

Dietary Benefits of *Baphia nitida* Stem Bark and Antimicrobial Effect on Some Pathogens

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Abstract. The need to find a broad-based nutritional and antimicrobial therapy gave impetus to this investigation. The crude extracts of *Baphia nitida* stem bark were used to check for antimicrobial effect on selected micro-organisms for possible nutritional and therapeutic application. The stem bark components were extracted with four solvent systems using maceration. A synthetic drug gentamycin – 80 mg/mL (2 mL) was dissolved in 20 mL of distilled water to obtained 8 mg/mL, was used as a control. The crude extract exhibited an antibacterial effect on gram-positive organisms (*Staphylococcus aureus* and *Bacillus cereus*), and gram-negative organism (*Pseudomonas aeruginosa*, and *Escherichia coli*) at 2 mg/mL. These showed competitiveness with the 8 mg/mL of the control drug. The normal hexane fraction had an effect on *Staphylococcus aureus* but showed no effect on other bacteria. Minimum inhibitory concentration (MIC) showed a dose-dependent decrease in the effect for the crude extract. Ethyl acetate and methanol fraction had an effect on *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli* at varying concentrations - 20, 10, and 5 mg/mL. the initial screening of the *Baphia nitida* stem bark revealed alkaloids in the methanol-methylene chloride crude extract and methanol fraction only. Other solvents showed the alkaloids, flavonoids, saponins, terpenoids, glycosides, hydrogen cyanides and steroids respectively. Therefore, the presence of the phytochemicals implicates its antimicrobial effect and the basis for its efficacy as antimicrobial therapy.

Keywords: antimicrobial, *Baphia nitida*, nutrition, gram-positive, gram-negative

Introduction

The World Health Organization (WHO) suggests that herbal plants are any part, tissue or organ of a plant species with substances used for treating and curing diseases, or serves like templates for the manufacturing drugs (Nyananyo, 2006). About 70% of these traditional medicines are plants based, while an estimated 75-90% of the native people rely on herbs for their healthcare. In some African villages, Asia and Latin America, medicine from herbs are sold alongside vegetables (Lai and Roy, 2004). Herbal medicine forms the stepping stone for the introduction of synthetic drugs. With advances in technology, extraction of elemental constituent medicinal plants was isolated and research upon. These form a template for the production of other analog herbal medicine and the advent of plants therapies (Hazel *et al.*, 1999). The emergence of micro-organisms that are resistant to synthetic antibiotics, coupled with toxic nature of the residues of some synthetic drugs,

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this pave way for the exploration of plants with capacities to damage diverse strains of pathogens and their recalcitrant metabolites. Researchers have considered the use of medicinal plants as a reservoir of phytomedicine. Most of these active principles are second hand metabolites - products that are not too required by plants but are produced, secreted and kept in a body of a part as waste or as protective deposits (Marjorie, 1999). The fractionation and purification of plant's actives will remove impurities and improved the potency.

Though, some fractionation expands the spectrum of activity (Okoli and Iroegbu, 2005), while in others, it reduces the spectrum of activities by depending on the constituents of the crude extracts. This also suggests the antagonistically or synergistically interact when used as a consortium. *Baphia nitida* plant is a camwood. The Igbo tribe of Nigeria called it *Aboshi*, Yoruba *Irosun*, and Efik *Ubara*, respectively (Onwukeme and Lot, 1991). Camwood is a fast-growing smallest tree

that about 5 meters tall (Fig. 1a and b). These fruits are straight pods, 10-15 cm long and 12-16 mm wide, sharply pointed at both ends and with 2-4 brown, flat seeds. (Wee, 1990; Soladoye, 1985). The pulp is usually sweet to taste and silky (Etukudo, 2003). Most conventional analytical methods assess the *in vitro* effects of antimicrobial plants on the micro-organisms or determine the antimicrobial modifying enzymes of the plant extract. The clinical institute has provided the guidelines for media preparation, incubation factors and interpretation of results such as inhibition zone (IZ). Okoro (2012), *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *K. pneumonia* and *Pseudomonas aeruginosa* have been utilized for the antimicrobial tests with *Baphia nitida* roots. This research utilized *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and



Fig. 1. 1a and 1b showing the young and mature *Baphia nitida* plant with leaves.

Bacillus cereus. Tests organisms are selected based on their pathogenic significance in relation to ethno medicinal applications. These selected pathogens have been implicated in various diseases etiology. *Escherichia coli* is associated with gastroenteritis and food poisoning (Todar, 2007). *Bacillus cereus* causes chronic skin infections and responsible for a majority of foodborne illnesses. *Staphylococcus aureus* causes oral/dental caries infections and life-threatening diseases such as pneumonia, meningitis, sepsis etc. (Cimolai, 2008). *Pseudomonas aeruginosa* can reason oral infections and urinary tract infections (Scannapieco and Mylotte, 1996).

Materials and Methods

The *Baphia nitida* plant (stem bark) was used for this study.

Chemicals and reagents. The chemicals and reagents were analytical grade and products of British Drug House, (BDH, England). Langendorf's reagent (May and Baker, England).

Preparation of gentamycin (control drug). One ample gentamycin injection containing 80 mg/mL in 2 mL was purchased from the University of Nigeria, Nsukka pharmacy store. The 2 mL of the control drug (gentamycin) was emptied (diluted) in 20 mL of distilled water. An 8 mg/mL was obtained after calculation and used for the analysis of all the test organisms.

Test organisms. The American Type Culture Collection (ATCC) were obtained from the microbiology section of the bio-resources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Nigeria. They comprise of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 90000 and *Candida albicans* ATCC 90028, respectively.

Plant collection and identification

A 2.5 Kg fresh *Baphia nitida* (Camwood) stem barks were obtained from Orba in Udeno Local Government Area (LGA) of Enugu State, Nigeria on 13 April 2013. The plant samples were identified in the herbarium section of Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka.

Preparation of plant extracts. The stem barks were cut into pieces and air-dried for three weeks. They were ground into coarse powder with a grinder. The powder was kept in a closed container at ambient temperature until analysis was carried out.

Experimental protocol. A 12,000 mL of methanol-methylene chloride (MeOH/MeCl₂) was prepared as follows: a 6,000 mL of methanol and 6,000 mL of methylene-chloride was measured and added to 2500g of the coarsely powdered sample in the ratio of 1:1 and allowed to stand for 48 h before proceeding with extraction. After 48 h of maceration, the homogenate was filtered.

Purification of test organisms. The test bacterial strains were purified by re-isolating the discrete colonies in Mueller Hinton agar (MHA Oxoid), while their identities were reaffirmed after characterization by standard bacteriological method (Cheesbrough, 1984). Stock cultures were sustained in nutrient agar slants at 4 °C.

Screening of the plant extracts for antibacterial activity. A preliminary test was performed with *Baphia nitida* crude extract (methanol-methylene chloride) to ascertain its antibacterial activity. The extract was spot checked at 250 mg/mL using agar well diffusion technique. The test bacterium was seeded into a sterile MHA plate. Subsequently, a 2000 µg/mL was added into the wells of the MHA culture in triplicate. The plates were held for one hour at ambient temperature to allow diffusion of the extract in the agar and incubated at 37 °C for 24 h. The diameter of the inhibition zone (IZ) was recorded to the nearest millimeter.

Evaluation of minimum inhibitory concentration (MIC). The Minimum Inhibitory Concentration (MIC) for the extract and fractions against the test organisms were determined by the agar well diffusion method (NCCL, 1993). The extract was re-dissolved to achieve a 125 mg/mL and serially dilute a 2-fold stepwise to get a 3.91 mg/mL. A 100 µl of every dilution was added

into the wells of MHA plates already seeded with the test bacterial cells in duplicate. The incubation period was 24 h at 37 °C. The MIC was obtained as the lowest level of the extract indicating a clearer zone of inhibition. With macro-broth dilution technique, a double-fold serial dilution of the reconstituted extract was prepared in Mueller Hinton broth. Each dilution was seeded in duplicates with 100 µl of the suspension of the test bacterial strain to achieve a final concentration of 1×10⁶ CFU/mL for the gram-positive bacteria and 5×10⁵ CFU/mL for the gram-negative bacteria. All cultures were incubated at 37 °C for 24 h and a micro-broth MIC determined as the lowest level of inhibition by the extract at which the microorganism growth was not visible.

Analysis of data. The analyzed data are presented in mean ± standard deviation through analysis of variance, using the statistical package and service solutions (SPSS) version 20. The difference in means was considered significant at p < 0.05 degree of a confidence interval.

Results and Discussion

An investigation to determine the antimicrobial effect of *Baphia nitida* against selected micro-organisms was designed. After plant extraction with the four solvent systems, the preliminary phytochemical study of the crude methanol-methylene-chloride extract (MMCE) indicates a high level of alkaloids, flavonoids, and tannins.

Glycosides, terpenoids, carbohydrates including reducing sugars were moderate as present see Table 1. This represents the plant-chemical constituents of the *Baphia nitida* stem bark. Results suggest that reducing sugar

Table 1. Phytochemical constituents of *Baphia nitida* stem bark extracted with four solvents

Phytochemicals	Concentration			
	MeOH/CH ₂ Cl ₂ extract	n-hexane fraction	Ethyl acetate fraction	Methanol fraction
Alkaloids	3.64 ± 0.002 ^a	0.00±0.00	0.00±0.00	3.43 ± 0.004 ^b
Flavonoids	4.56 ± 0.002 ^a	2.57 ± 0.003 ^b	2.54 ± 0.002 ^b	2.65 ± 0.004 ^b
Steroids	0.41 ± 0.003 ^a	0.34 ± 0.004 ^b	0.34 ± 0.002 ^b	0.35 ± 0.001 ^b
Saponins	1.84 ± 0.004 ^a	1.75 ± 0.003 ^b	1.63 ± 0.003 ^b	1.78 ± 0.003 ^b
Glycosides	3.78 ± 0.003 ^a	3.48 ± 0.003 ^b	3.56 ± 0.003 ^b	3.58 ± 0.004 ^b
Terpenoids	0.33 ± 0.002 ^a	0.18 ± 0.003 ^b	0.29 ± 0.002 ^b	0.32 ± 0.002 ^a
Carbohydrates	1.91 ± 0.002 ^a	0.47 ± 0.003 ^b	0.00 ± 0.00	0.95 ± 0.001 ^c
Tannins	6.87 ± 0.004 ^a	0.00 ± 0.00	6.02 ± 0.003 ^b	6.72 ± 0.002 ^c
Hydrogen cyanide	0.08 ± 0.004 ^a	0.01 ± 0.003 ^b	0.07 ± 0.003 ^a	0.21 ± 0.004 ^c
Reducing sugar	304.35 ± 0.03 ^a	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values found in the same row but with different alphabets may differ significantly at (p < 0.05), n=3.

at 304.35 ± 0.003 mg/100g was the abundant constituent of the extract, while hydrogen cyanide (0.08 mg/100g) and terpenoids (0.33 mg/100g) were the lowest. The presence of bioactive compounds shows antimicrobial potentials as they regulate some physiological activities (Harbone, 1973). The different fractions; n-hexane fraction (nHf), ethyl acetate fraction (Ef) and methanol fraction (Mf), exhibited varying concentrations of the phytochemicals. Some alkaloids can relieve pain, while others may function as tranquilizers.

Tiwari *et al.* (2011), reported that alkaloids act by intercalating with cell and DNA of parasites. The protective effects of flavonoids include antiviral, anti-inflammatory and anticarcinogenic activities (Middleton *et al.*, 2000). (Trease and Evans, 2009; Okwu, 2001 and 2004) states that some biological influence of flavonoids includes the protection of humans against allergies, free radicals, platelet aggregation of micro-organisms, ulceration, hepatotoxins, and tumors. Flavonoids act by competing with the cell wall and binding to adhesins (Roopashree *et al.*, 2008). Tannins function in the prevention of microbial growth by precipitating their protein and hindering nutritional proteins of micro-organisms (Ogunleye and Ibitoye, 2003). Some tannin derivatives have bind adhesins. Though, other mechanisms of action include inhibition of an enzyme, deprivation of substrate, chelating the cell wall metal ions and damaging the organism cell membrane (Cowan, 1999). Tannins are used on inflamed surfaces. (Stephen *et al.*, 2009; Sodipo *et al.*, 1991) reported tannin antioxidants properties of nitida plant. Saponins were present in trace amount in the stem bark. Saponins are known for membrane permeability. This result aligned with (Okon *et al.*, 2013), reports on the existence of trace amounts of saponin in the root of *Baphia nitida*. Okwu (2001) applauded the relevance of steroids as a basal material for the synthesis of sex hormones. These bioactive compounds are responsible for the antimicrobial activity observed. The antimicrobial activities (inhibition zone diameters) of the *Baphia nitida* stem bark on the test micro-organisms for different fractions of solvent. The results obtained indicate considerable variations in antimicrobial activity between extract and fractions. The extracts with MMCE and methanol fraction were more potent than the other fractions. A similar observation was reported by (Alabi *et al.*, 2013; Kowalski and Kedzia, 2007). This was due to soluble phenols and polyphenols in the methanol-containing extract and methanol fraction. However, the

crude extract and two other fractions (Ef and Mf) revealed moderate antibacterial potentials against the employed gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria at a working concentration of 2mg/mL with their different zones of inhibition 0-19 mm see Fig. 2.

The diameter of inhibition of the extracts was significantly lower ($p < 0.05$) compared positive control (gentamycin). The diameter of the inhibition zone of the *Staphylococcus aureus* was significantly higher for the extract compared to the control.

The diameter of the inhibition zone of the extracts was considerably lower ($p \leq 0.05$) compared positive management (gentamycin). The diameter of the inhibition zone of the *Staphylococcus aureus* was considerably higher for the extract compared to the management.

The MMCE, Ef and MF unconcealed pronounced zones of inhibition on *Staphylococcus aureus* compared to different check micro-organism. The explanation for the various sensitivity between gram-positive and gram-negative micro-organism could also be because of the morphological variations between the micro-organisms. Gram-negative micro-organisms have an outer lipid membrane carrying the structural lipo-polysaccharide elements. This makes the plasma membrane impermeable to lipotropic solutes, whereas porins

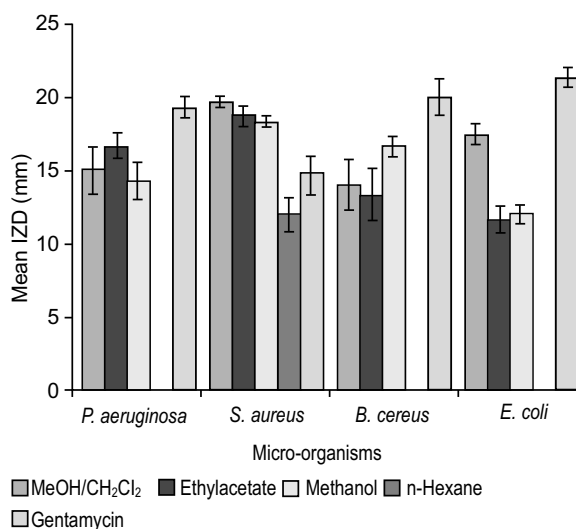


Fig. 2. Diameter of inhibition zone of *Baphia nitida* on micro-organisms.

represent a selective barrier to the hydrophilic solutes with a degree of exclusion limit - 600Da (Nikaido and Vaara, 1985). The gram-positive micro-organism would be inclined as a result of its outer peptidoglycan layer that isn't a selective pervious barrier (Scherrer and Gerhardt, 1971). The inhibition indicates attainable remedy for a variety of unwellness from minor skin infections equivalent to abscesses, and wounds, to life-threatening diseases equivalent to blood disease (Jill and David, 2004). The positive management (gentamycin) smothered the expansion of the entire tested micro-organism see Fig. 3.

This shows the MIC increase within the region of the crude extract and fractions on *Pseudomonas aeruginosa*. The MIC of the extract and fractions were considerably not up to that of the positive management (gentamycin). And, the MIC of different fractions was considerably lower compared to positive management.

Normal hexane fraction was only potent on *Staphylococcus aureus*, while providing no activity on *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Escherichia coli*. *Staphylococcus aureus* was the most susceptible to both the extract and three fractions. The susceptibility corresponds to that reported by Okoro (2013), which showed consistent inhibition activity on *Staphylococcus aureus* at varying concentrations, from 0.0625 mg/mL to 1.0 mg/mL (Onwukaeme, 1991), shown in Fig. 4, 5, and 6, separately. The most active extracts that (inhibited a diameter ≥ 12 mm) were

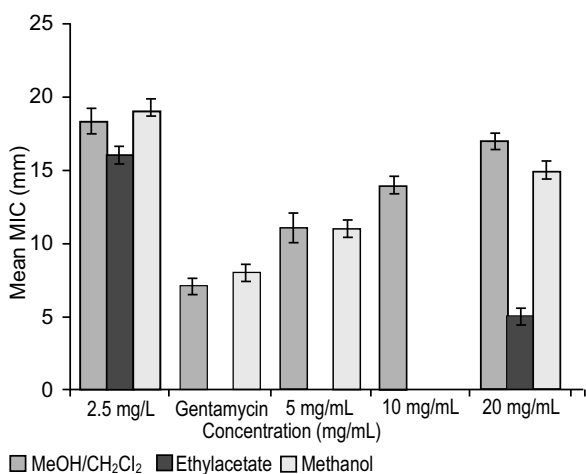


Fig. 3. Minimum inhibitory concentration of *Baphia nitida* against *Pseudomonas aeruginosa*.

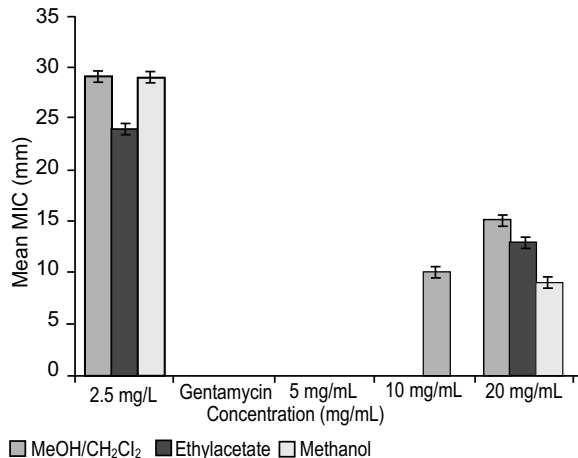


Fig. 4. Minimum inhibitory concentration of *Baphia nitida* against *Staphylococcus aureus*.

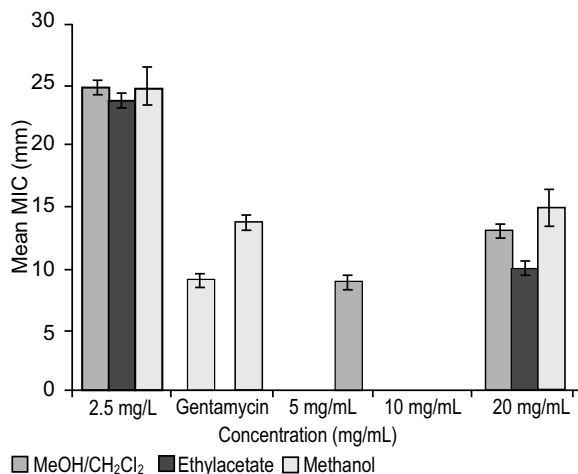


Fig. 5. Minimum inhibitory concentration of *Baphia nitida* against *Bacillus cereus*.

assayed for the MIC. The MIC result revealed a dose-dependent increase in the activity of the raw extract and fractions on all the test bacteria. MMCE exhibited its activity against *Pseudomonas aeruginosa* at all concentrations (20 mg/mL, 10 mg/mL, 5 mg/mL and 2.5 mg/mL).

The MIC Fig. 4 shows no activity at lower concentration (2.5mg/mL and 5mg/mL) for both the crude extract and fractions on *Staphylococcus aureus*. The MIC of the extract and fractions were significantly lower than that of the positive control (gentamycin). Also, the MIC of the various fractions were significantly lower than that of the crude extract at 20mg/mL.

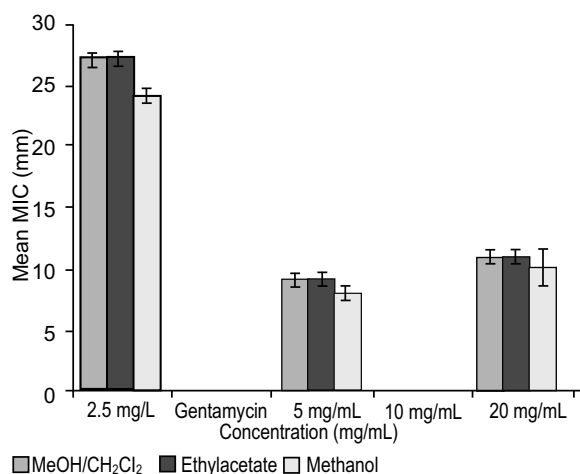


Fig. 6. Minimum inhibitory concentration of *Baphia nitida* against *Escherichia coli*.

A concentration-based increase in the activities of the crude extract and fractions on *Bacillus cereus* can be observed in Fig. 5. The MIC of the extract and fractions were significantly lower than the positive control (gentamycin). The MIC of the methanol fraction was significantly higher than the crude extract at 5mg/mL, 10mg/mL, and 20mg/mL concentrations.

The MIC shows a concentration-based increase in the activities of the crude extract and fractions on *Escherichia coli*. The MIC of the extract and fractions were significantly lower than the positive control (gentamycin).

Thereafter, none of the extract and fractions exhibited any activity at lower concentrations on the remaining test bacteria. The concentration of the positive control (gentamycin) inhibited all the test organisms. Gentamycin is known to bind bacterial ribosome thereby preventing protein synthesis (Alexander and Strete, 2001). Comparatively, the methanol-methylene chloride extract and the fractions of the plant possess good antibiotic properties since they contain both bioactive substances as opposed to the pure active constituents in the synthetic medicines.

Conclusion

The antibacterial activity exhibited by the *Baphia nitida* stem bark was good in various respects. The bacterial strains that were used for this investigation are majorly implicated in enteric disturbances. The fractionation of

the crude extract with different solvents help revealed how some bacterial strains lost susceptibility to the fractions, while others showed improved susceptibility. Thus, antibacterial activity justifies the utilization of the plant in traditional practice for the treatment of ailments.

Conflict of Interest. The authors declare no conflict of interest.

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