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Full Length Research Paper

Antimicrobial activity of *Androstachys johnsonii* Prain

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Extracts of leaf, root, bark and soil leachates of *Androstachys johnsonii* screened for antibacterial activity had a significant inhibitory effect on most Gram-positive bacteria tested. Gram-negative bacteria were resistant to most extracts. Of the Gram-negative bacteria tested, 1% leaf extract significantly inhibited the growth of all bacteria tested and both 1% root and bark extract inhibited only one bacterial strain. At the concentration of 1 mg/ml, soil extracts showed less inhibitory activity against the bacteria tested. The growth of three out of five Gram-positive bacteria was inhibited by leaf extracts. At 0.1 mg/ml, four Gram-positive bacteria were inhibited. In both 0.01 and 0.1 mg/ml of leaf extracts, all Gram-negative bacteria were uninhibited. Root extract did not inhibit the growth of four Gram-positive bacteria at 0.01 mg/ml and two Gram-positive bacteria at 0.1 mg/ml, but had a noticeably higher level of activity against all Gram-positive bacteria, than bark and soil extracts. From the results obtained in this study, we conclude that the crude extracts of *A. johnsonii* exhibit significant antibacterial activity.

Key words: Antimicrobial activity, *Androstachys johnsonii*, bacteria.

INTRODUCTION

Many plant secondary metabolites have an allelopathic effect on microorganisms (Rice, 1995) (Figure 1a). Surprisingly, it is unclear whether phenols and other secondary metabolites have a role in protecting plants from disease *in vivo* (Harborne, 1993), but, it has been demonstrated that anthraquinones extracted from different species of *Aloe* exhibit antibacterial activity by inhibiting nucleic acid synthesis in *Bacillus subtilis* (Levin et al., 1988). For many years it has been known that emodin has a direct antibacterial activity (Anke et al., 1980; Kitanaka and Takido, 1986). Emodin was found to inhibit nine soil bacterial species (Le Van, 1984). In the presence of emodin, the cells of these bacteria developed aberrant morphological forms, especially enhanced length (Le Van, 1984).

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Czygan, 1993). Plants still continue to be almost the exclusive source of drugs for the majority of the world's population (Ody, 1993). Recently, Molotja et al. (2011) studied the antifungal activity of root, bark, leaf and soil extracts of *Androstachys johnsonii*. Not much information on the antibacterial activity of compounds isolated from *A. johnsonii* is available. With the lack of knowledge of antibacterial activity of secondary metabolites found in *A. johnsonii*, this study was undertaken to screen *A. johnsonii* for antibacterial activity. In this study, the antibacterial activity of acetone extracts of root, leaf and bark of *A. johnsonii* against five Gram-positive and five Gram-negative were studied.

MATERIALS AND METHODS

Leaves, root, bark and soil from *A. johnsonii* were collected during the active growth of the plants (spring and summer seasons) from Makuya Nature Reserve (South Africa) and air dried at room temperature. The collected plant specimen was identified with the

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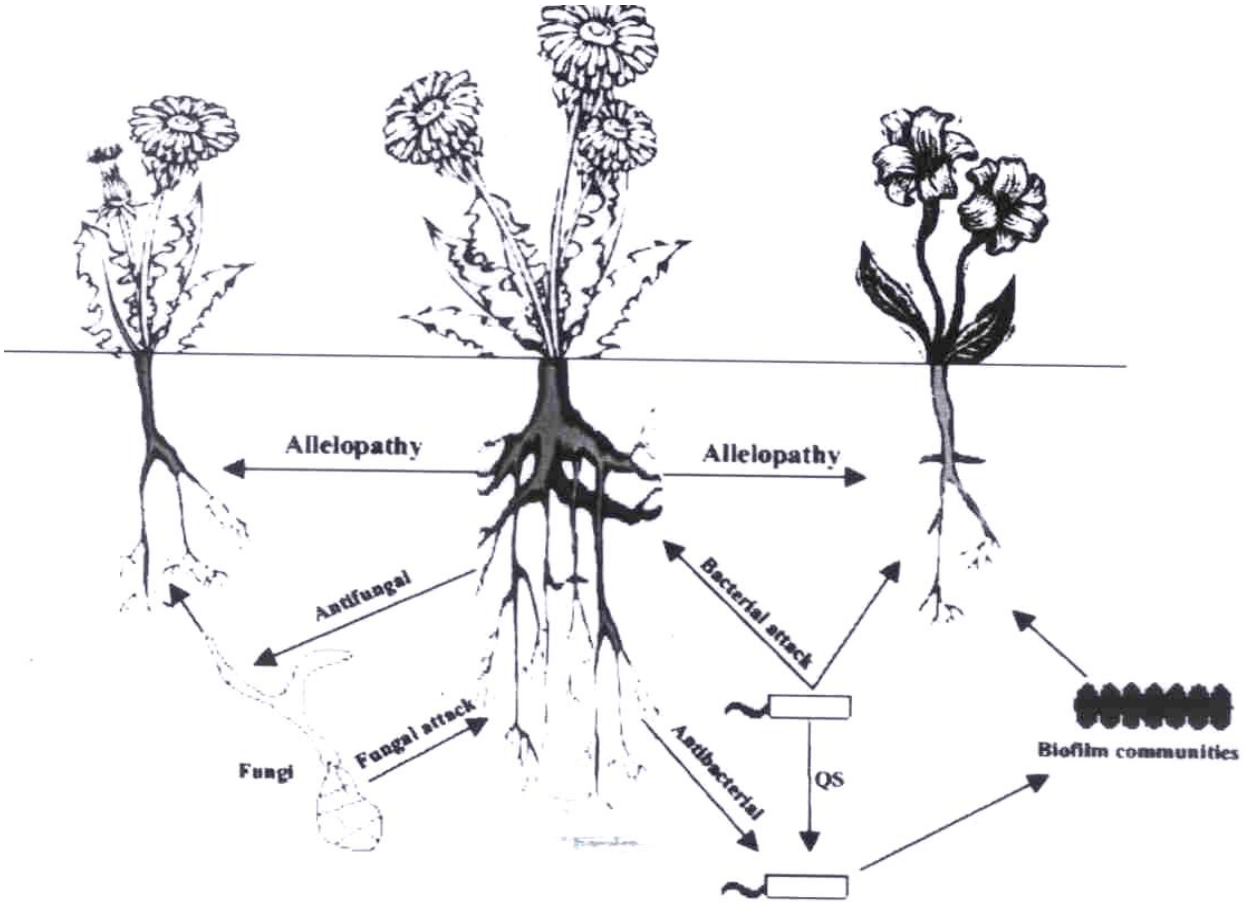

Figure 1a. Illustration of antibacterial and antifungal activities of secondary metabolites excreted from roots (Czygan, 1993).

help of floristic work of southern Africa, especially Dyer (1975). The collected plant specimen was prepared and deposited at the University of Venda Herbarium (Voucher specimen no. MMG001), in the Department of Botany. Each part (50 g) was soaked in acetone and shaken for 24 h. The mixture was then filtered through Whatman no. 2 filter paper under suction to obtain the extract. Extracts were concentrated to dryness under reduced pressure at 40°C with a rotary evaporator. The extracts were serially diluted to obtain a concentration range of 1.0 to 0.01 mg/ml in acetone and stored for further use.

Bacterial strains

Ten (10) bacteria species (Table 1) were obtained from the Department of Microbiology, University of Venda. Each organism was maintained on nutrient agar and an inoculum was recovered for testing by growth in nutrient broth for 24 h. Before streaking, each culture was diluted 1:10 with fresh sterile nutrient broth.

Antibacterial assay

Aliquots of 100 µm of each extract of 1.0 to 0.01 mg/ml concentrations in acetone were added to 5 ml of nutrient agar medium in Petri dishes and swirled carefully before congealing. The organisms were then streaked in radial patterns on agar plates (Mitscher et al., 1972). Plates were incubated at 37°C and examined after 24 h. Complete inhibition of growth was required for an extract to be declared bioactive. Three replicates were used per treatment. A blank containing only nutrient agar and 2% acetone served as controls (Meyer and Afolayan, 1995).

RESULTS

Extracts of leaf, root, bark and soil leachates of *A. johnsonii* screened for antibacterial activity had a significant inhibitory effect on most Gram-positive bacteria tested (Table 1). Gram-negative bacteria were resistant to most extracts (Figures 1a to e). Of the Gram-negative bacteria tested, 1% leaf extract significantly inhibited the growth of all bacteria tested and both 1% root and bark extract inhibited only one bacterial strain. At the concentration of 1 mg/ml, soil extracts showed less inhibitory activity against the bacteria tested.

The growth of three out of the five Gram-positive bacteria was inhibited by leaf extracts at the low minimum inhibitory concentration (MIC) of 0.01 mg/ml level. At 0.1

Figure 1b. Antibacterial activity of root extract (Number 1-10 represent bacterial strain).

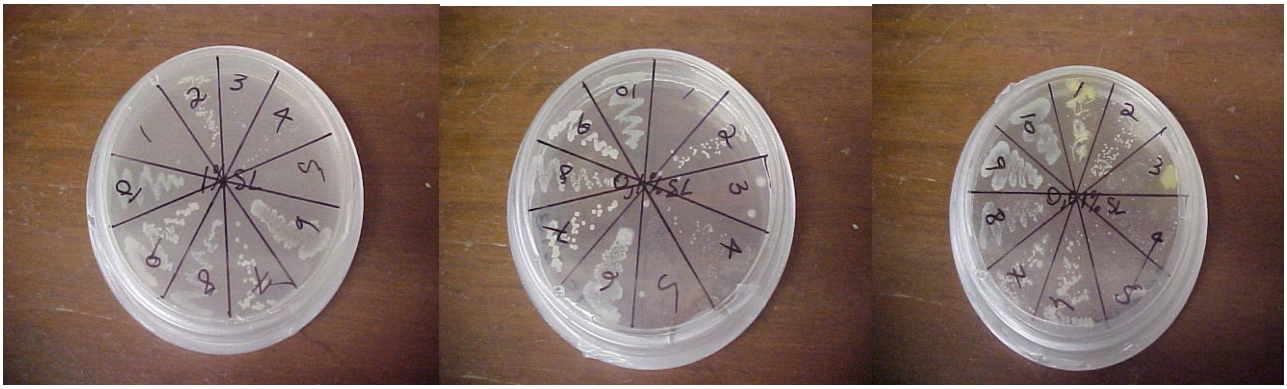

Figure 1c. Antibacterial activity of soil extract (Number 1-10 represent bacterial strain).

Figure 1d. Antibacterial activity of leaf extract (Number 1-10 represent bacterial strain).

mg/ml, four Gram-positive bacteria were inhibited. In both 0.01 and 0.1 mg/ml of leaf extracts, all Gram-negative bacteria were uninhibited. Root extract did not inhibit the growth of four Gram-positive bacteria at 0.01 mg/ml and two Gram-positive bacteria at 0.1 mg/ml, but had a noticeably higher level of activity against all Gram-positive bacteria, than bark and soil extracts at the MIC of 1 mg/ml, the highest dilution used in this investigation.

DISCUSSION

1 mg/ml of *A. johnsonii* leaf leachates showed inhibition against both Gram-positive and negative bacteria tested.

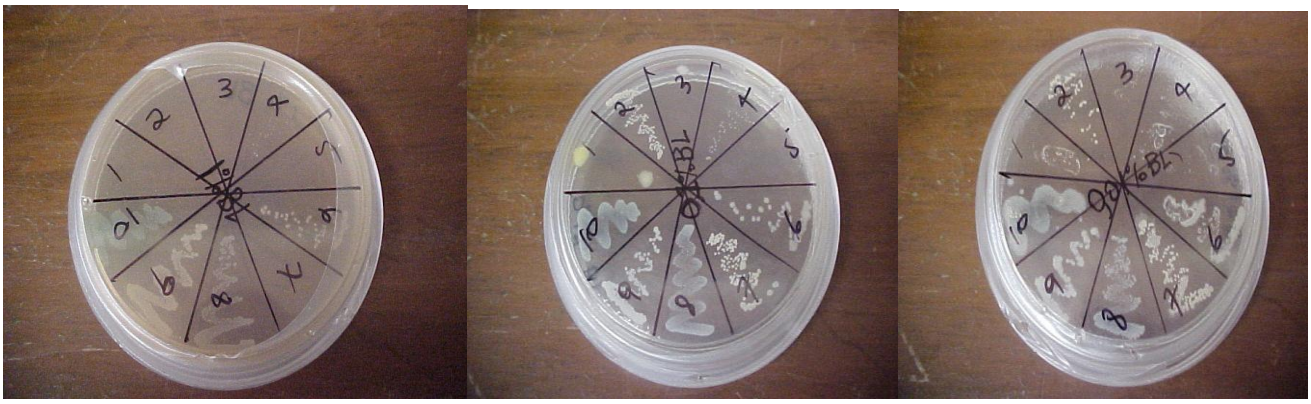

Figure 1e. Antibacterial activity of bark leachates.

Figure 1f. Illustration of positive and negative controls (Number 1-10 represent bacterial strain).

Table 1. Antibacterial activity of leaf (LL), root (RL), bark (BL) and soil (SL) extracts of *A. johnsonii*.

Bacteria		0.01 mg/mL				0.1 mg/mL				1 mg/mL			
		LL	RL	BL	SL	LL	RL	BL	SL	LL	RL	BL	SL
Gram positive	1	na	na	0.01	na	0.1	0.1	na	0.1	1.0	1.0	1.0	1.0
	2	na	na	na	na	0.1	na	na	na	1.0	1.0	1.0	na
	3	0.01	0.01	0.01	na	0.1	0.1	0.1	0.1	1.0	1.0	1.0	1.0
	4	0.01	na	na	na	na	na	na	na	1.0	1.0	na	na
Gram negative	5	0.01	0.01	0.01	na	0.1	0.1	0.1	na	1.0	1.0	1.0	1.0
	6	na	na	na	na	na	na	na	na	1.0	na	na	na
	7	na	na	na	na	na	na	na	na	1.0	1.0	1.0	na
	8	na	na	na	na	na	na	na	na	1.0	na	na	na
	9	na	na	na	na	na	na	na	na	1.0	na	na	na
	10	na	na	na	na	na	na	na	na	1.0	na	na	na

1, *Bacillus cereus*; 2, *Staphylococcus aureus*; 3, *Streptococcus pneumoniae*; 4, *Enterococcus faecalis*; 5, *Bacillus subtilis*; 6, *Escherchia coli*; 7, *Enterobacter cloacae*; 8, *Salmonella typhi*; 9, *Klebsiella pneumoniae*; 10, *Pseudomonas aeruginosa*; na, not active; LL, leaf leachate; RL, root leachate; BL, bark leachate; SL, soil leachate.

Leachates of other *A. johnsonii's* parts, which are roots, bark and soil showed great inhibition against Gram-positive bacteria. The negative results obtained against Gram-negative bacteria were not unexpected as; in general, this class of bacteria is more resistant than the Gram-positive bacteria (Tomas-Barberan et al., 1988). Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria (Burn, 1988). Access of most compounds to the peptidoglycan layer of the cell wall is hindered by the outer lipopolysaccharide layer. This explains the resistance of Gram-negative strains to the lytic action of most extracts exhibiting activity.

Antibacterial extracts from tested parts of *A. johnsonii* can be assumed to be useful to the producing plant in warding off infectious diseases. The infecting microorganisms are usually the same as those infecting higher animals (Turnbull and Kramer, 1991), and there is therefore compelling reason to suppose that anti-infective agents could be active against human or veterinary pathogens.

Infectious diseases caused by *Pseudomonas aeruginosa* are among the most difficult to treat with conventional antibiotics (Levison and Jawetz, 1992). The growth of *P. aeruginosa* was inhibited at 1 mg/ml by leaf leachates. This plant may thus, be a source which could yield drugs that could improve the treatment of infectious diseases caused by this organism.

The activity of *A. johnsonii's* leaf extracts against *S. aureus*, another human pathogen, qualifies this plant for further investigation of its bioactive compounds. Strains of *Escherichia coli* have been identified which are capable of colonizing the gastrointestinal tract and producing potent enterotoxins (Kwon-Chung and Bennett, 1992). The pathogenesis of the resulting illness resembles that of cholera. Outbreaks of *E. coli* are characterized by prolonged illness, high mortality and morbidity and by the ease and rapidity with which infections spreads (Turnbull and Kramer, 1991).

Bacillus species are common microbes found in most natural environments including soil, water, plant and animal tissues. While most *Bacillus* species are regarded as having little pathogenic potential, *B. cereus* has been known to act as primary invader or secondary infectious agent in a number of diseases and has been implicated in some cases of food poisoning (Turnbull and Kramer, 1991). Therefore, the compounds present in some parts, especially leaves, of this plant might help to combat these microbes.

Conclusions

From the results obtained in this study, we conclude that the crude extracts of *A. johnsonii* exhibit significant antibacterial activity. This probably explains why extracts from this tree can be used by the indigenous people of South Africa, most especially people of the far Limpopo Province (South Africa), where these trees are found to be dominant. The extracts can be used against a number of infectious diseases. Consequently, we proposed a detailed study of this plant in order to determine its pharmacological effects, active compounds as well as their mechanism of action.

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