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Full Length Research Paper

Antimicrobial activity of *Solanum tomentosum*

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Acetone, methanol and water extracts from the leaves of *Solanum tomentosum* were investigated for their antimicrobial activities. Growth inhibition was determined using agar dilution assays against ten selected bacterial and three fungal species. Acetone and methanol extracts were active against the Gram positive and Gram negative bacteria at a concentration of 5 mg/ml. None of the extracts inhibited the growth of *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumonae* and *Penicillium notatum*. The activities of the extracts on the test fungi were generally low. Methanol extracts was particularly suppressive to the growth of fungi with inhibitory percentage ranging from 47.22 to 50.56% on *Aspergillus niger* and *Fusarium oxysporum*.

Key words: *Solanum tomentosum*, Solanaceae, antimicrobial, antibacterial, antifungal.

INTRODUCTION

There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. The number of resistant strains of microbial pathogens is growing since penicillin resistance and multiresistance pneumoccoci caused a major problem in South Africa in 1977 (Maurer-Grimes et al., 1996; Elloff, 1998). This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious medical problem (Marchese and Shito, 2001; Poole, 2001). This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic protypes (Maurer-Grimes et al., 1996; Rabe and van Staden, 1997; Afolayan, 2003).

Solanum tomentosum L. (Solanaceae), known as snake apple, is a shrub that grows up to 60 cm high. It occurs on roadsides, undisturbed soils and rocky grasslands in the coastal belt of South Africa. The stems are densely covered with shiny reddish-brown prickles and bears clusters of brightly orange berries on the stems. Ethnomedical information from the indigenous people of the Eastern Cape Province revealed that extract from the plant is used by the Bantu tribe as a remedy for syphilis, sore throat, toothache and for the treatment of boils (Batten and Bokelmann, 1966). There is however, no report on the antimicrobial property of *S. tomentosum* in the literature. Yet, species of the *Solanum* subgenus *leptostemonum* are known for their possession of medicinal flavonoids (Silva et al., 2002, 2004). According to Mathekga and Mayer (1998), *in vitro* antimicrobial screening methods could provide the needed preliminary observations necessary to select among crude extracts, those with potentially useful properties for further chemical and pharmacological investigations. This study was aimed at investigating the antimicrobial property of S. *tomentosum* by preliminary bioassay screening.

MATERIAL AND METHODS

Plant material

The plant material was collected from a natural population in Alice, Eastern Cape Province of South Africa and a voucher specimen was prepared and deposited in the Griffen Herbarium of the University of Fort Hare.

Extract preparation

Portions of the air-dried leaves were extracted separately in acetone, methanol and water for 24 h. The extracts were filtered

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Table 1. Antibacterial activity of *Solanum tomentosum*.

Bacteria	Gram +/-	MIC[a] (mg/ml)			Antibiotics (µg/ml)	
		acetone	MeOH	Water	Str	Chl
Bacillus cereus	+	na[b]	5.0	na	< 2	< 2
Staphylococcus epidermidus	+	na	na	na	< 2	< 2
Staphylococcus aureus	+	5.0	5.0	na	< 2	< 2
Micrococcus kristinae	+	na	5.0	na	< 2	< 2
Streptococcus pyrogens	+	na	5.0	na	< 2	< 2
Escherichia coli	-	na	na	na	< 2	< 2
Salmonella pooni	_	5.0	5.0	na	< 2	< 2
Serratia marcescens	_	5.0	na	5.0	< 2	< 2
Pseudomonas aeruginosa	_	5.0	na	na	< 5	< 20
Klebsiella pneumonae	_	na	na	na	< 2	< 2

[a] Minimum inhibitory concentration
[b] not active at concentration ≤ 5 mg/ml.
Str: Streptomycin; Chl: Chloramphenicol

using a Buchner funnel and Whatman No.1 filter paper, concentrated to dryness under reduced pressure with a vacuum evaporator at 40°C, and stored at 4°C until further use. Before use, each extract was re-suspended in their respective extractant to yield 50 mg extract residue per ml solvent.

Antibacterial testing

Ten bacterial species used in this study were obtained from the Department of Microbiology, Rhodes University. Bacterial species were maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth (biolab No.2) for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan and Meyer, 1997; Grierson and Afolayan, 1999).

The bacteria were streaked in radial pattern on the agar plates (Afolayan, 1995). Plates were incubated at 37°C and examined after 24 and 48 h. Complete suppression of growth by specific concentration of an extract was required to be declared active (Sindambiwe et al.,1999; Mathekga et al., 2000). Each extract was tested at 5.0, 1.0, 0.5, and 0.1 mg/ml. Blank plates containing only nutrient agar and another set containing nutrient and 2% acetone or methanol served as controls. Acetone and methanol have been reported to be non-toxic to the organisms at 2% (Meyer and Afolayan, 1995; Mathekga and Meyer, 1998).

Antifungal testing

Potato dextrose agar (PDA) was prepared and autoclaved before the addition of the extracts. Extracts were filtered through 0.22 µm syringe-filted filters, to remove possible contaminants, before mixing with the molten agar (at 45°C) to final concentrations of 5.0, 1.0, 0.5, and 0.1 mg extract residue per ml, and poured into Petri dishes. Each plate was swirled carefully until the agar began to set and left overnight for the solvent to evaporate. Blank plates containing PDA or 2% extractant served as controls.

Three fungal species were obtained from the Department of Microbiology, Rhodes University. Each culture was maintained on PDA and was recovered for testing by sub-culturing on fresh PDA for 3 days at 25°C. The prepared plates were inoculated with plugs obtained from the actively growing margin of the fungi plates and incubated at 25°C for 5 days (Afolayan and Meyer, 1997). The diameter of the fungal growth was measured and expressed as percentage growth inhibition of three replicates. Significant differences within the means of the treatments and the controls were calculated using the LSD statistical test at 5% probability (Steel and Torrie, 1996).

RESULT AND DISCUSSION

Acetone, methanol and water extracts from the leaves of *S. tomentosum* showed antibacterial activities (Table 1). However, little or no activity was observed from the water extracts. Generally, acetone and methanol showed broad spectra of activity against the tested organisms. The activities of acetone extract was found to be higher on Gram negative than Gram positive bacteria, while methanol extract displayed more activity on Gram positive bacteria. The action of *S. tomentosum* on *Bacillus cereus* and *Staphylococcus aureus* is noteworthy. *Bacillus cereus* is a human pathogen whose infections are amongst the most difficult to treat with conventional antibiotics (Mathekga et al., 2000). The susceptibility of *Pseudomonas aeruginosa* to the extract of this plant may be a pointer to its potential as a drug that can be used against this organism. Infections caused by *Pseudomonas* species such as mastitis are often difficult to combat (Salie et al., 1996). It is of interest to note that, the growth of *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonae* were not inhibited by the extracts at the tested concentration. In this study, the acetone and methanol extracts were more active than the water extracts. Traditionally, however, plant extracts are prepared with water as infusions,

Table 2. Antifungal activity of *S. tomentosum*

Extract	Conc. (mg/ml)	Percentage inhibition		
		F. oxysporum	***A. niger***	***P. notatum***
Acetone	5.0	38.33^{c}	41.67^{c}	9.44^{b}
	1.0	34.44^{cb}	36.11^{c}	0.00^{a}
	0.5	30.00^{b}	19.45^{b}	0.00^{a}
	0.1	28.33^{b}	19.45^{b}	0.00^{a}
	Extractant	0.00^{a}	0.00^{a}	0.00^{a}
	Control	0.00^{a}	0.00^{a}	0.00^{a}
Methanol	5.0	50.56^{c}	47.22^{c}	38.33^{c}
	1.0	33.33^{b}		
	0.5	32.22^{b}	5.55^{a}	5.56^{ab}
	0.1	30.55^{b}	2.77^{a}	0.00^{a}
	Extractant	0.00^{a}	0.00^{a}	0.00^{a}
	Control	0.00^{a}	0.00^{a}	0.00^{a}
Water	5.0	36.56^{c}	22.22^{b}	0.00^{a}
	1.0	22.22^{b}	8.33^{b}	0.00^{a}
	0.5	19.45^{b}	0.00^{a}	0.00^{a}
	0.1	16.67^{a}	0.00^{a}	0.00^{a}
	Extractant	0.00^{a}	0.00^{a}	0.00^{a}
	Control	0.00^{a}	0.00^{a}	0.00^{a}

Values are means of percentage growth inhibition of three replicates. Values within a column followed by the same superscript are not significantly different at P<0.05 according to LSD test.

decoction and poultices; therefore it would seem unlikely that the traditional healer is able to extract those compounds which are responsible for activity in the acetone and methanol extracts.

The results of antifungal assays of *S. tomentosum* are presented in Table 2. The extracts showed the least activity on the growth of these organisms at 5 mg/ml. However, methanol extracts suppressed the growth of *Fusarium oxysporum* and *Aspergillus niger* with inhibition percentages ranging from 47.22 to 50.56%. *F. oxysporum* is a phytopathogen that causes vascular wilt and damping off in plants which could result in substantial stand reduction and yield loss (Gerlach, 1954; Kishi, 1974). *A. niger* was reported to be resistance to dichloromethane, aqueous and methanolic extracts of 14 plants used for traditional medicine in Paraguay (Portillo et al., 2001). In this investigation, however, methanol extracts suppressed the growth of *A. niger* significantly. Water extract did not show any appreciable activity on the growth of the fungi at 5 mg/ml or lower, except on *F. oxysporum* which was weakly (36.56%) suppressed by the extract. *Penicillium notatum* showed no growth inhibitions even at 5 mg/ml which was the highest concentration of extract used in this investigation. These results signify the potential of *S. tomentosum* as a source of therapeutic agents which may provide leads in the ongoing search for antimicrobial botanicals. The findings also validate the claims for the use of this plant in traditional medicine by the Xhosas of the Eastern Cape.

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