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Antimicrobial Potency of *Pentaclethra Macrophylla* Seed Extract on Seven Selected Pathogens

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ABSTRACTS: The antimicrobial efficacy of extracts of *Pentaclethra macrophylla* in ethanol, methanol and water was determined against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella species*, *Salmonella typhi*, *Aspergillus niger* and *Candida albicans* using paper disc and hole diffusion methods. The results revealed that the growths of test organisms were inhibited by extracts used. The least inhibitory zone (25.0mm) was best recorded in ethanol extract against *A. niger* and *C. albicans*. The minimum inhibitory concentration of the extracts ranges between 62.5-250mg/ml on the test isolates. However, the antimicrobial potency of *Pentaclethra macrophylla* was more prominent against bacterial isolates than fungal isolates. These findings lend more weight to the use of *Pentaclethra macrophylla* seed for therapeutic purposes.

KEYWORDS: *Pentaclethra macrophylla*, antibacterial, microorganism

INTRODUCTION

Traditional medicine in many countries of the world could be traced back to antiquity. Among the diseases that have been successfully managed traditionally are malaria, epilepsy, infantile convulsion, diarrhea, dysentery, gonorrhoea, flatulence, tonsillitis, bacterial and fungal infections, mental illness and worm infections (Sofowora, 1996). Medicinal uses of these plants range from the stem, leaves and seeds to the use of extracts from whole plant. The use of antimicrobial agents for control of infection is almost entirely a development of this century. The need for new antimicrobial agents is closely associated with the problem of emergence of strains that are resistance to most present day antibiotics (Ibekwe *et al.*, 2000).

Pentaclethra macrophylla commonly called African oil bean belongs to the family fabaceae. They are trees which can be found in tropical African countries especially Cameroon, Cote d'Ivoire, Democratic Republic of Congo, Ghana, Niger, Nigeria, and Togo (Ladipo *et al.*, 1993). All the parts of the plant are used for various domestic and wild animals and human

ailments (Akah *et al.*, 1999). Antimicrobial property and the fixed oil extracted from the seeds are used in the preparation of formulation against pruritus, worms and dysentery (Gugnani and Ezenwanze, 1985, Kamanzi *et al.*, 2002).

The use of *Pentaclethra macrophylla* seed extract as antimicrobial substance is new and no tradition has been handed down in this regard. It has been shown that *Pentaclethra macrophylla* seed extract has a spectrum of efficacy and does not damage both the internal and external environments. It is a new age antibiotic antifungal, antinoceptive, anti-inflammatory. (Okorie *et al.*, 2006).

The fact that microorganisms develop resistance to many drugs has created a situation where some of the common and less expensive antimicrobial agents are losing effectiveness (Montetiore *et al.*, 1989). It is therefore imperative that common microorganisms be tested against the efficacy of the extract of *Pentaclethra macrophylla* with a view to studying the efficacy of the extracts and possible further investigations of this extract to find its pharmacological uses.

MATERIALS AND METHODS

The African oil bean (*Pentaclethra macrophylla*) seeds were purchased from a local market in Abeokuta, Ogun-state, Nigeria. Pure isolates of *Salmonella typhi*,

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Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella spp* were obtained from University College Hospital (UCH) at Ibadan while *Aspergillus niger*, *Candida albican* were obtained from Microbiology laboratory, University of Agriculture and all were maintained in stock culture medium. The bacteria were subcultured onto nutrient broth and incubated overnight at 37°C while the fungal isolates were subcultured into yeast extract and incubated at 25°C for 72hrs.

Preparation of the Extract: The seeds of *Pentaclethra macrophylla* were sun dried for 5-7 days. They were later dried at 45°C for 3-4hrs to eliminate moisture and later pulverized using mortar and pestle. Extraction was done using cold water, hot water, ethanol 60% and methanol. Twenty grammes (20g) of the powder were dissolved in 100ml of cold water; hot water; ethanol and methanol. The suspended solutions were left to stand for 24 hours and filtered. The filtrates were labeled accordingly and stored at room temperature. Two Laboratory Processes used for this test were filter paper disc impregnated with extract and open hole diffusion method.

Hole Diffusion Method: Pour plate of the test organisms were prepared adding 1ml to 25ml of molten nutrient agar. A sterilised cork borer of 8mm diameter was used to make wells on the inoculated plate and each well was filled with 0.05ml of each extract. The plates were incubated at 37°C for 24 hours and examined for zones of inhibition around the wells. The zones of inhibition were measured from the edge of the wells to the end of inhibition zone, and results were recorded.

Use of Filter Paper Discs Impregnated with Extract. Filter papers were cut out into circular disc of 8mm in diameter. These circular pieces were impregnated with extracts from different solvents. The discs were

allowed to stay in the extract for 24hours. Prepared nutrient agar was seeded with each of the test pathogen. The filter paper discs were placed on each plate. The plates were incubated at 37°C for 24hrs, for bacterial isolates and 30°C for 72hrs for fungal isolates. Zones of inhibition were measured and recorded.

Minimal Inhibitory Concentration: Different concentrations of the extract were prepared using nutrient broth as diluents to obtain the following concentrations: 250mg/ml, 125mg/ml, 62.5mg/ml, and 31.25mg/ml. Three drops of over-night broth culture of the test organisms were inoculated into the different concentrations and incubated at 37°C for 24 hours. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the minimal inhibitory concentration (MIC). Yeast extract broth was used instead of nutrient broth to culture fungi.

Minimal Bactericidal Concentration
The tubes that showed no visible growth from the MIC test was subcultured onto nutrient agar at 37°C for 24 hours for bacteria and potato dextrose agar (PDA) was used to culture fungi at 30°C for 72 hours. The lowest concentration of the extracts yielding no growth was recorded as the minimal bactericidal concentration (MBC).

RESULTS

The diameter of zones of inhibition of the test organism using the ethanol, hot water, methanol and cold water extracts in hole diffusion method of the seed extracts are presented in Table 1. The highest zone of inhibition was observed in ethanol extracts against *E. coli* (25.0mm) while lowest inhibition was in methanol extracts against *A. niger* and *C. albicans* with zone of inhibition diameter of 1.0mm.

Table 1:
Antimicrobial activities of *Pentaclethra macrophylla* seed extracts using hole diffusion method.

Solvent	Zone of inhibition (mm)						
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>Klebsiella sp.</i>	<i>S. typhi</i>	<i>A. niger</i>	<i>C. albican</i>
Ethanol 60%	25.0	20.0	12.0	10.0	8.0	6.0	4.0
Methanol	15.0	7.0	5.0	3.0	3.0	1.0	1.0
Hot water	19.0	17.0	8.0	6.0	5.0	1.0	2.0
Cold water	23.0	19.0	9.0	7.0	7.0	3.0	2.0

Table 2:Antimicrobial activities of *Pentaclethra macrophylla* seed extracts using filter paper disc method.

Solvent	Zone of inhibition (mm)						
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>Klebsiella sp</i>	<i>S. typhi</i>	<i>A. niger</i>	<i>C. albicans</i>
Ethanol	7.0	3.0	-	2.0	4.0	2.0	2.0
Methanol	2.0	1.0	-	-	2.0	1.0	1.0
Hot water	4.0	1.0	-	1.0	2.0	1.0	1.0
Cold water	5.0	1.0	-	1.0	3.0	1.0	1.0

Table 3:Minimum Inhibitory Concentration and Minimum Bacteriocidal Concentration of Ethanol Extracts of *P. macrophylla* against the test Organisms.

Concentration	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>Klebsiella sp.</i>	<i>S. typhi</i>	<i>A. niger</i>	<i>C. albicans</i>
MIC (mg/ml)	62.5	62.5	125	125	125	250	250
MBC(mg/ml)	125	125	250	>250	>250	>250	>250

Table 4:Minimum Inhibitory Concentration and Minimum Bacteriocidal concentration of Aqueous extract of *P. macrophylla* against the test Organism

Concentration	<i>E.coli</i>	<i>S. aureus</i>	<i>Pseudomonas sp</i>	<i>K. aeruginosa</i>	<i>S. typhi</i>	<i>A. niger</i>	<i>C. albicans</i>
MIC (mg/ml)	250	125	250	250	250	ND	250
MBC(mg/ml)	>250	250	>250	>250	>250	ND	>250

The results obtained using paper disc diffusion method in Table 2 probably gave a poor result because the filter paper acted as a barrier between the seed extracts and the organisms. No visible zone of inhibition was observed and recorded against *Pseudomonas aeruginosa* in any of the extract used with this method.

Tables 3 and 4 show the minimum inhibitory concentration and minimum bactericidal concentration of ethanol and aqueous extracts of the *P. macrophylla* seed extracts. The ethanol extracts showed activities against the seven test organisms with the highest activity against *E. coli* and *S. aureus* (MIC = 62.5mg/ml, MBC = 125.0mg/ml) and the lowest against *A. niger* and *C. albicans* (MIC = 250mg/ml, MBC >250mg/ml).

Aqueous extracts had no activity against *A. niger* up to the highest concentration of 250mg/ml with MBC greater than 250mg/ml. *E. coli*, *Pseudomonas aureginosa*, *Klebsiella spp*, *S. typhi*, and *C. albicans* had the MIC of 250mg/ml and MBC greater than 250mg/ml

From the results obtained, it could be observed that ethanol was the best solvent for extracting antimicrobial substance from the seed of *P.*

macrophylla. (Table 1 and 2). This is because the zones of inhibition produced against the microorganisms were highest with ethanol extract. It could be seen from Table 1 and 2 that, *P. macrophylla* extracts differ in their antimicrobial effectiveness depending on the solvent used. These results agree favourably with the study of Oloke and Kolawole (1982) that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvents used. Table 1 shows that methanol is the worst solvent of the solvents used in extracting *P. macrophylla* seeds. The widest inhibitory zone (25.0mm) in the hole diffusion method was demonstrated by *P. macrophylla* against *E. coli* for the ethanolic extract. But, this value dropped to 23mm, 19mm and 15mm respectively when cold water, hot water and methanol were tested against the same bacterium.

The antibacterial properties exhibited by *P. macrophylla* confirm the report of Akah *et al.*, (1999) and Kamanzi *et al.*, (2002), that traditional doctors have successfully used *P. macrophylla* to cure gonorrhoea, dysentery and convulsion as the extract was found to be active against the enteric pathogenic organisms tested in this study.

When filter paper and hole diffusion methods were compared, the latter was found more sensitive for evaluating antimicrobial activities of *P. macrophylla* extracts (Table 1 and 2). The zone of inhibition was 25.0mm against *E. coli* when hole diffusion method was used whereas, it was 7.0mm for filter paper disc method (Table 1 and 2).

Toda *et al.*, (1991), suggested that in hole diffusion method, there is a better contact between the medium and organisms, but filter paper disc may act as a barrier between the extract and the organisms. There may not be proper diffusion and total release of active components adsorbed by the discs into the media, this could be the reason why the result got in Table 2 using this method was poor. The best antifungal activity (6.0mm) was recorded in *P. macrophylla* ethanolic extract against *A. niger* (Table 1), this suggests that the plant is a promising antifungal agent.

Generally, the observed values for all other extracts against pathogenic fungi were low. The minimum inhibitory concentration (MIC) values of ethanol and aqueous extracts varied from 62.5mg/ml to 250mg/ml respectively (Table 3 and 4). The MIC against *S. aureus* in *P. macrophylla* ethanol extract was similar to that of *E. coli*. The MIC against *P. aeruginosa*, *Klebsiella*, and *S. typhi* are the same in *P. macrophylla* ethanol extract. The MIC against *A. niger* of *P. macrophylla* aqueous extract was not detectable (Table 4). The minimal bactericidal concentration (MBC) values of ethanol and aqueous extracts varied from 125mg/ml to > 250mg/ml respectively (Table 3 and 4). This study has shown that different solvents (ethanol, water, and methanol) have been used *in-vitro* to inhibit the growth of some disease-causing bacteria and fungi. It can therefore be suggested that *P. macrophylla* seed extract is a promising antimicrobial agent. The antimicrobial potency of the extract increased with increased concentration. Although this investigation did not involve human and animal administration of the extract, caution is required when it is taken for cure of disease. Further works will however need to be done on assessing the pharmacological significance of this extract's antibiotic activities.

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