

Short Communication

Screening of the leaves of three Nigerian medicinal plants for antibacterial activity

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The leaves of three plants, *Alstonia boonei*, *Morinda lucida* and *Petiveria alliacea*, and latex of *A. boonei* were screened for antibacterial activity. In evaluating antibacterial activity, both aqueous and ethanol extracts of the plants were used. Agar well diffusion method was used to determine the antibacterial activity of the plants. Among the bacterial screened, *Pseudomonas aeruginosa* was the most resistant bacterial strain, while *Flavobacterium* sp., the most susceptible one. *M. lucida* extract was active against all the tested bacteria. Therefore, this plant can be selected for further investigation to determine its therapeutic potential. The latex of *A. boonei* was not active against any of the bacteria tested.

Key words: Antibacterial activity, medicinal plants, *Alstonia boonei*, *Morinda lucida*, *Petiveria alliacea*, Nigeria.

INTRODUCTION

The use of plants as source of medicine in treating disease is an ancient practice. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands of indigenous plants dating back to pre-history (Cowan, 1999). The widespread use of herbal remedies and health care preparation, such as those described in the ancient text like the Bible and the Vedas, has been traced to the occurrence of natural product with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines (Nair et al., 2005). In recent times, attention has been reverted back to plants as sources of therapeutic agents due to their higher properties. These include among others reduced cost, relative lower incidence of adverse reactions compared to modern conventional pharmaceuticals (Karachi, 2006), and ready availability.

The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno-medicinal plants in Nigeria and Africa. Nevertheless, the increased incidence of diseases for which there is yet an effective remedy is a driving factor for more screening programmes. Diseases like Acquired Immune Deficiency Syndrome (AIDS), multiple sclerosis, Parkinson's disease, Alzheimer disease and certain cancers still pose a major challenge to modern chemotherapeutic agents. It is in this context that the latex of *Alstonia boonei* and leaves of *A. boonei*, *Morinda lucida* and *Petiveria alliacea* were

screened for antibacterial activity.

A. boonei De Wild belongs to the family Apocyanaceae. This tree is 30 m high and 3 m in girth, with straight trunk, deeply fluted at the base and the branches are whorled (severally radiating from the trunk at the same level especially in the young trees). The leaves are simple whorled and are confined towards the ends of the twigs to give a rather small crown (Burkill, 1985). The bark is grayish, rough and has small scattered lenticels. Its slash is thick, granular, mottled yellow and exudes a copious white latex. Traditionally, the infusion of the stem bark is drunk as a remedy for snake-bite, and also for arrow poison. It is also used for treating fever and the infusion of root, stem bark and leaves are drunk as remedy against asthma.

Petiveria alliacea belongs to the family, Phytolaccaceae, a herb used in tropical America and established in Southern Nigeria. It is used as a remedy for whooping cough and the plants leaves are prepared as poultice for tumours.

Morinda lucida is a medium size tree with a crooked hole and rather short twisted branches. It belongs to the family, Rubiaceae. The bark is rough, grey in colour, flaking off in irregular patches. Its leaves are about 7-15 cm long by 3.5 - 7.5 cm broad, and flowers are white with a narrow glabrous corolla-tube about 2.5 cm. It is used as an astringent and antiseptic for ulcerating abscess, exudates is rubbed on affected area. In some parts of Nigeria, its stem bark, roots and leaves which are bitter

Table 1. Plants used in this study.

Plant	Family	Part of plant used	Uses
<i>Alstonia boonei</i> De Wild	Apocynaceae	Leaf Latex	Liver compliant, malaria. Swellings caused by filarial worms.
<i>Morinda lucida</i> Benth	Rubiaceae	Leaf	Treating sore, abscesses, Chancre, Leprous maculare, fever, ringworm.
<i>Petiveria alliacea</i> (L)	Phytolaccaceae	Leaf	Tumours, febrile, venereal diseases, Whooping cough inflammatory conditions.

Table 2. Effects of *Morinda lucida* aqueous leaf extract on test bacteria at different concentrations.

Bacteria	Zone of inhibition (mm)			
	250 mg/ml	500 mg/ml	750 mg/ml	1000 mg/ml
<i>Staphylococcus aureus</i>	-	4	8	14
<i>Salmonella typhi</i>	-	4	8	15
<i>Klebsiella pneumoniae</i>	-	7	10	16
<i>Pseudomonas aeruginosa</i>	-	-	-	-
<i>Esherichia coli</i>	5.5	8	12	18
<i>Bacillus subtilis</i>	8	10	16	23
<i>Flavobacterium</i> sp.	9	11	20	25

- = No inhibition.

are astringent used in the treatment of fever, malaria, jaundice and dysentery (Burkil, 1997).

MATERIALS AND METHOD

Preparation of extracts

The leaves of three flowering Plants *A. boonei*, *M. lucida*, and *P. alliacea* were collected from the nursery section of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria between December, 2002 and April, 2003. They were dried and hammer milled. Identification of plants was carried out using available information at the Botany and Microbiology Department's herbarium.

Hammer milled samples (100 g) were soaked in 500 ml each of water and absolute ethanol for 24 h and filtered through another 500 ml of either water or absolute ethanol and the combined filtrates were evaporated in water bath maintained between 60 - 70°C. The extracts obtained were placed in sterile labelled containers.

The latex of *A. boonei* was obtained by making incision on the bark of the tree after sterilizing with alcohol. The white latex was collected in labelled sterile bottle and then transferred to the laboratory. The latex was utilized the same day to avoid deterioration.

Bactericidal screening

The pure cultures of two gram-positive and five gram-negative bacteria were obtained from the medical and parasitology laboratory of the University College Hospital, Ibadan. These include *Bacillus subtilis*, *Staphylococcus aureus*, *Esherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Flavobacterium* sp. They were collected in slants in McCartney bottles containing nutrient agar. They were then stored until required.

A known weight of each extract (ethanol and water) was dissolved

in a little volume of sterile distilled water or ethanol to give the desired concentration of extract in milligram (mg). The bacterial suspensions were cultured in peptone water for 24 h. 0.2 ml of 10⁶ cfu/ml was used as inoculum for all except for *B. subtilis* which inoculum was 0.2 ml of 10⁴ cfu/ml. Each inoculum was mixed with 20 ml of nutrient agar in Petri dishes. Wells (6 mm in diameter) were punched in the agar medium using sterile stainless cork borer before being filled with 0.1 ml of plant extracts or solvent. The plates were incubated for 24 h at 37°C and the diameter of any resulting zone of inhibition was measured. Each combination of bacteria and extracts was repeated twice.

One milliliter of diluted latex of *A. boonei* was mixed with 20 ml of nutrient agar at 45°C. 0.1 ml of inoculum was spread in the solidified agar with latex and then incubated at 37°C for overnight. Inocula were prepared by growing test bacteria in peptone water for 24 h at 37°C. Other plates containing bacterial suspension with no latex were also incubated. Tests were performed in duplicates.

RESULTS AND DISCUSSION

Table 1 shows the list the plants used in this study with information on their traditional applications. Table 2 summaries the result obtained from screening of aqueous leaf extract of *M. lucida* at different concentrations. *P. aeruginosa* was resistant to this extract at all concentrations considered, while the other test bacteria were susceptible. Judging by the diameter of the zones of inhibition obtained, *Flavobacterium* sp., the causative agent of infant meningitis (Topley and Wilson, 1975), was the most susceptible organism to this extract. This is probably why in folk medicine the infusion of leaf is used as medicine for young children. The infusion of the leaf is usually given along with other herbs as a tonic from few

weeks of birth till weaning period (Burkil, 1997). In general, for all susceptible organisms, activity increased as concentration of extract increased.

The latex of *A. boonei* was not potent against any of the organisms tested. It was observed that the microbial colonies were larger than the colonies in control plates in all bacteria tested. No doubt, the latex must have served as enrichment medium to the tested bacteria. Similarly, both aqueous and ethanol extract of *A. boonei* was not potent against the bacteria. A similar result was obtained by Anani et al. (2000) who reported that methanolic leaf extract of *A. boonei* was not potent against all the bacteria tested. However, the stem bark of *A. boonei* is potent against bacteria tested (Adomi, 2006).

P. alliacea was not potent against the bacteria tested as well. However, Musahsearch Laboratory (<http://www.rabimusah.com/organosulfur.htm>) reported that this plant has antimicrobial effects; hence its use in folk medicine.

Conclusion

Aqueous leaf extract of *M. lucida* was the only active extract among the ones screened for antibacterial activity. Additionally, there is still need to study the two previous plants which were not potent in this study. The fact that a biological activity was not detected does not mean that the plant is uninteresting. It may contain natural compounds with activities or useful compounds which can be modified to provide potent therapeutics (Hostettmann et al., 2000).

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