EVALUATION OF ANTIBACTERIAL PROPERTIES OF *XYLOPIA AETHIOPICA* LEAVES EXTRACTS AGAINST SOME GRAM POSITIVE AND NEGATIVE BACTERIAL ISOLATED FROM AQUATIC ANIMALS

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ABSTRACT
Resistance to antibiotics by antibacterial agents resulted in the consideration for natural products with novel antibacterial activity that will help to manage or control the menace caused by resistant organisms. Therefore, this study was carried out to assess the curative potential effects of Xylopia aethiopica leaves extracts on seven fish pathogens: Aeromonas hydrophila, Staphylococcus iniae, Bacillus subtilis, Pseudomonas aeruginosa, Samonella typhi, Escherichia coli, and Streptococcus pyogenes using pour plate method. The antibacterial activity and minimum inhibitory concentration of ethanolic and methanolic extracts of X. aethiopica leaves were determined using standard methods. The ethanolic leaf extracts of X. aethiopica revealed antibacterial properties against all the pathogens tested except S. iniae and S. pyogenes while the methanolic leaf extracts displayed antibacterial properties against all the pathogens tested. The ethanolic and methanolic leaf extracts of X. aethiopica exhibited the highest antibacterial activities against Bacillus subtilis (20.50 mm) and Salmonella typhi (20.00 mm) respectively. No antibacterial effects were recorded in the negative control (distilled water). The minimum inhibitory concentration of ethanolic and methanolic leaf extracts of X. aethiopica was 450 µg/ml and 225 µg/ml respectively. This study attests to the possibility of using X. aethiopica leaf extracts in the treatment of bacterial infections in fish.
Keywords: *Xylopia aethiopica;* Minimum inhibitory concentration; Fish diseases; Antibacterial; Aquatic animals

INTRODUCTION

Intensive fish farming has promoted the growth of several bacterial diseases, which has led to an increase in the use of antimicrobials (Defroirdt *et al*., 2004). There have been various attempts to provide lasting solutions to fish diseases caused by microbial agents known as fish pathogens. This is because they affect the growth of fish production especially culture fisheries with consequent adverse effects on the industry's economic development (Ibrahim *et al*., 2011). The uses of drugs such as antibiotics have been exploited in different means to see if they can overcome this problem. However, attempts to control or prevent this problem using conventional antimicrobials and other chemo-therapeutics have been generally unsuccessful (Bello *et al*., 2013). The repeated and uncontrolled use of antibiotics and hormones in aquaculture to improve immunity, growth and reproduction performance either by application through feed or water has led to the development of antibiotic-resistant pathogens (Bello, 2014). Medicinal plants possess immuno-modulatory and antioxidant properties, leading to antibacterial activities. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Nascimento *et al.,* 2000). *Xylopia aethiopica* or Ethiopian pepper as it is usually called is an angiosperm belonging to the family Annonaceae and it is among the species that thrive in the evergreen rain forests of tropical and subtropical Africa. *Xylopia aethiopica* is used in the treatment of cough, biliousness, bronchitis, rheumatism, dysentery, malaria, uterine fibroid, amenorrhea, boils, sores, wounds and cuts among others. However, there is little or no information about its utilization in fish as an antibacterial agent. Hence, this study evaluated the potential curative effects of *Xylopia aethiopica* leaf extracts as bio- antibacterial agent for fish pathogens.

MATERIALS AND METHODS

Plant Collection, Identification and Preparation

Xylopia aethiopica was obtained in the botanical garden of Olusegun Agagu University of Science and Technology, Okitipupa, and was identified in the Department of Biological Sciences (Botany Programme), Olusegun Agagu University of Science and Technology, Okitipupa. *Xylopia aethiopica* leaves were air-dried for 4 weeks and the air-dried leaves were grounded with a hammer mill. The extraction of *X. aethiopica* leaves was done as described by Olusola *et al*., (2019). 180g of the fine powder of the plant leaves were soaked in 1000 ml of ethanol and methanol for 72 hours. The plant leaves were properly mixed with ethanol and methanol and filtered at regular intervals (every 3 hours) using a sterile muslin cloth after which the extraction was obtained, air-dried and stored at 25°C until required.

Antimicrobial Assay and Detection of Antimicrobial Activity

The bacteria isolated from *Clarias gariepinus* juveniles and *Oreochromis niloticus* were *Aeromonas hydrophila*, *Pseudomonas aeruginosa, Streptococcus pyogenes Salmonella typhi* and *Staphylococcus iniae*. The isolation and characterization of bacteria using biochemical tests was carried out at the Microbiology Laboratory, Olusegun Agagu University of Science and Technology, Okitipupa. The pure cultures were labelled, sub-cultured on nutrient agar slants and nutrient broth(s), and preserved in the refrigerator at 4°C until it is required for study. A well diffusion assay as described by (Bello *et al*., 2013) 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 indicating of bacteria). Wells of 10 mm diameter were cut into these agar plates using cork borer and 0.2ml of this plant extract was placed into each well (Bello *et al*., 2013). Distilled water was used as negative control and the plates were examined for zones of inhibition, which was scored positive, if the width of the clear zone was 10 mm or longer. The diameter of the inhibition zones was taken to be proportional to the logarithm of the antimicrobial compounds in *X. aethiopica* leaves.

Minimum Inhibitory Concentration

The minimum inhibitory concentration of *X. aethiopica* leaves was carried out using a doubling dilution of 1800 µg / ml of *X. aethiopica* leaves extract was made in 2 ml volume of broth to 7 µg / ml. One row of the test was inoculated with 0.02 ml of 1 in 10 dilutions of the overnight 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 (Olusola *et al*., 2019).

Phytochemical Screening

The phytochemical screening was carried out using the standard testing procedures as described by Olusola *et al*. (2017).

RESULTS

Phytochemical Analysis *X. aethiopica* leaves

The results of phytochemical screening of *X. aethiopica* leaves for primary and secondary metabolites revealed the presence of saponins, glucosinolates, flavonoids, phenol, protein and amino acids in the plant while Triterpenes and steroids were absent in *X. aethiopica* (Table 1).

Table 1: Phytochemical screening of *Xylopia aethiopica* leaves

Parameter	*Xylopia aethiopica*
Saponins	+++
Phenol	++
Tannin	+
Flavonoids	++
Glucosinolates	+
Triterpenes and steroids	-
Protein and amino acids	++

Keys: +++ present in high quantity, ++ moderately present, + present in low quantity, - negative or not present

Determination of antibacterial activities of *X. aethiopica* leaves

The result of ethanolic leaf extracts of *X. aethiopica* revealed antibacterial properties on the pathogens isolated from *C. gariepinus and O. niloticus* except for *Streptococcus pyrogenes* and *Staphylococcus iniae* while the methanolic leaf extract of *X. aethiopica* showed antibacterial properties against all tested pathogens. The widest zone of inhibition diameter was recorded in *B. subtilis* and *S. typhi* while the least inhibition zone was recorded in *A. hydrophila* and *B. subtilis and S. pyogenes* in ethanolic and methanolic extracts respectively (see Table 2).

Table 2: Antibacterial activities (diameter of zone of inhibition, mm) of methanolic and ethanolic leaf extracts of *X. aethiopica*

Method of extraction	Pathogens	*Xylopia aethiopica*	Control (distilled water)
Ethanol	*Bacillus subtilis*	20.50 ± 0.02	-
	Pseudomonas aeruginosa	15.00 ± 0.00	-
	Streptococcus pyogenes	-	-
	Escherichia coli	16.00 ± 0.30	-
	Salmonella typhi	17.50 ± 0.05	-
	Aeromonas hydrophila	12.50 ± 0.30	-
	Staphylococcus iniae	-	-
Methanol	*Bacillus subtilis*	10.00 ± 0.00	-
	Pseudomonas aeruginosa	15.00 ± 0.02	-
	Streptococcus pyogenes	10.00 ± 0.01	-
	Escherichia coli	11.50 ± 0.02	-
	Salmonella typhi	20.00 ± 0.00	-
	Aeromonas hydrophila	12.50 ± 0.30	-
	Staphylococcus iniae	18.50 ± 0.03	-

Minimum Inhibitory Concentration (MIC) of *X. aethiopica* Leaves

The doubling dilution method was used to determine the minimum inhibitory concentration of ethanolic and methanolic leaf extract of *X. aethiopica* against seven isolated bacteria from *C. gariepinus* and *O. niloticus*. This study revealed that the minimum inhibitory concentration of the ethanolic and methanolic leaf extracts of *Xylopia aethiopica* is 450 µg/ml and 225 µg/ml respectively (Tables 3).

Table 3: Minimum inhibitory concentration (µg/ml) assay of methanolic and ethanolic extracts of *X. aethiopica* leaves

	Ethanolic extract of *Xylopia aethiopica* leaves								
Pathogens	**1800**	**900**	**450**	**225**	**112**	**56**	**28**	**14**	**7**
B. subtillis	-	+	+	+	+	+	+	+	+
P. aeruginosa	-	-	+	+	+	+	+	+	+
S. pyogenes									
E. coli	-	+	+	+	+	+	+	+	+
S. typhi	-	-	+	+	+	+	+	+	+
A. hydrophila	-	-	+	+	+	+	+	+	+
S. iniae									
	Methanolic extract of *Xylopia aethiopica* leaves								
B. subtillis	-	+	+	+	+	+	+	+	+
P. aeruginosa	-	-	+	+	+	+	+	+	+
S. pyogenes	-	-	-	+	+	+	+	+	+
E. coli	-	-	+	+	+	+	+	+	+
S. typhi	-	-	-	-	+	+	+	+	+
A. hydrophila	-	-	+	+	+	+	+	+	+
S. iniae	-	-	+	+	+	+	+	+	+

Keys: - inhibition, + no inhibition, *B. subtilis = Bacillus subtilis, P. aeruginosa = Pseudomonas aeruginosa, S. pyogenes = Streptococcus pyogenes S. typhi = Salmonella typhi, A. hydrophila = Aeromonas hydrophila, E. coli = Escherichia coli, S. iniae = Staphylococcus iniae*

DISCUSSION

Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens (Olusola *et al*., 2013). Extracts obtained from various parts of the plant through *in vitro* studies have confirmed their anti-microbial activity in aquaculture (Olusola *et al*., 2017). The results of the phytochemical screening of *X. aethiopica* leaves revealed the presence of saponins, glucosinolates, flavonoids, phenol, protein and amino acids in the plant while Triterpenes and steroids were absent in *X. aethiopica* and this study was similar to the report of John – Dewole *et al*., (2012) who reported the presence of saponins, glucosinolates, tannins, and phenol in *X. aethiopica* leaves. Medicinal plants are used as antimicrobials in animal feed and aquaculture as therapeutic agents (Bello, 2014). The result of the study revealed antibacterial properties of the ethanolic leaf extract of *X. aethiopica* on the pathogens isolated from *C. gariepinus and O. niloticus* except for *Streptococcus pyrogenes* and *Staphylococcus iniae* while the methanolic leaves extract of *X. aethiopica* showed antibacterial properties against all tested pathogens. This study was aligned with the report of Konning *et al.* (2004) reported that methanol extract of dried *Xylopia aethiopica* fruit showed some antimicrobial activity against gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria. Yadav and Khan, (2012) reported antibacterial activity of ethanolic extract of medicinal plants screen showed antibacterial activity and inhibited the growth of bacterial strains such as *P. aeruginosa, B. cereus* and *E. coli* which was similar to the present study. The reason for high antibacterial activity in *X. aethiopica* leaves might be due to the presence of phyto-constituents available in the plant which initiates steps against contagious bacteria. The results of a minimum inhibitory concentration of *X. aethiopica* leaves extracts indicate that 450 µg/mL and 225 µg/mL for ethanolic and methanolic extracts respectively are required to inhibit the seven clinically isolated fish pathogen and these results agree with the work of (Bello *et al*., 2013) who reported inhibitory effect on two herbal plants against the pathogen tested.

CONCLUSION

This study revealed that *X. aethiopica* leaf extracts had antibacterial properties against seven pathogens tested. However, this study justifies the use of *X. aethiopica* leaves as therapeutic and antibacterial agents to treat fish pathogenic diseases.

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