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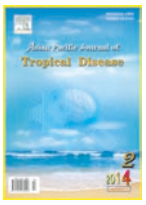

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Antimicrobial activities of *Alstonia boonei* stem bark, a Nigerian traditional medicinal plant

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ABSTRACT

Objective: To determine the *in vitro* antimicrobial activities of various solvent extracts of stem bark of *Alstonia boonei*, a Nigerian traditional medicinal plant against some microorganisms of food and clinical importance.

Methods: The antimicrobial activities of crude solvent extracts of stem bark were determined using well in agar diffusion method against *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* and *Kluyveromyces sp*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration were determined to establish the antimicrobial potential of extracts.

Results: The antimicrobial results revealed that ethanol extract produced maximum zone of inhibition (23.73 mm) against *E. coli*. All the extracts had no inhibitory activity on *Salmonella typhi* and *Pseudomonas aeruginosa* at the lowest concentration tested (3.2 mg/mL). MIC was determined at various concentrations and the least MIC (5.8 mg/mL) was produced by the crude ethanol extract on *E. coli*, while the least minimum bactericidal concentration (20 mg/mL) was also produced by the ethanol extract on *E. coli*. Ethanol and chloroform extracts had the highest and least inhibitory effects on the isolates, respectively.

Conclusions: The present study has revealed that the solvent extracts of *Alstonia boonei* stem bark possess potent antimicrobial activity that can be harnessed. It may also be a new source of antimicrobial compounds that could be used to combat drug resistance which has become a global challenge.

1. Introduction

Plants in recent times have become a valuable source of natural products for maintaining human health[1], especially in the developing countries where traditional medicine is the main source of medical care for a great proportion of the population[2-5]. The use of plants for treating various diseases is not new to man where they are used mainly at the primary health care level[6], and popular observations on the use and efficacy of these medicinal plants significantly contribute to the disclosure of their therapeutic properties[7]. Several countries in Africa and other parts of the world have continued to encourage screening programmes of plants used in traditional medicine in order to authenticate their antimicrobial activities and possible inclusion in primary health care[8-11], and several plants have been reported worldwide to possess antimicrobial activities[12-17].

Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans[18]. Despite efforts made by pharmaceutical companies to produce new and more effective antimicrobial drugs resistance to these antimicrobials, which continues to increase and remains a major global problem[19,20]. Thus, the

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demand for more drugs from plant sources is continuously increasing, thereby, necessitating the screening and investigation of more plants for bioactive antimicrobials, determination of their safety and efficacy[21-23].

Alstonia boonei De Wild (*A. boonei*) is a medicinal plant of West African origin, popularly known as Egbu in Igbo language. *A. boonei* De Wild belongs to the family Apocynaceae[24]. The species are scattered all over the world of which two are indigenous to Africa[25]. In some African countries, *A. boonei* is considered a sacred tree and worshiped in the forest, thus human beings in those countries do not eat its parts[26]. The bark of this tree has been found to possess anti-rheumatic[, anti-inflammatory, analgesic/pain-killing, anti-malaria/antipyretic, antidiabetic (mild hypoglycaemic), anti-helminthic and antimicrobial properties[24,27-30]. Traditionally, the stem bark and root/root bark are used for treatment of some infections and ailments in Nigeria[24]. The plant parts are rich in various bioactive compounds such as echitamidine, Nα-formylechitamidine, boonein, loganin, lupeol, ursolic acid, and β-amyrin among in which the alkaloids and triterpenoids form a major portion[25,26,31,32].

The use of plant parts in traditional medicine is a common practice in Nigeria[33-35]. Due to the indiscriminate use of antibiotics against pathogens of clinical importance, development of drug resistance has been on the increase these days, and it is possible that solution to this problem could be found in extracts of plants commonly used in traditional medicine. This work therefore aims at determining the antimicrobial properties of the extracts of stem bark of *A. boonei* on some microorganisms of food and clinical importance.

2. Materials and methods

2.1. Plant collection and identification

Fresh stem barks of *A. boonei* were collected from Umuguma, Owerri West Local Government Area of Imo State, Nigeria. The taxonomy of the plants were identified and authenticated. They were cleaned to remove sand and other extraneous materials.

2.2. Sample preparation and extraction procedure

The fresh stem barks were air dried for about four weeks and ground into fine powder under aseptic conditions using a mechanical grinder. About 20 g of the fine powder was weighed into 250 mL of ethanol (95%), methanol and chloroform in conical flasks. These were covered, shaken every 30 min for 6 h, and then allowed to stand for about 48 h. The solutions were subsequently shaken and filtered using Whatman filter paper. The filtrates were evaporated to dryness using a rotary evaporator (Model type 349/2, Corning Inc. USA) and stored at 4 °C until required for antimicrobial assay.

2.3. Preparations of dilutions of crude extract for antibacterial assay

The methods of Selvamohan *et al*., and Amole and Ilori were adopted with some modifications[3,36]. The crude extracts were dissolved in 5% dimethylsulphoxide and further diluted to obtain 200.00, 100.00, 50.00, 25.00, 12.50, 6.25 and 3.20 mg/mL concentrations. These were stored at 15 °C until required.

2.4. Test microorganisms

The organisms *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Salmonella typhi* (*S. typhi*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were isolated from clinical samples obtained from patients at the Federal Medical Centre, Owerri; while *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Kluyveromyces* sp. were isolated from fruits. They were reisolated, identified using standard methods and the pure cultures subcultured on nutrient agar (CM0 003, Oxoid) slants for bacteria and potato dextrose agar (CM0 139, Oxoid) for yeasts. They were stored at 4 °C until required for the study.

2.5. Evaluation of antimicrobial activity

The agar diffusion method as described by Amole and Ilori was adopted for the study[36]. A total of 15 mL of molten nutrient agar (CM0 003, Oxoid) and potato dextrose agar (CM0 139, Oxoid) were seeded with 1.0 mL of standardized broth cultures of bacteria and yeasts, respectively (approximately 1.0×10^7 CFU/mL) by introducing the broth cultures into sterile Petri dishes, incorporating the molten agar, rotating slowly to ensure uniform distribution of the microorganisms, and then allowed to solidify on a flat surface. Three holes were made in the plates (about 5.0 mm diameter) using a sterile cork borer and equal volumes of the extracts were transferred into the holes using a Pasteur's pipette. Two Petri dishes containing a particular microorganism were used for each concentration of the extract.

The plates were allowed to stand for 2 h for pre-diffusion of the extract to occur[35], and were incubated at 37 °C for 24 h.

At the end of incubation the plates were collected and zones of inhibition that developed were measured. The average of zones of inhibition was calculated.

The minimum inhibitory concentration (MIC) was determined using the method described by Rivera *et al*[37]. Each bacterial strain was grown in nutrient broth (CM0 067, Oxoid), while yeasts were grown on potato dextrose broth (CM0 962, Oxoid). Antimicrobial activity of plant extracts was evaluated in test tubes with screw cap. Each test tube was filled with 5 mL of sterile brain heart infusion broth medium for bacteria and potato dextrose broth for yeasts. The standardized broth cultures of the organisms were inoculated into the media with a sterile inoculating loop (approximately 1.0×10^7 CFU/mL). Inoculated test tubes were incubated at 37 °C for 24 h. The MIC of each sample was determined by measuring the optical density in the spectrophotometer (620 nm) (Perkin Elmer, USA) comparing the sample readout with the non-inoculated medium. All the samples were prepared in triplicates. Minimum bactericidal concentration (MBC) was determined using the method of Koochak *et al*[38]. The treated broth cultures from test tubes showing no visible growth in MIC assay were cultured on Mueller Hinton agar (CM0 337, Oxoid) plates for bacteria and potato dextrose agar for yeasts. The least concentration (highest dilution) of the extracts that inhibited colony formation on a solid agar medium after incubation at 37 °C and 30 °C for 24 h and 72 h, respectively for bacteria and yeasts was considered as MBC. The clinical strains were also tested for their sensitivity against the solvents used for the extraction of the stem barks.

The data obtained from the study was subjected to statistical analysis using ANOVA and the means were separated using Fisher's least significant difference. Microsoft Excel 2007 was used for data processing.

3. Results

The antimicrobial activity of stem bark of *A. boonei* on the isolates is shown in Tables 1-3. The result showed that the antimicrobial activities of the plant extracts increased with increase in concentration of crude extracts. However, the ethanol extracts generally produced higher antimicrobial effects on the isolates. The extracts showed prominent antimicrobial activity against *E. coli*, *S. aureus*, *S. cerevisiae* and *Kluyveromyces* sp. The chloroform extract had the greatest effect on *Kluyveromyces* sp., while the ethanol and methanol extracts had the greatest effect on *E. coli*.

The highest zone of growth inhibition was produced by the ethanol extract on *E. coli* (23.730 mm) at 200.00 mg/mL concentration, while the least was produced by the

Table 1
Mean zone of growth inhibition (mm) by methanol extracts of *A. boonei* stem bark.

Concentration of extract (mg/mL)	Microorganisms						LSD
	E. coli	*S. aureus*	*S. typhi*	*P. aeruginosa*	*S. cerevisiae*	*Kluyveromyces* sp	
3.20	5.78±0.02^{c}	4.81±0.01^{d}	–	–	6.83±0.04^{b}	6.90±0.00^{a}	0.052
6.25	8.45±0.00^{c}	7.29±0.10^{d}	4.42±0.03^{f}	5.76±0.05^{e}	8.93±0.02^{a}	8.64±0.00^{b}	0.088
12.50	11.60±0.00^{a}	10.40±0.00^{b}	7.45±0.00^{d}	8.82±0.03^{c}	11.35±0.00^{a}	11.49±0.01^{a}	0.670
25.00	14.75±0.00^{a}	13.24±0.05^{c}	10.61±0.01^{e}	11.93±0.01^{d}	14.61±0.01^{b}	14.75±0.00^{a}	0.055
50.00	17.63±0.02^{a}	16.40±0.00^{c}	13.78±0.00^{e}	14.81±0.01^{d}	17.62±0.02^{a}	17.27±0.03^{b}	0.060
100.00	19.51±0.01^{a}	18.56±0.05^{c}	15.91±0.01^{e}	16.30±0.00^{d}	19.24±0.01^{b}	19.50±0.00^{a}	0.114
200.00	22.76±0.02^{a}	21.40±0.00^{d}	18.27±0.04^{e}	19.45±0.00^{e}	22.50±0.00^{b}	22.35±0.00^{c}	0.127

±: Standard deviation of three determinations; ^{a,b,c,d,e,f}: Means with different superscript in the same row are significantly ($P<0.05$) different; –: Not detected, LSD: Least significant different.

Table 2
Mean zone of growth inhibition (mm) by chloroform extracts of *A. boonei* stem bark.

Concentration of extract (mg/mL)	Microorganisms						LSD
	E. coli	*S. aureus*	*S. typhi*	*P. aeruginosa*	*S. cerevisiae*	*Kluyveromyces* sp	
3.20	4.77±0.04^{c}	3.16±0.00^{d}	–	–	5.40±0.00^{b}	5.79±0.01^{a}	0.116
6.25	6.81±0.14^{c}	5.65±0.00^{d}	3.46±0.02^{f}	4.61±0.01^{e}	7.69±0.01^{a}	7.45±0.00^{b}	0.046
12.50	8.75±0.00^{c}	8.54±0.08^{d}	5.41±0.01^{f}	7.63±0.02^{e}	10.43±0.02^{b}	10.50±0.03^{a}	0.021
25.00	10.80±0.14^{c}	10.63±0.02^{d}	7.52±0.00^{f}	9.61±0.01^{d}	12.34±0.00^{b}	12.62±0.01^{a}	0.024
50.00	13.85±0.01^{c}	13.72±0.02^{d}	10.65±0.04^{f}	12.76±0.02^{e}	15.62±0.03^{b}	15.85±0.01^{a}	0.024
100.00	16.76±0.05^{c}	16.34±0.08^{d}	13.56±0.05^{f}	15.82±0.03^{e}	18.22±0.03^{b}	18.61±0.01^{a}	0.054
200.00	19.49±0.01^{c}	19.50±0.00^{c}	16.29±0.01^{e}	18.75±0.01^{d}	21.67±0.03^{b}	21.54±0.08^{a}	0.116

±: Standard deviation of three determinations; ^{a,b,c,d,e,f}: Means with different superscript in the same row are significantly ($P<0.05$) different; –: Not detected; LSD: Least significant different.

Table 3
Mean zone of growth inhibition (mm) by ethanol extracts of *A. boonei* stem bark.

Concentration of extract (mg/mL)	Microorganisms						LSD
	E. coli	*S. aureus*	*S. typhi*	*P. aeruginosa*	*S. cerevisiae*	*Kluyveromyces* sp	
3.20	6.450±0.000^{c}	5.870±0.040^{d}	–	–	6.710±0.010^{b}	7.500±0.000^{a}	0.116
6.25	9.400±0.000^{b}	8.600±0.000^{d}	3.390±0.010^{f}	4.450±0.000^{e}	9.250±0.000^{c}	9.700±0.000^{a}	0.173
12.50	11.650±0.000^{b}	10.920±0.000^{d}	7.610±0.010^{f}	8.910±0.000^{e}	11.500±0.000^{c}	11.820±0.020^{a}	0.116
25.00	14.810±0.001^{a}	13.750±0.000^{b}	8.950±0.010^{d}	8.660±0.060^{e}	13.520±0.100^{c}	13.760±0.050^{b}	0.024
50.00	18.370±0.100^{a}	17.560±0.050^{b}	13.910±0.010^{f}	14.810±0.080^{e}	17.830±0.020^{b}	17.420±0.240^{c}	0.035
100.00	21.750±0.070^{a}	20.600±0.000^{c}	16.750±0.000^{f}	17.620±0.010^{e}	20.460±0.020^{d}	20.660±0.020^{b}	0.019
200.00	23.730±0.040^{a}	22.240±0.000^{c}	18.600±0.000^{f}	19.920±0.030^{d}	22.680±0.000^{b}	22.650±0.000^{b}	0.117

±: standard deviation of three determinations; ^{a,b,c,d,e,f}: Means with different superscript in the same row are significantly (P<0.05) different; – : not detected; LSD : least significant different.

crude ethanol extract on *S. typhi* (3.390 mm) at 6.25 mg/mL concentration. It was also observed that the extracts (methanol, chloroform and ethanol) did not have any inhibitory effect on *S. typhi* and *P. aeruginosa* at 3.2 mg/mL concentration.

Statistical analysis of the values obtained for each of the organisms revealed that most of the values at each concentration were significantly different (P<0.05) from each other.

Results of the MIC of extracts on isolates are shown in Table 4. The least MIC was produced by the crude ethanol extract on *E. coli* (5.80 mg/mL), while the highest value was obtained with chloroform extract with a value of 27.61 mg/mL on *S. cerevisiae*. Statistical analysis also revealed that all the values were significantly different (P<0.05) from each other.

Table 4
MIC of extracts of *A. boonei* stembark (mg/mL).

Microorganisms	Methanol	Chloroform	Ethanol	LSD
E. coli	8.71±0.01^{5,b}	10.72±0.07^{6,a}	5.80±0.07^{5,c}	0.045
S. aureus	23.85±0.00^{1,c}	27.60±0.00^{1,a}	25.91±0.01^{1,b}	0.031
S. typhi	7.51±0.01^{6,b}	13.79±0.01^{5,a}	6.90±0.07^{5,c}	0.038
P. aeruginosa	19.65±0.07^{3,b}	25.74±0.01^{2,a}	12.65±0.07^{4,c}	0.146
S. cerevisiae	20.63±0.01^{2,b}	27.61±0.01^{1,a}	18.54±0.01^{2,c}	0.028
Kluyveromyces sp	15.84±0.01^{4,b}	18.70±0.00^{4,a}	14.57±0.01^{3,c}	0.028
LSD	0.119	0.032	0.02	

^{a,b,c}: Means with different superscript in the same row are significantly (P<0.05) different; ^{1,2,3,4,5,6}: Means with different superscript in the same column are significantly (P<0.05) different; ±: Standard deviation of three determinations; LSD: Least significant different.

The MBC values of extracts are presented in Table 5. The least value (20.00 mg/mL) was observed on *E. coli* by ethanol extract while the next in value (25.00 mg/mL) was shown by ethanol and methanol extracts on *S. typhi*, *S. cerevisiae* and *Kluyveromyces* sp. The highest value (100.00 mg/mL) was produced by chloroform extract on *S. aureus*, *P. aeruginosa* and *Kluyveromyces* sp. Some of the values were not significantly different (P>0.05) from each other, either among the extracts or among the isolates.

Table 5
MBC of extracts of *A. boonei* stembark (mg/mL).

Microorganisms	Methanol	Chloroform	Ethanol	LSD
E. coli	50.00±0.70^{1,a}	50.00±0.70^{2,a}	20.00±0.70^{1,b}	13.720
S. aureus	50.00±0.70^{1,b}	100.00±0.70^{1,a}	50.00±0.70^{1,b}	1.609
S. typhi	25.00±0.70^{2,c}	50.00±0.00^{2,a}	25.00±0.70^{2,b}	1.447
P. aeruginosa	50.00±0.70^{1,b}	100.00±0.70^{1,a}	50.00±0.70^{1,b}	1.440
S. cerevisiae	50.00±0.70^{1,a}	50.00±0.70^{2,a}	25.00±0.70^{2,b}	2.490
Kluyveromyces sp	50.00±0.70^{1,b}	100.00±0.70^{1,a}	25.00±1.41^{2,c}	13.110
LSD	2.81	2.56	3.44	

^{a,b,c}: Means with different superscript in the same row are significantly (P<0.05) different; ^{1,2}: Means with different superscript in the same column are significantly (P<0.05) different; ±: Standard deviation of three determinations; LSD: Least significant different.

4. Discussion

For centuries medicinal plants have been the main source for drugs in many countries and it is estimated that at least 25% of all modern medicines are derived either directly or indirectly from medicinal plants[4]. The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotics[39]. *A. boonei* is commonly found in the rain forest regions of Nigeria along with other kinds of plant species with good medicinal properties. The increase in bacterial diseases is becoming common in Africa especially in Nigeria, due to development of antibacterial drug resistance arising from misuse and abuse of antibiotics. The search for alternative sources for chemotherapeutic agents to combat the problem is therefore a global challenge.

The crude plant extracts tested in this study showed antimicrobial activity (bactericidal and bacteriostatic) on all isolates tested *viz.*, *E. coli*, *S. aureus*, *S. typhi*, *P. aeruginosa*, *S. cerevisiae* and *Kluyveromyces* sp. However, differences were observed between their antimicrobial activities. These differences could be attributed to the differences in the chemical composition and amount of the bioactive compounds extracted by the solvents. These compounds usually accumulate in different parts of the plant[4,37], and such secondary metabolites have been found to produce many effects including antibacterial and antiviral

properties[40,41]. The plant has been found to be rich in various bioactive compounds which include echitamidine, Nα-formylechitamidine, boonein, loganin, lupeol, ursolic acid, and β-amyrin, of which the alkaloids and triterpenoids form a major portion[25,26,31,32].

Among the solvent extracts, ethanol extract showed the highest antimicrobial activity with MIC of 5.80 mg/mL against *E. coli* and 6.90 mg/mL against *S. typhi*. This is worthy of note, thus partial purification of the crude extract could increase the antimicrobial activity since some of the components in the extracts could be antagonistic to other components responsible for the observed antimicrobial activity. Such natural plant metabolites are important as potential antimicrobial crude drug and source for natural compounds that can be used as new anti-infection agents[42], and probably in food preservation. However, the crude solvent extracts (methanol, chloroform and ethanol) did not have any inhibitory effect on *S. typhi* and *P. aeruginosa* at 3.2 mg/mL concentration. It may be that the concentration of extracts applied is too low to inactivate the bacterial isolates.

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes which has become a global challenge. Previous study showed the antimicrobial potentials of ethanol extracts of this plant[24].

This present study has revealed that ethanol and methanol extracts of *A. boonei* displayed considerable inhibitory activity against the test isolates which are food pathogens and food spoilage organisms. Thus, such extracts could be applied as antimicrobial preservatives in food. Experimental data available in this line is minimal.

Modern drugs in use today have undergone various levels of experiments to determine the safety and efficacy unlike some herbal preparations. Majority of persons that take these herbal remedies assumes that they are inherently safe because it is natural[26]. It is therefore important to conduct further studies on the extracts to ascertain their toxicological properties and develop standard methods for assuring safety and efficacy, since in sufficiently high doses they can cause serious harm to humans. Without well-documented information on these and phytochemical characteristics of the bioactive compounds the utilization of these natural resources from Africa will be difficult.

This study has shown that methanol, ethanol and chloroform extracts of *A. boonei* stem bark possess antimicrobial activities against the tested microorganisms, although the ethanol extract appears to be more active. The results of this study therefore support and justify the traditional use of the studied plant part in the treatment of some bacterial infections in Nigeria. Thus, the plant extracts have a great potential as antimicrobial compounds against microorganisms. Further work aimed at isolation, purification and characterization of the active compounds should be initiated thus leading to development of new antimicrobials that could help combat the problem of drug resistance being experienced globally.

Conflict of interest statement

We declare that we have no conflict of interest.

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