



Contamination Of Some Common Medicinal Plant Samples And Spices By Fungi And Their Mycotoxins

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Abstract

From ancient time herbal medicines have been used for curing of various fatal diseases in human beings world wide. In recent studies medicinal values of herbs have been extensively recognized. The potential fungal contamination of medicinal herbs affects its quality due to the presence of mycotoxins. Mycotoxins are secondary metabolites of fungi identified in many agricultural products and medicinal herbs screened for toxigenic fungi. The ultimate exposure and toxicities of mycotoxins can be diversely influenced by endogenous food components in different commodities of the medicinal herbs. The fungal pathogen is one of the major causes of serious diseases in growing crop because of poor health and biodeterioration of seeds. To realize this aspect, the study has been undertaken on ten medicinal plants belonging to different families. *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Mucor* sp. and *Rhizopus* sp. fungi have been isolated from the different parts of the medicinal plants. After aflatoxins analysis, presence of aflatoxin was proved in all samples. The present study suggests that detection of fungi and aflatoxins poses a risk for consumer's health and it is necessary to check the herbal drugs before allowing distribution for public use.

Key words: Aflatoxin, *Aspergillus*, Medicinal Plants, Fungal Contamination

Introduction

Since prehistorically periods plants have been used for medicinal purposes. Medicinal plants are considered as rich resources of pharmacopoeial, non-pharmacopoeial or synthetic drugs. In all over the world WHO has documented about 20,000 plant species which are used for medicinal purposes. In India, over 6000 plants are used in traditional, folk and herbal medicine, representing about 75 % of the medicinal needs of the world [1, 2], in which only 2500 plant species are documented as herbal medicines [3]. WHO has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for herbal medicine, which provide a detailed description of the techniques and measures required for the appropriate cultivation and collection of medicinal plants. Despite such guidelines, there is still a lacuna between this available knowledge and implementation, because producers, handlers and processors of herbal drugs are not much aware of WHO's

guidelines and they continue their work as before without any quality control measures. This produces inferior quality of herbal drugs with lots of contaminants like heavy metals, pesticides and microbes. The unscientific methods of harvesting, collection, storage and transport of herbal drugs in unhygienic conditions, are the main causes considered to make both, raw materials as well as herbal drugs prone to microbial infections leading to several human diseases. Practices used in harvesting, handling, storage, production and distribution contaminate medicinal plants by various fungi, which may be responsible for spoilage and production of mycotoxins [1-15]. Considering this situation the present study on the mycoflora of stored herbal parts used in Ayurvedic medicines, started as a first step to fill up this major lacuna in the field of Ayurvedic Pharmacopodia. Medical herbs have chemical compounds for biological functions, including defense against many diseases in human beings. These chemicals work on the human body in the same as pharmaceutical drug so can be beneficial and have harmful side effects. However, contaminated chemicals in medicinal herbs seriously affected the value of herbal products and also affected the human health [4,5].

Mycotoxins are secondary metabolites produced by fungi that grow naturally in foodstuffs [6-11]. Toxicogenic fungi may contaminate foodstuffs in the most different phases of production and processing, from cultivation to transport and storage. Diseases caused by mycotoxins are called mycotoxicoses. They are diffuse syndromes that cause lesions mainly in organs such as liver, kidneys, digestive tract, respiratory organs, genital organs, and nervous system, epithelial tissue (skin and mucous membranes) depending on the type of the toxin [12-15]. More than 400 types of mycotoxins have identified in the world to date. Among mycotoxin, Aflatoxin (AFs), Ochratoxin A (OTA), Fusarium (FBs), Zearalenone (ZEA) and Deoxynivalenol (DON) are most frequently detected mycotoxin in herbal medicine.

Aflatoxins are mycotoxins produced by fungi in the genus *Aspergillus*, species *A. flavus*, *A. parasiticus* and *A. nomius* [16]. These fungi contaminated can occur either in harvest or in the post harvest and storage stages. Climatic changes, pH, Relative humidity of 80-85% and Temperature around 30°C poor storage and damage from insects or harvest processing make them more susceptible to mycotoxin contamination [6,17,18].

Nowadays, 18 similar compounds are called aflatoxins. However, the most important in medical terms are types B₁, B₂, G₁ and G₂ [6]. Aflatoxin B₁ (AFB₁), besides being the most frequently found in plant substrates has the greatest toxigenic power. Aflatoxins B₂ (AFB₂), G₁ (AFG₁) and G₂ (AFG₂) have about 50, 20 and 10% of AFB₁ toxigenic power, respectively [19]. Biotransformation is a process by which the body transforms foreign substances (xenobiotics) in new chemical compounds (metabolites), that is, a process in which the initial compound is modified to be eliminated by the biological system [20]. After oral ingestion, AFB₁ is efficiently absorbed and biotransformed before urinary and fecal excretion.

The fungal contaminates has been reported to affect the chemical composition of the raw materials and thus, decreases the medicinal potency of the herbal drugs (Roy, 2003). Mycotoxins produced by these fungal contaminants causes several ailments of liver, kidney, nervous system, muscular, skin, respiratory organs, digestive tract, genital organs etc [22, 23]. Mycotoxin contamination beyond the WHO permissible limits in some cases has been observed earlier studies [24]. The present study is an effort to identify the fungal and mycotoxin contaminants of crude herbal drugs that are most commonly used in the manufacture of various commercial drugs and which can be toxic to human health, if any.

Material and Methods

The aflatoxin elaboration study was carried out on ten different medicinal herb samples collected from Bareilly districts of Uttar Pradesh. Samples of each medicinal herb were collected in polythene bags and stored at 28°C. One gram powdered samples was diluted in 10 ml sterile 0.1% aqueous peptone. 200µL of this suspension (dilution factor 10-1) was inoculated onto PDA medium and spread plate method [25]. Direct plating was also performed by placing small pieces of unsterilized samples on the medium without dilution. The plates were incubated at 28°C for 7-8 days. The fungal colonies were counted and the total fungal load was calculated as colony forming unit (CFU) per gram of the sample. The different fungal isolates were sub cultured on PDA till pure cultures were obtained. The isolated fungi were identified macroscopically and microscopically by their culture and morphological characteristics. These pure cultures were further analysed for toxin production and transferred to SMKY liquid medium [26]. All the collected samples were subsequently extracted chemically for the natural occurrence of aflatoxin [27]. Quantitative estimation of aflatoxin was done on TLC plates using TEF

solution (toluene: ethyl acetate 90%: formic acid, 6: 3: 1, v/v), whereas quantitative estimation of aflatoxin (B1, B2, G1, G2) was calculated spectrophotometrically [28]. Identification of aflatoxin in the contaminated samples was confirmed chemically by the treatment of trifluoroacetic acid and by spraying 25% sulphuric acid [29].

Results and Discussion

Table 1: Fungal contamination in medicinal herb.

S. no.	Sample	Part used	Fungal load (cfu/g)	Isolated fungi	Toxin
1	<i>Asparagus racemosus</i>	Root	4.2×10^4	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Aspergillus peraciticus</i> <i>Penicillium citrinum</i> <i>Fusarium oxysporum</i>	AFB ₁ , AFB ₂ , AFG ₁ , AFG ₂
2	<i>Chlorophytum borivilianum</i>	Root	2.5×10^4	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Aspergillus terreus</i> <i>Penicillium chrysogenum</i> <i>Mucor sp.</i>	AFB ₁ , AFB ₂ , AFG ₁ , AFG ₂
3	<i>Cinnamomum verum</i>	Bark	6.9×10^3	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Penicillium citrinum</i> <i>Fusarium moniliforme</i>	AFB ₁ , AFB ₂ , AFG ₁ , AFG ₂
4	<i>Elettaria cardamomum</i>	Seed Pod	6.5×10^3	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Penicillium chrysogenum</i> <i>Fusarium moniliforme</i>	AFB ₁ , AFB ₂ , AFG ₁ , AFG ₂
5	<i>Glycyrrhiza glabra</i>	Root	5.0×10^3	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Aspergillus terreus</i> <i>Penicillium citrinum</i> <i>Fusarium moniliforme</i>	AFB ₁ , AFB ₂ , AFG ₁ , AFG ₂
6	<i>Illicium verum</i>	Fruit	1.2×10^2	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Penicillium chrysogenum</i> <i>Fusarium moniliforme</i>	AFB ₁ , AFB ₂ ,

7	<i>Mesua ferrea</i>	Flower bud	7.3×10^3	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Aspergillus terreus</i> <i>Penicillium citrinum</i>	AFB ₁ ,
8	<i>Terminalia beleria</i>	Fruit	2.8×10^2	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Penicillium chrysogenum</i> <i>Fusarium oxysporum</i>	AFB ₁ , AFB ₂ , AFG ₁ ,
9	<i>Terminalia chebula</i>	Fruit	1.9×10^2	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Aspergillus terreus</i> <i>Penicillium citrinum</i> <i>Fusarium moniliforme</i> <i>Mucor sp.</i> <i>Fusarium</i>	AFB ₁ , AFB ₂ ,
10	<i>Withania somnifera</i>	Root	3.2×10^4	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Aspergillus terreus</i> <i>Penicillium citrinum</i> <i>Fusarium oxysporum</i> <i>Fusarium moniliforme</i> <i>Mucor sp.</i> <i>Rhizopus sp.</i>	AFB ₁ , AFB ₂ , AFG ₁ , AFG ₂

In this study, the presences of fungal population isolated from the medicinal herbs are shown in table 1. In all case, a total of 10 species of fungi belonging to 5 genera were isolated and identified. Six fungal species were isolated from *Asparagus racemosus*, *Chlorophytum borivilianum*, *Glycyrrhiza glabra* samples had a moisture level 7.1%, 7.6%, 8.3%; Five species from *Chlorophytum borivilianum*, *Elettaria cardamomum*, *Illicium verum*, *Mesua ferrea*, *Terminalia beleria* with a moisture content 5.8%, 5.6%, 6.1%, 6.5%, 6.2%; Eight species from *Terminalia chebula* had a moisture content 8.3%; Nine species were isolated from *Withania somnifera* samples with a moisture content 8.7%. The moisture content of all the samples was below 9%, which is the optimum condition of the storage. The great number of species was held to the genus *Aspergillus*. Five species were observed namely: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus* and *Aspergillus peraciticus*. Two species were recorded *Penicillium* (*Penicillium citrinum*, *Penicillium chrysogenum*) and *Fusarium* (*Fusarium oxysporum*, *Fusarium moniliforme*). Single specie was observed namely *Mucor sp.* and *Rhizopus sp.* The results showed that 70% of the samples have a fungal lode above the permissible limit of the World Health Organization [30]. A contamination limit of 1×10^3 cfu/g has been set by WHO for yeasts and molds in medicinal plants.

The most prevalent fungi isolated from the medicinal plant samples were *A. flavus*, *A. fumigatus*, and *A. niger*. Postulated that the contamination of feedstuffs with fungal species was as a result of natural extraneous contamination by dust following storage in humid conditions [31]. Fungi fall into two ecological categories: field and storage fungi. Field fungi were observed to invade developing or mature seed while it is on the plant, the major field fungi genera are: *Alternaria*, *Helminthosporium*, *Fusarium*, and *Cladosporium*. On the other hand, storage molds are those encountered on plants at moisture conditions routinely found in stored products, these fungi are principally species of *Aspergillus* and *Penicillium*.

Two fungal *Aspergillus* and *Penicillium* spp. were most dominant. In all examined medicinal herbs samples was in accord with the results of [32-36]. Who stated that *Aspergillus* and *Penicillium* spp. were the main components of cardamon, cinnamon, fennel, coriander, cumin, black cumin, and white pepper, all of which are common in the food industry. They found a high degree of contamination in all samples. *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. orchaceus*, *A. candidus*, *A. sydowi*, *Chaetomium dolicholichum*, *F. moniliforme*, *Penicillium oxalicum*, *Alternaria*, *Curvularia*, and *Rhizopus* from the seeds of *Amomum subulatum*, *Coriandrum sativum*, *Cuminum cyminum*, *Foeniculum vulgare*, *Piper nigrum*, *Cinnamomum zeylanicum*, and from the bark of *Acacia catechu*, all of which are commonly used drug plants [37-38]. Earlier reported that species of the genera *Aspergillus*, *Penicillium* and *Fusarium* were the most abundant fungi present in medicinal herbs [39]. Examination of 84 medicinal plants and spices revealed *A. flavus*, *A. parasiticus*, *F. oxysporum*, and *P. viridicatum* as the most commonly occurring contaminant [42]. Other genera, such as *Fusarium*, *Mucor* and *Trichoderma*, were also reported as dominant fungi present in medicinal plants and herbal drugs [39, 43]. *Aspergillus niger* has been reported as a frequent contaminant in medicinal plants [39, 44, 45]. Fifty-eight out of the 63 samples were contaminated, while five were free from fungal contamination. The samples *Mesua ferrea*-II and *Terminalia chebula*-III had the highest fungal load, i.e., 5.0×10^4 cfu/g. A total of 187 fungi were isolated, out of which 28 were toxigenic which included 19 aflatoxin-producing *Aspergillus flavus* and 9 citrinin-producing *Penicillium citrinum* [46]. Especially *Aspergillus flavus* was the most frequent *Aspergillus* species yielded in all examined medicinal plant samples in this investigation. This was in accordance with the results of Roy and Chourasia (1990), who stated that *A. flavus* was the main contaminant of different herbal drug samples [40].

Table 2: Quantitative estimation of Aflatoxin production by *A. flavus* in medicinal herbs

S. no.	Medicinal herbs	Aflatoxin B ₁ µg/gm-1	Aflatoxin B ₂ µg/gm-1	Aflatoxin G ₁ µg/gm-1	Aflatoxin G ₂ µg/gm-1
1	<i>Asparagus racemosus</i>	2.35	1.26	0.86	0.21
2	<i>Chlorophytum borivilianum</i>	1.63	0.75	0.38	0.18
3	<i>Cinnamomum verum</i>	1.53	0.66	0.09	0.07
4	<i>Elettaria cardamomum</i>	1.25	0.62	0.19	0.05
5	<i>Glycyrrhiza glabra</i>	1.20	0.58	0.17	0.03
6	<i>Illicium verum</i>	0.65	0.26	-	-
7	<i>Mesua ferrea</i>	1.54	-	-	-
8	<i>Terminalia beleria</i>	0.68	0.27	0.09	-
9	<i>Terminalia chebula</i>	0.42	0.16	-	-
10	<i>Withania somnifera</i>	1.98	1.03	0.94	0.32

As shown in Table 2, 156 isolates of *Aspergillus flavus* was isolated from ten medicinal herbs samples and 103 isolates was positive to toxigenic and 53 isolates was nontoxigenic. Out of 21 isolates of *A. flavus*, isolated from twenty seed samples of *Asparagus racemosus* 17 isolates were found to positive to aflatoxin producing ability. *Asparagus racemosus* have highest isolates of *A. flavus* ie. 17. Whereas *Withania somnifera*, *Chlorophytum borivilanum*, *Mesua ferrea*, *Cinnamomum verum*, *Glycyrrhiza glabra*, *Elettaria cardamomum*, *Terminalia belleria*, *Illicium verum* and *Terminalia chebula* have all isolates of *A. flavus* respectively. It is clear from the table that aflatoxin B1, B2, G1, G2 were found in seeds of *Asparagus racemosus*, *Cinnamomum verum*, *Withania somnifera*, *Chlorophytum borivilanum*, *Glycyrrhiza glabra*, *Elettaria cardamomum*. In sample of *Terminalia belleria* aflatoxin B1, B2, G1 were recorded. In *Illicium verum* and *Terminalia chebula* aflatoxin B1 B2 were observed. Only aflatoxin B1 in *Mesua ferrea*; All samples were found to be affected from Aflatoxin B1. The results also showed that maximum concentration of aflatoxin B1 (2.35 $\mu\text{g gm}^{-1}$); aflatoxin B2 (1.26 $\mu\text{g gm}^{-1}$) in *Asparagus racemosus*; aflatoxin G1 (0.94 $\mu\text{g gm}^{-1}$) and aflatoxin G2 (0.52 $\mu\text{g gm}^{-1}$) in *Withania somnifera*.

Halt reported that the presence of high moisture content along with higher fungal counts in amla, bahera, tejpatta and ashwagandha samples justify favorable impact of high moisture and temperature on the fungal growth of stored herbal drugs [41-42]. Species of *Aspergillus* and *Penicillium* dominating the mycoflora of stored herbal drugs with acidic pH level were already reported earlier [44, 47-48]. The present investigation indicates that in Bareilly region the contamination of crude herbal drugs with *Aspergillus*, *Penicillium* and *Fusarium* species is cosmopolitan. This might be due to unavailability of proper harvesting and storage facility, without temperature and moisture control, which expose them to microbial infection. Therefore, it is essential to scrutinize these herbal raw materials before processing for the presence of contaminants and the best quality raw materials should be allowed to use for the preparation of herbal drugs. Moreover, after processing these herbal drugs should also be tested for presence of toxigenic moulds and mycotoxins in order to reduce the risk for consumer's health. Presence of *A. flavus* is of main concern because fungus is a potent aflatoxin producer. The aflatoxins are reported to be carcinogenic, hepatogenic, nephrogenic, hepato carcinogenesis and cause various nervous disorders [49-56].

In humans, acute aflatoxicosis is manifested by vomiting, abdominal pain, pulmonary edema, coma, convulsions, and death with cerebral edema and fatty involvement of the liver, kidneys, and heart [57]. The toxic and carcinogenic effects of aflatoxin B1 are intimately linked to both the rate of activation and detoxification at the primary and secondary levels of metabolism, in a similar way to chlorinated hydrocarbon [58]. Several reports are available on aflatoxins contaminating raw materials of plant origin and herbal drugs which support the detection of aflatoxins in analyzed samples during present investigation [38, 40, 59-64].

Conclusion

The present investigation of different medicinal herbs reveals that some herbs are good substrate for *Aspergillus flavus* infestation and production of aflatoxins with potential hazard to the health of consumers. Therefore, there is an urgent need to prevent the entrance of such contaminated crude drug into direct use. To meet this demand, people would have to be aware of these contaminated herbal drugs and use the processed trade mark drug and should take advantage of modern storage system that improves the quality of crude herbal drug and decreases the probability of fungal contamination.

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