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Antibacterial Effects of *Vernonia amygdalina* Delile (Bitter leaf) Extract on Some Bacterial Flora Isolated from *Chrysichthys nigrodigitatus* Lacepede

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ABSTRACT

Vernonia amygdalina (bitter leaf), a perennial shrub of family *Asteraceae* grows throughout tropical Africa, and it possesses numerous phytotherapeutic properties as antimicrobial (antibacterial, antifungal, and antiplasmodial), antioxidant, hypoglycemic /anti-diabetic, anti-tumor and other properties. The sensitivity of bacterial isolated from *Chrysichthys nigrodigitatus* catfish specie to extract of *Vernonia amygdalina* and its inhibitory effects was investigated. Extract of *Vernonia amygdalina* shrub as anti-bacteria was investigated on eight species of bacteria flora isolated from *Chrysichthys nigrodigitatus* silver catfish. Leaves of *Vernonia amygdalina* were grinded and squeezed to release extract. Antibiotics sensitivity tests of *Vernonia amygdalina* to water (1 mg / ml) leave extract to bacterial flora was carried out. Bacteria species were streaked on nutrient agar, and cloudiness of streaked area reveals that the bacteria is sensitive to the fluid extract while clearness of streaked area reveals that bacteria is resistant to the fluid extracts. Data obtained were subjected to analytical analysis and descriptive statistics such as and bar charts. Result obtained revealed variations in sensitivity profile. *Streptococcus faecalis* was the most sensitive organism to leave extract with the zone of inhibition of 4.00mm and the least sensitive organism to the extract was *Staphylococcus aureus* with zone of inhibition of 0.8 mm at the same concentration, (1mg / ml) (*V. amygdalina* / water). Results revealed gram-negative bacteria were more sensitive to *Vernonia amygdalina* extracts which aid improved animal immune system against diseases.

Keywords: Anti-bacteria effect, Bacteria flora, *Vernonia amygdalina*, Fish species

Introduction

Medicinal plants are important in disease prevention, and are utilized as food (Sofowora *et al.*, 2013). *Vernonia amygdalina*, of Asteraceae family is a small shrub of tropical Africa. It is a food vegetable which grows typically to a height of 2 – 5 m under a range of ecological zones in Africa. It possesses leaves that are elliptical and up to 20 cm long. Its bark is rough and commonly called bitter leaf, (Oyeyemi *et al.*, 2017). *V. amygdalina* has biologically active compounds which include saponins and alkaloids (Muraina *et al*., 2010), terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and anthraquinone, edotides and sesquiterpenes, (Sabiuab *et al.*, 2017).

V. amygdalina plant: leaves, stem, extract and barks have been utilized in varying health care and medicinal purpose, culinary and curative purpose. It functions well as an antibiotic against drug resistant microorganisms, as it possesses antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities, (Olga *et al.*,

2017). Several investigators had reported that plants contain antibacterial or antimicrobial substances (Ijeh and Adedokun, 2006 and Oyeyemi *et al.,* 2017).

According to Ijeh and Ejike, (2011), *V. amygdalina* provides enhancement of the immune system. Linlin *et al.,* (2018), reported that *V. amygdalina* extracts may strengthen the immune system through many cytokines regulation; and that it possesses invitro anthelminthic anti-parasitic properties. Hence, *V. amygdalina* extract have various medicinal values and the inhibitory effects of the leaves extract against the pathogen can introduce the plant as potential herb for drug development for the treatment of ailments caused by these pathogens.

Fish, regarded as safe, nutritious and beneficial (WHO, 2007) has been known to taking bacteria into their gut from water sediment and food. Both fresh and brackish water fishes can keep human pathogenic bacteria mostly coliforms (*Escherichia coli*), (Abidemi-Iromini, 2017); which are large group of bacteria occurring and indicating presence of harmful disease-causing organisms in fish environment (Gogoi and Sharma, 2013).

Chrysichthys nigrodigitatus silver catfish of family Bagridae, Lacepède, 1803 is widely distributed in fresh and brackish water of West Africa (Emmanuel and Osibona, 2013). It is a demersal potandromous scale-less fish which reside in shallow and slow-moving waters i.e lakes, over mud and fine bottom, in rivers and in swamps. *C. nigrodigitatus* shrives well in tropics at temperature range from 22 ^{0}C to 28 ^{0}C, and pH 6.0 to 7.2 (Soyinka and Kassem, 2008). It is a substrate brooder that exhibits parental care; it possesses varying feeding habit with age and size; an omnivore, but generalized predator. *C. nigrodigitatus* is a good acceptable fish food protein source of high economic value, and has been adopted for aquaculture, (Uneke *et al.,* 2015).

The consumption of fresh silver catfish (*Chrysichthys nigrodigitatus*) and existence of pathogenic bacteria are of public health significance since bacteria isolated from fish samples are a function of bacteria found in the environment depending on exposure to external influences and pollutants, (Phan *et al.*, 2011; Abidemi-Iromini and Fofah, 2016).

Hence, effect of *V. amygdalina* extracts on some bacteria flora isolated from *C. nigrodigitatus* were investigated to see anti-bacteria impact of the extracts on the bacteria for cheap phytotheraphy and public health purpose.

Materials and Methods

Collection of Sample for Bacterial Isolation and Serial Dilution of Fish Sample

Chrysichthys nigrodigitatus fish collected from brackish water environment, and were dissected and subjected to bacteriological examination according to standard procedure by: Wedemeyer *et al.*, (1976), FAO and NACA, 2001). 1 g gram of the fish sample was collected from muscle, gill and intestine of the fish and mashed together using pestle and mortal. 1 g of mashed fish samples were put into test-tube containing 9 ml distilled water. This was mixed vigorously for 60 seconds to achieve homogenous substance. Serial dilution up to 10^5 were made with distilled water. One ml of the serially diluted sample (10^5) was then dispensed aseptically into sterilized petri dishes.

Isolation of Bacteria

Nutrient Agar (28g) was prepared according to standard manufacturer's specification. The culture medium was autoclaved at 120^{o} C for 15 minutes. The agar was allowed to cool down before it was poured into petri-dishes containing 1 ml serially diluted (10^{5}) fish sample using pour plate technique to culture the fish sample for bacteria isolation. The sample petri-dishes were left to solidify, and then turned upside down inside incubator for 24 hours at 37^{o}C. Observation of colonies began after 24 hours.

Culture of Pure Bacteria Isolates

Pure culture of probable bacterial were cultured in Nutrient agar for another 24 hours at 37^{o}C, and pure isolates obtained were stored on slants of Nutrient Agar and stored in refrigerator at 4^{o} C. Inoculums from these sources were used for the study as desired.

Bacterial Characterization and Identification

Bacterial colonies characterizations were carried out according to Cheesbrough, (1985). The available were observed after 18-24 hours of incubation for shape, surface texture, colour, size, transparency and edge elevation. Isolates were Gram stained to differentiate into Gram negative and Gram positive using microscopic examination of stained preparation. Motility test was carried out by hanging drop preparations of isolates on cavity slides, and examined microscopically. Other biochemical reactions including coagulase, catalase and sugar fermentation were intensified. One percent of sugars such as glucose, sucrose, lactose, maltose and others were used in a basal fermentative medium to determine ability of organisms to utilize appropriate carbon sources signified by acid production or change in colour of medium and production of gas in Durham tube provided.

Purification of Isolates

Distinct colony was inoculated and sterilized in another sterilized medium as pure culture, and incubated at 37^{o} C for 24 hours.

Preparation of Aqueous Extracts from *Vernonia amygdalina*

Fresh *Vernonia amygdalina* leaves were collected from botanical garden, Federal University of Technology, Akure, Ondo State, Nigeria. The leaves were taken to Fisheries and Aquaculture Department, where the leaves were washed under flowing water and grinded using pestles and mortal, to release the aqueous substances. The aqueous extracts were squeezed out respectively into a conical flask using mesh net; and filtered using Whatman No. 1 filter paper.

Preparation of bioassay as inoculum for antibacterial test

Bio-assayed for antimicrobial activity were made as inoculum, using 1 ml of the herbs extracts was respectively pour plated with 28 g nutrient agar preparation into petri-dishes the gel in the petri-dishes were allowed to cool before pure isolated bacterial were streaked onto the petri-dishes and incubated at 37^{o} C for 24 hours during sensitivity test, and antibiotics' disc was used as control.

Statistical analysis

The data were analysed using analytical and descriptive statistics. Tables, pie and bar charts were used to show bacterial load and inhibition effects.

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Results

Frequency of Occurrence of Isolated Bacterial flora

Bacteria load on *Chrysichthys nigrodigitatus* from Lagos lagoon indicated (78.00±0.26 x10^5). Occurrences of bacterial flora isolated from fish are *Streptococcus faecalis* (7), *Escherichia coli* (8), *Aeromonas hydrophylla* (3), *Proteus mirabillis* (3), *Staphylococcus spp* (10), *Pseudomonas spp* (7), *Klebsiella spp* (2) *and Salmonella spp* (1). Also, figure 1 revealed the percentage occurrence of bacterial flora isolated from the fish

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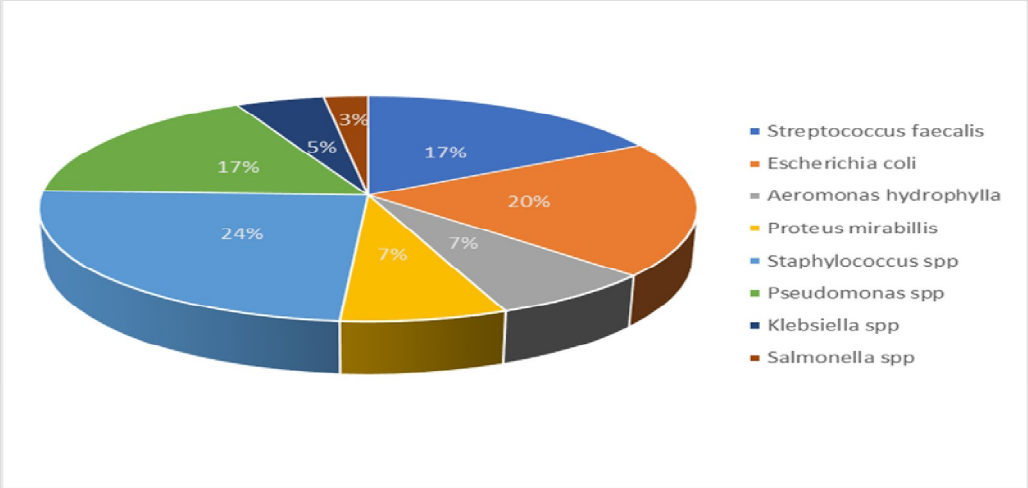

Figure 1: Percentage occurrence of bacteria flora

Antibacterial effect of *Vernonia amygdalina* (Bitter leaf) on Isolated Bacterial Flora

Antibacterial effect of *Vernonia amygdalina* was investigated on bacterial isolates, the following bacteria were isolated at the end of the research; *Streptococcus faecalis, Escherichia coli, Aeromonas hydrophylla, Proteus mirabillis, Staphylococcus spp, Pseudomonas spp, Klebsiella spp and Sallmonella sp*p.

The antibiotics sensitivity tests of *V. amygdalina* to water at (1 mg / ml) leave extract to bacterial flora revealed variations in sensitivity profile. The aqueous extract of the herb created layers on bacteria streaked in the petri-dishes, and this layer is known as the layers of inhibition of the herb on the bacteria and also shows its effectiveness on the bacteria. Cloudiness of streaked area reveals that the bacteria are sensitive to the fluid extract while clearness of streaked area reveals that the bacteria are resistant to the fluid extracts. *Streptococcus faecalis* was the most sensitive organism to leave extract with the zone of inhibition of 4.00mm and the least sensitive organism to the extract was *Staphylococcus aureus* with zone of inhibition of 0.8 mm at the same concentration, (1mg / ml). Result of the research also showed as revealed in (Table 1) the sensitivity profile of the bacterial flora to *V. amygdalina* extract, and (Figure 2) also showed percentage of zone of inhibition of the bacteria flora.

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Table 1: **Sensitivity Profile of Bacterial Flora to *V. amygdalina* Extract**

Bacteria Flora	Sensitivity Test at 1 mg / ml	Zone of inhibition (mm)
Escherichia coli	S	3.0
Staphylococcus aureus	S	0.8
Proteus mirabilis	Highly resistant	NI
Streptococcus faecalis	S	4.0
Pseudomonas aeruginosa	S	NI
Areomonas hydrophylla	R	N1
Klebsiella spp	Highly resistant	NI
Salmonella spp	R	3.0

R = Resistant; S = Sensitive, NI = No Inhibition (n =8)

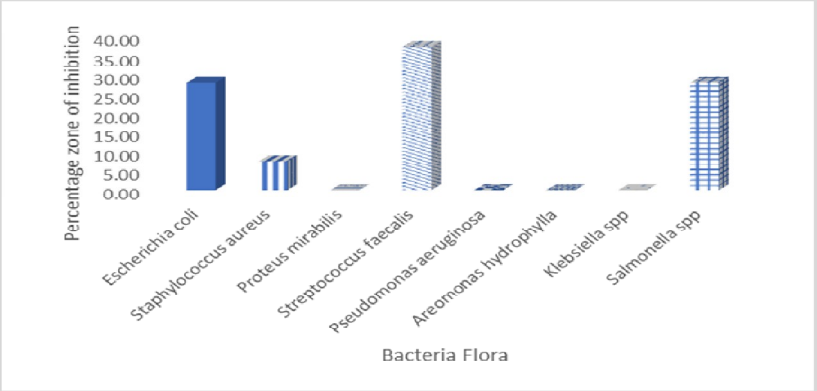

Figure 2: Percentage zone of inhibition of Bacteria flora exposed to aqueous *Vernonia amygdalina*

Discussion

Occurrence of bacterial in fish species revealed pathogenic and faecal contamination condition, hence, indicating polluted environment which can result in disease condition. This nonetheless is due to unabated use of the water body and human interference; and faecal bacteria subsequent to anthropogenic activities are crucial to aquatic health to prevent chronic status. The result obtained as indicated in (figure 1) above showed percentage load of bacteria isolates with feacal contaminants and indicated environmental pollution status, hence corroborated the work of (Templar, *et al*., 2016) which reported sewage contamination of urban waterways which is one of major environmental, hence, this is major health concern to public health officers.

The results from this research on *Vernonia amygdalina* (bitter leaf) extracts revealed that the plant extract has antibacterial effect on some of the organisms most especially aquatic organisms. Result of the research corroborated with findings of Ifeoluwa, *et*

al., (2018), which revealed that the leaves extract of *V. amygdalina* are effective against dysentery, gastrointestinal disorder and has some antimicrobial and antiparasitic activity. Also, Akortha and Nwachukwu (2009) reported antimicrobial inhibition effect of ethanol extracted of *V. amygdalina* on pathogenic bacterial strain of *Escherichia coli, Klebsiella species, Pseudomonas aeruginosa* and *Staphyloccolcus aureus*. Leafy vegetables are high moisture, low acid produce, which support the growth of a range of microorganisms, risk of contamination by bacteria that cause food poisoning, especially those for example *Staphyloccolcus aureus* and *E. coli* which produce heat stable toxins that may not be destroyed by heat treatment such as cooking (James and Kuipers, 2003). The plant extracts had profound activities against both Gram-positive and Gram- negative bacteria. There was however more activity against the Gram-negative organism than the Gram-positiveas discovered by (Evbuomwan *et al.*, 2018). More so, Mai-Prochnow *et al.*, (2016) suggested that the difference in susceptibility of Gram positive and Gram-negative bacteria to various antimicrobial agents probably depends on structural differences in their cell walls.

However, this result revealed that there was variation in the degree of antibacterial activities of the extracts, as not all the isolates show high sensitivity to the aqueous extract of the bitter leaf. This indicated that susceptibility of bacterial strains depends also on the extraction processes, either aqueous or ethanoic and this was in agreement with (Akortha and Nwachukwu, 2009) on different sensitivity level of bacterial strain to different extracts of *V. amygdalina.* Also, Lewis and Elvin-Lewis, (1995) reported that organic solvents (alcohols) are extensively used for crude extraction before other organic purification to obtain active compounds. Activity of ethanolic extracts enables more activity against bacterial isolates than water extracts. This may be due to the higher volatility of the ethanol which tends to extracts more active compounds from the samples than water, (Ibekwe *et al.*, 2000).

Conclusion

The use of *Vernonia amygdalina* plant extract as anti-bacteria agent against bacteria flora yielded more activities against Gram-negative organism than the Gram-positive. And it can serve as natural antibiotic agents and immune booster against disease (dysentery) caused by such susceptible bacteria.

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