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In vitro antibacterial activities of the seed extract of *Picralima nitida*

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Abstract

The in vitro antibacterial activities of *Picralima nitida* seed extracts were studied against selected pathogenic bacteria. Seeds were extracted using ethanol, methanol and water. Among the three solvents used, seed extracts of ethanol and methanol were more effective against pathogenic bacteria, where the minimum inhibitory concentration (MIC) ranged between 31.0mg/l to 75.0mg/l and inhibition zone diameter (IZD) of between 11mm to 19mm. All the extracts were ineffective against *P. aeruginosa* and *S. typhi.* The antimicrobial activities of the seeds of this indigenous medicinal plant are discussed in this paper.

Keywords: Antibacterial activity, *Picralima nitida*, Seeds

Introduction

Various bacteria have been implicated in the aetiology of infections in different parts of the world especially in the tropics, with high prevalence of infection due to ignorance, unhealthy socio-cultural and religious practices, lack of public amenities, poor sanitation, poverty and inadequate access to health care (Udokpoh *et al*., 2005). Drug resistance, fake drug syndrome and high cost of newer effective drugs have been the major factors affecting the poor populace, thus making their choice of herbal remedies inevitable and economical (Okokon *et al*., 2007). *Picralima nitida*, family Apocynaceae, has varied usage in Nigeria and other West African sub region. Many traditional medicine practitioners have claimed to use the leaves, seed or stem-bark as treatment for various fevers, hypertension, jaundice, gastro-intestinal disorders, vomiting and for malaria. Various parts of the plant have been reported to be effective antipyretic, antihypertensive, hypoglycaemic and antitussive (Oliver 1960, Dalziel 1961., Ayensu 1978., Iwu 1993., Okokon *et al.,* 2007). The plant's seeds have been reported to contain alkaloids like akuammine, akuammicine, akuammidine, picratidine, akuammigine, pseudoakuammig-ine, picraline and picralicine (Guyledouble 1964; Moller *et al.,* 1972; Arens *et al.,* 1982; Ansa *et al.,* 1990). However, there is a paucity of scientific report on the antibacterial properties of the plant; hence the reason for this work which was to evaluate the in vitro antibacterial activity of *Picralima nitida.*

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Experimental

Plant material and extraction. The pods of *Picralima nitida* were collected in August, 2006 from a homestead in Ubulu, in Oru west LGA of Imo State, Nigeria and was identified and authenticated by the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at the Faculty of Pharmacy Herbarium.

The seeds of *P. nitida* were extracted by maceration in ethanol, methanol and water. The plant parts were first dried at room temperature (27.2°C) and pulverized to powder using a mechanical grinder (Corona). A 20.0 g amount of the pulverized seed was soaked, separately, in 80 ml of ethanol, methanol and water. The preparations were filtered and concentrated *in vacuo* using rotary evaporator (Buchi, CH-920 Laboratorium Technic, Flak/SG, Switzerland) and their percentage yield value were calculated. The dried extracts were exposed to ultra violet rays for 24 h and checked for sterility by streaking on nutrient agar plate. The yields were 2.62 %, 1.98% and 1.63% (w/w) for ethanol, methanol and water respectively (Table 1). The extracts were stored in a refrigerator at 4^0C until used for the experiment reported in this study.

Test Microorganisms. Standard typed cultures of *Staphylococcus aureus* NCTC 6571, *E. coli* NCTC 10418, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* NCTC 8853, *Salmonella typhi* NCTC 8571 were obtained from Pharmaceutical Microbiology laboratory Faculty of Pharmacy, University of Uyo. All test strains were re-isolated three successive times on Mueller Hinton agar, MHA (oxoid). Identity was confirmed by standard bacteriological methods (Buchanaan & Gibbons 1974, Cowan 1985, McFaddin 1985).

Antimicrobial assay. Antimicrobial assay was performed using the agar-well diffusion technique. Standardized inoculum (5 x 10^5 cfu/ml) of each test bacterium was spread on to sterile Muller Hinton agar (MHA) plates so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0 mm was used to bore wells in the agar plates. Different concentrations; 12.5, 25, 50 and 100mg/l were prepared by redissolving the extracts in the same solvent which was used in the extraction. Subsequently, a 100 µl volume of the extracts were introduced in triplicate wells of the surface inoculated MHA plates. The plates were allowed to stand for 1 h or more for diffusion to take place and then incubated at 37°C for 24 h. The zone of inhibition was recorded. Only extracts exhibiting apparent zone of inhibition were chosen for further evaluation. Minimum inhibitory concentration (MIC), which was determined as the lowest concentration of plant extracts inhibiting the growth of the organism, was determined based on the readings.

Results and Discussion

The study was conducted to assess antibacterial activity of *P. nitida* seed extract on common bacteria species within the study area and to compare with standard drug. The result shows inhibition diameter of ≥13.5.0mm for ethanolic extract and ≥11.5mm for methanolic extract for *Staphylococcus aureus* and *E. coli* (Table 2). Aqueous extract shows inhibition diameter of 11.5mm at extract concentration of 100mg/l for only *Staphylococcus aureus*. There was no inhibition for *P. aeruginosa* and *S. typhi*. However, there was 13.5mm diameter inhibition for *B. subtilis* at 100.0mg/l of ethanolic extract. Minimum inhibitory concentration (MIC) for the three solvent extracts range between 31.0mg/l and 75.0mg/l with ethanolic extract having the least (31.0mg/l) and aqueous extract having the highest (75.0mg/l).

Table 1: Yield of extracts from ethanol, Methanol, cold water

	Ethanol	Methanol	Water
Yield(g) ±SD	0.52 ± 0.007	0.40 ± 0.005	0.33 ± 0.015
Yield (%)	2.62	2.00	1.63

Table 2: Inhibition zone Diameter of (mm) *Picralima Seed Extracts* for antibacterial activity

Source	Extract Conc.	Solvent	Inhibition Zone Diameter (mm)				
			S. aureus NCTC 6571	*E. coli* NCTC 10418	*B. subtilis* NCTC 8853	*P. aeruginosa* ATCC 27853	*S. typhi* NCTC 8571
P. nitida seed extract	100 mg/l	Ethanol	19.0	16.0	13.50	-	-
		Methanol	15.0	11.5	-	-	-
		Water	11.0	-	-	-	-
	50 mg/l	Ethanol	13.5	-	-	-	-
		Methanol	12.0	11.0	-	-	-
		Water	-	-	-	-	-
	25 mg/l	Ethanol	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-		-	-	-
	12.5 mg/l	Ethanol	-	-	-	-	-
		Methanol	-	-	-	-	-
		Water	-	-	-	-	-
Streptomycin (0.04mg/l)			39.5	31.5	24.5	38.0	45.5

Values are the average of at least three determinations. - Not active;

Table 3: Comparison of Inhibition zone diameter (IZD) (mm) between *P. nitida* seeds extracts and standard drug (streptomycin)

	S. aureus NCTC 6571	*E. coli* NCTC 10418	*B. subtilis* NCTC 8853	*P. aeruginosa* ATCC 27853	*S. typhi* NCTC 8571
Ethanol extract	19.0	16.0	13.50		-
Methanol extract	15.0	11.5	-	-	-
Water extract	11.0	-	-	-	-
Streptomycin (0.04mg/l)	39.5	31.5	24.5	38.0	45.5
% of ethanolic extract to streptomycin	48.10%	50.79%	55.10%	-	-

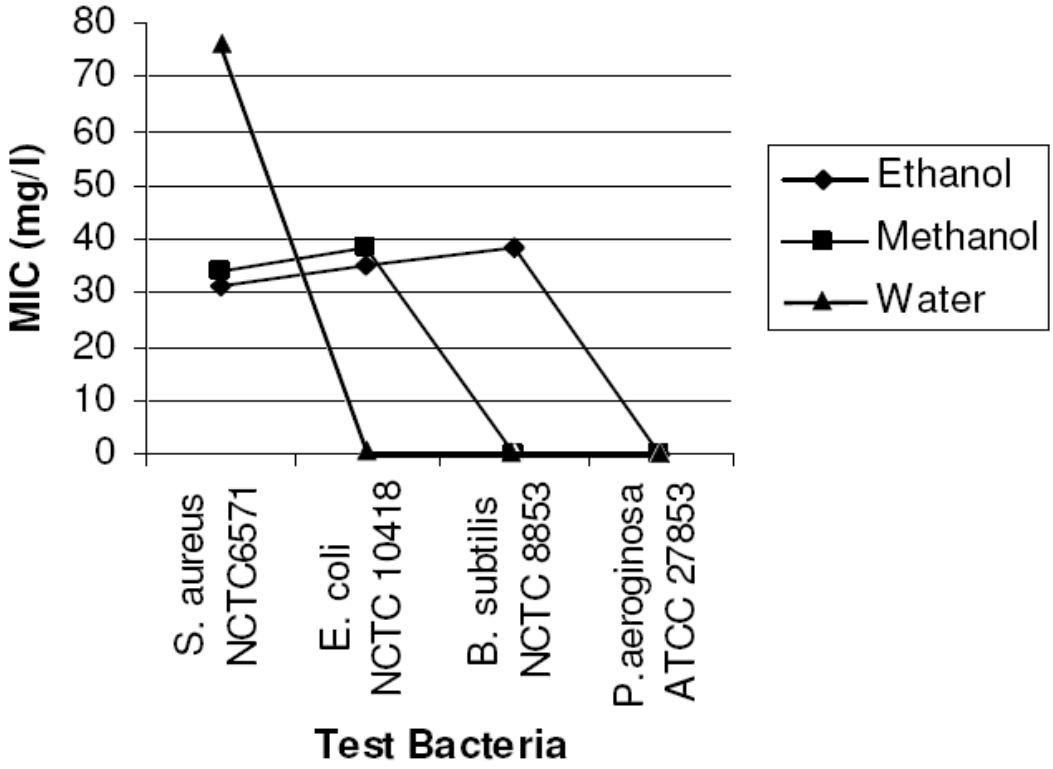

Fig. 1: MIC of *P. nitida* extracts on test bacteria

This result supports the highest (19.0mm) inhibition zone diameter (IZD) obtained from ethanolic extract and the lowest (11.0mm) from aqueous extract (Table 3).

The ethanolic, methanolic and aqueous extracts of *P. nitida* seeds showed considerable antimicrobial activity against S*taphylococcus aureus, Bacillus subtilis*, and *Escherichia coli* .This finding corroborates the work of Nkere and Iroegbu (2005). Amongst the organisms tested, *S. aureus* proved to be the most susceptible. This finding is consistent with the susceptibility of the microbe to different plant extracts by some other researchers (Arora and Kaur 1999, Digraki *et al.,* 1999, Okemo *et al.* 2001, Madamombe and Afolayan 2003), and justifies its ethnomedicinal usage, especially with *S. auerus* being implicated in many diseases affecting the rural populace (Okemo *et al.,* 2001). Table 3 shows a high percentage inhibition zone diameter (≥ 48.10 %) of ethanolic seed extract of *P. nitida* compared with a standard drug (Streptomycin). The result of the work confirms high in vitro antibacterial activities of the seed extracts. These findings corroborate previous findings (Burkil 1985, Fakeye *et al.*, 2000, Nkere and Iroegbu 2005, Okokon *et al., 2007*) and further justify the usage of the drug in ethnomedicinal practices.

Further work needs to be done to identify the active ingredients responsible for the observed activities. However, compounds detected in the seeds include saponins, tannins, flavonoids, terpenoids, alkaloids and these groups of compounds from previous reports could exhibit antibacterial activities.

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