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Evaluation of antibacterial activities of some Nigerian medicinal plants against some resistant bacteria pathogens

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The search for the healing properties of plants is an ancient idea that has remained even till date. In this work, the antibacterial activity of leaf extracts of *Corchorus olitorius, Pterocarpus santaliniodes, Pentaclethra macrophylla* and *Azadirachta indica* was tested against resistant strains of *Staphylococcus aureus, Escherichia coli, Klebsiella* species and *Streptococcus* species using agar well diffusion method. The following concentrations of 100, 50, 25 and 12.5 mg/ml of aqueous, ethanol and methanol leaf extracts were used against the test organisms. Results of this study reveal that all the leaf extracts had antibacterial activity against the test organisms at various concentrations (in particular: at 100 and 50 mg/ml) but the aqueous leaf extracts had higher inhibitory effect for all of them. However, little inhibitory effect was observed with the methanol and ethanol leaf extracts. Our findings justify the therapeutic use of these plants by traditional healers in most part of Nigeria for the treatment of infections caused by these bacteria. Medicinal plants have unlimited possibilities to produce putative compounds for the development of novel drugs to curtail the upward trend in bacterial resistance, thus the need for sustained research towards this objective.

Key words: Plant extracts, microorganisms, resistance, herbal medicine, Nigeria.

INTRODUCTION

Herbs have been used for many centuries as medicine for the treatment of infectious diseases (Roja et al., 2006; Zhan et al., 2007; Barhour et al., 2004). Traditional healers have used plants with medicinal properties to prevent or cure infectious diseases for centuries even till date. Some herbal plants with antibacterial activities including *Pentaclethra macrodphylla* (oil bean plant), *Azadirachta indica* (Neem plant), *Persea gratissima* (Avaocado leafs), *Corchorus oiltorius* (locally called Ahiara by the Igbo speaking tribe of Nigeria) and

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Pterocarpus santaliniodes have been investigated and found efficacious in the traditional treatment of some infectious diseases (Izzo et al., 2009; Buwa et al., 2006; Adeleke et al., 2009; Zakaria et al., 2005). The rising level of resistance of pathogenic microorganisms (including *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Streptococcus* spp.) to available conventional drugs is a source of worry to public health practitioners, and this has given great impetus to the search for new antimicrobials that can contain and possibly alleviate the menace of microbial drug resistance. Antimicrobial properties of medicinal plants against some known pathogens (bacteria and fungi inclusive) have been studied extensively, and the results of these researches are very promising to the development of new agents to fight resistance amongst microbes (Pamplona-Roger, 2002; Abbiw, 1990; Abu-Hadid et al., 1994; Adeleke et al., 2009; Zakaria et al., 2005). Plant extracts still remain a veritable tool which can be harnessed by the biopharmaceutical industries to develop better antimicrobial agents that can be used for the effective treatment of infectious diseases globally. In this work, we investigated the antibacterial activities of leaf extracts of *Corchorus olitorius, Pterocarpus santaliniodes, Pentaclethra macrophylla* and *Azadirachta indica* against resistant strains of *S. aureus, E. coli, Klebsiella* and *Streptococcus* species.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *C. olitorius, P. santalimiodes, P. macrophylla* and *A. indica* were collected from Ezza South Local Government Area of Ebonyi State, Nigeria and they were identified by Professor S.C.C. Onyekwere, a taxonomist at the Department of Applied Biology, Ebonyi State University, Abakaliki.

Preparation and extraction of plants materials

The leaves of each of the plant was washed with sterile water to remove debris, cut into pieces and allowed to dry under mild sunlight. The dried leaves were pulverized using mechanical grinder. Hundred grams (100 g) of each plant leaves were extracted using 98% ethanol, methanol, cold and hot water respectively according to a previously used method (Nwafor et al., 2007).

Test organisms

All bacterial isolates used for this study were provided by the culture collection unit of the Microbiology Laboratory Department of Federal Teaching Hospital Abakaliki, Ebonyi State, Nigeria. The test organisms included resistant strains of *S. aureus, E. coli, Klebsiella* spp. and *Streptococcus* spp. All isolates were sub-cultured on growth media, purified and identified by standard microbiological identification techniques (Cheesbrough, 2002).

Determination of antibacterial activity

Antibacterial activity of the plant extracts was performed by the agar well diffusion method on Mueller-Hinton (MH) agar using standard procedure as previously described (Nwafor et al., 2007). Standard inoculum of the test organisms (adjusted to 0.5 McFarland turbidity standards) was seeded evenly on the MH agar plate. Using a standard six (6) mm cork borer, four (4) wells were made in the agar plate. Suitable concentrations of each of the plant extracts (100 mg/ml) were respectively added into the wells. The plates were allowed to stand for 30 min for pre-diffusion, and then incubated at 37°C overnight. The inhibition zone diameters were measured in millimeters to determine antibacterial activity of the respective plant extracts.

RESULTS

Table 1 shows the antibacterial activity of the cold water extracts of the plants. The highest inhibition zone diameter was observed with the cold water extract of *P. macrophylla* at 100 $mgml^{-1}$ while the lowest inhibition zone diameter (4 mm) was recorded at 25 $mgml^{-1}$ concentrations with the same plant extracts (Table 1). Results of the hot water extracts are shown in Table 2, and they show that the leaf extracts of *A. indica* and *P. macrophylla* had inhibition zone diameter of 18 and 16 mm against *Klebsiella* species and *E. coli* at 100 and 50 $mgml^{-1}$, respectively (Table 2). Also, at herbal concentration of 25 and 12.5 $mgml^{-1}$, the majority of the test organisms were resistant except with *A. indica* and *P. macrophylla* that had activity against *Klebsiella* spp., *E. coli,* and *Streptococcus* spp. (Table 2). Table 3 shows the results of the antibacterial activity of the methanol extracts of all the plants. It was observed that at 50, 25 and 12.5 $mgml^{-1}$, concentrations, the methanol extracts of the plants were not active against the test organisms except with *Klebsiella* species. The highest inhibition zone diameter (26 and 24.75 mm) recorded was with methanol extracts of *P. macrophylla* leaves against *Klebsiella* species at concentrations of 100 and 50 $mgml^{-1}$, respectively (Table 3). The sensitivity patterns of the test bacteria isolates with ethanol extracts of the plant used in this study are shown in Table 4, and these results revealed that *S. aureus* was susceptible to the four plant extracts at 100 $mgml^{-1}$. *Klebsiella* species was also susceptible to *P. macrophylla* at 100 and 50 $mgml^{-1}$, and this was followed by *E. coli*. The ethanol extracts of the other three medicinal plants had no antibacterial activity against the rest of the test organisms.

DISCUSSION

The medicinal plants including *C. olitorius, P. santaliniodes*, *P. macrodphylla, A. indica* are frequently used traditionally in most Nigerian communities for the treatment of some ailments such as cough, tooth ache, inflammation, wounds, stomatitis and some fungal infection (e.g. candidiasis). The antibacterial activity of these medicinal plants that warrants their usage for meeting most primary health care needs has been attributed to

Table 1. Inhibition zone diameter (mm) of cold water extracts of the four medicinal plants used at different concentrations.

Bacteria Isolate	Medicinal plants used	Concentration ($mgml^{-1}$) of cold water extracts			
		100	50	25	12.5
S. aureus	*P. macrophylla*	21	20	18.50	17
	C. oliforus	11	10	-	-
	A. indica	12	11.75	10	8
	P. santaliniodes	16	11	10	-
*Klebsiell*a spp.	*P. macrophylla*	15	14	-	-
	C. oliforus	-	-	-	-
	A. indica	16	13	12.50	-
	P. santaliniodes	14	10	8	-
E. coli	*P. macrophylla*	20	18.75	17	15
	C. oliforus	10	-	-	-
	A. indica	10	9.45	-	-
	P. santaliniodes	12	10	10	-
Streptococcus spp.	*P. macrophylla*	12	9	4	-
	C. oliforus	-	-	-	-
	A. indica	15	14.50	-	-
	P. santaliniodes	13	10	8	-

Table 2. Inhibition zone diameter (mm) of hot water extracts of the four medicinal plants used at different concentrations.

Bacteria Isolate	Medicinal plants used	Concentration ($mgml^{-1}$) of cold water extracts			
		100	50	25	12.5
S. aureus	*P. macrophylla*	12	8	-	-
	C. oliforus	12	11	-	-
	A. indica	11.79	11	--	-
	P. santaliniodes	14	10	-	-
Klebsiella spp.	*P. macrophylla*	11	-	-	-
	C. oliforus	-	-	-	-
	A. indica	18	16	15	11.75
	P. santaliniodes	12	10	-	-
E. coli	*P. macrophylla*	18	16	10	-
	C. oliforus	10	10	-	-
	A. indica	10	9	-	-
	P. santaliniodes	11	9	-	-
Streptococcus spp.	*P. macrophylla*	12	10	-	-
	C. oliforus	-	-	-	-
	A. indica	14.75	12	9	-
	P. santaliniodes	11	10	-	-

the presence of some active constituents in their extracts (Osuagwu et al., 2007; Roja et al., 2006; Nwafor et al., 2007 Kraus et al., 1981). The results of this study indicate that the extracts of *P. macrophylla A. indica, C. olitorius* and *P. santaliniodes* had antibacterial activity on clinical isolates of *S. aureus*, *Klebsiella* species, *E. coli* and *Streptococcus* species at different concentrations of the extracts. Results obtained from this study revealed that the aqueous plant extracts (cold water and hot water) of *P. macrophylla* (oil bean plant) and *A. indica* (Neem) showed high antibacterial activity against *S. aureus* and *E. coli* which are major etiologic agents of bacteraemia, septiceamia, osteomyelitis, furuncle and gastroenteritis.

These findings however, are in line with similar studies

Table 3. Inhibition zone diameter (mm) of methanol extracts of the four medicinal plants used at different concentrations.

Bacteria Isolate	Medicinal plants used	Concentration ($mgml^{-1}$) of methanol extracts			
		100	50	25	12.5
S. aureus	*P. macrophylla*	-	-	-	-
	C. oliforus	10	10	-	-
	A. indica	16	-	-	-
	P. santaliniodes	11	-	-	-
Klebsiella spp.	*P. macrophylla*	26	24.75	19	16
	C. oliforus	-	-	-	-
	A. indica	-	-	-	-
	P. santaliniodes	10	-	-	-
E. coli	*P. macrophylla*	-	-	-	-
	C. oliforus	-	-	-	-
	A. indica	13	-	-	-
	P. santaliniodes	10	-	-	-
Streptococcus spp.	*P. macrophylla*	-	-	-	-
	C. oliforus	10	10	-	-
	A. indica	15	-	-	-
	P. santaliniodes	-	-	-	-

Table 4. Inhibition zone diameter (mm) of ethanol extracts of the four medicinal plants used at different concentrations.

Bacteria Isolate	Medicinal plants used	Concentration ($mgml^{-1}$) of ethanol extracts			
		100	50	25	12.5
S. aureus	*P. macrophylla*	12	9	-	-
	C. oliforus	10	10	-	-
	A. indica	12	-	-	-
	P. santaliniodes	12	-	-	-
Klebsiella spp.	*P. macrophylla*	10	7	-	-
	C. oliforus	-	-	-	-
	A. indica	-	-	-	-
	P. santaliniodes	-	-	-	-
E. coli	*P. macrophylla*	13	11	-	-
	C. oliforus	-	-	-	-
	A. indica	13.75	-	-	-
	P. santaliniodes	10	-	-	-
Streptococcus spp.	*P. macrophylla*	-	-	-	-
	C. oliforus	-	-	-	-
	A. indica	13	-	-	-
	P. santaliniodes	-	-	-	-

conducted by Abu-Nadid et al. (1994) and Adegoke et al. (2004) in which they reported that the aqueous extracts of these plant extracts had antimicrobial activity, and can be used for prophylactic applications especially against enteric fever, chronic cystitis and gastroenteritis. Zakaria et al. (2005) also showed that extracts of these plants possess antibacterial activity that can be comparable to some of the available conventional antibiotics. *P. santaliniodes* (locally called Gbengbe by the Yoruba, Nturukpa by the Igbos and Mututi by the Hausas in Nigeria) is eaten as a vegetable in virtually all parts of Nigeria, and it helps in giving bulk to the intestinal bowels in addition to its strong antimicrobial activity. Results of the antibacterial activity of *P. santatiniodes* obtained from our study showed that the leaf extract of this plant at 100 and 50 $mgml^{-1}$ concentrations had inhibitory effect against all the test isolates. Similar antibacterial activity of *P. santatiniodes* against clinical pathogens has also been reported in some Nigerian states (Adeleke et al., 2009; Osuagwu et al., 2007). Bioactive products of the Neem

plant have been used in the treatment of various ailments since time immemorial (Sasidharan et al., 1998; SaiRam et al., 2000). In this work, the antibacterial activity of *A. indica* (the Neem plant), which is generally used as a chewing stick in most Nigerian communities, was investigated. The hot water extract of *A. indica* exhibited maximum inhibitory activity against the test organisms. This was followed by the cold water, methanol and ethanol extracts. Extracts of *A. indica* were found to be effective against *S. aureus,* a pyrogenic bacterium known to play a significant role in invasive skin diseases including superficial and deep follicular lesion and food poisoning (SaiRam et al., 2000).

Similar antimicrobial activity of *A. indica* has also been reported by previous studies both within and outside Nigeria (Nwafor et al., 2007; SaiRam et al., 2000; Saidharan et al., 1998). Conclusively, the findings in this work validate the traditional use of these plants in Nigerian rural communities for treating a variety of infections caused by the test organism. The plants holds potential in the improvement of health care delivery in Nigeria; thus there is need for further molecular research to isolate their actual bioactive components to be compounded into dosage forms for therapeutic purposes.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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