

Antimicrobial Activities of Leaf and Stem Bark Extracts of *Blighia sapida*

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Abstract

Antimicrobial investigation of the leaf and stem bark extracts of *Blighia sapida* was carried out against pathogenic bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Shigella dysenteriae*) and fungi (*Candida albicans*, *Microsporum canis*, *Aspergillus niger* and *Aspergillus fumigatus*). The two extracts did not exert antifungal effect on any of the tested fungal species, at the concentrations used in this study but exhibited activity against *S. aureus* and *B. subtilis* but not against *E. coli* and *S. dysenteriae*. The stem bark extract was more potent than the leaf extract. The highest stem bark extract concentration (200 mg/ml) resulted in inhibition zone diameter (IZD) of 14.33 ± 0.57 and 15.00 ± 0.00 for *S. aureus* and *B. subtilis* respectively, whereas the same concentration of the leaf extract resulted in IZD of 10.66 ± 1.15 and 9.33 ± 0.98 for *S. aureus* and *B. subtilis* respectively. Minimum inhibitory concentrations (MIC) of 12.5 mg/ml, and 100 mg/ml were obtained for the stem bark extract assayed against *B. subtilis* and *S. aureus*, respectively. With the same extract, minimum bactericidal concentrations of 25 and >100 mg/ml were obtained for *B. subtilis* and *S. aureus* respectively. Phytochemical screening revealed the presence of saponins, anthraquinones, cardiac glycosides and flavonoids in both extracts. Alkaloids, tannins, phlobatannins and terpenes were also detected in the leaves. Results obtained, explains why parts of this plant are used traditionally for the treatment of various infections.

Keywords: *Blighia sapida*, antimicrobial activity, inhibition zone diameter, extract, phytochemicals

1. Introduction

The prevalence of diseases of microbial origin remains high and is of serious concern especially in the tropics. Microbial infections can lead to serious and sometimes life-threatening complications. The high cost of newer and effective drugs along with other factors, has made the choice of herbal remedies against these infections inevitable and more economical (Okokon et al., 2007).

Since earliest times, plants have been a valuable source of natural products for maintaining human health (Sher, 2009). According to the World Health Organization (WHO), medicinal plants would be the best source of obtaining a variety of drugs. Results of many recent studies have demonstrated the promising potentials of medicinal plants used in various traditional and complementary systems for the treatment of human diseases.

Blighia sapida is a medicinal plant that belongs to the family Sapindaceae. It is also referred to as ackee or ackee apple. The trunk diameter can reach 12 m and it supports a dense crown of spreading branches over 182 cm above the ground. The ackee is indigenous to the forests of most West African countries where the fruits are rarely eaten but are used for other purposes. In Ghana for instance *Blighia sapida* is used as ornamental plants and also as shades. The green fruits of this plant produce lather when mixed with water and are therefore used for laundry while the seeds find use in soap making because of their oil content. Some parts of this plant are also used for medicinal purposes e.g the juice from the leaf is used to treat conjunctivitis. This plant species has been used by the Centre for Scientific Research into Plant Medicine (CSRPM), Ghana, for the treatment of diarrhea, for over 20 years. The antidiarrheal activity of the stem bark of *B. sapida* has also been documented by Antwi et al. (2009). The traditional use of *B. sapida* for the treatment of dysentery, yellow fever, eye sore, burns, wounds, skin sore etc has also been reported (Etukudo, 2003).

This paper presents the reports of the antimicrobial potentials of the ethanolic leaf and stem bark extracts of *B. sapida* against some pathogenic bacteria and fungi of medical importance.

2. Experimental

2.1 Collection of Plant Materials

Fresh leaves and stem bark of *B. sapida* used in this study were obtained from Itak Ikot Akap village, Ikono Local Government Area, Akwa Ibom State, Nigeria in 2010. They were identified by Prof. Rufus Ubom, of the Department of Botany and Ecological studies, University of Uyo, Nigeria and a voucher specimen with herbarium No. UUH 69(a) was deposited in the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

2.2 Preparation of Extracts

The plant parts were separately washed and air-dried at room temperature ($28 \pm 2^\circ\text{C}$). When dried, they were ground into powder using crusher machines. Five hundred grams (500 g) of the leaf and stem bark powder, were separately macerated in 1.8 and 1.5 liters of 50% ethanol respectively for 72 hours with periodic stirring. This was followed by repeated filtration using sterile muslin cloth, non absorbent cotton wool and Whatman filter paper No.1, in order to remove the marc. The filtrates were concentrated in vacuo at 40°C using a rotary evaporator (Bibby Sterlin Ltd, England, RE. 2000) after which they were lyophilized using a lyophilizer (Aqua Lyovac GT2, Germany). The percentage yield of each extract was determined by comparing the weight of the yield and the initial weight of the powder extracted. The extracts obtained were preserved in the refrigerator at a temperature of 4°C prior to use.

2.3 Phytochemical Screening

Phytochemical screening was carried out using the methods described by Harborne (1984), Evans (2002) and Sofowora (2006).

2.4 Test Microorganisms

The test organisms used in this study were *Shigella dysenteriae*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* (bacteria) and *Aspergillus niger*, *Aspergillus fumigatus*, *Microsporium canis* and *Candida albicans* (fungi). They were all clinical isolates obtained from the Pharmaceutical Microbiology laboratory of the Faculty of Pharmacy, University of Uyo, Nigeria. They were purified through repeated subculturing and characterized using the methods of Collins and Lyne, (1970) and Murray (1985). Confirmed isolates were subsequently stored in agar slants in the refrigerator at 4°C .

2.5 Antimicrobial Assay

The agar-well diffusion technique (Udobi & Onaolapo, 2009) was employed in this assay. Standardized inoculum using the 0.5 McFarlands standards (1×10^8 cfu/ml) of each test bacterium was spread on to plates containing sterile Mueller Hinton Agar. The plates were allowed to dry and a sterile cork borer of 6 mm diameter was used to bore wells in the agar plates. For the antifungal assays, standardized inoculums using the 0.5 McFarlands standard (1×10^8 cfu/ml) of each test fungus was spread on to plates containing sterile Sabouraud Dextrose Agar. The plates were left to dry and a sterile cork borer of 6 mm diameter was used to bore wells in the agar plates. From a stock solution of 200 mg/ml of each extract obtained by dissolving the extract in sterile distilled water, varying concentrations of the extracts were prepared to obtain 100, 80 and 60 mg/ml of each extract. Each extract concentration was separately introduced to fill the wells. The plates were allowed to stand for one hour for diffusion to take place and then were incubated at 37°C for 24 hours for assays involving bacterial species and 28°C for 72 hours for assays involving fungal species. The positive control consisted of streptomycin, the standard antibacterial drug, and the antifungal drug nystatin at concentrations of 0.04 and 1mg/ml respectively while the negative control experiments involved adding sterile distilled water in the agar wells.

The zone of inhibition for each experiment was recorded and the mean inhibition zone diameter was determined in each case.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

Based on the results obtained from the antimicrobial assay, the MIC was determined only for the ethanolic stem bark extract using *S. aureus* and *B. subtilis*. Broth dilution method was employed in the experiment. A stock solution (200mg/ml) of the stem bark extract was prepared. From the stock solution, extract concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.5625 mg/ml were obtained by serial dilutions. Standardized inoculum (1×10^8 cfu/ml) of the test bacterial species (*S. aureus* and *B. subtilis*) were separately introduced into test tubes containing the different concentrations of extract. Control experiments were also set up using sterile nutrient broth and test organism without the extract. All tubes were covered with sterile cotton wool and incubated at 37°C for 24 hours.

At the end of experiment (24 hrs) the tubes were examined for turbidity. Clear tubes were recorded as negative (no growth) and turbid tubes were recorded as positive (growth).

2.7 Determination of Minimum Bactericidal Concentration (MBC)

This bioassay was carried out to determine the bactericidal effect of the ethanolic stem bark extract of *B. sapida* on *S. aureus* and *B. subtilis*. The spread plate method was adopted for this study (Cheesbrough, 2004). Emphasis was on the tubes without growth. A loopful from each of these tubes was subcultured on sterile Mueller Hinton Agar plates. The plates were incubated for 48 hours at 37 °C, after which the plates were observed for growth. The MBC was determined by the plate with the lowest concentration of extract that showed no growth.

2.8 Data Analysis

Data obtained from this study were analyzed using the statistical tools of Microsoft Excel.

3. Results

3.1 Phytochemical Screening and Extract Yield

The percentage yield values of the leaf and stem bark extracts of *B. sapida* used in this study were 2.95 and 2.18% respectively. Alkaloids saponins, tannins, phlobatannins, flavonoids, terpenes, cardiac glycosides and combined anthraquinones were detected in the leaf of *B. sapida*, whereas only saponins, flavonoids, combined anthraquinones and cardiac glycosides were detected in the stem bark.

3.2 Antimicrobial Activity

Table 1 depicts the antibacterial activity of the ethanolic leaf extract and the standard drug streptomycin. It shows that the leaf extract had no activity against the two test Gram negative bacteria (*E. coli* and *S. dysenteriae*) at the various test concentrations, but had activity against the Gram positive bacteria (*S. aureus* and *B. subtilis*) only at 200 mg/ml. The antibacterial activities of the ethanolic stem bark extract of *B. sapida* and streptomycin are presented in Table 2. The stem bark extract inhibited the growth of *B. subtilis* at concentrations 60 mg/ml and higher whereas it inhibited the growth of *S. aureus* only at 100 and 200 mg/ml. No inhibition was observed for *E. coli* and *S. dysenteriae* at the various test concentrations.

There was no antifungal activity against any of the fungal species when the ethanolic leaf and stem bark extracts of *B. sapida* were assayed. Only the standard drug nystatin inhibited the growth of the fungal species with mean values of inhibition zone of 22 mm, 18 mm, 20 mm and 22 mm for *C. albicans*, *M. canis*, *A. niger* and *A. fumigatus* respectively. In all the control experiments (without extract and standard drug) there was steady growth.

Table 3 depicts the Minimum Inhibitory Concentration (MIC) of the ethanolic stem bark extract of *B. sapida* for susceptible bacterial species. The MIC values obtained were 12.5 mg/ml and 100 mg/ml for *B. subtilis* and *S. aureus* respectively.

The Minimum Bactericidal Concentration (MBC) of the ethanolic stem bark extract is presented in Table 4. Results obtained indicated that the MBC for *S. aureus* was > 100 mg/ml, while 25 mg/ml was recorded for *B. subtilis*.

4. Discussion

The low yield values of extracts used in this study may be due to the employed method of extraction (maceration). Maceration has been reported to result in low yield of extracts (Ibrahim et al., 1997). However, it was preferred to other methods because it does not require heating and as such serves to protect thermolabile phytoconstituents that may be present.

Medicinal plants are widely used in African communities for the treatment of different types of bacterial and fungal diseases. They are rich in a wide variety of secondary metabolites, with antimicrobial properties (Sher, 2009). *B. sapida* leaf and stem bark extracts tested positive to some of these plant metabolites. Thus, the antimicrobial activity reported in this investigation can be assigned to the presence of these plant metabolites. It is interesting to note that fewer metabolites were detected in the stem bark extract than the leaf extract, yet it performed better than the leaf extract. The higher inhibitory activity demonstrated by the stem bark extract corroborates the report of Sukumar et al. (1991), that the activity of phytochemical compounds on target species varies with respect to plant parts from which they are extracted, among other factors. Screening did not involve quantitative analysis of the extract to ascertain plant metabolites that were abundant, moderate or trace. The screening only revealed the presence or absence of groups of phytoconstituents present in the extract. The exact nature/identity of each phytoconstituent was not revealed. Thus some of the phytoconstituents detected in the

leaf may have been present in trace amount or may have been different in type from those detected in the stem bark. For Instance saponins often occur as complex mixtures and according to the structure of the aglycone or sapogenin, two kinds of saponins are recognized, the steroidal and the pentacyclic type (Evans, 2002). Also various classes of alkaloids exist, but a basic nitrogen is the unifying factor (Evans, 2002; Sofowora, 2006). Each phytoconstituent is a group of compounds, each compound differing in structure and chemical properties. The elucidation of the structure of the active principle(s) in each extract will give a clearer picture of their mechanism of action.

The results of antimicrobial sensitivity test revealed that there was no activity against the tested fungal species at the extract concentration used. Thus fungal species used in this study demonstrated physiological resistance to the leaf and stem bark extracts of *B. sapida*. The absence of antifungal activity using the extracts agrees with the report of Duraipandiyan et al. (2006), who documented that ethanolic leaf extract of *B. sapida* had no antifungal activity against *C. albicans*.

The ethanolic leaf and stem bark extracts of *B. sapida* demonstrated inhibitory activity against Gram positive bacteria (*S. aureus* and *B. subtilis*), but the Gram negative bacteria (*E. coli* and *S. dysenteriae*) were resistant to the two extracts. This may be due to the fact that the Gram negative bacteria possess sophisticated cell wall which does not allow permeation of external agents (Brown, 1975). However, further investigation is required to substantiate this view.

The extracts though active to some extent against the Gram positive bacterial species did not compare favourably with streptomycin, the standard drug used (Tables 1 and 2). Streptomycin is a broad spectrum antibiotic, which is active against both Gram positive and Gram negative bacteria. The lower inhibitory activity of the extracts when compared with the standard drug may be attributed to the fact that the extracts used were in their crude form. It is anticipated that better results would be obtained with purified fractions of the extracts.

Analysis of variance revealed significant difference ($P < 0.05$) in potency between leaf and stem bark extracts on sensitive bacteria with the stem bark extract being more potent. Only the minimum inhibitory concentration (MIC) of the stem bark was determined, because of aforementioned results earlier obtained.

In coincidence with the negative effect of the extract on Gram negative bacteria, Antwi et al. (2009), reported that the antidiarrheal activity of aqueous and ethanolic stem bark extracts of *B. sapida* was as a result of the ability of the extracts to inhibit intestinal motility and enteropooling effect. This implies that the usefulness of the leaf and stem bark extracts of *B. sapida* in the treatment of dysentery in folklore is not due to its antibacterial effect on toxin producing bacteria that are associated with diarrhea, such as *E. coli* and *S. dysenteriae*, rather it is due to the ability of the extract to inhibit intestinal motility.

Results obtained from this study have provided an insight into the reasons for the usage of these plant parts for the traditional treatment of sores, burns, dysentery, wounds and other infectious diseases especially those where *S. aureus* is incriminated.

Table 1. Inhibitory effect of hydroethanolic extract of *B. sapida* leaves on test bacterial species with streptomycin as positive control

Test Organisms	Inhibition Zone Diameter (mm)				
	200 mg/ml	100 mg/ml	80 mg/ml	60 mg/ml	Streptomycin
<i>Staphylococcus aureus</i>	10.66 $\pm$ 1.15	0.00	0.00	0.00	24.66 $\pm$ 0.57
<i>Bacillus subtilis</i>	9.33 $\pm$ 0.98	0.00	0.00	0.00	22.33 $\pm$ 2.51
<i>Escherichia coli</i>	0.00	0.00	0.00	0.00	10.33 $\pm$ 0.57
<i>Shigella dysenteriae</i>	0.00	0.00	0.00	0.00	15.00 $\pm$ 0.00

The values are the mean $\pm$ S.D of the three measurements of inhibition zone diameter.

Zero values indicate no inhibition.

Concentration of streptomycin used = 0.04 mg/ml.

Table 2. Inhibitory effect of hydroethanolic extract of *B. sapida* stem bark on test bacterial species with streptomycin as positive control

Test Organisms	Inhibition Zone Diameter (mm)				
	200 mg/ml	100 mg/ml	80 mg/ml	60 mg/ml	streptomycin
<i>Staphylococcus aureus</i>	14.33 $\pm$ 0.57	8.66 $\pm$ 1.15	0.00	0.00	24.66 $\pm$ 0.57
<i>Bacillus subtilis</i>	15.00 $\pm$ 0.00	14.00 $\pm$ 0.00	12.00 $\pm$ 0.00	11.00 $\pm$ 1.10	22.33 $\pm$ 2.51
<i>Escherichia coli</i>	0.00	0.00	0.00	0.00	10.33 $\pm$ 0.57
<i>Shigella dysenteriae</i>	0.00	0.00	0.00	0.00	15.00 $\pm$ 0.00

The values are the mean $\pm$ S.D of the three measurements of inhibition zone diameter.

Zero values indicate no inhibition.

Concentration of streptomycin used = 0.04 mg/ml.

Table 3. The minimum inhibitory concentration (MIC) of hydroethanolic extract of *B. sapida* stem bark assayed against *Staphylococcus aureus* and *Bacillus subtilis*

Concentration (mg/ml)	Test Organisms	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
100	-	-
50	+	-
25	+	-
12.5	+	-
6.25	+	+
3.125	+	+
1.5625	+	+

Key: - = Clear (no growth); + = turbid (growth).

Table 4. The minimum bactericidal concentration (MBC) of hydroethanolic extract of *B. sapida* stem bark assayed against *Staphylococcus aureus* and *Bacillus subtilis*

Concentration (mg/ml)	Test Organisms	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
100	+	-
50	+	-
25	+	-
12.5	+	+

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References

- Antwi, S., Martey, O. N. K., Donkor, K., & Nii-Ayitey Okine, L. K. (2009). Antidiarrhoeal Activity of *Blighia sapida* (Sapindaceae) in Rats and Mice. *Journ. Pharmacol. Toxicol.*, 4(3), 117-125. <http://dx.doi.org/10.3923/jpt.2009.117.125>
- Brown, M. R. (1975). A Question of Resistance. *Pharm. Journ.*, 215, 239-242.

- Collins, C. H., & Lyne, P. M. (1970). *Microbiological Methods* (13th ed.). Butterworth and Co. Ltd, pp. 414-427.
- Cowan, S. T. (1985). *Manual for the Identification of Medical Bacteria* (2nd ed.). Cambridge University Press, England.
- Duraipandiyar, V., Ayyaner, M., & Ignacimuthus, S. (2006). Antimicrobial Activity of Some Ethnomedicinal Plants used by Paliyar tribe from Tamil Nadu, India. *BMC Compl. Altern. Med.*, 6(35). <http://dx.doi.org/10.1186/1472-6882-6-35>
- Ekpo, M. A., & Etim, P. C. (2009). Antimicrobial Activity of Ethanolic and Aqueous Extracts of *Sida acuta* on Microorganisms from skin infection. *Journ. Med. Pl. Res.*, 3(9), 621-624. Retrieved from <http://www.academicjournals.org/JMPR>
- Etukudo, I. (2003). *Ethnobotany. Conventional and Traditional Uses of Plants*. The Verdict Press, Uyo. 191.
- Evans, W. C. (2002). *Trease and Evans Pharmacognosy* (15th ed.). (pp. 135-150). W.B. Saunders Company Ltd.
- Harborne, J. B. (1984). *Phytochemical Methods*. Chapman and Hall, London, pp. 166-226. <http://dx.doi.org/10.1007/978-94-009-5570-7>
- Ibrahim, M. B., Owonubi, M. O., & Onaolapo, J. A. (1997). Antimicrobial effects of extracts of leaf, stem and root bark of *Anogiessus leicarpus* on *Staphylococcus aureus* NCTC 8190, *Escherichia coli* NCTC 10418 and *Proteus vulgaris* NCTC 4636. *Journ. Pharm. Res. Develop.*, 2, 20-26.
- Murray, P., Baron, E., Pfaller, M., Tenover, F., & Tenover, R. (1985). *Man. Clin. Microbiol.* (pp. 67-72). Washington, D. C.: ASM Press.
- Okokon, J. E., Antia, B. S., Igboaso, A. C., Essien, E. E., & Mbagwu, H. O. C. (2007). Evaluation of anti-plasmodial activity of ethanolic seed extract of *Picralima nitida*. *Journ. Ethnopharmacol.*, 4514, 1-4.
- Sher, A. (2009). Antimicrobial Activity of Natural Products from Medicinal Plants *Glo. Journ. Med. Sci.*, 7(1), 72-78.
- Sofowora, A. (2006). *Medicinal Plants and Traditional Medicine in Africa* (pp. 150-156). Spectrum Books Limited, Ibadan.
- Sukumar, K., Perich, M. J., & Boobar, L. R. (1991). Botanical Derivatives in Mosquito Control: *A Rev. Journ. Amer. Mosq. Cont. Assoc.*, 7, 210-212.
- Ubulom, P., Akpabio, E., Udobi, C. E., & Mbon, R. (2011). Antifungal Activity of Aqueous and Ethanolic Extracts of *Picralima nitida* seeds on *Aspergillus flavus*, *Candida albicans* and *Microsporum canis*. *Res. Pharm. Biotech.*, 3(5), 57-60.
- Udobi, C. E., & Onaolapo, J. A. (2009). Phytochemical analysis and antibacterial evaluation of the leaf, stem bark and root of the African locust bean (*Parkia biglobosa*). *Journ. Med. Pl. Res.*, 3(5), 338-344.
- Udokpoh, A. E., Peace, M., Ubulom, E., Arnold, C., Sinyefori, A., Edoho, J., & Ekaete, I. (2009). In vitro antibacterial activities of the seed extract of *Picralima nitida*. *Journ. Pharm. Bioresour.*, 6(2), 1-5.
- World Health Organization. (2006). *Traditional Medicine Strategy*. (p. 260). Switzerland: WHO Geneva.