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Research Article
Phytochemical Antibacterial Effect of *Borrerial verticillata* Extract on Bacteria Isolated from Some Fish Species of Ogbese River

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Abstract

Background and Objective: Increased human interference on aquatic habitat resulted in pollution exposing fish to microbes pathogenic to man; hence, creating public health awareness. The aim of this study was to investigate the health status and bacterial occurrence in fishes, water and sediments of Ogbese river and *Borrerial verticillate* plant sensitivity against isolates. **Materials and Methods:** Gills, skins and intestines of *Mormyrus rume*, *Parachanna obscura* and *Clarias gariepinus* fishes of economic importance in Ogbese river were examined for bacterial occurrence using standard bacteriology measure. Serial dilution (10^4) in water was carried out; 1 g each of sample was cultured by pour plating on nutrient agar and incubated at 37°C for 24 h and anti-bacteria effect of aqueous *Borrerial verticillata* herbs on isolated bacteria were assessed using its zone of inhibition to know its natural antibacterial effectiveness. Data collected were subjected to descriptive and analytical statistics. Growth and bacteria data were subjected to one-way analysis of variance (ANOVA) and significant differences ($p>0.01$) between mean were separated by Duncan Multiple Range test using Computer Software SPSS version 20.0. **Results:** Bacteria load was highest in gills of *Mormyrus rume* and *Parachanna obscura* and highest in intestine of *Clarias gariepinus*. A total of 10 bacteria flora were isolated from fish samples and environment: *Micrococccus* spp., *Bacillus subtilis, Proteus vulgaris, Streptococcus faecalis, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeruginosa, Escherichia coli* and *Micrococcus luteus*. Antibacterial effectiveness of aqueous *B. verticilata* on isolated bacteria, revealed *Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeruginosa, Escherichia coli* sensitive and *Serratia marcescens* was the most sensitive to aqueous *B. verticilata*. **Conclusion:** Aquatic environment and microbes depicted health status of fish as well as man and *B. verticillate* leaf extracts have proved sensitivity against some isolated bacteria in Ogbese river for public health purpose.

Key words: *Borreria verticillate*, bacteria occurrence, *Clarias gariepinus*, Ogbese river, phytochemical sensitivity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The microbial communities of an aquatic environment are a function of environmental factors[1]. The structure of water column may has greater production of phytoplankton above the thermocline in some places and below in other. This is a function of bacteria community and the complex relationship as influence by nutrient cycle and environmental factors which can cause eutrophication[2]. Aquatic environments are easily polluted by wastes from homes, farmlands and industries which expose aquatic biota e.g., fish to danger. The types of micro-organisms associated with fish depend on the aquatic habitats of fish and are known to be affected by factors (e.g., salinity level and bacterial load) of the habitat [3,4]. Fish takes large number of bacteria into their gut from water sediment and food. It has been well known that fresh and brackish water fishes can harbor human pathogen bacteria particularly the coliform group[5].

However, infection due to microbial contamination does and may not result in disease condition, but environmental stresses may alter balance between potential pathogens and their hosts[6]. The bacterial pathogens are of two groups: non-indigenous and indigenous pathogens. These are more directly related to turnover of organic carbon as production in water columns is dependent on concentration and availability of dissolved organic compounds[7]. In sediments, there are gradients in bacterial concentration, hence, productivity of bacteria is a better indicator of trophic status than abundance[8].

Several studies have been done in different aquatic ecosystem on assessing influencing factors of the aquatic environment on microbial characteristics and population[9,10]. Bacteria often occur in parts of fish such as; scales, gills, gut and alimentary tract. The bacteria present on the body or internal organs of fish indicate the extent of pollution of aquatic ecosystems. Faecal polluted water bacteria contamination may include *Aeromonas* sp., *Shigella* sp., *Salmonella* sp., the coliforms etc. these microbes enter the fish through different channels such as; wounds, ingestion and engulfment processes[11].

Plants are prospective source of antimicrobial agents, as they are rich in a wide variety of secondary metabolites. They are eco-friendly unlike the artificial antibiotic and they possessed properties of alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils[5,12,13]. Several works have been done with plant source as antibacterial agent[14-16].

Herbs are sources of safer and cheaper chemicals important in disease control due to their antioxidant and anti-microbiological activities like anti-stress, promoting growth, appetizing, tonic, immune-stimulation, aphrodisiac and antimicrobials. Several works have done include engaging use of herbal medicine in combating microbial activities. Plants use-age as in aquatic environment is a cheap antibiotic eco friendly agent against isolated bacteria from aquatic environment[7,17,18].

Indiscriminate exposure of fish in aquatic environmental is becoming unseemly on the increase; exposing fish to bacterial infection. Control of bacteria in environment is kin in public health as it reduces fish shelf-life, resulting in food poisoning[19]. Therefore, optimum growth of fish and environmental health surveillance is important for biota and consumer health[20]. This study investigated bacteria occurrence and load on some fish species: *Parachanna obscura, Mormyrus rume* and *Clarias gariepinus* (skins, gills and intestines), water and sediments of Ogbese river, southwest, Nigeria.

MATERIALS AND METHODS

The study was carried out on fishes from Ogbese river. Figure 1 shows the map of Ogbese river.

Sample collection: About 60 Live *Mormyrus rume, Clarias gariepinus* and *Parachanna obscura* fish species were obtained during wet season (April-July, 2016) from Ogbese river, Ondo state in south west of Nigeria. Fish sample was transported alive in plastic containers (covered with net) to the Fisheries and Aquaculture, Federal University of Technology, Akure Ondo-state.

Meristic measurement of fish: Length (cm) of fish species were measured using graduated meter rule and weight (g) of fish species were measured using Mettler Toledo electric weighing balance (Model PB8001).

Growth pattern of fish species: Length-Weight Relationship of fish were assessed on *Parachanna obscura, Mormyrus rume* and *Clarias gariepinus* fish respectively using equation:

$$W = aL^b$$

where, W is weight (g) of fish, L is length (cm) of fish, 'a' is intercept and 'b' is slope [regression coefficient which lies between 2 and 4; while isometric (symmetric) growth is indicated at 3 and values other than 3 will indicate allometric growth i.e., values greater than 3 is positive allometric growth and values less than 3 is negative allometric growth]. Linear

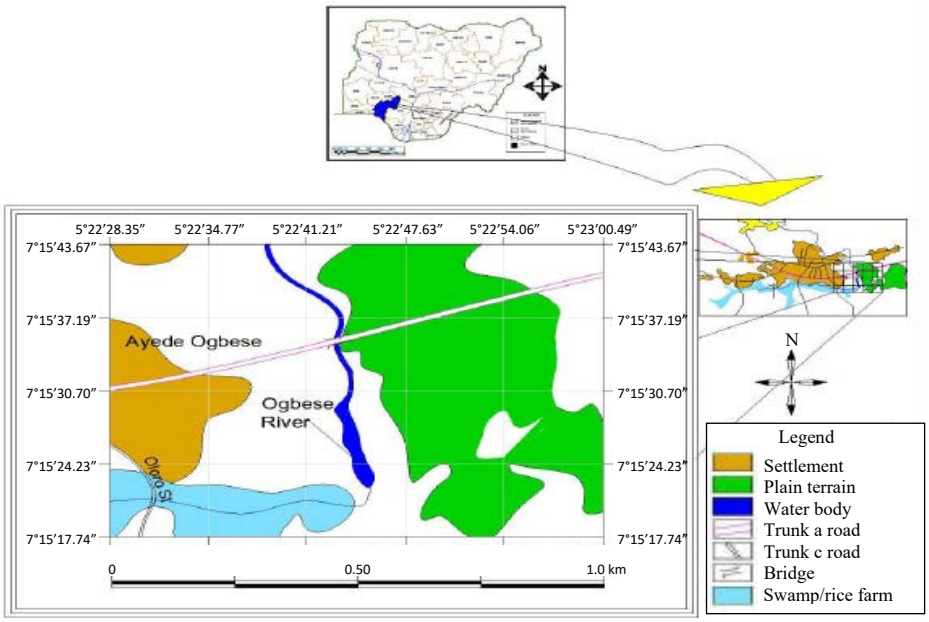

Fig. 1: Ogbese river, Ondo state, Nigeria
Source: Olawusi-Peters and is Ayo-Olalusi[21]

regression of graphy of length-weight relationship of fish species was plotted.

Condition factor: Condition factor of fish samples collected were determined using the formula:

$$K = \frac{100W}{L^3}$$

where, K is condition factor, W is weight (g), L is Length (cm), b is isometric value[22].

Bacteriological examination: The bacteriological media namely Nutrient agar (NA) was prepared according to manufacturer's instructions. About 28 g Nutrient agar (NA) powder and B.P.A were dissolved in 1 L of distilled water and autoclaved at 120°C for 15 min. According to standard methods, the media was sterilized at 121°C for 15 min in an autoclave (Fishers scientific, USA) and was poured into sterile disposable Petri dishes (Fishers scientific). The mixture was left to solidify the streak plates technique was used to obtain pure culture of bacterial from their colonies on nutrient agar. Samples from skin (1 cm^2), intestine and the gill were macerated aseptically in separate Petri dishes and homogenized in 100 mL sterile distilled water. The stock solution was serially diluted in three folds (10^{-3}). About 1 mL of the appropriate diluents were transferred into sterile Petri dishes and pour plate technique were used to enumerate the bacteria counts by adding NA in duplicate. These plates were incubated at 37°C for 24 h. Pure cultured of bacteria isolate were stored on slants of fleshly prepared nutrient agar at refrigeration temperature of 8±1°C. Standard characterizations of bacteria isolates were done using Berger's Manual of Systemic Bacteriology[23].

Collection, preparation and sensitivity test of bacterial isolates to *Borreria verticillata:* *Borreria verticillata* herbs were collected and air dried over 14 days to dry matter. These were milled using electric blending machine into fine powder. Aqueous solutions of powdery *B. verticillata* were prepared by soaking 100 g in 250 mL methanol in conical flasks for 14 days, the mixture was placed in a shaker machine for 5 h and stirred constantly at 50°C temperature. A rotary evaporator was used to extract methanol as the solvents. The extracts were filtered using filtered paper. An essential oil extracted from leaves were dried, was applied in each of the bacteria culture plate and inoculated at 37°C for 24 h for sensitivity test, antibiotics disc was used as control. Result showing cloudiness of streaked area revealed that bacteria are sensitive to the fluid extract while clearness of streaked area reveals that bacteria are resistant to the fluid extract[15].

Statistical analysis: Descriptive and analytical statistics were used to analyze data collected. Growth and bacterial occurrence data were subjected to one-way analysis of variance (ANOVA). Regression analysis was carried out on growth parameters. Frequency of bacteria (cfu) occurrence of

all samples were recorded as mean±standard deviation. The significant difference (p>0.01) between mean were separated using Duncan Multiple Range test using Computer Software SPSS version 20.0.

RESULTS

Growth parameters: About three fish species indicated that *Clarias gariepinus* has highest regression coefficient, while *Momurus rume* has highest slope and isometric values. The three species indicated negative isometric which revealed above 80% relationship in weight gain to increase in length in the three fish species (Table 1).

Bacteriological examination: Bacteria species prevailed in water, sediment, skin, intestine and gill. Ten probable organisms were isolated which were pathogens capable of causing a variety of disease in man. The isolates include: *Micrococcus* spp, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis*, *Proteus vulgaris*, *Micrococcus luteus*, *Streptococcus feacalis*, *Serratia marcescens*, *Escherichia coli* and *Pseudomonas aeruginosa*. Frequency of bacterial occurrence (cfu mL^{-1}) in fish, water and sediment samples were indicated in Fig. 2.

Bacterial load in Obese water, sediments and fish species: Bacterial load over a period of 6 months in Ogbese river indicated mean value of 5.36±0.03 in water and 4.27±1.22 in sediment at serial dilution of 10^4 cfu mL^{-1}. Table 2 showed mean bacterial load from the skins, gills and intestines of fish species at 10^4 cfu mL^{-1}. Microbial load ranked highest on gill of *Mormyrus rume* highest in gill of *P. obscura* and highest in intestine of *C. gariepinus*. Figure 3 showed the percentage bacterial load among specimens from Ogbese water, sediments and fish species.

Antibacteria sensitivity tests of *Borreria verticillata*: Antibacteria sensitivity tests of aqueous concentrations of *Borreria verticillate* leaves extract revealed high variation in sensitivity profiles to tested bacterial flora. *Serratia marcescens* was most sensitive organism to the leaves extract among the five bacterial isolates that were sensitive to the plant extract (Table 3).

Table 1: Regression and Means±standard deviation of morphometric characteristics of *Clarias gariepinus, Parachanna obscura* and *Mormyrus rume* from Ogbese river

Parameters	Standard length (cm)	Weight (g)	Condition factor (K)	Regression coefficient (R^2)	Slope (a)	Isometric value (b)	Remarks
Clarias gariepinus	34.50±3.54	551.00±4.87	1.34	0.88	1.89	1.24	Negative allometric
Parachanna obscura	29.50±3.54	438.85±7.73	1.71	0.82	2.52	1.05	Negative allometric
Mormyrus rume	27.80±3.11	267.25±5.47	1.24	0.81	3.75	2.79	Negative allometric

Table 2: Bacterial load in fish species skins, gills and intestines from Ogbese river

Bacterial Load (10^4 CFU mL^{-1})	*Clarias gariepinus*	*Parachanna obscura*	*Mormyrus rume*
Fish Parts			
Skin	4.25±2.58^b	7.10±2.00^a	2.80±3.90^c
Gill	4.80±1.11^c	8.00±0.00^b	24.00±6.14^{a*}
Intestine	7.65±2.12^{a*}	7.10±3.44^a	1.00±0.00^b

Mean with the same superscript alphabet has no significant difference between the column and a*: significance difference in bacterial load (n = 60) (p>0.01)

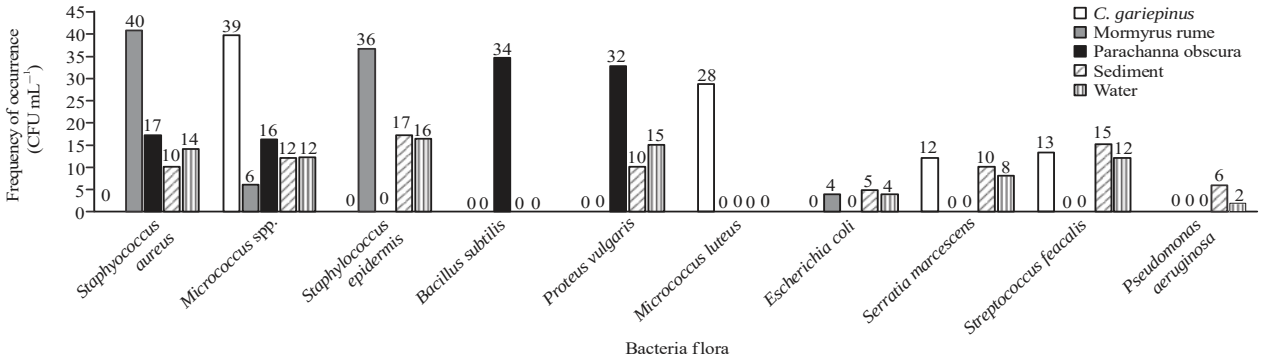

Fig. 2: Frequency and percentage of Bacterial Occurrence in Fish species, Sediment and Water of Ogbese river

Table 3: Sensitivity effect of aqueous *Borreria verticillate* (25 mg L^{-1}) leaves extract on isolated bacterial floral

Bacterial flora	Zone of inhibition (mm)	Sensitivity test
Micrococcus spp.	NI	R
Bacillus subtilis	NI	R
Proteus vulgaris	NI	R
Serratia marcencea	7.5	S
Staphylococcus aureus	5.5	S
Staphylococcus epidermis	5.5	S
Pseudomonas aeruginosa	5.0	S
Escherichia coli	5.0	S
Micrococcus luteus	NI	R
Bacillus cereus	NI	R
Streptococcus feacalis	NI	R

NI: No inhibition, S: Sensitive, R: Resistant

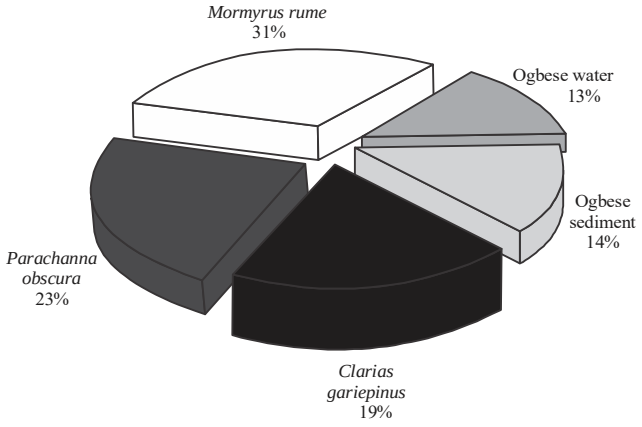

Fig. 3: Bacterial load (%) in Ogbese water, sediments and fish species

DISCUSSION

Fish species from Ogbese river showed normal growth rate, but contained pathogenic bacterial indicating feacal contaminants in water body and antibacterial effect of *Borreria verticillate* on the isolates indicated sensitivity of some bacterial to plant extract, while others resisted it. Growth parameter assessed on fish revealed normal growth and sequence in a freshwater environment[24] and result for bacteria colony forming unit (CFU) on bodies of the three species from Ogbese shows no significant differences on gill, intestine and skin as showed in Table 2, in which high bacteria load on the gill, intestine and skin of the three species in the first month compare to the bacteria load on the gill, intestine and gill of the three species in the second month[25].

Many investigators have isolated different species of bacteria from the skin of the fresh water fish (catfish) including *Bacillus subtilis* spp. from the skin of sea water fish[26-30]. *Proteus species* was isolated from some fresh water[31]. *Staphylococcus* spp. and *Escherichia coli* were isolated frequently from the skin of fresh water fish and reported skin of fresh water fish a permissible habitat of the bacteria[32].

Escherichia coli are often used as an indicator for faecal contamination; however, because of the ubiquitous nature of this organism in the tropics this association is questionable. Some strains of *E. coli* are capable of causing foodborne disease, ranging from mild enteritis to serious illness and death. These bacteria species may therefore; be opportunistic. Association of bacteria with specific fish disease has not been successful in *Clarias gariepinus* as this species is regarded as a rather resistant fish[33]. Most strains of *E. coli* are harmless and occasionally responsible for product recall. The harm less strain is part of the normal flora of the gut and can benefit their host by producing Vitamin K_2 and by preventing the establishment of pathogenic bacteria within the intestine[34]. Other experimental study revealed that *Pseudomonas* spp. caused peeling of the outermost skin and reduced appetite of the fishes which the skin peeling was not experienced in fish inoculated *E. coli* and *Proteus* sp[35].

The results on the use of *Borreria verticillata* revealed that it's an antibacterial on some of the organisms. The *B. verticillata* was effective against amoebic dysentery, gastrointestinal disorder and have antimicrobial and antiparasitic activity. Leafy vegetables are containing high moisture and low acid produce which supported the growth of a wide range of micro-organisms risk of contamination by bacteria that cause food poisoning especially those for example *Staphylococus aureus* and *Escherichia coli* which produced heat stable toxin that may not be destroyed by heat treatment such as cooking[36,37].

The plant extracts had profound activities against both Gram-positive and Gram-negative bacteria. Gram-positive was most sensitive than Gram-negative organism having that differences in susceptibility of Gram-positive and Gram-negative bacteria to various antimicrobial agents probably depends on structural differences in their cell walls[38] and use of organic solvents such as alcohol can increase sensitivity level in plant crude extraction before being retracted to obtain purified active compound using some other organic solvents[39,40]. Plants contained antibacterial or antimicrobial substances[41]. However, some isolates showed high sensitivity to the aqueous extract of the *B. verticillate*. The results revealed there was variation in degree of antibacterial activities of the plant extract. Hence, further investigation on effectiveness of *B. verticillata* using other extraction methods (i.e., alcohol) can be employed to assess degree of

bacterial susceptibility to the plant. While the plant extract can be used by fisher folks in disinfecting fishing equipment, it can also serve as additive in fish feed against bacterial infections.

CONCLUSION

From this study it was concluded that bacterial flora are natural components of natural aquatic ecosystem, which could cause alteration in normal functioning of biota, especially due to human interference. This may impair health status of fish and public health; hence, *Borreria verticillate* leaf extracts has revealed antibacterial sensitivities on some bacterial isolated in Ogbese river. The results therefore would enable farmers and fisher folk's access to cheap eco-friendly microbial control plants extracts to improve quality of fish; disinfectant of fishing gears and public health.

SIGNIFICANCE STATEMENT

This study discovered *Borrerial verticillate* antibacterial properties beneficial against *Escherichia coli*, *Staphylococcus aureus* and *pseudomonas* sp. pathogenic bacteria isolated from aquatic fish and environment. This study will help the researchers to uncover the critical areas of phytochemical importance towards healthy ecosystem functioning and public health that many researchers were not able to explore. Thus, a new theory on use of plant resources in aquatic ecosystem health management may be arrived at.

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