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E-mail: jzus@zju.edu.cn

Antibacterial potency of methanol extracts of lower plants

OJO O.O.†, AJAYI A.O., ANIBIJUWON I.I.

(*Department of Microbiology, Faculty of Science, University of Ado-Ekiti, Ado-Ekiti, Ekiti State 234030, Nigeria*)

†E-mail: walelugba@yahoo.com

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Abstract: Antibacterial potency of methanol extracts of three green lower plants, *Pneumatopteris afra*, *Platycerium bifurcatum* and *Nephrolepsis bisserata* was determined using agar dilution method on clinical strains of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp. and *Salmomelia typhi*. Antibacterial activities were observed at concentrations of 12.5, 25.0, 50.0 and 100.0 μg/ml. Their minimum inhibitory concentrations ranged from 12.5~100 μg/ml. Extracts of *P. afra* and *P. bifurcatum* were most active. Antibacterial activities observed with *N. bisserata* were less pronounced with no detectable activity at extract concentrations of 12.5 and 25.0 μg/ml. *E. coli*, together with *S. aureus* appeared to be the most susceptible of the test bacteria while *Klebsiella* spp. was least sensitive. The significance of our findings is discussed.

Key words: Lower plants, Extracts, Antibacterial activity, MIC (minimum inhibitory concentration)
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INTRODUCTION

In recent times, the search for potent antibacterial agents has been shifted to plants. However, the major part of the search has focused mainly on higher plants (Oyagade, 1998; Olukoya *et al*., 2003) while little attention is given to lower plants with possible antimicrobial properties. Most plants are medicinally useful in treating disease in the body and in most cases, the antimicrobial efficacy value attributed to some plants is beyond belief. Conservative estimates suggest that about 10% of all flowering plants on earth have at one time, been used by local communities throughout the world but only 1% have gained recognition by modern scientists (Kafaru, 1994).

Liverwort, mosses and hornwort are small, low-growing plants, which constitute the phylum bryophyta. They lack true stems, leaves and roots and modify their microclimate by conserving moisture, checking soil erosion on lilly slopes and serve as seedbed for forest cover. They are now increasingly used for diverse purposes including pollution control and as new sources of pharmaceuticals (Saxena and Saxena, 2002). Liverworts and mosses have been tested and used as fuel in developed countries like Finland, Sweden, Ireland, Germany, Poland and the Soviet Union (Baker and Hawkinson, 1993). Chinese traditional medicine named 40 kinds of bryophytes used to treat cardiovascular diseases, tonsillitis, bronchitis, cystitis and skin infections. It has also been shown that an extract of *Giganteum* can increase aorta blood transport by up to 30% in animals (Jorgnersteen, 1998). The aim of this study is to determine the antimicrobial potencies of three lower plants with a view to ascertaining their possible medicinal values.

MATERIALS AND METHODS

Plant material

Whole parts of *Pneumatopteris afra*, *Platycerium bifurcatum* and *Nephrolepsis bisserata* were collected in Ado-Ekiti, Ekiti State, Nigeria. Identification of plants was done at the herbarium unit, Department of Plant Science, University of Ado-Ekiti, Nigeria. Voucher specimens are deposited in this unit.

Preparation of extracts

In each case, powdered air-dried plant material was extracted with methanol. The crude methanol extract was prepared by maceration of plant material (100 g) with methanol (300 ml) (Alanis *et al*., 2005). The extract was filtered and evaporated to dryness in vacuum.

Bacterial strains

All bacterial strains used in this study were clinical strains. They are *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp. and *Salmonella typhi*.

Antibacterial testing

Antibacterial activity was measured using agar dilution technique. Briefly, the methanol extracts were dissolved in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Mueller Hinton agar (MHA, Sigma) in petridishes (100 mm×15 mm) to obtain final concentrations: 100, 50, 25 and 12.5 μg/ml. The solvent did not exceed 1% concentration and did not affect the growth of the organisms. All bacterial strains were grown in Mueller Hinton broth (MHB, Sigma) for 4 h at 37 °C. Bacterial suspensions with 0.5 McFarland standard turbidity, which is equivalent to 108 cfu/ml, were prepared by dilution with Mueller Hinton broth. The diluted inoculum was added to a Steer's replicator calibrated and incubated for 24 h at 37 °C. After incubation, all dishes were observed for microbial inhibition. The minimum inhibitory concentration (MIC) was determined as the lowest concentration that completely inhibited macroscopic growth of the organisms.

RESULTS

The results obtained in this study showed that the organisms were susceptible to the methanol extracts of the lower plants based on their minimum inhibitory concentrations. In general, the organisms showed the same susceptibility to methanol extracts of *P. afra* and *P. bifurcatum* with minimum inhibitory concentrations ranging from 12.50~100 μg/ml (Table 1); while they were least sensitive to *N. bisserata* (Table 1). *E. coli* showed the highest sensitivity to methanol extracts of *P. afra* and *P. bifurcatum*, followed by *S. aureus* while *Klebsiella* spp. are the most insensitive of all the organisms tested. *E. coli* also showed the highest sensitivity to *N. bisserata*. *Salmonella typhi* did not show any sensitivity to all the concentrations of *N. bisserata* and none of the organisms tested showed sensitivity to *N. bisserata* at concentrations of 25 μg/ml and 12.5 μg/ml.

Table 1 Minimum inhibitory concentration of methanolic extracts of *P. afra*, *P. bifurcatum* and *N. bisserata* (μg/ml)

	P. afra	*P. bifurcatum*	*N. bisserata*
E. coli	12.50	12.50	50.00
S. aureus	12.50	12.50	50.00
Klebsiella spp.	100.00	100.00	50.00
S. typhi	25.00	25.00	100.00

DISCUSSION

Due to the increasing prevalence of antibiotic-resistant pathogens in hospital and homes, deliberate search is in progress for alternative treatments to combat further spread of antibiotic resistant-pathogens (Olukoya *et al*., 2003). Different parts of higher plants have been screened for their potential antimicrobial properties but studies on the antimicrobial properties and activities of lower plants are very scanty (Oyagade, 1998; Sofowora, 1998).

This study has, however, revealed that lower plants also possess antimicrobial properties. The overall results showed that the organisms were susceptible to different concentrations of methanol extracts of the lower plants, which is a function of their antimicrobial property. This is consistent with other works (Oyagade, 1998; Jorgnersteen, 1998; Smith and Reynard, 1992). The antibacterial properties of the lower plants observed in this study could be attributed to chemicals including polygodial, norpiquisone and lunularin, all of which constitute the phytochemical components of lower plants (Smith and Reynard, 1992).

P. afra and *P. bifurcatum* were more active than *N. bisserata* as observed by their minimum inhibitory concentrations (12.5~100 μg/ml). Both plants showed exactly the same minimum inhibitory concentrations for the individual organisms. This implies that *P. afra* and *P. bifurcatum* could be better options for use as alternative treatment for infections that could be

caused by any of these organisms. No activity was detected for *N. bisserata* at 12.5 μg/ml and 25.0 μg/ml for all the organisms tested (Table 1).

The different susceptibilities of the individual organisms to different concentrations of extracts were noted. It was observed that *E. coli* and *S. aureus* were most sensitive to methanol extract of *P. afra* and *P. bifurcatum* at all concentrations as evident in the enhanced minimum inhibitory concentrations against these organisms (12.5~100 μg/ml). *S. typhi* follows immediately. *Klebsiella* spp. was most insensitive of all the test organisms. This study also showed that *S. typhi* was least sensitive to *N. bisserata* (*MIC*=50 μg/ml). The variation in the susceptibilities of these organisms could be attributed to the intrinsic properties of these organisms, which related to the permeability of their cell surface to the extracts. Further studies are needed to look into this aspect.

Finally, this study revealed that apart from higher plants, the lower plants also possess some antibacterial properties, which make them candidates for alternative drugs in the not too distant future.

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