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Original Article

Isolation and Identification of Fungal Contamination in Stored Medicinal Plants

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ABSTRACT

Objectives: The increase in the consumption of natural drugs has made their use a Public Health problem due to the possibility of access to products without adequate conditions of use. The concern with the quality of the natural products is due to the potential fungal contamination and the risk of the presence of mycotoxins.

Methods: The levels of fungal contamination were analyzed in 15 samples of *Saraca indica, Terminalia arjuna, Hemidesmus indicus* randomly collected from different places of Agra and nearby regions and mycoflora was isolated and identified with different methods.

Results: As a result of Mycological examination, 93% of the total samples examined, found to be contaminated with different fungi. A total of 13 different fungal species was isolated from all the three medicinal plant samples. The predominant mycoflora obtained was distributed in five different genera comprised of *Aspergillus, Penicillium, Alternaria, Rhizopus* and *Syncephalastrum.* The *Aspergillus* (71.95%) was observed as the most dominant genera recovered, followed by *Penicillium* (15.44%), *Rhizopus* (9.51%), *Alternaria* (1.67%) and *Syncephalastrum* (1.41%). Most of the identified fungal species like *Aspergillus, Penicillium* and *Alternaria* are reported to have the ability to produce mycotoxins like aflatoxins, ochratoxins, citrinin and alternaria toxins. The presence of a wide range of storage fungi indicates that the mould probably infects the crude herbal drugs during harvesting and post harvesting, processing i.e. mainly during drying, storing, transportation and processing.

Conclusion: On the basis of present investigations, it may be concluded that the contamination of raw materials is alarming, as these raw materials needs thorough inspection before being channeled to the drug industries and for public use.

Keywords: Medicinal plants, Mycoflora, Toxigenic fungi, Mycotoxins.

INTRODUCTION

Plants have been used in the prevention and treatment of disorders and diseases since ancient time. In spite of their origin, natural drugs should not be viewed by simple tools of folk medicine since they are a class of pharmaceutical products and should meet the requirements of quality safety and efficacy[1]. The advancement of synthetic medicine overshadowed the traditional herbal medicine for over 50 years. However, in the last years there was a progressive increase in the demand of herbal preparations of botanical origin as an alternative or complementary medicine due to economics, local and cultural factors[2].

Use of herbal medicines in Indo-Pak subcontinent is many centuries old. The peoples of this region believe herbal products are closer to nature and free of any side effect. A wide variety of herbs are found in Pakistan due to varied ecological conditions. But because of lack of coordination between stakeholders of the herbal trade, this sector has not been exploited in accordance with emerging needs and development in the discipline. Therefore, it is not playing its due role in the economics of the country. It is estimated that 60% of the population use herbal medicines prescribed by traditional practitioners due to non availability of medical health facilities in rural areas[3-5]. These medicinally important plants are facing serious problems of the fungal attack. Various pathogens adversely affect the medicinal plant parts and decrease the medicinal value of the part. It may be harmful to the human body while using these infected parts as a medicine. So identification of the infected fungi is important.

Discoloration, quality deterioration, reduction in commercial values as well as in therapeutic potential and mycotoxin production has been linked to moldy contaminated herbal drugs[6]. Unfortunately, the number of reports of people experiencing negative effects, caused by the use of herbal drugs, has also been increasing[7].

MATERIALS AND METHODS

Source of samples

A total 15 samples of 1. *Saraca Indica* Linn. (Ashoka) (n=5), 2. *Terminalia arjuna* (Roxb.) Wight & Arn. *(*Arjuna*)* (n=5), 3. *Hemidesmus indicus* (L.) R. Br. (Anantmula) (n=5) were collected from the different markets of Agra and nearby regions during the year 2013 – 14. The samples were packed in airtight polyethylene bags and were transported to the Department of Botany, School of Life Science, Khandari Campus, Agra, immediately from there they were stored at room temperature. All the samples were examined morphologically and the percentage moisture content was determined. Enumeration of mycoflora was determined in various culture media.

n= number of samples

Moisture content determination

A known weight of the samples were dried in an oven at 100 0C for 1-21/2 h to determine their moisture content on the basis of weight loss using the following formula[6]:

$$MC = [(W_1 - Wf)/W_1] \times 100$$

Where MC = moisture content, W_1 = Initial weight and Wf = final weight.

Isolation and identification of mycoflora

Fungi were isolated from different medicinal plant samples of, *Terminalia arjuna, Hemidesmus indicus* and *Saraca indica* on Potato Dextrose Agar and Czapek agar media. Identification of fungi was done on the basis of morphological and cultural characteristics as described by Ananthanarayan and Paniker[8].

Frequency of fungal species

The Frequency of different fungal species was assessed of calculated the frequency percentage. There values were obtained according to Girridher and Ready[9].

$$Frequency\ \ percentage\ \ = \frac{No.of\ observation\ in\ which\ a\ species\ appeared}{Total\ no.of\ observations} \times 100$$

RESULTS AND DISCUSSION

Moisture content of different medicinal plant samples

The moisture content was observed in all medicinal plants ranges form of 1% to 8%. However, it was noted that there was a significant difference between the mean moisture content of different medicinal plant samples. Highest mean moisture content was observed in *H. indicus* (8%) followed by *Saraca indica* (6%) the lowest mean moisture content was observed in *T. arjuna* (1%) are shown in table 1.

The presence of moisture content in medicinal plant samples justifies the favorable impact of fungal growth in store medicinal plants[10, 11]. Diverse abiotic factors operating in the processing and storage conditions as well as chemical constituents of the medicinal plants might have resulted in variation in mycopopulation in different substrates[12]. During the survey for sample collection, it was found that necessary precautions were not taken during processing and storage of these medicinal plant samples, in some places these medicinal plant samples were found under open environmental conditions and some ware under dark store rooms under unhygienic conditions. All these practices may contaminate these medicinal plants by exposing them to microbial infections.

Frequency of fungal contamination

The Mycological examination of all 15 samples of 3 different medicinal plants revealed that, 14 (93%) of the total analyzed samples was found to be contaminated with one or more fungal species.

All (100%) samples of *S. indica, T. arjuna, we* were found to be contaminated with one or more fungal species.

While in *H. indicus* 80% of the total samples analyzed was found to be contaminated with one or more fungal species. Which are shown in table 2.

Distribution of different fungal species in all samples of different medicinal plants

Analysis of different sample of all medicinal plants revealed that the frequency of contamination of *A. niger* is maximum (19.44%) followed by *P. janthinellum* (16.66%)*A. flavus* (8.33%), *A. brassicae* (8.33%), *A. pullulans* (8.33%), *Drechslera sp.(*8.33%) and contamination of *P. janthinellum* (2.77%) is minimum as shown in table 3.

Species of *Aspergillus* dominate the mycoflora of collecting samples of medicinal plants of *S. indica, T. arjuna,* and *H. indicus* where it was already reported that *Aspergillus* species dominating mycoflora of stored medicinal plants[13-18]. Hence, the presence of a wide range of fungi in these medicinally important medicinal plants showed that there was is a potential risk for mycotoxins contamination, especially during prolonged storage in poor storing conditions without temperature and moisture control[19, 20].

Distribution of different fungal species with in samples of different medicinal plants

By Analyzing the samples of different medicinal plants revealed that in S. indica medicinal plant samples were frequently contaminated with *Microascus cinereus* (40%), *Aspergillus flavus* (60%), *Emericella nidulans* (40%), *Alternaria brassicae* (60%), *Aspergillus niger* (40%), *Penicillium janthinellum* (60%) and in *T. arjuna* medicinal plant samples *Fusarium chlamydosporum* (40%), *Alternaria alternata* (40%), *Aspergillus niger* (60%), *Penicillium janthinellum* (20%), *Drechslera sp*. (60%),

Saccharomyces sp. (40%), and in *H. indicus* samples *Trichothecium roseum* (40%), *Penicillium citrinum* (20%), *Aureobasidium pullulans* (60%), *Aspergillus niger* (40%), *Penicillium janthinellum* (40%) were found are shown in table 4.

SUMMARY AND CONCLUSION

Fungal contamination of raw materials of medicinal plants is a major impediment preventing India from becoming an herbal giant. Therefore, fungal contamination of drugs, especially raw materials, should be prevented during storage. Plant materials used for medical purposes should be carefully stored and the growth of toxigenic fungi should be inhibited.

In the present study 15 samples of 3 different medicinal plants were collected from markets of Agra and nearby regions were taken to evaluate the presence of toxigenic.

Isolation and purification of mycoflora were done by serial dilution method, identification of mycoflora was done by a culture and microscopic characterization, detection of toxigenic fungi was done from isolated mycoflora by culturing them on activating medium.

Out of 15 samples of different medicinal plants 14 (93%) samples are found to be contaminated with one or more fungal species. All (100%) the samples of *S. indica, T. arjuna* were found to be contaminated with one or more fungal species, while 80% in the case of *H. indicus* and 60% in case of H. indicus was found to be contaminated with one or more fungal species.

The dominant mycoflora comprise of 13 different fungal species The maximum fungal species are of *A. niger* (19.44%) followed by *Penicillium janthinellum* (16.66%), *A. flavus* (8.33%), *A. brassicae* (8.33%), *A. pullulans* (8.33%), *Drechslera sp*. (8.33%), *Microascus cinereus* (5.55%), *Emericella nidulans* (5.55%) *Trichothecium roseum* (5.55%), *Fusarium chlamydosporium* (5.55%), *Alternaria alternata* (5.55%), *Saccharomyces sp.* (5.55%) and minimum fungal species are of *Penicillium citrinum* (2.77%).

Contamination of different medicinal plant samples represents the risk of contamination with mycotoxins. Therefore, these medicinal plants should be carefully stored and the growth of the naturally found toxic fungi should be inhibited.

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Table 1. Showing mean moisture content percentage in different medicinal plant samples

S. No.	Name of plants	Mean initial weight W_1 in gm.	Mean final weight Wf in gm.	Mean moisture content percentage
1.	*Saraca indica*	20	18.77 gm	6%
2.	*Terminalia arjuna*	20	19.77 gm	1%
3.	*Hemidesmus indicus*	20	18.37gm	8%

Table 2. Showing all samples of different medicinal plants contaminated with one or more fungal species

Name of plants	Sample No.	Name of fungus													Fungal contamination per sample
		Microascus cinereus	*Aspergillus flavus*	*Emericella nidulans*	*Alternaria brassicae*	*Aspergillus niger*	*Penicillium janthinellum*	*Trichothecium roseum*	*Penicillium citrinum*	*Aureobasidium pullulans*	*Fusarium chlamydosporum*	*Alternaria alternata*	*Drechslera sp.*	*Saccharomyces sp.*	
Saraca indica	1	+	-	+	+	-	-	-	-	-	-	-	-	-	3
	2	-	+	-	-	-	+	-	-	-	-	-	-	-	2
	3	+	+	-	-	+	-	-	-	-	-	-	-	-	3
	4	-	+	-	+	_	+	-	-	-	-	-	-	-	3
	5	-	-	+	+	+	+	-	-	-	-	-	-	-	4
Terminalia arjuna	1	-	-	-	-	-	-	-	-	-	-	-	+	-	1
	2	-	-	-	-	-	+	-	-	-	+	-	-	-	2
	3	-	-	-	-	+	-	-	-	-	+	-	+	-	3
	4	-	-	-	-	+	-	-	-	-	-	+	-	+	3
	5	-	-	-	-	+	-	-	-	-	-	+	+	+	4
Hemidesmus indicus	1	-	-	-	-	+	-	-	-	-	-	-	-	-	1
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	0
	3	-	-	-	-	+	+	+	-	+	-	-	-	-	4
	4	-	-	-	-	-	-	+	-	+	-	-	-	-	2
	5	-	-	-	-	-	+	-	+	+	-	-	-	-	3
Frequency of contamination in all samples		2	3	2	3	7	6	2	1	3	2	2	3	2	38

Table 3. Percentage Distribution of the fungal species detected in medicinal plants

S. No.	Fungi isolated	Number of isolated	Percentage
1.	*Microascus cinereus*	2	5.55%
2.	*Aspergillus flavus*	3	8.33%
3.	*Emericella nidulans*	2	5.55%
4.	*Alternaria brassicae*	3	8.33%
5.	*Aspergillus niger*	7 (2+2+3)	19.44%
6.	*Penicillium janthinellum*	6 (3+2+1)	16.66%
7.	*Trichothecium roseum*	2	5.55%
8.	*Penicillium citrinum*	1	2.77%
9.	*Aureobasidium pullulans*	3	8.33%
10.	*Fusarium chlamydosporum*	2	5.55%
11.	*Alternaria alternata*	2	5.55%
12.	*Drechslera sp.*	3	8.33%
13.	*Saccharomyces sp.*	2	5.55%
	Total	36	

Table 4. Percentage of samples contamination by different fungal species isolated from different medicinal plants

S. No.	Fungal Species	% of samples yielding different species of fungi		
		S. indica	*T. arjuna*	*H. indicus*
1.	*Microascus cinereus*	40%	0%	0%
2.	*Aspergillus flavus*	60%	0%	0%
3.	*Emericella nidulans*	40%	0%	0%
4.	*Alternaria brassicae*	60%	0%	0%
5.	*Aspergillus niger*	40%	60%	40%
6.	*Penicillium janthinellum*	60%	20%	40%
7.	*Trichothecium roseum*	0%	0%	40%
8.	*Penicillium citrinum*	0%	0%	20%
9.	*Aureobasidium pullulans*	0%	0%	60%
10.	*Fusarium chlamydosporum*	0%	40%	0%
11.	*Alternaria alternata*	0%	40%	0%
12.	*Drechslera sp.*	0%	0%	0%
13.	*Saccharomyces sp.*	0%	40 %	0%