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Full Length Research Paper

Antibacterial activity of aqueous and ethanol crude extracts of the root barks of *Alstonia boonei* and preliminary phytochemical test Of *Morinda lucida*

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The antibiotic activity of aqueous and ethanol extracts of the root bark of two plants *Alstonia boonei* De wild and *Morinda lucida* locally used for treating different diseases especially fever were studied against seven bacteria. Crude extracts obtained from *A. boonei* were not potent against any of the bacterial tested. *Bacillus subtilis* was the most sensitive organism to the ethanol root bark extracts of *M.lucida* while *Pseudomonas aeruginosa* was the least sensitive. Difference in antibacterial activity of these plants show that different bioactive components are present in each species. Preliminary phytochemical test of *M. lucida* plant parts showed presence of saponins, anthraquinone, cardenolides, alkaloids but absence of tannins.

Key words: Antibacterial activity, preliminary phytochemistry, *Morinda lucida, Alstonia boonei.*

INTRODUCTION

Antibiotics are naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria, generally at low concentrations (Brooks et al., 2007). Antibiotics was actually first discovered in 1896 by a French medical student named Ernest Duchesne. His work was forgotten until Alexander Fleming accidental rediscovery in 1928 (Prescott et al., 2008).

Antibiotics though rediscovered by Alexander Fleming in (1928) did not find wide usage until 1940s. Countless lives have been saved since then. The success of antibiotics against disease causing microbes is among modern medicines' great achievements. However, this kind of drug is beginning to loose its usefulness due to the development of resistance on the part of microbes.

One of the impact of antibiotic resistance is the emergence of microbes which are difficult to treat, which may eventually lead to increase cost of disease management and in the long run lead to increased morbidity and mortality.

The use of medicinal plants for therapy is an ancient practice. Though much work has been done on ethno medicinal plants, there is still need to seek plants with medicinal value to combat diseases. Diseases such as Acquired Immune Deficiency Syndrome (AIDS), multiple sclerosis, cancer still pose a major challenge to modern chemotherapeutic agents. The emergence of multi drug resistance tuberculosis (MDR TB) and even extremely drug resistant tuberculosis (XDR TB) is a signal for the need to search for more therapeutic agents. World Health Organization estimates that 500,000 people world wide have MDR TB, while the frequent fatal XDR TB has been detected in 46 countries. It is in this view that further studies on the antibacterial activity of the root bark of *Alstonia boonei* and phytochemical test of *Morinda lucida* was done as a follow up of the other parts of the plants which have earlier been investigated (Adomi, 2006; Adomi, 2008).

Alstonei boonei

A. boonei De wild belongs to the family Apocyanaceae. The tree is 39 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

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whorled and are confined towards the end 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 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 is drunk as remedy against asthma.

Morinda lucida

This 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. Slash is yellow. Leaves are about 7 – 15 cm long by 3.5 – 7.5 cm broad; 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. Its stem bark roots and leaves which are bitter are astringent, and used in the treatment of fever, malaria, Jaundice and dysentery (Burkill, 1997).

MATERIALS AND METHODS

Collection of plant materials

The rootbark of *A. boonei* and *M. lucida* were obtained from the nursery section of the Department of Botany and Microbiology and the Botanical garden, University of Ibadan, Ibadan. They were sun-dried and hammer milled. Identification of plants was carried out using available information at the Botany and Microbiology Department's herbarium.

Preparation of extracts

Pulverized root bark were extracted using water and ethanol

The aqueous extraction was done by weighing 100 g of powdered plant into flat bottom flask containing 500 ml of de-ionized water. The flask was swirled to mix. The flask was left to stand on the laboratory bench for 24 h. After which the mixture was filtered with Whatman filter paper No 1 to obtain clear solution of extract. The filterate was then evaporated to dryness under waterbath regulated within 60 – 70°C the solid crystal/oily extract obtained was weighed and then kept in sterile labelled McCartney bottles and stored at 4°C. The ethanol extract was obtained using the same method.

Collection and maintenance of test organisms

Clinical isolates of *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Flavobacterium sp.* and *Salmonella typhi* were collected from the Medical and Parasitology Laboratory, University College Hospital, Ibadan Nigeria. They were collected in McCartney bottles containing nutrient agar slants and stored at 4°C in labelled bottles until required. Further subcultures were carried out at 2-weekly intervals to maintain the viability of these organisms.

Inoculum

The microorganism were inoculated into peptone water and incubated for 24 h at 37°C. Serial dilution was prepared from the stock cultures up to the eighth dilution. The inoculum used was 0.2 of 106 cfu/ml for all the organisms except for *B. subtilis* where the inoculum of 0.2 of 104 cfu/ml was used. Each inoculum was mixed with 20 ml of nutrient agar after sterilization and cooling to 55°C

The agar well diffusion technique

This was carried out using the method of Kela and Kufeji (1995). The plates containing nutrient agar was seeded with inoculum obtained as described above. Wells of six millilitre diameter was punched using a sterile stainless steel punch before being filled with plant extract diluted within a range of 250, 500, 750 and 1000 mg. Standard antibiotics (ampiclox and chloramphenicol) were used as positive control, while the solvents (water and Ethanol) were the negative control. Plates were left on bench for one hour before incubation at 37°C for 24 h to allow pre-diffusion of the extracts (Esimone et al., 1998). The resulting zone of inhibition was measured using a ruler. Each combination of extracts was repeated twice.

Preliminary phytochemical screening

Preliminary phytochemical tests *of M.lucida* was carried out to confirm the presence of alkaloids using Drengenduff's test and Mayer's test, presence of cardenolides was confirmed by using Keller-Killani test and Kedde test, anthraquinones was tested by using chloroform/ammonia test while saponins, and tannins were confirmed using standard phytochemical methods as described by Sofowara (1993) and Trease and Evans (2002).

RESULTS AND DISCUSSIONS

Table 1 shows the results obtained from the preliminary test of the plant extracts. Both the aqueous and ethanol extracts of *A. boonei* were not active on the bacteria tested. *A. boonei* is used locally for the treatment of diseases such as fever and malaria (Burkill, 1997), Omoregbe et al. (1996) reported that ethanol extract of *A. boonei* was active against *S. typhi* and *S. paratyphi* the causative agents of typhoid fever. Similarly, the aqueous extracts of the stem bark of the plant was active against the two gram-positive and five gram-negative bacteria tested (Adomi, 2006). Asita and Campbell (1990), reported the antibacterial activity of the smoke extract of the wood of this plant against *S.aureus*. However, the evidence of activity is lacking in this study. The lack of response of the test bacteria to the root bark extract of *A. boonei* could be attributed to a reduced level of bioactive component. Seasonal variation, among other reasons can affect the chemical composition of the plant and thus its biological activity. In most cases, maximum accumulation of chemical constituents occurs at the time of flowering which then declines at the beginning of the

Table 1. Preliminary screening of crude root bark extracts of *M. lucida* and *A. boonei* for antibacterial activity.

			Test Bacteria						
Plant	**Type of extract**	**Concentration (mg/ml)**	***S. aureus***	***S. typhi***	***P. aeruginosa***	***B. subtilis***	***E. coli***	**Flavobaterium sp**	**K pneumonia**
Apocynaceae									
Alstonia									
Boonei									
Root	Ethanol	1000-	-	-	-	-	-	-	-
	Aqueous	1000-	-	-	-	-	-	-	-
Rubiaceae									
Morinda									
lucida									
Root	Ethanol	1000+	+	+	+	+	+	+	+
	Aqueous	1000+	+	-	-	-	-	+	-

+ potent
-not potent.

Table 2. Effect of aqueous root extracts of *M. lucida* and antibiotics.

Bacteria	**Zone of inhibition (mm) Concentration (mg/ml)**				**Standard**	**Antibiotic**
	250	**500**	**750**	**1000**	**A**	**B**
Staphylococcus aureus	-	6	8	10	22.50	17.00
Salmonella typhi	-	-	-	-	30.00	26.00
Klebsiella pneumoniae	-	-	-	-	25.00	18.50
Pseudomonas aeruginosa	-	-	-	-	21.50	19.00
Esherichia coli	-	-	-	-	25.00	20.50
Bacillus subtilis	-	-	-	-	25.00	28.00
Flavobacterium sp	-	5	8	16	24.00	10.00

- No inhibition. Concentration of antibiotics 250mg/ml. A-Chloramphenicol B-Ampiclox

fruiting stage (Mendonca-Filho, 2006). Makinde et al. (1994) observed this in their reports on *M. lucida* leaf extract against *Plasmodium berghei berghei in* mice. This may indicate that at certain period of the year, the bioactive component present in some plant specimen may have reduced to minimum level such that when the plants is tested again, contrary results may be obtained. This could explain why certain plants are used within certain period of the year for effective cure of disease in ethno medicine. State of maturity is another factor. It was reported that younger leaves of tropical rainforest plants contained secondary metabolites that were either present in very little quantities or totally absent in matured leaves. The extracts from these younger leaves showed better biological activity when tested for anticancer activity and against *B. subtilis* and *Artemia salina* (brime shrimp) (Kursar et al., 1999).

In contrast, *M. lucida* (Tables 2 and 3), crude extracts displayed appreciable activity against the bacteria tested compared to standard antibiotics used. *M. lucida* has a wide usage in traditional medicine. It is used for treating yellow fever and other forms of fever. It also has application in the treatment of sore, abscesses, chest complaints and other diseases (Burkill, 1997). In this study, ethanol crude extract was very active against the test bacteria. The zones of inhibition produced by the crude extracts are comparable to those obtained from the standard antibiotic which lack impurity of any sort. *B. subtilis* was the most sensitive organism to this extract.

Whilst Akinyemi et al. (2000) study showed that aqueous extract of *M. lucida* was potent against *S. typhi* and *S. Paratyphi.* In this study, the ethanol extract of root bark was more potent compared to the aqueous crude extracts of the plant (Tables 2 and 3).

The preliminary phytochemical results (Table 4) of *M. lucida* stem, root bark and leaf show that saponins were expressed in all parts of the plant. Anthraquinones were present in the stem bark and root bark of the plant as reported by Adesida and Adesogan (1972), and Adesogan (1972). While in the report of the stem bark of

Table 3. Effect of ethanol root extracts of *M. lucida* and antibiotics.

Bacteria	Zone of inhibition (mm) Concentration (mg/ml)				Standard	Antibiotic
	250	500	750	1000	A	B
Staphylococcus aureus	-	10	12	18	22.50	17
Salmonella typhi	-	8	11	12	30.00	26
Klebpsiella pneumoniae	10	12	14	18	25.00	18.5
Pseudomonas aeruginosa	-	-	8	12	21.50	19
Esherichia coli	12	14	16	18	25.00	20.50
Bacillus subtilis	13	15	17	19	25.00	28
Flavobacterium sp	6	8	10	12	24.00	10

- No inhibition. Concentration of antibiotics 250 mg/ml. A-Chloramphenicol B- Ampiclox.

Table 4. Preliminary phytochemical test of *M. lucida* extracts.

S/N	Constituents/Test	Leaves	Stem	Root
1		**Alkaloids**		
	Drengendoffs	+	+	-
	Mayers	+	-	-
2		**Cardenolides**		
	Keller-killani test	+	+	+
	Kedde test	+	+	+
3	Antraquinones			
	Chloroform/ammonia test	-	+	+
4		**Saponins**		
	Frothing test	+	+	+
5		**Tannins**		
	Ferric chloride	_	_	_

M. lucida tested by Fasola and Ogunyomi (2005), anthraquinones were absent in both old and new stem bark of *M. lucida* tested. Alkaloids were also present in the leaves, stem but absent in root bark. Cardenolides were present in the three parts of the plant. In this present study however, tannins were absent in all the parts of plant examined. This also agrees with the reports of Fasola and Ogunyomi (2005) for the stem bark of *M. lucida.*

Conclusion

This study has shown that plants used in traditional medicine have antibacterial activity but bioactive components of the plant may vary. The characterization of the active component of *M lucida* may lead to commercialization of the plant for large scale use.

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