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Fungal Contamination and Mycotoxin Detection of Powdered Herbal Drugs

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Forty-nine powdered herbal drugs were analyzed for their mold profile and for the potential presence of three mycotoxins (aflatoxin, sterigmatocystin, ochratoxin A). *Aspergillus* and *Penicillium* species were predominant, but *Rhizopus, Mucor, Cladosporium,* and *Aureobasidium* spp. were also found in a few samples. Mycotoxins were not detected in any samples, and only one isolated culture was found to be a mycotoxin producer on laboratory media.

Herbal drugs are crude preparations of various kinds of medicinal plants. In other words, a herbal drug is a dried medicinal plant or any part thereof, such as leaf, stem, root, flower, or seed. Herbal medicine has a long history, probably extending over 2,000 years, and is quite popular with many people, particularly Asians and Northern Europeans. Herbal drugs were probably introduced to Japan from ancient China about 1,500 years ago. Even though the pharmaceutical effect of all of these drugs has not been thoroughly investigated in recent years, the low level of side effects has increased demand for the drugs from consumers. In Japan, herbal drugs are given to patients as a hash or powder, singly or mixed with other medicines. Most of them are boiled with water to make a decoction just before oral administration, or fine powders of the herbal drugs are mixed with other types of solid medicines without heating. Since they are natural products, the drugs are quite often deteriorated by microorganisms before harvesting and during handling and storage. Quality control to prevent growth of fungi and bacteria is essential for processors.

As a result of fungal contamination, the risk of mycotoxin production, especially aflatoxin, should be taken into consideration in the manufacturing process.

At present, some investigators (6-9, 14) have reported fungal population of the herbal drugs and several kinds of spices.

This study was designed to further the identification of the fungal flora of powdered herbal drugs, to identify mycotoxin production of the fungal isolates, and to assay for natural occurrence of mycotoxins.

MATERIALS AND METHODS

Samples. Herbal drugs manufactured in compliance with the specifications of the Japanese Pharmacopeia-IX (13) were sampled from drugstores in Tokyo between August and December, 1975. A total of 49 samples of the drugs belonging to 13 species were examined for their mycological profiles. Geographical source of each sample was uncertain, but some domestic samples were probably included. Commercial and medicinal names are as follows: powdered cinnamon bark (Cinnamoni Cortex Pulveratus), powdered cininidium (Cinidii Rhizoma Pulveratum), gardenia fruits powder (Gardeniae Fructus Pulveratus), powdered Japanese peony roots (Paeoniae Radix Pulverata), powdered plactycodon (Placticodi Radix Pulverata), powdered licorice roots (Glycyrrhizae Radix Pulverata), powdered *Atractylodes japonica* rhizome (Atratylodis Lanceae Rhizoma Pulveratum), powdered ginger (Zingiberis Rhizoma Pulveratum), powdered phellodendron bark (Phellodendri Cortex Pulveratus), powdered coptis (Coptidis Rhizoma Pulveratum), powdered scutellaria roots (Scutellariae Radix Pulverata), powdered *Atractylodes* rhizome (Atractylodis Lanceae Rhizoma Pulveratum), and powdered zanthoxylum (Zanthoxyli Fructus Pulveratus). All samples had been powdered by the processors. Before sampling for mycological examination, the drugs were mixed manually in plastic bags to insure homogenicity.

Mycological examination. To determine the number and kinds of fungi present, 10-g samples of each were taken by a spatula directly into sterilized flasks. Prior to this step, the flasks, containing 90 ml of 0.05% water agar and about 20 ml of glass balls (diameter about 5 mm), were autoclaved at 121°C for 15 min. Before the agar hardened, 1-ml portions of each sample were poured and spread on the surface of media in petri dishes. The dishes were incubated at 25°C until the colonies of fungi could be counted and identified. Two kinds of media, potato dextrose agar (PDA) plates containing 100 μg of chloramphenicol

per ml and 20% glucose-PDA plates for isolation of xerophilic fungi, were used in this experiment. All isolates were maintained on PDA slants.

Mycotoxin production of the isolates. Production of aflatoxin, sterigmatocystin, and ochratoxin A was chemically determined with representative isolates of *Aspergillus flavus, A. versicolor,* and *A. ochraceus,* respectively. Test strains were produced by growing the organism on PDA slants for approximately 7 days at 25°C until well sporulated. The spores were harvested by adding 10 ml of a sterilized 1% solution of Driwel (polyethylene glycol sorbitan monooleate; Fuji Photo Film Co., Ltd., Tokyo). A loopful of the spore suspension (approximately 10^3 spores) was inoculated into each test tube containing either 10 ml of synthetic low salt (11) broth for aflatoxin production or 1 ml of yeast extract sucrose (3) broth for sterigmatocystin and ochratoxin A production. All tube cultues were held stationary in a slantwise position and incubated at 25°C for 8 days for the culture of aflatoxin-producing and ochratoxin-producing strains and 12 days for the sterigmatocystin-producing strains.

Mycotoxin analysis. A 10-ml sample of chloroform was added to the culture. The mixture was shaken by horizontal shaker for 5 min, then kept in a water bath maintained at 40°C for approximately 5 min. The chloroform layer was passed through an anhydrous sodium sulfate funnel, and then concentrated to 0.5 ml using a flash evaporator.

Cleanup of aflatoxins, ochratoxin A, and sterigmatocystin in the extract was done according to conventional methods (4, 10).

Mycotoxin assays were conducted by thin-layer chromatography using Adsorbosil-1 (Applied Science Laboratories, Inc., State College, Pa.) plates, which were developed in chloroform-acetone (9:1) for aflatoxins, in benzene-acetic acid (9:1) for ochratoxin A according to the method of Eppley (4), and in benzene-methanol-acetic acid (90:1:5) for sterigmatocystin as described by Naoi et al. (10). The intensities of fluorescence of the separated mycotoxin spots were measured by fluorodensitometer (model MPF-2A, Hitachi Ltd., Tokyo).

Detection of natural occurrence of mycotoxin in the drugs. Natural occurrence of aflatoxin, sterigmatocystin, and ochratoxin A was chemically examined in each 50-g sample of herbal drug using the method described by Eppley et al. (4, 5) and Naoi et al. (10).

Measurement of moisture content of the drugs. Moisture content of the samples was measured by the Kett infrared moisture meter (Kett Science Institute, Tokyo).

RESULTS AND DISCUSSION

Names of the drug samples, number of samples tested, percent frequency of occurrence of fungi, and range and average of the colonies isolated are given in Table 1.

As the result of mycological examination, cinnamon bark was found to have the lowest frequency of fungal-contaminated samples (37.5%; 3 samples out of 8 examined; moisture content averaged 11.2%); all the other samples had a frequency of 100% (the range of moisture contents was from 8.6 to 13.0%). The samples of cinnamon bark averaged 10 colonies of fungi per g, varying from 3 to 73/g. Powdered coptis and powdered *Atractylodes* rhizome contained a great number of fungi, 6,494 and 1,382/g on the average, respectively. The other 10 drugs averaged 10^2 to 10^3 fungal colonies per g.

Genera of fungi isolated from the samples are shown in Table 2. Among the fungi so far iden-

TABLE 1. *Fungal counts on powdered herbal drugs*

Herbal drug	No. of contaminated/no. examined	Fungal counts per g	
		Range	Avg
Powdered cinnamon bark (Cinamoni Cortex Pulveratus)	3/8	3–73	10
Powdered cininidium (Cinidii Rhizoma Pulveratum)	6/6	13–894	229
Gardenia fruits powder (Gardeniae Fructus Pulveratus)	6/6	5–340	129
Powdered Japanese peony roots (Paeoniae Radix Pulverata)	5/5	144–1,069	382
Powdered plactycodon (Placticodi Radix Pulverata)	5/5	10–75	36
Powdered licorice roots (Glycyrrhizae Radix Pulverata)	4/4	125–414	232
Powdered *Artractylodes japonica* rhizome (Atractylodis Lanceae Rhizoma Pulveratum)	3/3	60–1,113	418
Powdered phellodendron bark (Phellodendri Cortex Pulveratus)	3/3	168–795	391
Powdered ginger (Zingiberis Rhizoma Pulveratum)	3/3	43–1,000	393
Powdered coptis (Coptidis Rhizoma Pulveratum)	2/2	188–12,800	6,494
Powdered scutellaria roots (Scutellariae Radix Pulverata)	2/2	1,286–1,478	1,382
Powdered *Atractylodes* rhizome (Atracytolodis Lanceae Rhizoma Pulveratum)	1/1	161	161
Powdered zanthoxylum (Zanthoxyli Fructus Pulveratus)	1/1	3	3

TABLE 2. *Distribution of fungal genus in powdered herbal drugs*

Fungus	Herbal drug													Total isolates (%)
	Cinnamon bark	Cinidium	Gardenia fruits	Japanese peony roots	Platycodon	Licorice roots	*Atractylodes japonica* rhizome	Phellodendron bark	Ginger	Coptis	Scutellaria roots	*Atractylodes* rhizome	Zanthoxylum	
Aspergillus group		1,133	676	1,523	96	676	764	1,028	877	3,438	2,241	133		12,585 (47.0)
A. niger		906	36	724	38	393	100	876	783	538	2,130	75		6,599 (24.6)
A. glaucus			561	786	41	269	651	38	78			58		2,482 (9.3)
A. flavus		10	5		11	6	5	111	3	1,900	58			2,109 (7.8)
A. nidulans		161		10	3			3	5					182 (0.7)
A. versicolor		53	14	3			8		5					83 (0.3)
A. ochraceus					3	8					48			59 (0.2)
A. terreus			45						3		5			53 (0.2)
A. ustus		3	15											18 (0.1)
Aspergillus spp.										1,000				1,000 (3.8)
Penicillium		60	18	2,579	28	269	145	120	45	9,500	525	3		13,290 (49.6)
Mucor	78	40	31	39			3			50				241 (0.9)
Rhizopus		38	3	8	35	15	3	21	5			25		153 (0.6)
Cladosporium					13	45								58 (0.2)
Aureobasidium			10		30									40 (0.1)
Other fungi	3	8	34	13	3	15	35		3				3	435 (1.6)
Total	81	1,279	772	4,167	205	1,020	1,268	1,169	930	12,988	2,764	161	3	26,807

tified, those belonging to *Aspergillus* and *Penicillium* have been predominant in most of the drugs, and *Mucor, Rhizopus, Cladosporium,* and *Aureobasidium* were found in a few samples. It is apparent that species of *Penicillium* predominated from powdered Japanese peony roots (2,579 colonies per g) and powdered coptis (9,500 colonies per g), although identification to species was not performed. *Aspergillus* mostly occurred in powdered coptis (3,438/g), powdered scutellaria roots (2,241/g), powdered Japanese peony roots (1,523/g), and powdered cininidium (1,133/g). Among the *Aspergillus, A. niger* group was the most frequently encountered and widely distributed group in the drugs, accounting for 24.6% of the total isolates. The *A. glaucus* group and *A. flavus* group were the next most prevalent groups, comprising 9.3 and 7.8% of the total isolates, respectively.

Suspected toxigenic fungi belonging to the genus *Aspergillus* were found in a few kinds of drugs at low levels. These frequencies were 7.8% *A. flavus* group, 0.3% *A. versicolor* group, and 0.2% *A. ochraceus* group, of the total number of *Aspergilli* isolated. Thirty-five strains of *A. flavus* group, 23 strains of *A. versicolor* group, and 11 strains of *A. ochraceus* group, selected at random from the drug samples, were tested to determine their ability to produce mycotoxins such as aflatoxin, sterigmatocystin, and ochratoxin A. Among the 69 isolates assayed, only one strain of *A. flavus* was found to be an aflatoxin-producing strain (aflatoxin B_1, 0.15 μg/ml). No mycotoxin-producing strain has been detected so far from the other strains of fungi tested.

Similar studies conducted by Udagawa et al. (14) indicated that *Aspergillus* was a main component of the mycoflora of herbal drugs and that *A. niger, Eurotium* (*A. glaucus*), and *A. flavus* were the most prevalent species among the *Aspergillus.* They also stated that two strains of *A. flavus* from ephedra (Ephedrae Herba) were found to be aflatoxin producers (B_1, 4.6 μg/ml and 6.4 μg/ml). Matsushima et al. (8, 9) examined fungal growth on 30 kinds of herbal drugs under different moisture conditions and showed that *A. glaucus, Penicillium variabile, P. frequentans, A. mangini, A. awamori, A. niger, A. ochraceus,* and *Rhizopus* were predominant, in that order, as moisture content increased.

From these studies and our present investigation, it is conceivable that *A. niger* and *A. flavus* are molds that should be considered in relation to fungal contamination and mycotoxin production.

Natural occurrence of the three mycotoxins was examined in a total of 49 drug samples by means of the chemical analysis method, as reported by several investigators (4, 5, 10). No mycotoxin-contaminated samples were detected.

Over the past two decades, it has been well documented that fungal-contaminated food and feedstuffs in which mycotoxin is produced are often responsible for animal mycotoxicosis as a result of ingestion of the toxin-contaminated materials. Because many medicines are made of material of plant and animal origin, there may be a risk of mycotoxicosis occurring in patients after oral administration; this can be avoided by good practices. Additionally, unsterilized medicines are occasionally contaminated with large numbers of microorganisms, including pathogenic and nonpathogenic ones (6, 7). Other opportunistic infections in the category "drug-borne" have occurred sporadically in Europe and the United States (1, 2, 12). Diseases caused by fungi such as *A. fumigatus* and *Candida albicans* are typical examples of so-called opportunistic pathogens; therefore, fungal contamination of drugs, especially raw materials, should be prevented as much as practicable during the course of manufacturing.

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