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ANTIBACTERIAL, PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF THE LEAF AND ROOT BARK EXTRACT OF *BAPHIA NITIDA* ON BACTERIA ASSOCIATED WITH WOUND AND ENTERIC INFECTIONS.

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ABSTRACT

The antibacterial, phytochemical and antioxidant properties of the leave and root bark extract of *Baphia nitida* on bacteria associated with wound and enteric infections was screened. The test organisms were *Escherichia coli, Staphylococcus aureus* and *Salmonella enterica*. The extracts were screened for their phytochemical composition using standard method and antioxidant properties was done using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay. The extracts were prepared with absolute methanol and distilled water using cold maceration method. The antibacterial susceptibility test was carried out using the disc diffusion method with Muller Hinton agar. *Salmonella enterica* showed a high susceptibility to the leaves extracts but was resistant to the root extracts. *Escherichia coli* and *S. aureus* showed resistance to both the leaves and root extracts. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were carried out using the tube test method with nutrient broth. The minimum inhibitory concentration and minimum bactericidal concentration of the methanolic extract of the leaves on *S. enterica* was shown to be 12.5 mg/ml and 50 mg/ml respectively while the water extract was seen to be 25 mg/ml and 100 mg/ml respectively. The Phytochemical analysis showed that saponins, glycosides, flavonoids, sterols, terpenes,

alkaloids and tannins were present. The aqueous extract of the root bark had the highest concentration of saponins while the methanol extract of the leave had the least concentration of saponins. The extracts produced a concentration dependent increase in antioxidant activity, at 400 µg/ml concentration, the methanolic extact *Baphia nitida* leave produced the highest mean percentage antioxidant activity of 51.26 while the aqueous extract of Baphia nitida root had the least activity of 11.92 compared to ascorbic acid. It is therefore recommended that the leave extract of *Baphia nitida* could be used as an alternative for the treatment of *Salmonella enterica* related infection.

KEYWORDS: Antibacterial, *Baphia nitida*, antioxidant, phytochemical, extract, wound.

INTRODUCTION

Medicinal plants have been used throughout human history for the treatment of disease conditions Plants have the ability to synthesize a wide variety of chemical compounds that perform important biological functions and defend against attack by predators such as insects, fungi, and herbivorous mammals as well as treating infections caused by bacteria.[1]

There is an increasing demand for medicinal plants and plant products as alternative to orthodox medicines especially in developing countries.[2] The need therefore for more potent, safe and affordable drugs have led to intensified research into herbal drugs, the result of which is the introduction of new herbal preparation for therapeutic uses.[3]

Baphia nitida is practically glabrous (leaf and branchlets). It is a tropical plant ubiquitous to the tropical rainforest. It is a shrub up to 9 m tall with glabrous to dense pubescent branchlets. The leaves alternate with petiole 1 – 4 cm long and prominently thickened at base and at top. The flowers are auxiliary fascicles and white with a yellow center. The fruit is a compressed pod and pointed at both ends. The seeds are brown 1 – 1.5 cm in diameter and circular in outline.[4] The name of *Baphia nitida* is camwood and among the Igbo, Yoruba and Efik of Nigeria is known as Aboshi, Irosun and Ubara respectively.[5] The leaves are used as fodder and in southern Ghana; it is propagated in livestock rearing areas because of its good palatability and high protein content. In Nigeria, the seeds are eaten by Igbo people and the twigs are used as chewing stick.[4]

The prevalence of pathogenic microorganisms that are resistant to modern antibiotics has been predominant in the past years.[6] Diseases and their causative agents which were

controlled by antibiotics before now are becoming resistant and this has led to the search of new sources of antibiotics which is of immense concern to medical practitioners.[7] Several researchers have considered the importance of medicinal plants as reservoir of phytomedicine, as these plants contain substances that can be used for therapeutic purposes. Their advantages over orthodox medicine are: environmentally friendly, easily available, cheap, safer, curative and have antimicrobial properties.[8] Traditional medicine has remained the most affordable and easily accessible source of treatment in the health care system of certain communities while in some communities local therapy is the only means of medical treatment.[9]

Plants have therefore been used in traditional medicine for years and more than 80% of the world populace still depends on traditional medicine for their health care needs.[10] Most of the prescribed medicines in developed countries are derived from plants. Plants known for treating ailments are thereby screened for their antimicrobial properties based on their folkloric uses. Antibiotic specificity is used to determine the efficacy of these plants for use as antibiotics. The most basic laboratory measurement of the activity of an antimicrobial agent against an organism is the Minimum Inhibitory Concentration (MIC) and the lowest concentration of a specific antimicrobial agent that kills 99.9% of cells of a given strain of bacteria being tested as the Minimum Bactericidal Concentration (MBC) are some of the procedures used to screen these plants.[11]

The Minimum Inhibitory Concentration (MIC) is a quantitative test which determines the lowest concentration of a specific antimicrobial agent needed to prevent the growth of a specific microorganism in vitro. It is determined by examining the test organism's ability to grow in broth cultures containing different concentrations of the antimicrobial agent. The Minimum Bactericidal concentration (MBC) is determined by assaying for organisms in those tubes for the MIC test that showed no growth. Thus an antimicrobial agent can be bacteriostatic for one organism and bactericidal for another.[12] These antimicrobial agents with known MIC and MBC are the drug of choice in treating infections and diseases. Under laboratory conditions, efficacy testing of antimicrobial agent depends on the type of organism and its source.

In Nigeria, the local use of natural products as source of treatment cannot be overlooked due to the large number of the country's population and the inadequacy of our health care system. The country is equally very rich in medicinal plants. A large number of these medicinal

plants are used in treatment and cure of diseases caused by microorganisms.[13] Investigation of folk medicine has therefore resulted in the discovery of the potential applications of medicinal plants like *Baphia nitida.*

The study is aimed at determining the antibacterial, antioxidant and phytochemical activities of *Baphia nitida* plant extracts on bacteria associated with wound and enteric infections.

MATERIALS AND METHODS

Source of Plant and Test Organisms

Samples of the leaf and root of *Baphia nitida* were collected and identified by Mr. A.O Ozioko, a Taxonomist at the Bioresource Development and Conservation Programme (BDCP), located at Aku Road, Nsukka, Enugu State, Nigeria. After authentication the leaves and roots were collected in bulk, air-dried and ground into coarse powder with a manual blender (Corona China). Each powdered sample was kept in an air tight polythene bag and labeled properly for further use.

The test bacterial isolates (*Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus*) were obtained from the Microbiology Laboratory Federal Medical Centre Umuahia, Abia State, Nigeria. They were purified, sub-cultured and re-identified to ensure purity of the isolates.

Confirmation of Bacterial Isolates

The isolates were confirmed using some biochemical tests such as catalase test, coagulase test, indole test, citrate test, methyl red – Voges Proskaurer reaction, urease test, H2S Production and carbohydrate fermentation tests.[14, 15, 16]

Preparation of Crude Extracts

A weighed portion of each sample 20g, was mixed with 180ml of each of the solvents (Methanol and water) in separate flasks. The mixture were separately shaken very well and then allowed to stand for 48 hours at room temperature. After this, the mixtures were separately shaken again and filtered through Whatman No.1 filter paper and the filtrates were collected in separate labeled beakers which were previously weighed. The entire filtrate from each sample was evaporated to dryness in an oven at 40 ^{0}C and the beakers were left in a dessicator to allow adhering condensing vapour to be removed. The filtrates were stored at 4 ^{o}C until required for use.[17]

Determination of the Antioxidant Property of *Baphia nitida*

The antioxidant properties of the extracts were carried out. The free radical scavenging property of the extract was analyzed using 2, 2 - diphenyl - 1 -picrylhydrazyl (DPPH) photometric assay.[18]

Determination of the Phytochemical Property of *Baphia nitida*

The phytochemical screening involved the simple chemical test to detect the presence of secondary metabolites in the extract. The fraction was subjected to phytochemical spot using the method of.[19]

Antimicrobial Susceptibility Testing

The antibacterial activity of the extract was evaluated using *Staphylococcus aureus*, *Salmonella enterica* and *Escherichia coli*. The ability of the various extract to inhibit growth of the clinically significant bacteria isolates was determined using the disc diffusion method as described by.[20] This was done by incorporating a constant volume of 0.2 ml of 100 mg/ml of each extract as well as 50 µg/ml of a standard antibiotic solution (ciprofloxacin) into sensitivity disc prepared with Whatman No.1filter paper. The filter paper was perforated to obtain disc of 5 mm diameter after which they were sterilized at 160 ^{0}C for 2 hrs. After incorporation of the extracts the disc were placed in the oven at 40 ^{0}C for 30 min to enhance diffusion of extract into the filter paper. 0.2 ml of diluted inocula were introduced into Petri dish and swabbed evenly before placing the sensitivity discs. The plates were incubated at 37 ^{0}C and examined after 24 hrs. The disc was placed in duplicates and the diameter zone of inhibition was measured using a plastic ruler and the mean diameter zone of inhibition was recorded. The zone of inhibition is referred as the clearly visible zone of inhibition across a diameter disregarding the diameter of the disc.

Determination of Minimum Inhibitory Concentration (MIC) of the Crude Extract

The minimum inhibitory concentration is the lowest concentration of antimicrobials that will inhibit the visible growth of microorganisms after 24 hrs incubation. The MIC was determined using the broth method. Here the extracts were diluted in a twofold dilution to get concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1.56 mg/ml, 0.78 mg/ml and 0.39 mg/ml. Two milliliters of sterile nutrient broth was poured into sterile test tubes and then inoculated with 0.1ml of prepared inocula, after which 0.1 ml of each concentration of the different extracts were added to the tubes and incubated for 24 hrs at 37 ^{0}C to determine the Minimum Inhibitory Concentration.

One of the tubes served as the negative control as it contained only the nutrient broth and the inocula.[21]

Determination of Minimum Bactericidal Concentration (MBC) of the Crude Extract

The minimum bactericidal concentration is the lowest concentration of antimicrobials that will kill microorganisms after 24 hrs incubation. The MBC is determined by sub culturing the tubes with no visible growth on a fresh nutrient broth.

RESULTS

The antibacterial activity of the leaf and root bark extract of *Baphia nitida* was examined using two solvents methanol and distilled water and three clinical isolates (*Salmonella enterica, Staphylococcus aureus* and *Escherichia coli*).

Table 1 shows the antibacterial activity of the different extract on the test organisms. The result shows that the leaf extract exerted inhibitory activity against *Salmonella enterica* with the mean zone of inhibition of the methanolic extract as 12.00 ± 2.00 mm and 10.00 ± 2.00 mm for the aqueous extract but *Salmonella* was resistant to the root bark extracts. *Staphylococcus aureus* and *Escherichia coli* were resistant to both the leaf and the root bark extracts.

Table 2 shows the MIC and MBC values of the leaf extract against *Salmonella enterica.* It was seen to be 12.5 mg/ml and 50 mg/ml respectively of the methanol extract with 25mg/ml and 100m g/ml respectively for the aqueous extract.

Table 3 shows the phytochemical properties of the extracts. Saponins, alkaloids, terpenes, tannins, flavonoids and glycosides were seen to be present in all extracts.

Figure 1 shows the mean antioxidant properties of the extracts. At the highest concentration of 400 µg/ml, the methanolic leaf extract was seen to have the highest activity of 51.26 ± 1.58 while the aqueous extract of the root bark had the least activity with mean percentage antioxidant activity of 11.92 ± 2.75 compared to ascorbic acid which is a standard solution.

Table 1: The mean values of the zone of inhibition diameter (mm) of leaf and root extract of *Baphia nitida* against test organisms.

	S. enterica	*E. coli*	*S. aureus*
Methanol extract(100 mg/ml)			
BNL	12.00 ± 2.00	0.00 ± 0.00	0.00 ± 0.00
BNR	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Water extract (100 mg/ml)			
BNL	10.00 ± 2.00	0.00 ± 0.00	0.00 ± 0.00
BNR	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Antibiotics (50 mg/ml)			
Ciprofloxacin	40	30	12

Key: BNL = *Baphia* nitida leaf, BNR = *Baphia* nitida root

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Baphia nitida* Leaf Extract on *Salmonella enterica*

	S. enterica
Methanol extract	
MIC (mg/ml)	12.5
MBC (mg/ml)	50
Water extract	
MIC (mg/ml)	25
MBC (mg/ml)	100

Table 3: Phytochemical Properties of Root Bark and Leaf Extract of *Baphia nitida*

	Saponin	Glycoside	Flavonoid	Sterol/Terpenes	Alkaloid	Tannin
Methanol						
BNL				+	+	+
BNR				+	+	+
Water						
BNL	++	+	+	+	+	+
BNR	+++	+	+	++	+	+

Key: + = present, ++ = highly present, +++ = excessively present, BNL = *Baphia nitida* leaf, BNR = *Baphia nitida* root

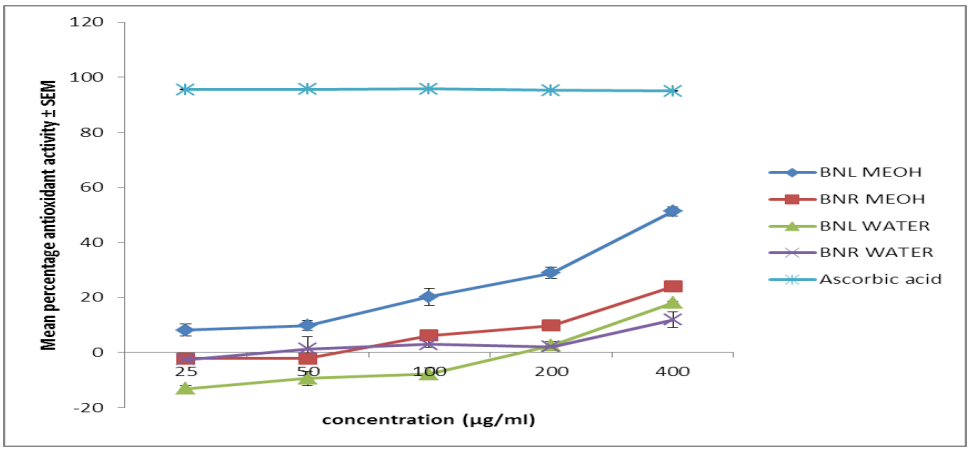

Fig. 1: Antioxidant Properties of Root Bark and Leaf Extract of *Baphia nitida*

DISCUSSION

The sensitivity of the test organism against the aqueous and methanol extract of the leaf and root bark of *Baphia nitida* was used for the study. Activities revealed that *Salmonella enterica* was susceptible to the leaf extracts and resistant to the root extracts while *E. coli* and *S. aureus* were resistant to both the root bark and the leaf extract of the plant.

The result of the antibacterial activity of *B. nitida* is not in conformity with with the report of previous studies.[6] recorded that *E. coli* and *S. aureus* were susceptible to *Baphia nitida* leaf with zone of inhibition diameter of 15 mm and 14 mm respectively and MIC of 37.5 mg/ml and 75 mg/ml respectively.,[20] also recorded that the *E. coli, S. aureus, S. enterica* were susceptible to the methanol and aqueous extract of the root bark of *Baphia nitida*. He stated that *S. aureus* was more susceptible to the extracts and thus indicates its use for the possible treatment of a range of illness from minor skin infection such as pimple, impetigo, boil to major illness as wound diseases and that the inhibition of *E. coli* suggests a possible treatment of infantile gastroenteritis, tourist diarrhea and heamorrhagic diarrhea. The differences in the result obtained may be due to the resistance pattern of bacterial strain which is of utmost important in view of the necessity for the world wide study of antibacterial resistance which has recently been associated with major disease outbreak.[22]; [23] The level of antibiotic agent resistance may be attributed to on the misuse and abuse of antibiotics in the environment. Consequently most antibiotics are given in hospitals without clear evidence or adequate medical instructions. Most of the time toxic broad spectrum antibiotics are administered in place of narrow spectrum drugs before culturing and sensitivity testing placing the patient at a very high risk and side effects, super infection and the detection of drug resistant mutants.[22]; [23]; [6] Another possible reason for the variation of this report from the previous reports may be due to the differences in the sources of the materials used. Plants may grow well in different environmental conditions but may fail to produce the same phytoconstituents. The nature, quality and quantity of secondary metabolites of plants are affected by rainfall, temperature, length of day light and altitude.[24] Light determines the amount of glycosides or alkaloids present in a plant. Also high quantity of rainfall can lead to the loss of water-solubles from leave and root of plants by leaching. This commonly affects some plants producing alkaloids, glycosides and even volatile oil.[24]; [25] The antibacterial activities of plants were affected by the phytochemical composition. Several phytochemical constituents have been demonstrated to possess antibacterial activity.[26]

Baphia nitida plant are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids and saponins which have been found to have *in-vitro* antimicrobial properties.[27]

This activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Lipophilic flavonoids may also disrupt microbial membranes.[28]

The mode of antimicrobial action of tannin may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport e.t.c. also form complex with polysaccharides.[29] There is also evidence for direct inactivation of microorganisms. Tannin in plants has also been seen to modify the morphology of gem tubes of *Crenipellis pernicosa,* inhibit insect growth and disrupt digestive events in ruminant animals.[30] The result of the phytochemical analysis and antioxidant activity of *B. nitida* were in agreement with the report of previous workers. They demonstrated that antioxidant activity of the extract could help to counteract the effects of oxidative stress posed by disease conditions and enhance the patient's recovery.[31]

This study therefore shows that the aqueous and methanol extract of *Baphia nitida* leaves can be used for the treatment of *Salmonella enterica* related infections. It is therefore interesting to note that the treatment of sudden fever, headaches, diarrhea, stomach upsets, nausea and vomiting also known as food borne diseases (Salmonellosis)[28] is possible with the use of extracts from *Baphia nitida* leaves as it contains active component that inhibit the growth of *Salmonella enterica*.

CONCLUSION

This study has thus shown that extracts from *Baphia nitida* leaves could be used to discover natural bioactive product that may serve as lead for the development of new pharmaceuticals that address therapeutic needs for *Salmonella* related infection.

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