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Mycoflora and mycotoxins in some important stored crude and powdered herbal drugs

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ABSTRACT

Stored samples of crude herbal drugs and processed powders of *Emblica officinalis* (Amla), *Terminalia bellirica* (Baheda), and *Terminalia chebula* (Haritiki) sold in the market of Gwalior, were analyzed on different fungal culture media for the incidence of fungi and their related mycotoxins. During morphological examination of collected samples a remarkable change was observed in the colour and appearance of fruit and powdered samples. About 88% of the fruits and powdered samples were found to be contaminated with 1199 isolates of different fungi belonging to the genera *Aspergillus, Penicillium,Helminthosporium, Rhizopus, Syncephalastrum, Alternaria,* and *Curvularaia*. A total of 11 fungal species classified under five different genera were isolated from the fruit samples, whereas, only nine species belonging to five different genera were isolated from the powdered samples. About 25% of fruits and 12.5% of powdered samples were found contaminated with different mycotoxins like aflatoxins, citrinin, and sterigmatocystin. The presence of toxigenic fungi and mycotoxins may make these crude as well as powdered herbal drugs hazardous for human health. Therefore, prior to their use, one needs to assure the quality control and decontamination of these herbal drug preparations.

Key words: Herbal drugs, fungal contamination, mycotoxins, *Aspergillus*

INTRODUCTION

Herbal drug is a preparation of stem, bark, root, rhizome, leaves, flowers, fruits and seeds of medicinal plants, used to prevent and treat diseases of humankind. Over 8000 plant species have been reported to prepare some 25,000 formulations, to treat various ailments (Dubey, 2004). Herbal drugs are preferred to cure diseases because of better cultural acceptability, compatibility with the human body with lesser side effects (Kamboj, 2000). Considering the adverse effects of synthetic drugs the western populations are now looking for natural remedies, which are safe and effective (Shivanna, 2004). Fruits of *Emblica officinalis* Gaertn. (Amla), *Terminalia bellirica* (Gaertn.) Roxb. (Baheda) and *Terminalia chebula* Retz. (Haritiki)are used in traditional Ayurvedic medicine, while the mixture of these three fruits named as Trifla churn, is prescribed for the various stomach problems like constipation or indigestion, dyspepsia, anemia, impurity of blood, hyperlipidaemia, skin diseases, excessive heat, and irritation of eyes (Juss, 1997). Raw materials of this medicinally important drug are stored under unhygienic conditions after harvesting, before marketing or further processing. The unscientific methods of harvesting, collection, storage of raw materials, processing and poor storage of herbal drugs, retailed in market openly in unhygienic conditions, are the main causes considered to make both, raw materials as well as herbal drugs prone to fungal infections (Essono *et al.*, 2007). The fungal contaminates has been reported to affect the chemical composition of the raw materials and thereby, decreases the medicinal potency of the herbal drugs (Roy, 2003), whereas secondary metabolites produced by these fungal contaminants causes several ailments of liver, kidney, nervous system, muscular, skin, respiratory organs, digestive tract, genital organs, etc. (Durakovic *et al.*, 1989, Muntanola, 1987 and Purchase, 1974). The present study is an effort to identify the contaminants of crude and powdered herbal drugs, especially fungal flora which can be toxic to human health, if any.

MATERIALS AND METHODS

Sample Collection

A total of 25 sun dried freshly stored fruit samples of and 25 Powdered of *E. officinalis*), *T. bellirica* and *T. chebula* were collected in late December, 2007 and January, 2008 from different sites of Gwalior city. These samples were stored at room temperature after the collection. All the collected (Fruit and powdered) samples were examined morphologically with the help of magnifying lens and fungal flora was isolated on different culture media.

Mycological analysis

Enumeration of fungi was performed by pour plating method for powdered and small pieces of fruit samples were inoculated, using potato-dextrose agar (PDA) and Czapek dox agar media (Mandeel, 2005). The culture plates were incubated upside down at 28 ± 2 ºC for 5-7 days and observations were recorded at various intervals. Pure culture of different isolated fungi were prepared by transferring the isolated colony on Czapek dox agar media culture media with the help of inoculating loop and incubated for 4-5 days.

Identification of fungi

After 6-7 days of inoculation, fungi growing on fruit and powdered samples were isolated and identified primarily on the basis of their morphological and cultural characteristics and then stained with lactophenol cotton blue and identified microscopically with reference to standard texts (Ananthanarayan and Paniker, 1999, Gilman, 1975). The percent relative density of different fungal species isolated in each sample was calculated (Agrawal *et al.*, 1980; Verma and Dubey, 2001). Percent frequency of occurrence of mycobiota on individual raw materials of herbal drug samples was also calculated by the method of Mandeel (2005).

Mycotoxins analysis

About fifty gram (50 gm) of the fruit and powdered samples were finely ground, extracted with chloroform following the method of extraction as described by Singh (1988). Of the extracted samples 50µl of chloroform extract were spotted on TLC plates (20×20 cm glass plates, coated with 0.25mm layer thickness of silica gel G). The chromatogram was developed at room temperature, in TLC chamber containing a solvent system composed of benzene, methanol and acetic acid (24:2:1). Visualization was performed under UV light at 365 nm (Singh, 1988). Qualitative detection of mycotoxins was done on the basis of their fluorescence and Rf values (Scott *et al.*, 1970).

RESULTS AND DISCUSSION

Morphological Examination

During morphological examination of collected samples a remarkable change was observed in the colour and appearance of fruit and powdered samples. Black spots were observed on the surface of old stored fruit samples as compared to freshly collected samples. A change in the colour and texture was observed in case of the powdered samples. Light brown and light greenish colour with fine powdered form was observed in fresh samples of Amla, Baheda and Haritiki powders, whereas solid clumps with darker shade were appeared in the old stored samples.

Mycological Analysis

About 88% fruits and powdered samples of *E. officinalis* (Amla), *T. bellirica* (Baheda), and *T. chebula* (Haritiki) were found to be contaminated with 1199 different fungal isolates. The isolated species of fungi belonging to the genera *Aspergillus, Penicillium, Helminthosporium, Rhizopus, Syncephalastrum, Alternaria,* and *Curvularaia*. *Aspergillus* and *Penicillium* were the most common fungal genera found in both, raw and powdered samples.

Fungi associated with Fruit samples

A total of 309 fungal isolates were recorded from 25 fruit samples, out of which 131 isolates were recorded from *T. bellirica* and 94 from *T. chebula*. On the other hand, about 84 isolates were from *E. officinalis*. A total of 11 fungal species classified under 5 genera isolated from the fruit samples, which

included 2 species of Zygomycotina, 9 species of Ascomycotina and remaining one was from Deuteromycotina. The species are listed in table 1 along with their colony forming unit (CFU) per gram of sample and their percentage frequency. The mzximum number of species belonged to the genus *Aspergillus,* with six species viz. *A. niger, A. flavus, A. fumigatus, A. parasiticus, A. versicolor,* and *A. nidulens,* while three species to *Penicillium* namely *P. rubrum, P. citrinum* and *P. chrysogenum* and only one species of each viz. *Helminthosporium, Curvularaia, Alternaria* and *Rhizopus* were observed.

Relative density of fungal species (% age) was calculated to determine the abundance of isolated genus among all fruit samples. The highest percentage of relative density was shown by *A. niger* (42.39%), followed by *A. flavus* (17.79%) and *A. fumigatus* (15.85%), *A. parasiticus* and *Penicillium rubrum* (5.17% each) among the fruit samples. The lowest relative density was recorded in the range of 4.85-0.97% (Figure 1).

Fungi associated with powdered samples

A total of 890 fungal isolates were recorded from 25 powdered samples, out of which maximum number of isolates were recorded from *E. officinalis* (452), followed by *T. bellirica* (286), where as 152 were recorded from *T. chebula*. Altogether nine species belonging to five genera were isolated from the powdered samples which included one species from Zygomycotina, six species from Ascomycotina and remaining two species of Deuteromycotina. The results presented in table 2 shows the fungal species along with their colony forming unit (CFU) per gram of sample with their percentage frequency isolated from powdered samples. The genus *Aspergillus* and *Penicillium* were two most predominant genera encountered, with three species each, while all remaining fungi had only one species. Among all the samples, highest percentage of relative density was shown by *A. niger* (29.88%), followed by *P citrinum* (27.86%), P. rubrum (12.356%) and *A. parasiticus* (12.11%) and *A. flavus* (6.74%). The lowest relative density ranged from 4.49-0.11% (Figure 1).

Mycotoxins analysis

In the present study, we analysed the fruit and powdered samples of *Emblica officinalis* (Amla), *Terminalia bellirica* (Baheda), and *Terminalia chebula* (Haritiki) for the natural occurrence of mycotoxins during their storage. During mycotoxin assay, fluorescent colors on TLC indicate the presence of six mycotoxins, in which blue colors refer to aflatoxin B, green for aflatoxin G, yellow for citrinin, while orange or red may indicate the presence of sterigmatocystin (Scott *et al*., 1970). The results revealed that 48% fruit samples and 28% powdered samples were found contaminated with different mycotoxins (figure 2). Highest percentage of baheda fruits (50%) was found contaminated with AFB2 mycotoxin, whereas, in amla and hatiritki the percentage of contaminated fruits was 11.11 & 13% respectively, on the other hand it was 25% in powdered samples of baheda. Simultaneously, AFB1 & G2 mycotoxins were detected in 22.22% of Amla fruits. Maximum 37.5% & 25% samples of powdered baheda were showing the presence of Sterigmatocystin and AFB1 mycotoxins, respectively. Only 12.5 % samples of baheda (fruits & powder) and haritiki powder have shown the presence of Citrinin mycotoxin. Among all fruits and powdered samples, AfG1 was detected only in 12.5% Haritki Powdered samples (Figure 2).

The incidence of various moulds and mycotoxins in raw fruit and powdered samples revealed that fungi contaminate the herbal drugs during storage of raw materials as well as during the storage of processed final products (Eufuntoye, 2004, Bugno *et al.*, 2006).The findings shows that the raw material are heavily contaminated by different fungal species, some of which are toxigenic. Long term storage of phytomedicines and their raw materials, in unfavorable conditions is one of the greatest factors, to promote fungal contamination of herbal drugs. During the survey it was observed that botanical raw materials or these processed products are stored under uncontrolled environmental conditions like temperature, moisture and relative humidity over the years, and often contain a mixture of other plant raw materials,

thus adversely affecting their bioefficacy and promote fungal contamination. There are several fungal genera which are contaminating herbal drugs during storage. The presence of a wide range of storage fungi in herbal drugs indicates that considerable improvements could be made during harvest, drying, processing and post processing storage (Giridhar and Ready, 1997; Hitokoto *et al.*, 1978; Aziz *et al.*, 1998; Dutta and Roy, 1987; Chauhan, 2004). Most of the identified fungal species in this study like *Aspergillus, Penicillium*, *Helminthosporium* and *Alternaria* are reported to have ability to produce various mycotoxins, such as aflatoxins, ochratoxins, helminthosporium toxins, in medicinal herbs (Hitokoto *et al.*, 1978; Aziz *et al.*, 1998; Bugno *et al.*, 2006). The presence of Aflatoxins, citrinin and sterigmatocystin in herbal drug samples analysed under present investigation, indicates the hazardous nature of these herbal drugs, which can be harmful to the users. Aflatoxin is reported to cause aflatoxicosis, a toxic hepatitis leading to jaundice which in severe cases proves fatal (Shephard, 2004; Lewis *et al.*, 2005).

CONCLUSION

The presence of mycotoxin producing fungal species of *Aspergillus, Penicillium, Alternaria, Helminthosporium,* in stored fruit and powdered market samples of *Emblica officinalis* (Amla), *Terminalia bellirica* (Baheda), and *Terminalia chebula* (Haritiki) revealed that these herbal drugs are not acceptable for human consumption. Presence of Aflatoxins is also a matter of great concern because once the raw materials are contaminated with aflatoxin they are not fit for use. Because, even routine boiling will also not be able to detoxify them since aflatoxins have been reported to be heat stable up to 269^{o}C (Frazier and Westhoff, 1988). The persons who are involved in harvesting, storage, processing and post processed storage of these herbal drugs are required to take stringent precautions in order to present the user with healthy and potential herbal drugs.

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REFERENCES

Agrawal, G.P., Thakur, M.K. and Awasthi, S. 1980. Studies on the wheat grain storage on Madhya Pradesh "Fungi associated with different varieties of freshly harvested wheat rains". *Nat Acad Sci Lett.* **3**:195–197.

Ananthanarayan, R. and Paniker, C.K.J. 1999. A Text Book of Practical Microbiology. Orient Longman, Hyderabad.

Aziz, N.H., Youssef, Y.A., Moheye, Z. EL-F and Lofty, A.M. 1998. Contamination of some medicinal plant samples and spices by fungi and their mycotoxins. *Botanical Bulletin of Academia Sinica*, **39**:279-285.

Bugno, A., Adriana, A.B.A., Tatiana, C.P., Terezinha, A.P. & Myrna, S. 2006. Occurrence of toxigenic fungi in herbal drugs. *Brazillian Journal of Microbiology*, **37**: 47-51.

Chauhan, R.K.S. 2004. Mycotoxins the "Hidden Killers" in Food and Feed: An Overview and Strategy for Future. *Journal Indian Botanical Society*, **83**: 11-21.

Dubey, N. K., Kumar R. and Tripathi, P. 2004. Global promotion of herbal medicine: India's opportunity. *Current Science,* **86**: 37- 41.

Durakovic, S., Galic, J. and Pajnovic, P. 1989. Toxic and cancer metabolites of moulds in food and fodder. *Hrana I ishrana,* **30**(2): 71–100.

Dutta, R.K. and Roy, A.K. 1987. Mycoflora associated with *Strychnos* seeds and deterioration of their active principles under storage. *Indian Phytopathology,* **40**: 520-524.

Efuntoye, M.O. 2004. Fungi associated with herbal drug plants during storage. *Mycopathologia,* **136**:115-118.

Essono, G., Ayodele, M., Akoa, A., Foko, J., Olembo, S. and Gock, J. 2007. Aspergillus species on cassava chips in storage in rural areas of southern. Cameroon: their relationship with storage duration, moisture content and processing

methods. *African Journal of Microbiology*, 001-008.

Frazier, W.C. and Westhoff, D.C.1988. Food Microbiology. 4th ed. International edition,

Gilman, J.C. 1975. A manual of soil fungi. Oxford & IBH Publishing Corporation, New Delhi, Bombay, Calcutta. II edition.

Giridher, P. and Ready, S.M. 1997. Incidences of mycotoxin producers on spices from Andhra Pradesh. *Journal Indian Botanical Society*, **76**: 161-164.

Hitokoto, H., Morozumi, S., Wauke, T., Saka, S., and Kurata, H. 1978. Fungal contamination & mycotoxin detection of powdered herbal drugs. *Applied and Environmental Microbiology*, **36**: 252-256.

Juss, S.S. 1997. Triphala D: The Wonder Drug. *Indian Med. Gazette*, **131**:194-196.

Kamboj, V.P. 2000. Herbal medicines. *Current Science*, **78**: 35-39.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Roger, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A.M., Misore, A. 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives*, **113**: 1763-1767.

Mandeel, Q.A. 2005. Fungal contamination of some imported species. *Mycopathologia*, **159**: 291–298.

Muntanola, M. 1987. General mycology. Beograd: NIRO. *Knjez evne novine*, 257–269.

Purchase, I.F.H. 1974. Mycotoxins. Amsterdam. *Elsevier*. 1–28.

Roy, A.K. 2003. Mycological problems of crude herbal drugs-Overview and Challenges. *Indian Phytopathology*, **56**: 1–13.

Scott, P. M., Lawrence, J.W. and Walbeek, W. 1970. Detection of Mycotoxins by Thin Layer Chromatography: application to Screening of Fungal Extracts. *Applied Microbiology*, **20**: 839-842.

Shephard, G.S. 2004. Impact of mycotoxins on human health in developing countries. *Food Additives and Contaminants*, **25**: 146-151.

Shivanna, K.R. 2004. Quality standards of indian medicinal plants. *Current Science*, **86**: 13-28.

Singh, P. L.1988. Studies on deleterious effects of mycotoxin contaminated pulses and its control. P.G. Department of Botany Bhagalpur, Bihar, India. The Allied Press, Bhagalpur. 5-19.

Verma, J. and Dubey, N.K. 2001. Efficacy of essential oils of Caesulia axillaries and Mentha arvensis against some storage pests causing bio-deterioration of food commodities. *International Journal of Microbiology*, **68**: 207–210.

Table 1. Percentage frequency of various fungi isolated from fruit samples

Name of fungus	**Amla**		**Baheda**		**Haritiki**	
	Average CFU/g	**% Frequency**	**Average CFU/g**	**% Frequency**	**Average CFU/g**	**% Frequency**
Ascomyotina						
Aspergillus. niger	1.66	88.88	2.66	100	1.28	87.5
A. flavus	1.32	66.66	2.83	25	0.33	12.5
A. fumigatus	0.49	22.22	1.46	62.5	1.74	50
A. parasiticus	0.99	22.22	1.66	12.5	1.66	12.5
A. versicolor	1.66	11.11	-	-	-	-
A. nidulens	0.33	11.11	-	-	-	-
Penicillium. rubrum	0.33	11.11	0.83	25	1.00	37.5
P. citrinum	0.33	11.11	1.33	12.5	0.66	12.5
P. chrysogenum	-	-	-	-	1.00	12.5
Zygomycotina						
Rhizopus sp.	0.33	11.11	1.83	25	1.66	12.5
Syncephalastrum sp.	1.33	11.11	-	-	-	-
Deuteromycotina						
Alternaria sp.	-	-	0.66	12.5	0.33	25

Table 2. Percentage frequency of various fungi isolated from powdered samples

Name of fungus	**Amla**		**Baheda**		**Haritiki**	
	Average CFU/g	**% Freq.**	**Average CFU/g**	**% Freq.**	**Average CFU/g**	**% Freq.**
Ascomyotina						
Aspergillus. niger	10.87	88.88	3.57	50	2.33	25
A. flavus	8.00	22.22	-	-	-	-
A. parasiticus	3.00	11.11	19.55	37.5	-	-
Penicillium. rubrum	2.83	22.22	6.99	50	5.66	50
P. citrinum	10.91	44.44	5.83	25	11.83	25
P. viridicatum	0.66	11.11	0.83	25	-	-
Zygomycotina						
Rhizopus sp.	6.33	11.11	-	-	-	-
Deuteromycotina						
Curvularia sp.	-	-	2.16	25	-	-
Helminthosporium sp.	1.00	11.11	5.16	25	-	-

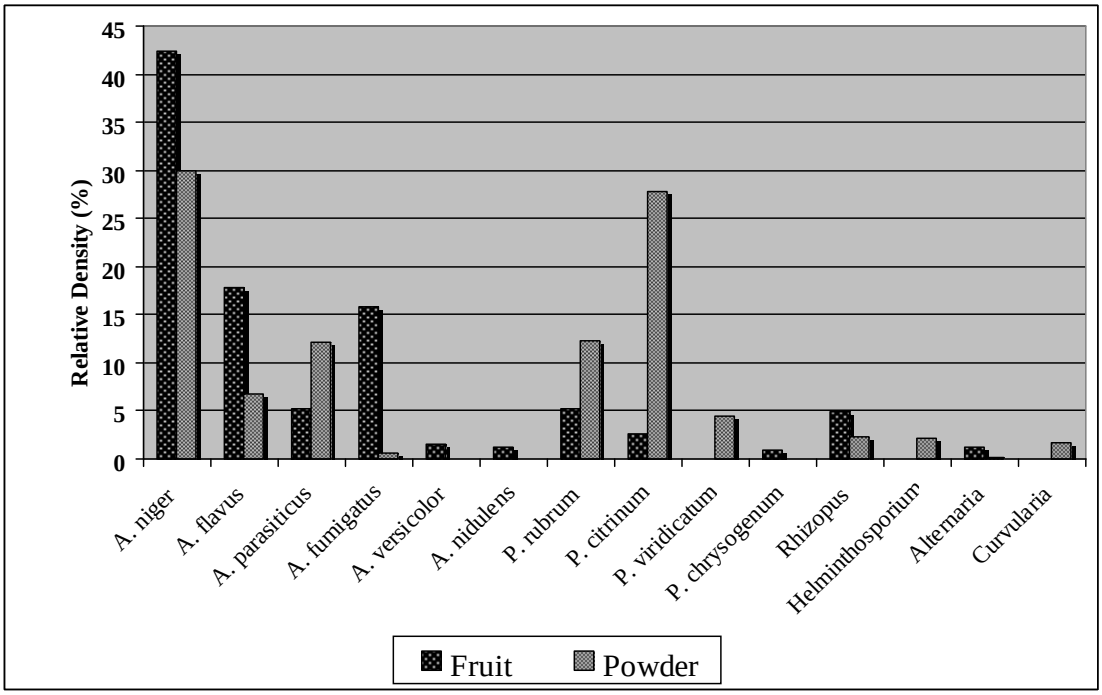

Figure 1. Relative density of mycoflora among all fruit and powder samples

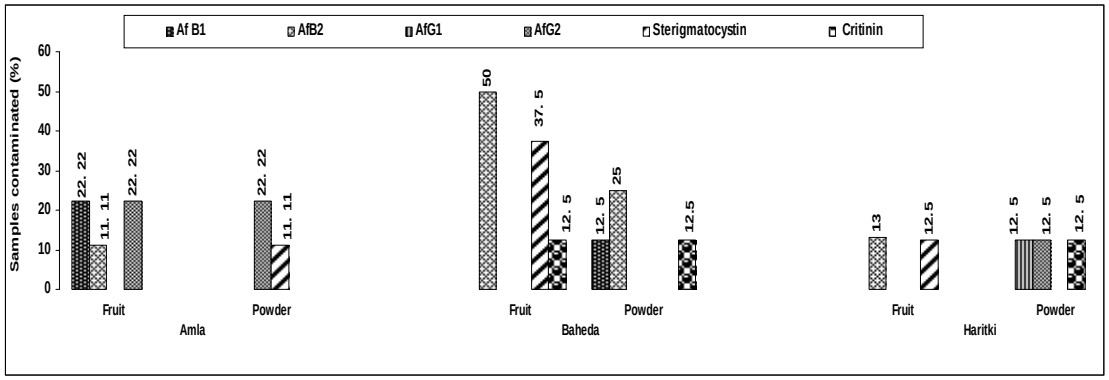

Figure 2. Distribution of mycotoxins in fruit and powdered samples