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ANTIMICROBIAL ACTIVITY OF THE AQUEOUS AND ETHANOLIC EXTRACTS OF THE STEM BARK OF *ALSTONIA BOONEI*

***O.O. Amole [1] and O. O. Ilori [2]**

[1]Lagos State University, College of Medicine, Department of Pharmacology, Ikeja, Lagos, Nigeria.
[2]University of Lagos, College of Medicine, Department of Pharmacology, Idi Araba, Lagos, Nigeria.

ABSTRACT

Aqueous and ethanolic extracts of the stem bark of *Alstonia boonei (*Apocynaceae), *AB,* were screened for antimicrobial activities against *Candida* species, *Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes, Bacillus subtilis* and *Staphylococcus aureus* using the agar diffusion method. The extracts of *AB* produced a concentration dependent antimicrobial activity. LD_{50} obtained by oral route was calculated to be 6.17g/kg. The results of this study showed that the aqueous and ethanolic extracts of *Alstonia boonei* are potent antimicrobial agent.

Keywords:- *Alstonia boonei,* antimicrobial activities, agar diffusion, phytochemicals.

INTRODUCTION

A large portion of the world population especially in developing countries depend on traditional system of medicine for treatment of a variety of diseases caused by microorganism(Ahmad *et al.,*1998; WHO, 1993, Akinmoladun, 2007). This has been attributed to two main factors, inaccessibility of modern drugs to many people in the rural areas and the economic factor. It is reported that at least 25% of the prescription drugs issued in United States of America (USA) and Canada were derived from or modeled after natural products (Farnsworth, 1994). In addition, antibiotics are sometimes associated with adverse effects on the host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions.

Therefore, the need for development of alternative antibacterial and antifungal agents for treatment of infectious diseases has arisen . *Alstonia boonei,* Apocynaceae, is a decidous plant abundant in rain forest regions of Senegal to western Cameroon extending across Africa to Egypt, Sudan, Uganda and Zaire. Ascribed local names include Awun (Nigeria), Sinupo (Ghana), Mujina (Uganda), Botuk (Cameroon), Emien (Ivory coast), and Kaini (Sierra leone). In Traditional African Medicine , *Alstonia boonei* is used for the treatment of chronic diarrhea and dysentery, fever, pain, intestinal disorders and as an antidote for strophanthus poison. In view of these facts, this study was conducted to investigate the antimicrobial activity of the aqueous and ethanol stem bark extracts of *Alstonia boonei.* No report of such study was found in the course of literature research.

***Corresponding author**

Dr. O.O. Amole
E mail id:- femiamole@yahoo.co.uk

Materials and Methods

Plant material

The plant material was collected from a farm in Oshogbo, Osun state, Nigeria. Botanical identification and authentication was done by Mr. T. O. Odewo, Senior Superintendent of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen with identification number FH1108275 was deposited in the herbarium of the institute.

Preparation of plant extract

The stem bark of AB was air dried and hammer milled. Hundred grams (100g) of the hammer milled sample was soaked in 500 ml of water for water extraction and 500 ml of 70% ethanol separately for 24hours. Then each was filtered through Whatman No. 1 filter paper. The resulting filtrates were then evaporated in water bath maintained at 45^{0c} to dryness. The resulting stock was then measured and the weight compared to that of difference between the sample before soaking and the dry weight after soaking. From the stock, various concentrations were obtained.

Experimental animals

Albino mice (15-25g) of either sex obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria were used for this experiment. The animals were fed with rodents chow and had free access to drinking water. All experiments were performed in compliance with institutional; and international policies governing the human and ethical treatment of experimental animals as contained in United States National Institutes for Health Guidelines (1985).

Acute toxicity test

Groups of mice of both sexes (5 per group) fasted for 12 h prior to the test were given AB at doses of 2.5, 5.0, 7.5 and 10g/kg. One group was orally administered distilled water. Animals in each group were observed for any signs of toxicity and mortality within 24 h. The LD_{50} was estimated by the logdose-probit analysis.

Antimicrobial studies

Both local and standard strains of six microorganisms were used. The microorganisms were gram positive and gram negatives. Five strains of each organism were used. The bacteria used include *Staphylococcus aureus*, *Streptococcus pyogenes Bacillus subtilis, Pseudomonas aeruginosa*, *Escherichia coli.*

Susceptibility test

This was done using the agar diffusion method of Boakye-Yiadom (1977). The organisms for use which were present on slopes in bottle were collected with wire loop and streaked on the Muller hinton agar for subculturing. The subcultured organisms were then incubated for 24 h and at the end of which the organisms were observed for growth. From the growth observed, they were collected with cool flamed wire loop into broth media measured into bottles The inoculation was then gently shaken. From the inoculum, 1 ml of the organism containing broth was measured and emptied into Petri dish. Twenty milliliters (20 ml) of the nutrient agar already sterilized at 121^{oc} was allowed to cool to 45^{oc} and was then poured on the inoculum in the Petri dish. It was then gently swirled for even mixing of the organism. The plates were then allowed to set, divided into four sectors and holes were bored into each sectors' middle using metallic hole borer. Into the hole present in each sector,30ul of following concentrations of the aqueous and ethanolic extract was aseptically transferred into each respective well: 1000, 500, 250 and 125mg/ml filling them to the brim. This was then allowed to diffuse for at least 2 hr. After this, they were taken to the incubator for incubation at 37^{oc} for 24 hr. The same process was carried out for the standard, chloramphenicol and ciprofloxacin. Ethanol and water were also used for the susceptibility test.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) count.

The minimum inhibitory concentration is a test aimed at determining minimum concentration of the extract that will inhibit the growth and activity of the microorganisms. This is usually within a 24hr period, The minimum bactericidal concentration (MBC) is aimed at determining the minimum concentration of the extract that will kill the microorganisms such that a concentration lower than this will not kill them. The MBC is usually taken in 48 and 72 hours period. For the MIC and MBC, serial dilution of each extract was carried out of the order 1/5, 1/10, 1/20, 1//40, 1/80, 1/ 160 and 1/320.

The value of each dilution in mg/ml was obtained. For each concentration, a volume of each was taken which is made up to 20 ml with a corresponding volume of nutrient agar, which together was poured into Petri dish and allowed to set after swirling. The agar plates were then taken to the oven for dryness at 60^{oc} for 20minutes. Then the microorganisms present in the broth media were then sub cultured to reduce their viable count to about 1 in 10^6. Each plate was then divided into 10 sectors. Onto each sector, 10ul of each organism was spotted. Then the plates were incubated after spotting for 24hr. The MIC was taken based on the inhibition of organisms spotted. The plates were then reincubated for further 24 and 48 hours for the count of MBC.

Statistical analysis

Values are reported as mean $\pm$SEM. Statistical analysis was carried out using Student's t-test to calculate significance of difference. P values <0.05 were considered significant.

Results and Discussion

The phytochemical screening of the aqueous and ethanolic extracts of *Alstonia boonei* gives the indication of bioactive constituents which are alkaloids, saponin, tannin, steroid, flavonoid and cardiac glycosides. These biologically active constituents of the extracts are indicators to its antimicrobial activity(Oliver,1960;Reynolds,1982). In addition to the phytochemicals present in the stem bark extract of AB, it has also been suggested that it also contains macro elements such as calcium, magnesium, sodium, potassium, phosphorus, iron, zinc, manganese, copper and cobalt to varying degrees(Akinmoladun,2007). Alkaloids are medicinally useful, possessing analgesic, antispasmodic and bactericidal effects. Tannins promote healing (Okwu and Okwu,2004; Oliver,1960).

Cardiac steroids are widely used in treating congestive heart failure (Okwu and Okwu,2004). Flavonoids lower risk of heart diseases, saponins also promote wound healing(Okwu and Okwu, 2004). Hence the medicinal effects of plants have often been attributed to the antioxidant activity of their phytochemical constituent(Thabrew *et al.,*1998). A synergistic relationship amongst phytochemicals has been adduced to be responsible for the overall beneficial effect derivable from plants(Liu,2004). It has been suggested that the mineral elements present in plants may play roles in the medicinal value of the plants(Akinmoladun,2007). Considering the study of both extracts – aqueous and ethanolic of AB, it is much evident that the ethanolic extracts contain more active constituents than the aqueous extract. This was why the inhibitory effect of the ethanolic extract was more than that of the aqueous extract. Though equal concentrations were used in both cases, the ethanol extract was more potent.

This can be alluded to the fact that ethanol is a better extractant than water. Ciprofloxacin has broader spectrum of antimicrobial effect than chloramphenicol. This was why the zone of inhibition average for ciprofloxacin was more than that for chloramphenicol of equal concentration (Table 1). The better antimicrobial effect of the ethanolic extract as seen from the result of ethanol in Table 2 is due not to the ethanol itself, but as a result of the active constituents of AB. This was why absolute ethanol only gave a mild inhibition at 100% whereas ethanol at 70% (used for the extraction), 40% and 10% gave no inhibition. The LD_{50} was estimated to be 6.17 g/kg, doses higher than this will have a lethal effect on 50% of the population. The highest zone of inhibition was given by the aqueous extract at a dose of 1000 mg/ml on *Candida* sp while ethanolic extract gave the highest at 1000mg/ml on *Bacillus subtilis* and *Pseudomonas aeruginosa.* Though the standards were used at much lower concentration, they gave higher inhibition

From Table 3, ethanol extract gave a minimum inhibitory concentration at 250 mg/ml for all organisms used except *Pseudomonas aeruginosa* which gives high resistance to this concentration, except when the dose concentration was increased to 500 mg/ml. The aqueous extract gave a MIC value of 500 mg/ml for all the organisms except for *Pseudomonas aeruginosa* where a concentration higher than 500 mg/ml is required to inhibit.

Experimental evidence also indicate that the extracts of their MIC value will give good minimum bactericidal concentration (MBC) at 48 hours. A 72 hour count will however be highly resisted, except at much higher concentrations.

Table 1. Average zone of inhibition of each organism (mm) to ciprofloxacin and chloramphenicol

Organism	**Ciprofloxacin (ug/ml)**				**Chloramphenicol (ug/ml)**		
	50	**40**	**30**	**25**	**50**	**40**	**30**	**25**
Bacillus subtilis	25.40±0.93	24.00±1.05	22.20±0.86	20.00±0.63	16.00±1.18	15.00±1.18	13.80±1.16	12.40±1.21
Escherichia coli	25.80±1.16	24.00±1.18	22.20±1.32	20.60±1.40	17.20±1.32	15.80±1.24	14.40±1.12	12.80±1.24
Staphylococcus aureus	31.00±1.20	29.00±0.68	28.00±0.71	26.00±0.73	18.00±0.95	17.00±0.86	16.00±0.86	15.00±0.93
Streptococcus pyogenes	32.00±1.00	31.00±0.93	29.00±0.66	26.00±1.10	20.00±0.93	18.00±0.86	17.00±1.00	16.00±1.00
Candida species	33.00±2.80	30.00±2.70	28.00±2.40	25.00±2.10	18.00±1.10	17.00±1.20	16.00±1.30	14.00±1.50
Pseudomonas aeruginosa	27.00±2.60	26.00±2.40	23.00±2.00	21.00±1.90	16.00±0.58	15.00±0.58	14.00±0.51	13.00±0.51

Values are mean ± S.E.M.

Table 2. Average zone of inhibition of each organism to ethanol (negative control) (mm)

Organism	Concentration			
	100%	70%	40%	10%
Bacillus subtilis	18.70± 0.68	-	-	-
Escherichia coli	16.20±0.55	-	-	-
Staphylococcus aureus	20.00±0.63	-	-	-
Streptococcus pyogenes	16.90±0.58	-	-	-
Candida sp	19.00±0.75	-	-	-
Pseudomonas aeruginosa	18.40±0.68	-	-	-

Values are mean ± S.E.M

Table 3. Average MIC values for aqueous and ethanolic extracts of *Alstonia boonei*

Organism	**Ethanol extract (mg/ml)**	**Aqueous extract (mg/ml)**
Bacillus subtilis	250	500
Escherichia coli	250	500
Staphylococcus aureus	250	500
Streptococcus pyogenes	250	500
Candida sp	250	500
Pseudomonas aeruginosa	500	1000

Table 4. Average zone of inhibition of each organism (mm) to aqueous and ethanolic extracts of *Alstonia boonei*

Organism	**Aqueous (mg/ml)**				**Ethanol (mg/ml)**			
	1000	500	250	125	1000	500	250	125
Bacillus subtilis	18.00±0.71	16.80±0.58	15.40±0.51	14.20±0.58	20.60±1.03	19.20±1.02	17.20±1.02	15.20±1.39
Escherichia coli	15.60±0.51	13.00±0.45	12.00±0.45	10.60±0.40	16.00±0.55	14.40±0.81	13.00±.0.55	11.80±0.49
Staphylococcus Aureus	18.00±0.68	17.00±0.86	15.00±0.93	14.00±1.20	19.00±1.20	17.00±1.10	16.00±1.20	14.00±1.40
Streptococcus pyogenes	16.00±0.68	14.00±0.75	13.00±0.75	12.00±0.80	18.00±0.24	17.00±0.24	16.00±0.55	15.00±0.75
Candida sp	19.00±1.20	17.00±1.10	16.00±1.10	15.00±1.10	20.00±1.40	18.00±0.84	17.00±0.86	15.00±1.20
Pseudomonas aeruginosa	19.00±0.60	18.00±0.60	17.00±0.60	15.00±0.55	21.00±0.98	19.00±0.75	17.00±0.53	16.00±0.63

Values are mean ± S.E.M.

CONCLUSION

The results obtained in this study suggest that the stem bark of *Alstonia boonei* possesses antimicrobial activity and that the constituents of the plant extract could be useful in the chemotherapy of some microbial infections

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