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Antifungal Activity of *Solanum pseudocapsicum*

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Abstract: Acetone, methanol and water extracts from the leaves, fruits and roots of *Solanum pseudocapsicum* were investigated for their antimycotic activities. The growth inhibition of the fungi was determined using the agar dilution assay against five fungal species. Acetone and methanol extracts showed significant growth inhibition of *Aspergillus niger* and *Penicillium notatum* with inhibitory activity ranging from 50.83 to 80.55% at 5.0 mg mL⁻¹. The growth of *Fusarium oxysporum* was markedly suppressed by methanol and acetone extracts from the leaves and the roots. None of the extracts suppressed the growth of *Aspergillus flavus* and *Candida albicans* at the tested concentrations. This study suggests a new potential application of *S. pseudocapsicum* as a fungicide.

Key words: *Solanum pseudocapsicum*, Solanaceae, medicinal plant, antifungal, biofungicide

Introduction

There has been an increasing incidence of fungal infections in recent years, largely due to an increase of AIDS-related opportunistic fungal pathogens and the emergence of resistance strains (Silva *et al.*, 2001; Afolayan *et al.*, 2002). Despite several available antimycotic drugs, the treatment of immunocompromised patients is still limited due to a number of factors. These include low drug potency, poor solubility of drugs, emergence of resistant strains and drug toxicity (McCutchen *et al.*, 1994; Li *et al.*, 1995; Nwosu and Okafor, 1995). This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections is a serious medical problem (Marchese and Shito, 2001; Poole, 2001). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototype (Rabe and van Staden, 1997; Afolayan, 2003; Aliero and Afolayan, 2006).

Solanum pseudocapsicum L. (Solanaceae), commonly known as winter cherry, is of widespread in the Eastern Cape Province of South Africa. It is used by the indigenous people for the treatment of acute abdominal pain, boils, gonorrhoea and as an aphrodisiac. Phytomedical investigations revealed that *S. pseudocapsicum* possesses hypertensive and anti-spasmodic (Dhar *et al.*, 1973), antiviral (Van Den Berghe *et al.*, 1978), hepatoprotective (Vijayan *et al.*, 2003) and antioxidant properties (Badami *et al.*, 2005). The genus *Solanum* is known to be rich in steroidal glycoalkaloids and sesquiterpenoids which are reported to have antifungal properties (Cipollini and Levey, 1997; Nagaoka *et al.*, 2001; Shamim *et al.*, 2004; Koduro *et al.*, 2006). There is however, no report available in the literature on the antifungal properties of extracts from the plant.

According to Mathekga and Mayer (1998), *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. This study aimed at investigating the antifungal property of *S. pseudocapsicum* by preliminary bioassay screening.

Materials and Methods

Plant Material

The aerial and underground parts of *Solanum pseudocapsicum* were collected from a natural population around Alice, South Africa. The plant was authenticated in the Department of Botany and a voucher specimen (Ali Med 01/05) was prepared and deposited in the Griffen Herbarium of the University of Fort Hare. This study was carried out in phytomedicine laboratory of the University of Fort Hare, South Africa in 2005.

Extract Preparation

Portions of the air-dried leaves, fruits and roots were extracted separately in acetone, methanol and water for 24 h. The extracts were filtered 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 the respective extractant to yield 50 mg extract residue per mL solvent.

Antifungal Testing

Adopting the method of Afolayan and Meyer (1997) and Erasto *et al.* (2006) Potato Dextrose Agar (PDA) was prepared and autoclaved before the addition of the extracts. Extracts were filtered through 0.22 µm syringe-filtered filters, to remove possible microbial 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 control.

Five 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. The diameter of the fungal growth was measured and expressed as percentage growth inhibition of three replicates (Afolayan and Meyer, 1997).

Significant differences within the means of the treatments and the controls were calculated using the LSD statistical test at 5% probability (Steel and Torrie, 1960). LC_{50} (the concentration at which there was 50% inhibition) was calculated by extrapolation.

Results and Discussion

The results of antifungal assays of *S. pseudocapsicum* are presented in Table 1. The majority of the extracts (67%) showed antimycotic activity against the test organisms at concentrations of 5 mg mL⁻¹ or lower. Extracts from the fruits had the highest mean growth inhibition on most of the fungi studied. It is of interest to note that all the extracts from the fruits suppressed the growth of *Aspergillus niger* and *Penicillium noctatum* with inhibition percentages ranging from 54.33 to 67.78%. However, methanol extract from the roots caused the highest growth suppression in *A. niger*, while the acetone extract from the leaves had 80.55% inhibition in *P. notatum*. The high percentage inhibition by the roots extracts might be due to sesquiterpenes which has been reported to be generally present in the roots of the genus *Solanum* and have high antifungal activity (Shamim *et al.*, 2004). *Aspergillus 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. Similar results were reported by Novarro Garcia *et al.* (2003) for the methanol extract from *Sedum oxypetalum* (Crassulaceae) which also

Table 1: Antifungal activity of *Solanum pseudocapsicum*. Values are means of percentage growth inhibition of three replicates

		Growth inhibition (%)					
Parts used	Conc. (mg mL ⁻¹)	<i>A. niger</i>			<i>A. flavus</i>		
		Acetone	Methanol	Water	Acetone	Methanol	Water
Leaves	5	38.89 ^a	37.78 ^b	61.11 ^c	24.44 ^e	9.44 ^b	0.00 ^a
	1	35.55 ^{ab}	37.26 ^b	21.11 ^b	13.89 ^b	0.00 ^a	0.00 ^a
	0.5	22.22 ^b	5.56 ^a	16.67 ^b	3.89 ^a	0.00 ^a	0.00 ^a
	0.1	0.00 ^a	1.11 ^a	0.00 ^a	6.11 ^{ab}	0.00 ^a	0.00 ^a
	Extractant	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	LC ₅₀ (mg mL ⁻¹)	>5	>5	3.89	>5	>5	>5
Fruits	5	54.33 ^c	62.22 ^d	61.11 ^c	19.45 ^b	31.11 ^c	3.87 ^b
	1	45.56 ^c	46.67 ^c	61.67 ^c	8.33 ^a	11.67 ^b	0.00
	0.5	20.55 ^b	41.67 ^{ab}	45.55 ^b	2.78 ^a	0.00 ^a	0.00
	0.1	3.33 ^a	31.67 ^b	0.00 ^a	2.78 ^a	0.00 ^a	0.00
	Extractant	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00
	Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00
	LC ₅₀ (mg mL ⁻¹)	3.03	1.86	1.79	>5	>5	>5
Roots	5	68.89 ^e	73.61 ^c	0.00 ^a	25.00 ^b	0.00 ^a	9.44 ^b
	1	61.95 ^c	50.83 ^b	0.00 ^a	8.33 ^a	0.00 ^a	0.00 ^a
	0.5	42.22 ^b	56.67 ^b	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	0.1	2.77 ^a	50.00 ^b	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	Extractant	0.00 ^a	1.11 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	LC ₅₀ (mg mL ⁻¹)	0.7	0.85	>5	>5	>5	>5

		Growth inhibition (%)					
Parts used	Conc. (mg mL ⁻¹)	<i>F. oxysporum</i>			<i>P. notatum</i>		
		Acetone	Methanol	Water	Acetone	Methanol	Water
Leaves	5	28.33 ^c	57.78 ^d	47.78 ^b	80.55 ^e	75.56 ^c	57.78 ^d
	1	27.22 ^c	43.89 ^c	46.00 ^b	65.56 ^d	57.78 ^b	44.44 ^c
	0.5	26.67 ^c	36.67 ^c	45.00 ^b	38.89 ^c	52.22 ^b	34.44 ^b
	0.1	13.89 ^b	17.78 ^a	43.89 ^b	16.67 ^b	8.89 ^a	34.44 ^b
	Extractant	3.87 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	LC ₅₀ (mg mL ⁻¹)	>5	2.76	>5	0.71	0.48	2.67
Fruits	5	30.56 ^c	43.89 ^c	45.00 ^c	67.78 ^d	66.67 ^c	66.67 ^d
	1	30.55 ^c	32.22 ^d	41.11 ^{ab}	54.44 ^c	60.00 ^c	58.33 ^c
	0.5	27.78 ^c	27.78 ^c	5.56 ^b	37.22 ^b	58.89 ^c	30.55 ^b
	0.1	18.33 ^b	21.11 ^b	33.89 ^b	32.22 ^b	43.33 ^b	0.00 ^a
	Extractant	0.00 ^a	0.55 ^a	0.00 ^a	1.11 ^a	0.00 ^a	0.00 ^a
	Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	LC ₅₀ (mg mL ⁻¹)	>5	>5	>5	0.87	0.27	0.85
Roots	5	51.11 ^d	41.67 ^d	48.33 ^c	79.44 ^d	63.89 ^d	0.00 ^a
	1	42.78 ^c	22.78 ^c	46.11 ^c	65.56 ^c	53.61 ^{cd}	1.11 ^a
	0.5	35.00 ^b	13.89 ^b	42.22 ^b	48.89 ^b	46.67 ^{ab}	9.44 ^a
	0.1	33.89 ^b	9.45 ^b	42.22 ^b	44.44 ^b	38.33 ^b	0.00 ^a
	Extractant	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
	LC ₅₀ (mg mL ⁻¹)	4.47	>5	>5	0.53	0.74	>5

Values within a column followed by the same superscript of the same extract are not significantly different at p<0.05 according to the LSD test. LC₅₀ values were calculated by extrapolation

inhibited the growth of *A. niger* significantly. The susceptibility of *Aspergillus niger* to the extract of *S. pseudocapsicum* is noteworthy, as the fungus has recently been implicated in cases of immunocompromised patients that frequently develop opportunistic and superficial mycosis (Ngane *et al.*, 2000; Portillo *et al.*, 2001; Silva *et al.*, 2001). Two fungal species, *Aspergillus flavus* and *Candida albicans* showed no growth inhibitions to all the 27 extracts at 5 mg mL⁻¹ which was the

highest concentration used in this investigation. The growth of *Fusarium oxysporum* was markedly suppressed by the methanol and acetone extracts with 51.11 to 57.78%, respectively. *Fusarium* species are known to produce mycotoxins mostly found as a contaminant in cereal grains (Rotter *et al.*, 1996; Placinta *et al.*, 1999; Goyarts *et al.*, 2006) which is responsible for reduced feed intake and decreased weight gain in animals at a lower dose levels (Rotter *et al.*, 1996). In this study, the acetone and methanol extracts showed a broad spectrum of activity against the fungal species tested and were more active than the water extracts. Traditionally, however, plant extracts are prepared with water as infusions, 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. This could be offset by the low concentration of the extracts used in the experiment (5.0 mg mL⁻¹ being the maximum concentration tested in this case), unlike the traditional herbal practitioners who apply the water extracts with no upper limit to their concentration. In this study, the extracts from the fruits display higher inhibitory activity than those of the leaves and roots against the fungi.

Although the fungal species used are not directly implicated as human pathogens, the aim of the antifungal testing of the extracts was to establish whether they exhibited antimycotic activity in general. These results signify the potential of *S. pseudocapsicum* as a source of antifungal therapies which may provide leads in the ongoing search for biofungicidal botanicals.

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