View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by AJOL - African Journals Online

African Journal of Biotechnology Vol. 10(30), pp. 5725-5727, 27 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2212
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Antifungal activity of root, bark, leaf and soil extracts of *Androstachys johnsonii* Prain

Georginah M. Molotja[1], Maanda H. Ligavha-Mbelengwa[2] and Ramakrishna B. Bhat[2]*

[1]Department of Science Foundation, University of Venda, Thohoyandou, Limpopo Province, South Africa.
[2]Department of Botany, University of Venda, Thohoyandou, Private Bag X5050, Limpopo Province, South Africa 0950.

Accepted 25 January, 2011

Extracts of leaf, root, soil and bark of *Androstachys johnsonii* Prain (commonly called Lembobo ironwood) screened for antifungal activity had a significant inhibitory effect on the most of fungi tested in this investigation. Of the four fungi tested in the present study *Fusarium solani* was significantly inhibited by all extracts (that is, 0.01% of leaf, root, bark; 0.1% leaf, root, soil and bark; 1% of leaf, root, soil and bark) of *A. johnsonii* from the lowest to the highest dilution. *Aspergillus flavus* was inhibited by all extracts except 0.01% soil and bark leachates, whereas *Aspergillus niger* was killed by root and soil extracts at all concentrations. The fourth fungus *Trichoderma harzianum* was inhibited by all extracts of *A. johnsonii* (that is, 0.01% of leaf, root and bark; 0.1% of leaf, root, bark and soil and 1% of leaf, root, bark and soil extracts) except 0.01% soil extract. Therefore, we conclude that the crude extracts of *A. johnsonii* exhibit significant antifungal activity. It would not be a wise idea to remove *A. johnsonii* and use the site for agricultural practices; instead this tree can be used as a medicine to treat diseases caused by fungi.

Key words: Antifungal activity, *Androstachys johnsonii,* leaf, bark, roots, soil.

INTRODUCTION

Androstachys johnsonii (*A. johnsonii*) is a large tree that can grow up to 20 m. It is an evergreen tree that belongs to the family Euphorbiaceae. The crowns are long and slender, the trunks are long, bare and straight when in dense stands, but in open stands, crowns are moderately spreading, irregular round and sparse with lateral branches fairly low down. The young twigs are covered by a white villose layer. The stems of trees that grow on sunny sides are grayish-white but stems of those growing under shade are nearly black. Barks are grooved longitudinally, resembling that of *Colophospermum mopane.*

Allelopathy is mediated by release of certain secondary metabolites by plant roots and plays an important role in the establishment and maintenance of terrestrial plant communities. These plant secondary metabolites have been isolated from various plants by many workers (Adeshina et al., 2009; Adeshina et al., 2010; El-Mahmood, 2009). It also has important implications for agriculture; the effects may be beneficial as in the case of natural weed control or detrimental, when allelochemicals produced by weeds affect the growth of crop plants (Callaway and Aschehoug, 2000). Recently, Bais et al. (2002), identified (±)-catechin as the root-secreted phytotoxin responsible for the invasive behavior of knapweed in the rhizosphere. Interestingly, (-)-catechin was shown to account for the allelochemical activity, whereas (+)-catechin was inhibitory to soil-borne bacteria and fungi (Bais et al., 2002).

Fungi are important because they help to process dead plant and animal matter through decay. Lots of string-like fungi live tangled up with a plant's roots and help to pass on nutrients. *Fusarium solani* is the most common *fusarium* species recovered in humans and animals (O'Donnell, 2000). This fungus is an atiologic agent in keratitis, endophthalmitis, cutaneous infections, burn patients, mycetoma, sinusitis and septic arthritis.

Trichoderma harzianum is a versatile fungus used commercially in variety of ways, including food and textile biocontrol agents and plant growth promotion. It is used for production of cellulases and other enzymes that degrade complex polysaccharides (Harman and Kubicek,

*Corresponding author. E-mail: bhatrb@yahoo.com, Bhat.Ramakrishna@univen.ac.za. Fax: + (27)-15-962-8648.

Table 1. Antifungal activity of leaf (LL); root (RL); bark (BL); soil (SL) extracts of *A. johnsonii.*

Fungi	0.01% (mg/ml)				0.1% (mg/ml)				1% (mg/ml)			
	LL	RL	SL	BL	LL	RL	SL	BL	LL	RL	SL	BL
A. flavus	+	+	-	-	+	+	+	+	+	+	+	+
F. solani	+	+	+	+	+	+	+	+	+	+	+	+
A. niger	-	+	+	-	-	+	+	-	-	+	+	-
T. harzianum	+	+	-	+	+	+	+	+	+	+	+	+

Key: LL, leaf leachates; RL, root leachates; SL, soil leachates; BL, bark leachates; +, active concentrations; -, not active concentrations.

1998). As noted, this fungus is used for the control of plant diseases (Chet, 1993). For many years, the ability of this fungus to increase the rate of plant growth and development especially, their ability to cause production of more robust roots has been known (Altomare et al., 1999). Recently, Altomare et al. (1999) found that, one strain increases the numbers of even deep roots at a meter below the soil surface.

Aspergillus flavus is a fungus that grows by producing thread like branching filaments known as hyphae. It can also be pathogenic on several plant and animal species. The fungus can infect seeds of corn such as maize kernels, peanuts, cotton and nut trees (Scheidegger and Payne, 2003). Payne (1998) coined out that, the growth of the fungus on a food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound. *A. flavus* is also the second leading cause of aspergillosis in humans.

Aspergillus niger is commonly found as saprophyte growing on dead leaves, stored grains, compost piles and other decaying vegetation. The primary uses are for production of enzymes and organic acids by fermentation. Fermentation to produce enzymes such as glycoamylase, pectinases, α-galactosidase, etc. may be carried out in vessels as large as 100 000 L (Staiano et al., 2005).

MATERIALS AND METHODS

Root (main and lateral), bark (from trunk and shoots), leaf (young and old) and soil of *A. johnsonii* and control of the same soil without *A. johnsonii* were collected from Thengwe village, Limpopo Province, South Africa, during summer and air dried at room temperature. Each pulverized part (10 g) was soaked in 100 ml 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. To obtain concentrations from 1% to 0.01%, firstly 10 g of each extracts (root, bark, leaf and soil) was diluted by adding 10 ml acetone. The pour plate method was used in this investigation.

Fungal isolates

Four fungal species (Table 1) were obtained from the Department of Microbiology and Plant Pathology, University of Pretoria. Each organism was maintained on nutrient agar (Biolab) and an inoculum was recovered for testing by growth on a potato dextrose nutrient agar (Biolab) for 24 h. The potato dextrose nutrient agar was prepared by dissolving 39 g (as per the instruction on the bottle) of potato dextrose powder in 1 L of distilled water. The mixture was boiled for 1 min to completely dissolve the powder. It was then, autoclaved at 121°C for 15 min.

Antifungal bioassay

The pour plate method was used in this investigation. The amended 5 ml of plant extracts were added to 15 ml of potato dextrose nutrient agar medium in Petri dishes and swirled carefully before congealing. Different concentrations were prepared to find out the degree of fungal growth. A negative blank containing nutrient agar and 2% acetone served as control (Mathekga et al., 2000). The prepared plates were inoculated with disks obtained from actively growing margins of fungi plates (that is, before spore formation) and incubated at 25°C in dark for three days. Plates were examined and complete suppression of growth was required for the extract to be declared bioactive. Three replications were used per treatment.

RESULTS AND DISCUSSION

Of the four fungi tested in the present study *F. solani* was significantly inhibited by all extracts of *A. johnsonii* from the lowest to the highest dilution. *A. flavus* was inhibited by all extracts except 0.01% soil and bark leachates, whereas *A. niger* was killed by root and soil extracts at all concentrations. The fourth fungus, *T. harzianum* was inhibited by all extracts of *A. johnsonii* except 0.01% soil extract.

This research has revealed that, *A. johnsonii* may be selected as one of plants utilized in traditional medicines. Results showed high activity of leaf extract of *A. johnsonii* against the four tested fungi. El- Mahmood et al. (2010) worked with the *Gmelina arborea* leaf extracts which showed higher activity than the stem bark extracts. Most leachates are antifungal at low MIC (Minimum inhibition concentration). The success of the ethnobotanical approach to drug discovery can no longer be questioned. Historical and current discoveries attest to its power (Cox, 1994). Medicinal plants are the “backbone” of traditional medicine (Farnsworth, 1994). The types of diseases or complaints treated are ailments of the digestive tract, general pain wound dressing and inflammations but this natural antifungal activity can be rapidly

lost because of seasonal changes presumably due to chemical or enzymatic degradation of the active compounds (Prusky et al., 1983). Even though *A. johnsonii* exclude other plant species from growing in its site, it represents a potential source of effective fungicide.

For a biologically active compound like fungicide to have activity it must first diffuse from its site of application, usually the exterior of the cell, to its site of action, often within the cell and then, partition itself onto the active site (Hansch and Lien, 1971). The rate of these events will depend on the lipophilicity of the compound. Once at the active site, the compound has some chemical and physical effect that accounts for its activity. There is a growing consensus that, in most systems, antifungal agents exert their toxicity by some membrane-associated phenomenon (Laks and Pruner, 1989), again indicating the possible importance of lipophilicity for their activity.

Adegoke et al. (2000) reported the inhibitory effect of limonene, the constituent of *Cinnamomum citrates* oil, against *A. flavus* and they confirmed that, limonene caused injury on membrane of susceptible organism. In other studies, clove and cinnamon powder were observed to promote the total inhibition of *A. niger* mycelial development in all doses tested (Hitokoto et al., 1980).

Conclusions

Results of this study showed that, *A. johnsonii* extracts had a significant inhibitory effect against the four fungi tested. We therefore conclude that, the crude extracts of *A. johnsonii* exhibited significant antifungal activity. It was pointed out in the previous study that, it would not be a wise idea to remove *A. johnsonii* and use the site for agricultural practices, but this tree can be used as a medicine to treat diseases caused by fungi. People from the far Limpopo Province where these trees are found dominant, can on the other hand benefit from this tree as an antifungal agent.

ACKNOWLEDGEMENTS

Sincere gratitude to the heavenly father who gave us power, strength and wisdom to overcome any difficulty throughout this research. Special thanks are due to Prof. ADM Mathekga for his enthusiasm and assistance throughout this study. Department of Microbiology, University of Pretoria (South Africa) is acknowledged for providing fungi isolates.

REFERENCES

Adegoke GO, Iwahashi H, Obushi K, Iwahashi Y (2000). Inhibition of food spoilage yeasts and of latoxigenic moulds by monoterpenes of the spice, *Aframomum danielli*. Flav. Fragr. J. 15: 147-150.

Adeshina GO, Okeke CE, Osuagwu NO, Ehinmidu JO (2009). Preliminary studies on antimicrobial activities of ethanolic extracts of *Ficus sycomorus* Linn and *Ficus platyphylla* Del. (Moraceae). Int. J. Biol. Chem. Sci. 3(5): 1013-1020.

Adeshina GO, Onaolapo JA, Ehinmidu JO, Odama LE (2010). Phytochemical and antimicrobial studies of the ethyl acetate extract of *Alchornea cordifolia* leaf found in Abuja, Nigeria. J. Med. Plants Res. 4(8): 649-658.

Altomare C, Norvell WA, Bjorkman T, Harman GE (1999). Solubilization of phosphates and micronutrients by the plant-growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol. 65: 2926-2933.

Bais HP, Walker TS, Stermitz FR, Hufbauer RA, Vivanco JM (2002). Enantiomeric dependent phytotoxic and antimicrobial activity of (±)-catechin; a rhizosecreted racemic mixture from *Centaurea maculosa* (spotted knapweed). Plant Physiol. 128: 1173-1179.

Callaway RM, Aschehoug ET (2000). Invasive plants versus their new and old neighbours: a mechanism for exotic invasion. Science, 90: 521-523.

Chet I (1993). Biotechnology in Plant Disease control. Wiley-Liss, New York, p. 373.

Cox PA (1994). The ethnobotanical approach to drug discovery. Strengths and limitations, In: CIBA Foundation Symposium 185. John Wiley and Chichester. New York. pp. 25-41.

El-Mahmood AM (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. J. Med. Plants Res. 3(7): 498-505.

El-Mahmood AM, Doughari JH, Kiman HS (2010). *In vitro* antimicrobial activity of crude leaf and stem bark extracts of *Gmelina arborea* (Roxb) against some pathogenic species of Enterobacteriaceace. Afr. J. Pharm. Pharmacol. 4(6): 355-361.

Farnsworth NR (1994). Ethnopharmacology and drug development, In: CIBA Foundation Symposium 185 Wiley and Chichester. New York. pp. 42-59.

Hansch C, Lien EJ (1971). Fungal sterols and the mode of action of the polyene antibiotics. J. Med. Chem. 14: 653-670.

Harman GE, Kubicek CP (1998). *Trichoderma* and *Gliocladium*, Vol.2 Enzymes, Biological Control and Commercial Applications. Taylor and Francis, London. p. 393.

Hitokoto H, Morozumi S, Wauke T, Sakai S, Kurata H (1980). Inhibitory effects of spices on growth and toxin production of toxigenic fungi. Appl. Environ. Microbiol. 39:818-822.

Laks E, Pruner MS (1989). Flavonoid structure/activity relation of flavonoid phytoalexin analogues. Phytochemistry, 28(1): 87-91.

Mathekga ADM, Meyer JMM, Horn MM, Drewes, SE (2000). An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespititium*. Phytochemistry, 53: 93-96.

O'Donnell K (2000). Molecular phylogeny of the Nectria haematococca-Fusarium solani species complex. Mycologia, 2: 919-938.

Payne GA (1998). Process of contamination by aflatoxin producing fungi and their impacts on crops. In: Mycotoxinsin Agriculture and food safety. Sinha KKBhatnagar D. Marcel Dekker, Inc. New York, p. 511.

Prusky MD, Keen NT, Eaks I (1983). Polygodial, an antifungal potentiator. Plant Pathol. 22: 189-192.

Scheidegger KA, Payne GA (2003). Unlocking the secrets behind secondary metabolism: A review of *Aspergillus flavus* from pathogenecity to functional genomics. J. Toxicol. Toxin. Rev. 22 (2-3): 423-459.

Staiano M, Paolo B, Mose`R, Sabato D`A (2005). Glucose biosensors as models for the development of advanced protein-based biosensors. Mol. Biosyst. 1: 354-362.