Antibacterial activity of methanolic extracts of *Terminalia avicennioides* against fish pathogenic bacteria

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Abstract

Human infections caused by pathogenic microbes transmitted from fishes through food chain are on the increase. Treatment of bacterial fish infections by herbal medicines is gaining popularity worldwide. *Terminalia avicennioides* is one of these medicinal plants, therefore, the crude methanolic extracts of stem bark, root bark and leaves of this plant were assayed against fish pathogens viz: *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi* and *Escherichia coli* obtained from fish suffering from the type of diseases caused by these pathogens. The antibacterial analysis of crude methanolic extracts showed the significant activity against the microbes. The MIC of root crude extract on *B. subtilis,* was 0.1µg/ml and 10µg/ml for *P. aeruginosa, K. pneumonia* and *E. coli.* It was not active on *S. typhi*. The MIC of leaves crude extract was 1µg/ml on all the fish *microbes*. The crude methanolic stem bark extract showed activity on only *E. coli* with MIC of 10µg/ml. The important phytochemical constituents like steroids, tannins and saponins were detected in the plant parts tested. The phytochemical constituents detected in *T. avicennioides* may be responsible for the observed bacterial activity of the plant and hence, its potential use as medicinal herb.

Keywords: Antibacterial activity, fish pathogenic bacteria, phytochemical constituents, *Terminalia avicennioides*

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Introduction

The healing herbs varies among the natives in various localities, many published report has shown the effectiveness of medicinal herbs against bacteria and other micro organisms. Thus plants are one of the solid bedrock for modern medicine to attained new principles (Evans *et al*., 2002). Closely 50% of modern drugs used for the treatment of infections are of plant origin. Plants which serve as food for man and other benefits are composed of enormous biological components most of which has medicinal properties (Magbabeola and Akiwande, 2006). Presence of certain phytochemical components such as alkaloids, saponins, polyphenols, anthraquinones, cardiac glycosides are the bioactive bases for the medicinal properties. For instance, these substances are responsible for the antimicrobial activity exhibited by the medicinal characteristics in plants (Ebana *et al*., 1993). The secondary metabolites are chemical substances used by plants for defence system and serve as the bioactive principles for various drugs in modern chemotherapy (Cragg and Newman, 2005). Investigation for new bioactive components found in plants against some microbes led to the screening and analysis of bioactive components in *Terminalia species* (Combretaceae). The frequent use of this plant in healing of microbial infections in Africa and in Asia has led to its choice for studying. Most of the species from Combretaceae family have been showed to contain antibacterial activities (Baba Mousa *et al*., 1999). Screening of the plant *Terminalia avicennioides* exhibit significant (P>0.05) activity against *S. aureus* (Mann *et al*., 2008). Most of the crude extracts of this plant genus shown Minimum Inhibitory Concentration (MIC) data of 0.08 mg/ml and some had MIC values as low as 0.02mg/ml (Masoko *et al*., 2005). Fish has served as food for man since the pre-historic era and still constitute an essential component of the diet in the universe (Leisner *et al*., 1995). The insufficient availability of animal protein in Nigeria, coupling with increase in human population has increase the cost of animal protein to a state that is almost beyond the capability of low income earner (Ezeri *et al*., 2001). The commodity is readily susceptible to microbial attack particularly bacteria due to this essential attributes (Adams *et el*., 1999). Bacterial fish disease and infections are very common and are one of the most difficult health problems to deal with. The bacteria are transmitted by fish that have made contact with other diseased fish (Douglas, 2007). The bacteria get contact with the fish body through the gill or skin. It can also stay on the surface of the body (Douglas, 2007). Fish is the best and cheapest source of protein especially for those that take from hand to mouth. Research has shown that fishes get infected by some

pathogenic bacteria which are transmittable to human being via food chain (Douglas, 2007; Miceal *et al*., 2007). Microbial infection caused by pathogens is a global health challenge for both human and other vertebrates. Orthodox medicines are beyond the reach of majority of rural dwellers. They also have some undesirable side effects. New drugs are urgently required to combat these pathogens. Since, traditional medicine is believed to be safer, accessible, and cheaper and with limited side effects. Researchers have focused on searching for new antimicrobial agents from plant origin. *Terminalia avicennioides* has been used traditionally in Nigeria and some West African countries for healing of diseases like wound, respiratory tract infections, trypanosomiasis, diarrhoea, dental caries, and skin infection etc; and its effectiveness against human bacterial infections has been established. Fish is consumed by most Nigerians. Since its infections cannot be overemphasized particularly those growth cases of fish related bacteria; it is therefore necessary to disinfect such fish so as to avoid the transfer of disease to humans via food chain. The antibacterial potentials of *Terminalia avicennioides* on human infection such as wounds, gastro-intestinal disorder and syphilis (Lewis and Elvin, 1977; Abdullahi *et al*., 2001); however, its antimicrobial activity on fish pathogenic bacteria has not been determined. The aim of this research is to screen the leaves, stem bark and root bark extracts of the *Terminalia avicennioides* for its antimicrobial activity against some fish pathogenic bacteria.

Materials and Methods

Plant Materials

The plant parts of *Terminalia avicennioides* such as stem bark, leaves and root bark were collected from farm land of Girls Day Secondary School, Kuta, Shiroro Local Government, Niger State. This plant was identified and authenticated by Mallam Ibrahim Muazzami of the Department of Medicinal Plant Research and Traditional Medicine of National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Abuja, Nigeria where a voucher specimen (NIPRDH 5735) was deposited at the herbarium unit of this same institution.

Preparation of plant materials

The plant parts were washed with portable water to removed sand and reduced other impurities. The parts were air dried at room temperature for about four weeks in order to reduce the moisture content in them. The plant parts were then pounded into powder form by means of sterile mortar and pestle which was carefully saved in containers for further use.

Preparation of plant extracts

500g each of plant parts powder were soaked in 70% of methanol for 72h. After extraction each solution was filter using whatman No1 filter paper. The filtrate was concentrated in a vacuum at 30ºC and was store at until when required.

Test organisms

Fresh chemical isolates of *Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis, Klebsiella preumonea* and *E. coli* isolated from fish suffering from these infections were collected from Microbiology Laboratory, Federal University of Technology Minna.

Bioassay

The antibacterial test was carried out using the agar diffusion method of Nair and Chanta (2005). The test organisms were inoculated on nutrient agar plates and were spread uniformly with the aid of sterile glass spreader. Wells of 4mm diameter was made on nutrient agar by the use of forceps sterilized by flaming. To each well 40mg/ml concentration of crude extracts of leaves, roots and stem bark were introduced. Control experiment by the use of Ampiclox was introduced into the well. The plates were inoculated at 38ºC for 24h. The inhibitory zones were recorded.

Determination of the Minimum Inhibitory Concentration (MIC) of the crude extracts

The serial dilution method was employed: 9ml of nutrient broth was dispensed into several numbers of test tubes. These were sterilized at 121ºC for 30 minutes. These were allowed to cool

to room temperature and were labeled 1 – 5. 1ml from the re-constituted extracts was introduced into the test tubes. 1ml of the standardized inoculums was introduced into the broth. The tubes were incubated for 24h and turbidity was observed. The test tubes with no visible growth was taken and recorded as MIC.

Determination of MBC

The tube with no visible growth was sub-cultured into a freshly prepared nutrient broth and incubated. The plates with visible growth were recorded as the (MBC).

Phytochemical screening

The phytochemical analysis of the plant crude extracts was performed using the methods explained by Sofowara (1982) and Trease and Evans (1983).

Thin layer chromatography of crude methanolic Extracts of *Terminalia avicennioides* stem barks, leaves and root barks extracts

Thin layer plates were used to carry out thin layer chromatography. A pencil lines was drawn near the bottom of each plate labeled to indicate the original position of the dropped methanolic crude extracts. Small quantity of the crude extracts was dissolved in methanol solvent. Concentrated light spot was made on the plate by means of capillary tube at a point of about 1cm from the base of the TLC plate. The plate was allowed to stand for some time to allow evaporation of the solvent. The TLC plate was then placed in developing tank chamber which contained a solvent or a mixture of solvents. The plate was positioned in such a way that the top of the slide rest against the wall of the tank, the developing tank was covered and left for some time so as to enable the mobile phase to the solvent front when the solvent front were advanced to the top pencil line, the slide were removed from the developing tank, the plates were then placed on a clean dry surface to dry proper. The TLC plates were then placed in an iodine tank for a given period of time to give a clear visible spots and then a boundary was drawn around

each visible spots using a pencil. The iodine vapour in the chamber oxidizes the substances in various spots to make the spots more clearly visible to the eyes. The results were then drawn and recorded.

Results and Discussion

Results

The table below show the results of phytochemical test carried out on the crude extracts of *Terminalia avicennioides*.

Table 1: Phytochemical analysis of the crude extracts of *Terminalia avicennioides*

Secondary metabolites	Test	Observation	Root extract	Stem extract	Leaf extract
Saponins	Frothing test	Persistent frothing	+++	+++	+++
Alkaloid	Mayers reagent, Wagners reagent	Orange-red ppt White buff ppt	–	–	–
Steroid	Salkowkii test	Reddish brown ring at interface	+++	+	+++
Tannins	Ferric chloride	Blue-Black or green ppt	+++	+++	+++
Glycosides	Fehling test	Orange-red	–	–	–
Phlobatannin	Filtrate + HCl	Red ppt	–	–	–
Flavonoid	Ferric chloride	Yellow ppt	–	–	–
Anthraquinone	Borntrager's test	Pink, red or violet colour	–	–	–

Key: + = Detected, – = Not detected

Table 2: Thin layer chromatography results of crude extracts of *T. avicennioides* stem barks, leaves and root barks

Crude extracts	Solvent system	Number of spots	RF	Sun light	Iodine chamber
Leaves crude extract	Pet ether/Ethyl acetate 1:1	2	0.89 0.59	Colorless Colorless	Golden brown Golden brown
	Pet ether/Ethyl acetate l 3:2	1	0.79	Colorless	Golden brown
	N-hexane/Ethyl acetate 3:2	2	0.36 0.36	Colorless Colorless	Golden brown Golden brown
	Methanol/chloroform/N-hexane1:1:1	1	0.68	Colorless	Golden brown
Stem crude extract	Pet ether/Ethyl acetate 1:1	1	0.59	Colorless	Golden brown
	Pet ether/Ethyl acetate 3:2	1	0.79	Colorless	Golden brown
	N-hexane/Ethyl acetate 3:2	2	0.38 0.38	Colorless Colorless	Golden brown Golden brown
	Methanol 100%	2	0.90 0.58	Colorless Colorless	Golden brown Golden brown
Root crude extract	Pet ether/Ethyl acetate3:2	1	0.79	Colorless	Golden brown
	N-hexane 3:2	1	0.38	Colorless	Golden brown
	Methanol100%	1	0.90	Colorless	Golden brown

Table 3: Antibacterial activity of crude methanol extracts (zone of diameter of inhibition measured in mm) (concentration of 40mg/ml)

Isolates	**Stem bark extract**	**Root bark extract**	**Leaves extract**	**Control**
Bacillus subtilis	–	18	13	20
Pseudomonas aeruginosa	–	19	18	30
Klebsiella pneumonia	–	18	17	17
Salmonella typhi	–	–	19	22
Escherichia coli	17	12	18	14
	Key: – = no zone of inhibition			

Table 4: Minimum Inhibitory Concentration (MIC) of crude methanol extracts (zone of diameter of inhibition measured in mm) (concentration of 40mg/ml)

Isolates	Stem bark extract	Root bark extract	Leaves extract	Control
Bacillus subtilis	_	0.1	1	0.01
Pseudomonas aeruginosa	_	10	1	0.1
Klebsiella pneumonia	_	10	1	0.01
Salmonella typhi	_	_	1	0.01
Escherichia coli	10	10	1	0.1
	Key: ⁻ = no zone of inhibition			

Table 5: Minimum Bactericidal Concentration (MBC) of crude methanol extracts (zone of diameter of inhibition measured in mm) (concentration of 40mg/ml)

Isolates	Stem bark extract	Root bark extract	Leaves extract	Control
Bacillus subtilis	_	1	1	0.01
Pseudomonas aeruginosa	_	10	10	0.1
Klebsiella pneumonia	_	10	1	0.1
Salmonella typhi	_	_	1	0.1
Escherichia coli	10	10	10	1
	Key: ⁻ = no zone of inhibition			

Discussion

The phytochemical screening of crude methanolic leaves, root bark and stem bark extracts of *Terminalia avicennioides* indicates the presence of tannins, saponins and steroids (table 1) as active components (Mann *et al* ., 2008), although alkaloid was not detected as reported by Mann *et al*. (2008). These active principles are responsible for their effectiveness against many microbes. These metabolites enable the plant parts to function as herbs or drugs by producing biological activity in animals and in humans. The presence of these secondary metabolites may be responsible for their wide use as traditional medicine in different part of West Africa including Kuta and its environs which agreed with report given by Ebana *et al*. (1993). The results of the susceptibility test of the crude extracts on some selected organisms (Tables 5, 6, and 7) showed that the crude methanolic leaves extract was active against all the fish microbes tested such as *B. subtilis, P. aeruginosa, K. pneumonia, S. typhi and E. coli*. The crude methanolic root bark extract was active on four pathogens but not active against *S. typhi*. The crude methanolic stem bark extract was only active on *E. coli*. From table 1, it can be observed that the steroids level in the crude methanolic stem bark extract was less when compared to the root bark and leaves extracts, this may be responsible for the ineffectiveness of the crude methanolic stem bark extract against *B. subtilis, P. aeruginosa, K. pneumonia* and *S. typhi*. It can also be observed from (Table 5) that Ampiclox which serve as control provides wider range of inhibition than the crude extracts against *B. subtilis, P. aeruginosa and S. typhi* but lesser zone of inhibition in the stem bark and leaves crude extract on *E. coli*. The minimum inhibitory concentration (MIC) of root bark extracts on *B. subtilis* was 0.1µg/ml; the remaining microbes such as *P. aeruginosa, S. typhi* and *E. coli* were 10µg/ml each. The MIC for leaves extract on the microbes was found to be 1µg/ml each. In control the MIC was 0.01µg/ml on *B. subtilis, K.*

pneumonia and *S. typhi* 0.1µg/ml in *P. aeruginosa* and *E. coli*. The crude methanolic stem bark extract was only active against *E. coli* with MIC of 10µg.ml (Table 6). The results of Minimum Bactericidal Concentration of crude methanolic extracts are summarized on the (Table 7) above.

The results of the various tests carried out in this investigation clearly showed the antibacterial potential of the crude methanolic extract of the leaves on *B. subtilis*, *P. aeruginosa*, *K. pneumonia*, *E. coli* and *S. typhi*. The crude methanolic root bark extract also demonstrated the antibacterial potential of the aforementioned pathogens except *S. typhi*. The crude methanolic stem bark extract exhibited only antibacterial potential on *E. coli*, which agreed with the report of Akinyemi *et al*. (2003).

The research has shown that the leaves and root bark extracts of T. avicennioides possess significant in vitro antibacterial activities against some of the bacteria implicated in the pathogenesis of fish infections. Such as Ulcerative syndrome, bacteria haemorrhagic, septicaemia, tail and fin rod, bacterial gill rot and dropsy caused by *P. aeruginosa*. The growth of *P. aeruginosa* was greatly inhibited by the two extracts with zone of inhibition of 18mm and 19mm respectively. The growth of this fish pathogen has also been inhibited by Ethanolic extracts *H. suaveolens* with zone of inhibition of 21mm (Renisheya *et al;* 2012). Also ethyl acetate extract of *C. linum* with zone of inhibition of 20mm (Vijayakumar, 2012). K. pneumonia incriminated as causative agent of lesions over body surface along with a typical mucosal polyp in the bucal cavity. Showing clinical signs of exophthalmia, skin discolouration with deep ulcers, frayed fins, damage gills, distended abdomen with calcified gonads and hemorrhages was susceptible to leaves and root bark extracts of *T. avicennioides* with zone of inhibition of 17mm and 18mm respectively. *K. pneumonia* has also been inhibited significantly with ethanolic extract of *H. suaveolens* with zone of inhibition of 17mm (Renisheya *et al; 2012*). The methanolic

medicinal plant couroupita guianensis inhibited the growth of *K. pneumonia* (NCIM2019 (Ramalakshmi, 2013). The growth of *Salmonella typhii* which is causative agent of *Salmonellosis* in fish, blisters and sores and eat away the fins and tails of multiple fish species was only inhibited by leaves extract of T. avicennioides. The activity of *salmonella typhii* has also been inhibited ny methanolic extract of *C. guianensis* (NCIM2019) (Ramalakshmi, 2013).

The crude extracts of leaves, root bark and stem bark of *T. avicennioides* exhibited significant activity on *E. coli* which is incriminated as causative agents of food borne diseases and severe sores on fish when passed unto human being via food causes diarrhea by releasing toxins called enterotoxigenic *E. coli* the zone of inhibitions are 18mm, 12mm and 17mm respectively. The fish pathogen has been acted by methanol extract of *P. odorata* with zone of inhibition of 8.0mm (Najia *et al;* 2011). Leaves extract and root bark extract of *T. avicennioides* has demonstrated significant activity on *B. subtilis* with zone of inhibition of 13mm and 18mm respectively. The growth of this pathogen has also been inhibited by the ethanolic extract of walnut leaves with zone of inhibition close to leaves methanolic extract of *T. avicennioides* i.e 12mm (Olusola *et al*; 2013). Also the chloroform extract of *E. agallocha* leaf showed maximum activity on *B. subtilis* with inhibitions zone of 10mm (Ravikumar, 2010). Although most studies revealed that *B. subtilis* has no pathogenic effect i.e does not cause diseases in fish. It has rather been reported that dietary *B. subtilis* cause an increase in the growth of tilapia *oreochromis niloticus* (Gunther *et al;* 2004). Furthermore, the addition of *Bacillus spp.* To the rearing water can increase survival and net production of channel cat fish, improving water quality (Queiroz *et al;* 1998). But if fish that has been infected with *B. subtilis* is taken by man may cause diarrhea, a type of hypersensitivity pneumonitis.

In conclusion, the result obtained from the phytochemical analysis revealed the presence of some secondary metabolites in the leaves, stem bark and root bark extracts of *Terminalia avicennioides*. The crude extracts exhibited some level of biological activity against fish pathogens such as *B. subtilis, S. typhi, K. pneumonia, E. coli* and *P. aeruginosa*. The susceptibility of these fish microbes to the extracts of this plant may be pointer to their potentials to formulate new and more potent antibacterial drugs of natural origin that can be used against these fish pathogens. The result also authenticates it use in herbal medicine. Based on the results obtained from the analysis carried out on the crude methanol extracts of *T. avicennioides*, it is recommended that further studies should be done using higher concentration or dosage of the extracts to test for bacterial activity because the higher dosage will give room for wider activity. The acute toxicity and animal tests should also be carried out.

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