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ISOLATION OF TOXIGENIC FUNGI FROM COMMERCIALLY AVAILABLE MEDICINAL PLANT MATERIAL

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Abstract: Sun-dried commercial samples of six medicinal plant species widely used in Sri Lanka for treatment of diseases were used to isolate saprophytic and pathogenic fungi. Twelve species of fungi were isolated from various parts of plants on potato dextrose agar plates after surface disinfection. *Aspergillus flavus* (yellow-green form), a notorious toxigenic fungus was isolated from all 6 plants. Other potentially toxigenic species present were *A. parasiticus, A. ochraceus, A. sulphureus,* and *Fusarium avenaceum.* Duration of storage and environmental conditions play a vital role in the growth and formation of mycotoxins by molds on/in plant products. Screening techniques for known mycotoxins and quality control methods are essential in reducing the likelihood of contamination of medicinal plant material.

Key words: *Aspergillus flavus,* fungi, *Fusarium,* medicinal plants, toxigenic fungi.

INTRODUCTION

Abrus precatorius Linn. (olinda), *Alysicarpus vaginalis* (Linn.) DC. (aswenna), *Asteracantha longifolia* Nees. (neera mulliya), *Cassia auriculata* Linn. (ranawara), *Mollugo cerviana* Seringe (patpadagam), and *Rauvolfia serpentina* (Linn.) Kurz (ekaweriya) are valuable medicinal plants widely used in the treatment of a variety of diseases. These plants occur in tropical regions of India, Sri Lanka, Thailand, Philippines, China and also in Africa and North America.

Aqueous extract of roots of *Abrus* relieves obstinate coughs and sore throats and is used as a snake-bite remedy. The leaves are used in the treatment of conjunctivitis, painful swellings and stomach complaints. In Sri Lanka, seeds are used to cure itch and sores and wounds due to biting of mammals and also in the treatment of diabetes.[1] *A. vaginalis* is used with other drugs for fever, dysentery, diarrhoea, and chronic malarial fevers.[1] In Sri Lanka, decoctions of the whole plant are used specifically for dissolving stones in the bladder and also as a diuretic and expectorant.[1] *C. auriculata* roots are used in decoctions for fever, diabetes, constipation and diseases of the urinary system. While leaves have laxative properties, dried flowers and buds are used as a substitute for tea in the case of diabetic patients. In Africa, bark and seeds of this plant are used for rheumatism, eye diseases and gonorrhoea.[1] *M. cerviana* has antiseptic properties and is also used to cure itch and other skin disorders. The extract of the plant is believed to promote the flow of lochial discharge in women.[1] *R. serpentina* is used for fever, cholera and to control blood pressure. In India, decoctions of roots are given to increase uterine contractions in childbirth.[1]

Some of the medicinal plants are restricted to special localities, from where they are collected, dried and then distributed to the rest of the country and stored for future use. Storage decay of plant products is a common occurrence in Sri Lanka. Proper storage facilities are not available in Sri Lanka thus increasing the possibility of medicinal plant contamination by naturally occurring saprophytic or pathogenic fungi. The levels of relative humidity and environmental temperature in tropical countries are precisely the conditions which favour fungal colonization of stored food material. This may result in the accumulation of toxic fungal metabolites, which can be hazardous to humans who consume mold contaminated products without knowing the presence of mycotoxins. Previous reports indicate the occurrence of *A. flavus* and *Fusarium* species on surface disinfected dried Sri Lankan medicinal plant parts of *Aerva lanata, A. vaginalis* and *Tribulus terrestris.*[2,3] These reports further indicate the capability of aflatoxin and *Fusarium* toxin production by the above mentioned fungi. Sclerotial and non sclerotial isolates of *A. flavus* (yellow-green form) from *A. lanata* and *A. vaginalis* produced aflatoxin B_1 levels upto 2800 µg/50 ml of culture filtrate when grown on a liquid (SMKY) medium and incubated at 25°C for 12 days.[2] The present study was conducted to determine the possibility of contamination of some commercially important stored medicinal plants (which are used in decoctions and other ayurvedic preparations) with saprophytic, toxigenic or pathogenic mycoflora.

METHODS AND MATERIALS

Plant material: Plants of *A. precatorius, A. vaginalis, A. longifolia, C. auriculata, M. cerviana* and *R. serpentina* were obtained from randomly selected medicinal plant retail stores in Gabo's Lane, Colombo. These plants had been collected from various localities in Sri Lanka, mixed, sun dried and stored in gunny bags at room temperature for 3-4 months. Five 100 g samples of each plant species were purchased from the retail stores. The five samples of each species were mixed prior to analysis.

Fungal flora: From each of the above mixed samples, 50 randomly selected pieces of stems, roots, leaves, seeds or flowers were surface sterilized for 5 min in 5% Clorox (0.025% NaOCl) and transferred to Potato Dextrose Agar (PDA) plates containing 0.1 mg/L Streptomycin.[2] The plates were incubated for 8-10 days at room temperature and the fungi that grew out were identified by comparing their morphology with published books/keys.[4-8] The frequency of occurrence was also calculated.

RESULTS

A number of fungal species which were previously known for mycotoxin production were isolated along with few pathogenic or saprophytic mycoflora from surface disinfected plant parts. A yellow-green form of *A. flavus*, a notorious toxigenic fungus was isolated from all 6 plants used in this study (Table 1). This fungus occurred at highest frequencies on roots of *R. serpentina* (84% of total colonies) and *A. precatorius* (50%). Olive coloured *A. flavus* species was isolated

from *A. precatorius*, *A. vaginalis* and *A. longifolia*. This mold occurred at 8% and 10% frequencies on stems and seeds of *A. longifolia* respectively. *Aspergillus parasiticus*, another toxigenic mold, was isolated only from *A. longifolia* at a frequency of 10%. *A. ochraceus* and *A. sulphureus* which have been reported to produce harmful secondary metabolites occurred in roots of *A. vaginalis* (10%) and *A. longifolia* (total frequency 24%). Besides potent *Aspergillus* species, *F. avenaceum* was isolated at a frequency of 4% from seeds of *A. longifolia*. Saprophyte *A. niger*, was isolated from 5 medicinal plants, and occurred at high frequencies on roots of *A. precatorius* (78%), seeds of *A. longifolia* (68%) and flowers/buds of *C. auriculata* (64%). Other non-toxigenic fungi present were *Neocosmospora vasinfecta, Anthostomella destruens, Curvularia pallescens* and *Rhizoctonia solani* (Tables 1 and 2).

Table 1: Fungi present in surface disinfected plant materials of *A. precatorius*, *A. vaginalis*, *A. longifolia* and their frequencies.

Fungi	Plant species				
	A. precatorius	*A. vaginalis*		*A. longifolia*	
	1[a]	2	1	3	4
A. niger	78[b]	6	6	18	68
A. flavus (yellow-green)	50	6	4	6	10
A. flavus (olive)	2	-	2	8	10
A. parasiticus	-	-	-	-	10
A. ochraceus	-	-	10	-	-
A. sulphureus	-	-	-	20	4
C. pallescens	-	-	-	28	44
F. avenaceum	-	-	-	-	4
A. destruens	-	10	-	8	-
N. vasinfecta	-	-	2	-	-
Peyronellae spp.	-	2	-	-	-
R. solani	-	64	28	-	-

a = plant part: 1 = root; 2 = leaves, seeds, and stems; 3 = stems; 4 = seeds; b = percentage colonies, based on colonies present in 50 pieces of plant tissue.

DISCUSSION

A previous study indicated the occurrence of sclerotial isolate of *A. flavus* (yellow-green) on surface disinfected leaves, flowers and stems of *Aerva lanata* and whole plant pieces of *A. vaginalis* at total frequencies of 35 and 100% respectively.[2] Another report indicated that out of 50 isolates of *A. flavus* obtained from different drug plant parts, 21 isolates were toxigenic.[9] It was reported that the frequency of *A. flavus* can be as high as 62% in surface disinfected winged bean seeds.[10] These results are not surprising since *A. flavus* can grow on a wide variety of crops including cereal grains, spices, pulses, vegetables and medicinal plants and also may produce many aflatoxins including aflatoxin B_1 and B_2. *A. flavus* therefore may grow readily on medicinal plant parts in tropical countries.[10]

Table 2: Fungi present in surface disinfected plant materials of *C. auriculata*, *M. cerviana*, *R. serpentina* and their frequencies.

Fungi	Plant species		
	C. auriculata	*M. cerviana*	*R. serpentina*
	5[a]	6	1
A. niger	64[b]	-	52
A. flavus (yellow-green)	4	2	84
A. flavus (olive)	-	-	-
A. parasiticus	-	-	-
A. ochraceus	-	-	-
A. sulphureus	-	-	-
C. pallescens	-	-	-
F. avenaceum	-	-	-
A. destruens	-	4	-
N. vasinfecta	-	-	-
Peyronellae spp.	-	34	-
R. solani	-	-	-

a = plant part: 1 = root; 5 = flower and flower bud; 6 = pieces from whole plant; b = percentage colonies, based on colonies present in 50 pieces of plant tissue.

In Sri Lanka, coconut oil and peanut products have been extensively tested and found to contain relatively high amounts of aflatoxins.[11] Processed coconut food products examined by Samarajeewa contained more than 50 µg/kg aflatoxin.[11] These levels are referred to as medium-high in the Tropical Products Institute classification of toxin levels.[11] Even though not very common and popular as *A. flavus, A. parasiticus, A. ochraceus* and *A. sulphureus* have been reported to be toxigenic molds which produce metabolites such as ochratoxin A,B,C, aflatoxins and other derivatives.[12] In a previous study *A. ochraceus* and *A. parasiticus* have been isolated from surface sterilized *Brassica* spp. and spices like pepper.[13,14] This is the first instance where *A. sulphureus* was isolated from a valuable tropical medicinal plant such as *A. longifolia*. A toxigenic fungal species, *F. avenaceum*, which was isolated from *A. longifolia* seeds has not been recorded in Sri Lanka before. *F. avenaceum* has a worldwide distribution as a pathogen that causes root, foot and ear rot of cereals and a large number of other hosts. This fungus has been isolated from overwintering cereals in the USSR and is particularly prevalent as a seed-borne organism in temperate areas.[15] *C. pallescens* has been reported to occur on surface disinfected rice seeds and seeds of *Brassica rapa* and *B. chinensis*.[16] Infections of *C. pallescens* are mainly responsible for the reduction of the germination of seeds.[16] *Rhizoctonia solani,* a pathogen which causes damping off in seedlings, is also responsible for sheath blight of rice.[17,18] The rest of the fungi isolated from the medicinal plants are not considered as important pathogens or toxigenic flora. *Anthostomella* spp. are widespread on dead leaves of many hosts,[19] whereas *Neocosmospora vasinfecta* occasionally infects roots of different crops causing wilt disease.[19]

Longer the period of storage, greater the opportunity for fungal growth and mycotoxin build up, particularly at high temperatures and humidity.[20] Cleanliness of storage containers is another important factor influencing mycotoxin production. For example, *A. flavus* can survive on residues left in storage containers which serve as a source of inoculum when fresh plant material is added to the containers.[20] Since many toxigenic fungi were isolated from dried, stored medicinal plants, the present study points out the need for proper storage facilities for medicinal plants. The best means of controlling the accumulation of aflatoxins in foods is to prevent the growth of fungi. This is best achieved by reducing moisture below 8% during storage. However, it is not easy to maintain such low moisture levels under humid tropical conditions.[11] Plants should be stored in special environments where temperature is low (< 13°C) and relative humidity is less than 75%.[21] Further, screening techniques for known mycotoxins are essential and standards/quality control methods have to be developed and employed before plant materials are distributed to retail or wholesale stores in Sri Lanka. One such screening technique would be to expose various levels (microgram amounts) of extracts prepared from randomly selected, dried and stored medicinal plants samples to mammalian cell lines. Depending on the amount of toxin present in plant extracts, various levels of cytotoxicity could be observed. The cytotoxicity of plant extracts could be compared with known toxin standards. Aflatoxin identification could be carried out by chromatographing various levels of plant extracts and by comparing with aflatoxin standards. These measures will reduce the likelihood of contamination of medicinal plant material by *Fusarium, Aspergillus* spp. or other toxigenic fungi.

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