton agar plates were inoculated with microorganism suspension, sterile filter paper discs, Whatman® 6mm in diameter were impregnated with 20µl microlitre of extract in triplicate. Water was used as negative control while local antibiotics was used as positive control they include Amoxycilline 25µg Chloramphenicol (30µg), gentamycin (10µg), Pefloxacin (5µg), Ciprofloxacin (10µg) Cotrimoxazole (25µg) others are Augmentin (30µg) and Ceftriazone (30µg.) The plants extracts and antibiotics placed on innoculated agar plate were run in triplicate and incubated at 37^0C for 24hrs. The diameter of the inhibition zone (mm) was measured after incubation. Inhibition zones >11mm are stated as "strong" 9 to 11 mm as "moderate" and < 9mm "weak" or susceptible activities. One way ANOVA and Duncan multiple Range test was used in order to evaluate the differences of the inhibition zones among the plant extracts and antibiotics.

III. RESULTS

Crude extracts obtained by ethanolic solvent of seven plants were screened for antibacterial activity against five fish pathogens. There was no inhibition zone in the negative control (water) the antibiotic was used as positive control the growth of *Streptococcus sp. Bacillus cereuss, Klebsiella sp. Enterobacter aerogenes and Escherichia coli was inhibited by Acalypha wilkesiana* extract at weak level <1mm). while L*eucaena leucocephala* inhibited the growth of *Klebsiella sp.* at weak level <1mm). Also *Sena alata* did inhibit at 0.5mm <1mm) considered weak. *Peperomia Pellucida* also inhibited the growth of *Enterobacter aerogenes* 0.5mm <1mm) considered weak activities. The ethanolic extract of *Acalypha wilkesiana* exhibited a broad-spectrum activity against both gram-positive (*Streptococcus sp. and Bacillus cereus*) and gram-negative bacteria (*Klebsiella sp., Enterobacter aerogenes and Escherichia coli*). The positive control (antibiotics) generally showed antibacterial activity to the tested micro-organism even though their zone of inhibition varied from 0.2mm to 1.22mm which was still considered as weak < 11mm).

IV. DISCUSSION

The highest inhibitory activity of 0.95mm <11mm was obtained from extract of L*eucaena leucocephala* which inhibited the growth of *Klebsiella sp. Acalypha wilkesiana* extract has a broad-spectrum activity against both gram-positive and gram-negative bacteria against the tested fish pathogens which agrees with earlier works by Akinde and Odebiyi (1987), Adesina *et al* (1980), Kabir *et al* (2005) and Oladunmoye (2006).*Peperomia pellucida* extract only inhibited the growth of *Enterobacter aerogenes*, though at a weak level . XU S et al (2006), Khan *et al.,* (2002) reported the antibacterial of *Peperomia pellucida* against numerous species of bacteria including *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.Sena alata* extract also inhibited the growth of *Klebsiella sp*. though at a weak level (0.5mm).Heavy antibiotics used in aquaculture needs to be reduced and replaced with alternative processes for treating fish diseases to avoid the emergence of antibiotic resistance in pathogenic and environmental bacteria (Sorum and L' Abee'-Lund, 2002; Cabello, 2006). Natural substances like thyme oil, clove oil and pine oil were used as alternative bio-herbicides and bio-pesticides in ecological agriculture (verschwels, 2005; Perez and Lewis, 2006).In addition, these medicinal plants may be used as potential and promising source of pharmaceutical agents against fish pathogens in organic aquaculture. This is inline with the screened plants and their antimicrobial activities. The medicinal herbs of *Acalypha wilkesiana, Peperomia pellucida, Leucaena lecocephala and Sena alata* which inhibit these bacterial pathogen out of the seven plant tested provides the aquaculturists with a promising management tool for control or treatment of fish disease.

Table 1. Antibacterial activity of used plant extracts, antibiotics and water control.

	Mean diameter of inhibitory zones (mm)			
Replicate	*Streptococcus. Sp*	*Bacillus cereus*	*Klebsiella sp.*	*Enterobacter aerogenes*	*E. coli*
Rx1a	-	-	0.95	-	-
Rx1b	-	-	0.96	-	-
Rx1c	-	-	0.94	-	-
Rx2a	-	-	0.5	-	-
Rx 2b	-	-	0.6	-	-
Rx 2c	-	-	0.4	-	-
Rx3a	0.74	0.5	0.4	0.4	0.7
Rx3b	0.75	0.6	0.5	0.5	0.5
Rx3c	0.76	0.7	0.6	0.6	0.3
Rx4a	-	-	-	0.7	-
Rx4b	-	-	-	0.4	-
Rx4c	-	-	-	-	-
Rx5a	-	-	-	-	-
Rx5b	-	-	-	-	-
Rx5c	-	-	-	-	-
Rx6a	-	-	-	-	-
Rx6b	-	-	-	-	-
Rx6c	-	-	-	-	-
Rx7a	-	-	-	-	-
Rx7b	-	-	-	-	-
Rx7c	-	-	-	-	-
Water control	-	-	-	-	-
RxA1	1.2	-	-	1.2	-
RxA2	1.3	-	-	1.1	-
RxA3	1.1	-	-	1.3	-
RxC1	0.5	0.6	-	-	-
RxC2	0.4	0.7	-	-	-

RxC3	0.6	0.5	-	-
-				
RxG1	0.6	-	0.9	1.1
0.8				
RxG2	0.6	-	0.8	0.7
1.0				
RxG3	0.9	-	1.0	0.9
0.9				
RxP1	0.2	1.1	-	-
-				
RxP2	0.3	1.2	-	-
-				
RxP3	0.1	1.0	-	-
-				
RxX1	1.0	1.2	-	-
-				
RxX2	0.9	1.1	-	-
-				
RxX3	1.1	1.3	-	-
-				
RxT1	0.6	-	-	1.2
-				
RxT2	0.5	-	-	1.1
-				
RxT3	0.7	-	-	1.3
-				
RxN1	-	-	-	0.5
-				
RxN2	-	-	-	0.4
-				
RxN3	-	-	-	0.6
-				
RxO1	-	-	-	-
0.6				
RxO2	-	-	-	-
0.5				
RxO3	-	-	-	-
0.7				

RxA1-A3=Amoxycilline,25µg, RxC1-C3=Chloramphenicol,30µg, G1-G3= Gentamycin,10µg, RxP1-P3= Pefloxacin, 5µg, RxX1-X3= Ciprofloxacin, 10µg, RxT1-T3= Cotrimoxazole, 25µg, RxO1-O3= Ceftriazone, 30µg, RxN1-N3= Augmentin, 30µg

TABLE 2: **Mean differences of the diameter of zones of inhibition of antibiotics and plant extracts against Gram – positive bacteria.**

	Diameter of inhibition zone (mm)								
Pathogenic bacteria	Antibiotics					crude plant extracts (100%)			
	AMX	CHL	GEN	PEF	CPX	COT	Acaly	Sena	Pep Leuc.
Strep. sp.	1.2±0.06^{a}	0.4±.06^{b}	0.7 ±.06^{a}	0.2±.06^{b}	1.0±.06^{b}	0.6±.06^{a}	0.75± .01^{a}	0± .00^{a}	0± .00^{a}	0± .00^{a}
Bac. cereus	0± .00^{b}	0.6±.0.06^{a}	0± .00^{b}	1.1±.00^{a}	1.2±0.06^{a}	0± .00^{b}	0.6±0.10^{b}	0± .00^{a}	0± .00^{a}	0± .00^{a}

Mean values bearing different superscripts (a, b) in the same column are significantly different but with the same superscripts are not significantly different ($p<0.05$)

AMX = Amoxycilline, CHL = Chloramphenicol, GEN = Gentamycin, PEF = Pefloxacin, CPX = Ciprofloxacin, COT = Cotrimoxazole. Sena = Sena alata, Pep = Peperomia, Leuc = Leucaena and Acaly = Acalypha

Table3: **Mean differences of the diameter zones of inhibition of antibiotics and plant extracts against Gram – negative bacteria.**

	Diameter of inhibition zone (mm)								
Path. bacteria	Antibiotics					crude plant extracts (100%)			
	AUG	CRO	GEN	COT	AMX	A	S	P	L
Kleb. sp.	0.0± .00[b]	0.0± .00[b]	0.9 ±0.06[a]	0± .00[b]	0± .00[b]	0.5±0 .06[a]	0.5±0 .06[a]	0.0± .00[b]	0.95± .01[a]
Enter. aer	0.5±.0.06[a]	0.0± .00[b]	0 .9±0.06[a]	1.2±0.06[a]	1.2±0.06[a]	0.5±0.06[a]	0±.00[b]	0.5± .06[a]	0±. 00[b]
E.coli	0.0± .00[b]	0.6± .06[a]	0.9±0.06[a]	0± .00[b]	0± .00[b]	0.5±0.06[a]	0± .00[b]	0.0± .00[b]	0± .00[b]

Mean values bearing different superscripts (a, b, c) in the same column are significantly different but with the same superscripts are not significantly different ($p<0.05$)
AUG = Augmentin, CRO = Ceftriazone, GEN = Gentamycin, COT = Cotrimozazole, AMX = Amoxycillin. S=Sena alata, P= Peperomia, L =Leucaena and A=Acalypha

Table 4 : Designation of studied plant extracts, their family names, parts used and solvent used.

Family and Plant species	parts used	Extract	Designation
FABACEAE			**R**x1a
Leucaena leucocephala	Leaves	Ethanol	Rx1b
			Rx1c
LEGUMNINOSAE	Leaves	Ethanol	Rx2a
(CAESALIPINACEAE)			Rx2b
Sena alata	Leaves	Ethanol	Rx2c
EUPHORBIACEAE			
Acalypha wilkesiana	Leaves	Ethanol	Rx3a
			Rx3b
			Rx3c
PIPERACEAE			Rx4a
Peperomia pellucida	Leaves	Ethanol	Rx4b
			Rx4c
EUPHORBIACEA	Leaves	Ethanol	Rx5a
Jatropha gossyppifolia	Leaves	Ethanol	Rx5b

	Leaves	Ethanol	Rx5c
LEGUMINOSAE	Leaves	Ethanol	Rx6a
Caesalipinae bonduc	Leaves	Ethanol	Rx6b
	Leaves	Ethanol	Rx6c
CUCURBITACEAE	Leaves	Ethanol	Rx7a
Momordica angustisepata	Leaves	Ethanol	Rx7b
	Leaves	Ethanol	Rx7c

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