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Original Article

Invitro antibacterial study on *Irvingia Gabonensis* (bush mango) against *Escherichia coli*

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ABSTRACT

Bacterial resistance to antibacterial drugs in the treatment of some bacterial infections has become a serious threat, consequently causing untold hardship to patients and a challenge to healthcare practitioners. The antibacterial activity of cold, hot water and ethanolic extract of stem bark of *Irvingia gabonensis* against *Escherichia coli* was evaluated using Agar-well and Disc diffusion method. Tetracycline was used as positive control while distilled water and ethanol as negative controls. The organism was susceptible to ethanol extract with the diameter zone of inhibition range between 15mm-25mm in well-in-agar method and 10mm-15mm in paper disc diffusion method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was 200mg/ml respectively. Saponins, flavonoids, tannins, cardiac glycosides, anthraquinones, phylobatanins and alkaloids are the phytochemical elements detected from the *I.gabonensis* bark stem extract. This study suggests that the bark extract of *I.gabonensis* has advantageous antibacterial properties against *E.coli*. Further exploration of these plant substances will possibly unveil its potential use for medication of ailments caused by the test organism.

KEYWORDS: Invitro Antibacterial, *Irvingia gabonensis*, *E.coli*

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INTRODUCTION

The search for a better and lasting treatment for most human diseases including microbial and parasitic infections has remained an active and interesting exercise since human existence. There seem to be no answer to this scourge with the emergence and reemergence of cases of resistant strains of pathogens [1]. However, the development of safe, effective and affordable drugs for treatment and prevention of major health problems in developing countries is mostly a neglected challenge partly due to limited basic knowledge and lack of adequate economic return on investment in pharmaceutical firms [2]. The rise of antibiotic resistant microorganism is one of the excessive issues in health care systems of the world, and infectious disease is the second maximum critical cause of death worldwide [3, 4]. In step with the World Health Organization, plants are sources of new compounds that have the potential to fight bacterial activity [3, 5]. Therefore, new drugs ought to be observed with a view to fight such diseases, and it is far important to discover new compounds which have antibacterial properties. Thus, there is a heavy reliance on traditional medicine in Nigeria and other developing countries. Tradition of many countries in the world originated from the age long links with nature and natural products from plant materials. These days, people who live near the forest use plant products to treat chronic illnesses. Concerning the above certainty, it's far profitable to display screened plant species that have the above attributes to synthesize new drugs [3].

Curiously, African mango (*Irvingia gabonensis*) bark stem extracts have documented inhibitory action against numerous bacteria and fungi [6, 7]. In Nigeria, extracts particularly from the bark are used to treat hernia and yellow fever and as an antidote for poisoning [8]. Kernels of *I.gabonensis* are used to treat diabetes. Preparations from the bark are rubbed on the body to alleviate pains and are applied to sores and wounds to aid healing and to alleviate toothache. Extract from stem bark is also used to treat diarrhoea [8]. Potential mechanism of action is membrane disruption by terpenoids and inactivation of microbial adhesion, enzymes and mobile envelope carrying proteins by means of ellagic acid-like compounds [6].

Escherichia coli is a gram-negative facultative anaerobic rod-shaped bacterium generally discovered within the intestine of heat-blooded organisms (endotherms) [8]. Most *Escherichia* strains are harmless, but a few serotypes can initiate extreme food poisoning in humans and are very often responsible for product recalls because of food infection [9]. The harmless strains are part of the normal flora of the intestine and can be of benefit by producing vitamin k and preventing pathogenic bacterial colonization of the intestine [10, 11, 12]. On the contrary, *E.coli* causes acute gastroenteritis mostly in infants but also in adults [13].This study aims to analyze the antibacterial property of extract from the stem bark of *I.gabonensis* against pathogenic *E.coli* implicated in diarrhoeal infection. In addition, the minimum inhibitory Concentration (MIC) and the minimum bactericidal concentration (MBC) were obtained.

MATERIALS AND METHODS

Collection of Plant substances and Processing: The stem bark of plant *I. gabonensis* (Bush mango tree) was collected from Umunahu village of Owerri North Local government location of Imo state, Nigeria. The plant substance was identified by Dr. Mbagwu F.N of Plant Taxonomies, Department of plant science and Biotechnology, Imo State University, Nigeria. The plant sample was transported to Microbiology Laboratory Unit for processing. At arrival the stem bark was washed with distilled water and air dried to reduce the bacterial load. This was preserved until regular weights were acquired and ground into high-quality powder and stored for further use.

Preparation of Extracts concentrations: Within reach, 7.5g of powdered *I. gabonensis* bark stems was extracted using 10ml of ethanol, hot water and cold water solvents respectively to obtain a concentration of 100mg/mil.

Qualitative Determination of Phytochemical components of Bark Extract of *Irvingia gabonensis*: The following phytochemicals was qualitatively determined in bark extract of *Irvingia gabonensis* namely: Tannin [10], alkaloids [11], saponin [12], and phlobatannins [14]. Anthraquinones [18]. Flavonoid and cardiac glycoside.

Purification and Re-identification of the Bacterial Isolates: The bacterial isolates (*E.coli*) was sub-cultured on nutrient agar plate and incubated at 37°C for 24 hours. The test isolate organism was re-identified with the use of gram staining and traditional biochemical test which included catalase test and Coagulase test.

Gram Staining: Gram staining is used to recognize and identify of gram positive bacteria and gram negative bacteria. A thin smear of isolate was made on a clean glass slide and allowed to air dry, then the slide was passed through a Bunsen flame for 3 times to heat fix. The slide was

flooded with crystal violent and it was allowed to stay for 30-60seconds. Thereafter it was washed off with distilled water, then the slide was flooded with Lugol's iodine and was allowed to stay for 30 seconds-1minutes, it was washed off with distilled water. Thereafter the slide was flooded with methanol (alcohol) and was washed away immediately with water then slide was flooded with safranin and was allowed to stay for 1-2 minutes and it was allowed to air dry for 10minutes. The dried slide was placed under microscope and was viewed at X100 (oil immersion) objective

Biochemical test: The subsequent biochemical test was achieved, Catalase test and Coagulase test [15], and Indole test.

Preparation of 0.5 McFarland standards: One percent chemically natural sulphuric acid was prepared with the aid of including 1mlof concentrated sulphuric acid on 99ml of distilled water in conical flask. One percent of barium chloride solution became organized in a different test-tube by means of including 0.5g of dehydrated Barium chloride to 50ml of distilled water. Then slowly, with steady agitation, 0.6ml of Barium chloride solution changed into added to 99.4ml sulphuric acid [15]

Starter Culture: This was mainly broth culture prepared from stock culture by inoculating the test organisms, 1ml into 9ml nutrient broth. The tube was then incubated at 37ºC for 18-24 hours before use.

Maintenance of Stock culture: This was aimed to maintain the viability of the organism. Slants were used for this purpose. Here, the organism was cultured on nutrient agar Slants, incubated at 37ºC for 24hrs and stored in the refrigerator.

Antibacterial Susceptibility Test: Well in Agar Method was carried out by preparing a molten agar solution and was allowed to cool, 0.2ml of 10^{-2} diluted cultures of organism was added and thoroughly mixed. The seeded agar was poured into a sterile Petri dish and allowed to set. Using a sterile cork borer with diameter of 6mm, appropriate number of wells were made in the seeded plates. Wells made were inoculated with different concentrations 200mg/ml, 100mg/l, 50mg/ml, 25mg/ml and 12.5mg/ml) concentrations of extracts preparations. The plates were allowed to stand at room temperature for 1 hour and thereafter, incubated at 37ºC for 24 hours. Diameter of zones of inhibition was measured. The solvents/diluents and antibiotic (Tetracycline 250mg/ml) was tested also as controls. Broth subculture standardized in line with National Committee for Clinic Laboratory general (NCCLS, 2002) by means of step by step including normal saline to evaluate its turbidity to McFarland popular of 0.5 that's approximately 1.0x10cfu/ml. The floor of the molten agar was allowed to dry and sterile cork borers were used to bore six holes of approximately 2.5cm equal length at the surface 0.1ml of the extract at distinct concentrations of 64p.cw/v, 56.0percent htw/v, 16.3 percent ethanol/v, and a hundred percent w/v became dropped into every hole and the plate became kept for about 1hour at room temperature and incubated at 37°C for 24hours.

Using disc diffusion method, Mackonkey bottle of Total viable count. Agar was melted and allowed to cool to 45-50ºC. 0.2ml of 10^{-2} diluted culture of the test organism (*E.coli*) was added to each melted agar and mixed thoroughly. The inoculated agar was poured into a sterile Petri dish and allowed to set firmly. With aid of a forceps, the disc contain the extracts concentrations were transferred onto the surface of agar such that all parts of the disc were then incubated at 37ºC for 24 hours after which they were observed for zones of inhibition. Diameter of zone of inhibition was measured nearest millimetre (mm). The experiment became repeated three instances and the mean diameter was obtained. Tetracycline (250mg/l) became tested as controls.

Minimum Inhibitory Concentration (MIC): This was decided with the aid of Agar dilution approach .One gram of the extract was dissolved in 10ml of diluents as incase of susceptibility test. Five (5ml) of extract solution of double strength medium Mueller-Hinton agar mixed at 45°C. Five (5ml) of mixture was transferred to a second tube containing 5ml of strength nutrient broth. This was mixed by a vortex device; this procedure was repeated to give five different concentrations. The last tube in the series contained no antibacterial agent and is a control for the viability of the test organism. Concentrations was poured aseptically into sterile Petri-dishes and dried at 37° for 1hour with the lid moderate raised. Standardized test microorganism (10cfu/ml) had been aseptically inoculated at the surface of the media/agar for every concentration of the test plant extract [16], incubated at 37°C for 24 hours The tubes were observed for turbidity or growth of the organism. The least concentration was taken as the Minimal inhibitory concentration.

Minimum Bactericidal Concentration (MBC): This was determined by transferring incubated organism from the concentration that showed no visible growth from the M.I.C determination into a sterile nutrient agar. These were incubated at 37°C for 72 hours. Absence of bacterial growth was detected. The least concentration without any

bacterial growth on the agar surface was recorded as the Minimal bactericidal concentration.

Qualitative assessment of phytochemical elements in *Irvingia gabonensis*: The phytochemical findings of bark stem extracts revealed the presence of cardiac glycosides, flavonoid and anthraquinones, alkaloid, tannins, phlobatan and Saponins. Phenols flavonoid, cardiac glycoside contents were done by Alkaline precipitation gravimetric methods [17]. The concentration of phenols was determined using the Folin-Ciocaltean Spectrophotometer [8]. Tannins content was determined by Folin Denis colormetric method of Kirk and Sawyer.

RESULTS

Table 1: Identification of Bacterial Isolate

Biochemical Test	Observation
Gram reaction	Gram negative
Catalase	Negative
Oxidase	Negative
Indole	Positive

Table 2: Characteristics/Percentage (%) yield of the Plant Extract

Plant Part	Solvent	Weight of Extract	Percentage Yield	Colour of Extract
***Irvingia gabonensis* Stem Bark**	**Cold water**	**29.9**	**64.4**	**Pale Yellow**
	Hot water	**26.0**	**56.5**	**Pale Yellow**
	Ethanol	**7.5**	**16.3**	**Dark Yellow**

Table 3.1: Antibacterial Susceptibility Test of *I.gabonensis* Stem Bark against *E.coli* Well-In-Agar Method- Diameter zone of Inhibition (mm)

Conc. (mg/ml)	Cold Water	Hot Water	Ethanol
200	**-**	**-**	**25**
100	**-**	**-**	**23**
50	**-**	**-**	**21**
25	**-**	**-**	**21**
12.5	**-**	**-**	**15**

NB: - =No Antibacterial Activity. Diameter of well = 6m

Table 3.2: Antibacterial Susceptibility Test of *I.gabonensis* Stem Bark against *E.coli* Paper Disc Method- Diameter Zone of Inhibition (mm)

Conc. (mg/ml)	Cold Water	Hot Water	Ethanol
200	**-**	**-**	**15**
100	**-**	**-**	**12**
50	**-**	**-**	**10**
25	**-**	**-**	**-**
12.5	**-**	**-**	**-**

N.B = No Antibacterial Activity. Diameter of well = 6mm
Control
Tetracycline (250mg/ml) = 27MM
Ethanol = -
Distilled Water = -

Table 4: Antibacterial Susceptibility Test of *I.gabonensis* Stem Bark against *E.coli* Tube Dilution Test (Determination of Minimum Inhibitory Concentration) Inhibitory Concentration)

Conc. (mg/ml)	Ethanol
12.5	**+**
25	**+**
50	**+**
100	**+**
200	**-**

N.B. + = Growth Observed (not turbid)
-= Growth observed (not turbid)
-

Minimum Bactericidal Concentration (MBC). Ethanol Extract = 200mg/ml.

Table 4: Phytochemical Screenings of Ethanolic Concentration of *I.gabonensis* Stem Bark

Phytochemical	Extraction content
Cardiac glycosides	++
Flavonoids	+++
Anthraquinones	++
Alkaloids	+++
Tannin	++
Phlobatannins	++
Saponins	+
Phenols	+++

+ = Detected amount present
++ = moderately present
+++ = noticeably present

DISCUSSION

The antibacterial activity of ethanol, cold water and hot water extracts of stem bark *Irvingia gabonensis* against *Escherichia coli*, an organism implicated in diarrhoeal infections was investigated. Ethanol, cold water and hot water were used as extract solvents. Cold water extract produced the most yields (64.4%) followed by hot water extract (56.5%) and the ethanol extract (16.3%).This may be as a result of the fact that active components of the stem bark are more soluble in organic solvents than polar solvents [21].

In this study, the antibacterial susceptibility screening tests using well-in agar method showed that cold water and hot water extracts of *I.gabonensis* stem bark had no antibacterial activity at the test concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml respectively. Only the ethanol extract demonstrated significant antibacterial activity against *E.coli* with a zone of inhibition in the range of 15-25mm in the well-in agar method. Paper disc diffusion method showed that both cold and hot water extracts of the plant sample were both inactive against the test organism as in the case with the well-in agar method. However, the ethanol stem bark extract showed slight activity for 200mg/ml, 100mg/ml and 50mg/ml concentrations respectively. The antibiotic, tetracycline (250mg/ml) as a positive control had a zone of inhibition of 27mm while distilled water and ethanol as negative controls had no zone of inhibition. The cold water and hot water extracts of the plant sample were both inactive against *E.coli* in the paper disc diffusion method as well as the well-in agar method. This could be due to the fact that the active compounds in the extract being insoluble in polar solvent [21] present in smaller concentration or does not exist in both the hot and cold water extracts.

The antibacterial activity recorded in this study agrees with previous report by Kuete *et al*. (2007) who stated that fractions and compounds isolated from the stem bark of *I.gabonensis* prevented the growth of 6 gram positive bacteria and 13 species of Gram negative bacteria including *E.coli* [6]. This study is also in line with that by Barry and Thomsberry. (1985) who reported the pharmacological activity of 2, 3, 8- tri-0-methyl ellagic acid isolated from the stem bark of *I.gabonensis* against Gram positive and Gram negative bacteria including *E.coli* [23]. However, Fadare and Ajaiyeoba (2008) stated that crude methanol extract of the stem bark of *I.gabonensis*

was inactive against *E.coli* and other organisms tested [24]. In their study, methanol was used as extract solvent and it might be that the active compounds in the extract are also insoluble in methanol.

The phytochemical screenings of *I.gabonensis* stem bark extract showed the presence of saponin, flavonoids, tannins, cardiac glycoside, anthraquinones, phlobatannins alkaloids and phenol [25].Therapeutic packages of stem bark of *I. gabonensis* have been extensively suggested in various studies [26, 27, 28, 29], while medicinal use of stem bark of *I. gabonensis* is presumably as a result of the presence of alkaloids and the synergistic effect of other bioactive agents that are available [30]. It has been reported that tannin can hasten up recovery of wound in an inflamed membrane; this may be due to its antiseptic nature in reducing bacterial infection in wounds [31]. In a previous study, saponin was mentioned with notable pharmaceutical importance due to its affinity to compounds like sex hormone, diuretic, calciferol (Vitamin D), steroids and cardiac glycoside [32].

CONCLUSION

The stem bark of *Irvingia gabonensis* (bush mango) contains chemical compounds which pose antibacterial activity against *E.coli*, the causative agent of diarrhoea, deadly dehydration, urinary tract infection and bladder infection. The antibacterial activity demonstrated by this plant therefore justifies its traditional medical use in the treatment of diarrhoea. Toxicological studies of this plant can be formulated into a useful drug for the treatment of *E.coli* infections.The prospect of finding a new antibacterial agent in the stem bark of *Irvingia gabonensis* is therefore apparent.

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