



Mycotoxigenic fungi in plant-based supplements and medicines

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The increasing consumption of plant-based products for health promotion or for disease treatment has generated public health concerns, since herbs or plants are in direct contact with soils and may easily be colonized by fungi. Many studies have shown the contamination of plant-based supplements and drugs with mycotoxigenic fungal species. *Aspergillus* and *Penicillium* species have been reported as the most frequent and dominant ones, with records of the occurrence of many mycotoxigenic species. The presence of different species raises the concern of multimycotoxin occurrence, while the increased consumption of these products increases exposure. This review aims to present updated data on the natural occurrence of mycotoxigenic fungi and their mycotoxins in plant-based supplements and medicines.

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Introduction

Phytotherapy is a field of science that uses plants either to treat diseases or as health-promoting agents [1]. These plant drugs are crude preparations of dried plants or any part thereof, such as leaf, stem, root, flower, or seed [2^{••},3[•]]. Plant-based dietary supplements (concentrated sources of vitamins, minerals, and other biologically active substances) are mostly formulated as infusions, oils, tablets and capsules containing dried ethanolic extracts of active compounds, which are used for health promotion or to aid drug treatments.

According to the World Health Organization [4], the use of phytotherapy is popular and the primary source of health care in countries with lower accessibility to

conventional medicine—Africa and some developing countries, but also in developed countries due to cultural influence—as in some Asian countries [4]—or as complementary therapy—being used by about 70% of the Australians and one third of the Americans [5,6]. The medicinal plants global market size is expanding and was valued at USD 71.19 billion in 2016 by Hexa Research [7]. This growth may be attributed to the belief that these products do not cause overdose toxicity and have fewer side effects.

However, it is important to highlight that plant-based products may also present toxicity and contamination (chemical, biological, radiological, and so on), since herbs and plants are in direct contact with soil, and common practices employed for their production do not avoid potential contamination. Plants and herbs can be contaminated with fungi either in the pre-harvest or in the post-harvest and storage stages [8[•],9], which may produce mycotoxins that remain in the product even after complete destruction of the fungi.

Mycotoxins are secondary metabolites produced by different fungi, mainly by toxigenic species of the genus *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium*. According to Do *et al.* [10], aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FB), zearalenone (ZEN) and deoxynivalenol (DON) are the most frequently detected in herbal medicines and can cause severe health problems like immunosuppression, carcinogenesis, genotoxic, hepatotoxic and nephrotoxic effects [11,12].

The present review addresses the occurrence of toxigenic fungi and their mycotoxins in plant-based supplements and medicines, to better understand the risk of multimycotoxin exposure from the consumption of these products.

Fungal occurrence in plant-based supplements and medicines

Since the middle of the 20th century, researchers have identified the presence of fungi in herbal drugs [13^{••},14]. Hitokoto *et al.* [15] evaluated 49 powdered herbal drugs for their mycobiota, being *Aspergillus* and *Penicillium* the dominant ones. *Aspergillus niger* group was the most frequently isolated and most widely distributed group (24.6% of the isolates), mostly occurring in powdered coptis (number of isolates of 3438/g), powdered scutellaria roots (2241/g), powdered Japanese peony roots (1523/g), and powdered cininidium (1133/g).

A survey on the fungal contamination of sun dried herbal drugs from Nigeria revealed a severe fungal contamination [16,17], where twenty-eight fungal species were isolated, with *A. niger*, *Aspergillus flavus*, *Fusarium verticillioides*, *Trichoderma viride*, *Penicillium expansum* and *Mucor fragilis* being the dominant ones.

The mycobiota and mycotoxin contamination of herbal drugs from Japan and India was also reported. Yamazaki *et al.* [18] found fungal infection in 17 out of 50 samples in Japan, and detected aflatoxin B1 (AFB1) and sterigmatocystin (STC). *Aspergillus* and *Fusarium* species (54 and 24%, respectively) were the most frequently isolated ones from Indian drugs, and mycotoxins were detected in 43% and 64% of the crude herbal drugs and of their finished commercial products, respectively [19]. AFB1 was the most frequently detected mycotoxin and was detected above tolerance levels fixed by the World Health Organization-WHO.

Halt [20] analysed the level of toxigenic moulds in 62 samples of medicinal plants and 11 samples of herbal tea. The dominant fungi detected were *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Absidia*, *Alternaria*, *Cladosporium* and *Trichoderma* species. *A. flavus*, a known producer of aflatoxins, was present in 11 (18%) and in 1 (9%) of the medicinal plants and herbal tea samples, respectively.

Medicinal plants (peppermint, chamomile flowers, tilio, anise and caraway) from the Egyptian market also presented contamination by *A. niger*, *Fusarium* spp. and *Penicillium* spp. which were detected with a frequency of 77% in packed tilio and 56% in caraway samples [21]. *A. flavus* was detected in all tested medicinal plants with the exception of packed tilio, and the highest percentage of infection was in peppermint (15.8%) followed by non-packed tilio (15.4%), as well as non-packed caraway (13.5%). Abou Donia [22] also identified *Aspergillus*, *Fusarium* and *Penicillium* in Egyptian medicinal plants, but aflatoxins were not detected.

In an evaluative study of the predominant mycobiota contamination of 65 different medicinal plants from Brazil, Bugno *et al.* [23*] identified 10 fungal genera and 55% of the samples exceeded the maximum fungal count limit determined by the US Pharmacopoeia (10^2 CFU/g). The genus *Aspergillus* was the most frequent one (179 isolates) followed by *Penicillium* (44 isolates), and these two genera were found in 90% and 40% of the samples analysed, respectively. Isolated strains included *A. flavus*, *A. niger*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Penicillium citrinum* and *Penicillium chrysogenum*. Almost 22% of these isolates were able to produce mycotoxins: 42.9% were aflatoxigenic strains, 22.4% were ochratoxigenic strains and 34.7% were citrinin-producing strains.

Ahmad *et al.* [24] screened plants from Pakistan and detected fungal contamination in 90% of tested samples, of which 70% exceeded the permissible limits; being opium poppy, liquorice root, and Indian rennet the most contaminated medicinal plants. The predominant fungal strains isolated belonged to *Aspergillus* (*A. flavus*, *A. niger* and *A. parasiticus*) and *Penicillium* species, and 31% of the 47 isolates tested were found to be toxigenic. The authors highlighted that these results could give rise to trade barriers, since 12% of Pakistani flora is used in medicines and more than 300 medicinal plants are traded all over the world [25].

Fungi were isolated from all samples of Pu-erh tea (made from the leaves and stems of the *Camellia sinensis* plant) which contains small amounts of lovastatin, a well-known medicine used for lowering cholesterol. A concentration of colony forming units of 10 to 2.6×10^6 /g was found, from 19 genera and 31 species. The most prevalent species were *Aspergillus acidus* and *Aspergillus fumigatus* [26].

Wu *et al.* [27] studied a previously isolated *Alternaria tenuissima* strain from the stem of *Tribullus terrestris* L., a medicinal plant and a dietary supplement used to lose weight all over the world, and found it to be a producer of mycotoxins (altertoxins II and IV).

Chinese functional foods presented 124 fungal strains distributed over four different genera [28], being *Aspergillus* and *Penicillium* strains the dominant ones, with an incidence of 66% and 15%, respectively. *Aspergillus* section *Nigri* (57%) was isolated more often, followed by *Aspergillus* sections *Flavi* (50%) and *Circumdati* (21%), with the most contaminated samples being Coix seeds. The latter three *Aspergillus* sections are well known for their mycotoxigenic potential.

Chen *et al.* [2**] assessed internal and superficial fungal contamination and multi-mycotoxin occurrence in medicinal seeds used as food in China. The superficial mycobiota on seed surfaces included 34 species (17 genera). *A. niger* and *Penicillium polonicum* were predominant (12% and 15%, respectively). The internal fungal incidence was also very high, with the isolation of 352 fungal strains, belonging to 12 genera and 27 species. *Chaetomium globosporum* was the most commonly identified fungus (23% of the internal fungal isolates), followed by *Microascus trigonosporus* and *Alternaria alternata* (12% and 9% of total internal fungal counts, respectively). Total fungal counts ranged from 6.5×10^1 to 8.1×10^4 /g on seed surfaces, being *Aspergillus*, *Penicillium*, *Mucor*, and *Fusarium* the four most predominant genera detected in association with all medicinal seeds (exception for spider flower seed), representing about 41%, 29%, 6% and 5% of the isolated strains, respectively.

In a recent study, Su *et al.* [29**] investigated the occurrence of fungi in eight root herbs used as medicines, dietary supplements and functional foods in China and other countries, and found that all 48 samples tested were contaminated with fungi (1844 isolates belonging to 25 genera). *Aspergillus* and *Penicillium* presented a frequency of 10% and 25%, respectively. The fungal counts ranged from 12 to 2.5×10^4 /g. Notoginseng and liquorice were highly contaminated (fungal counts $> 10^3$ /g); however, the fungal counts did not exceed the maximum limit of 10^5 /g set by the WHO. The authors also reported that among the 13 isolates of *A. flavus*, five isolates from Chinese yam, ginseng, astragalus, and liquorice were found to produce AFB1 and AFB2.

A comprehensive review on the occurrence of mycotoxigenic fungi in plant-based products is presented in supplementary Table S1.

Mycotoxin occurrence in plant-based supplements and medicines

The occurrence of mycotoxins in plant drugs has been widely published in several countries but Chinese medicines are the most studied [30].

Figure 1 presents data on the prevalence of different mycotoxins in reports published from 2000 [2**,11,24,29**,31–37,38**,39–55]. The main mycotoxins identified in plant-based supplements and medicines have been AFs, OTA, mycophenolic acid (MPA), STC and trichothecenes.

Discussion and conclusions

The technological advances of synthetic pharmacology in the last decades overlapped the use of natural medicine especially in American and European countries. However, in recent years, there was a progressive increase in the demand of herbs and preparations of botanical origin

(dietary supplements or drugs) as alternative or complementary medicine due to economic, social and cultural factors [1].

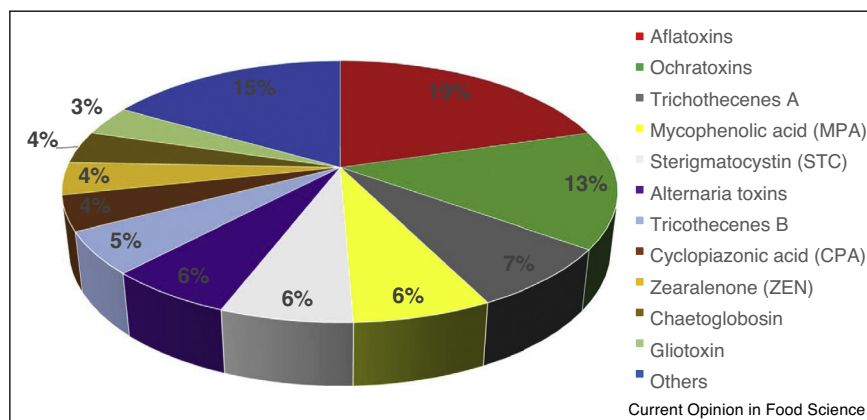
However, the use of natural herbal/plant products also has its associated risks, and among them fungal contamination and mycotoxins are public health concerns. Overall, many studies have shown that fungal contamination in plant-based supplements and medicinal herbs is quite frequent. Potential toxigenic species of *Aspergillus* and *Penicillium* have been reported as the most frequent and dominant ones, but the presence of toxigenic species of *Alternaria* and *Fusarium* may not be overlooked.

A. flavus and *A. niger*, known producers of aflatoxins and ochratoxin A, respectively, were often found with high incidence. In some studies, *A. niger* group represented more than 57% of the isolates, followed by *A. section Flavi* (50%) [28].

The levels of fungi and mycotoxin contamination found in plant-based ingredients throws light on the need for proper care and effective screening before their use. Thus, it is essential to highlight the need for deeper knowledge on the presence of fungi and mycotoxins (including masked forms and emergent metabolites) in plant-based raw materials widely used as ingredients by pharmaceutical companies or food industries.

Although there appeared to be a low risk to the consumers who occasionally use plant-based supplements and medicinal drugs, it is important to draw special attention to the potential risk of cumulative effects [56*]. The toxic effects resulting from the co-occurrence of multiple mycotoxins can be both additive and even synergistic, that is, the overall toxicity might be higher than the sum of individual toxicities [57]. These mycotoxin ‘cocktails’ have an underestimated toxicological hazard.

Figure 1



Main mycotoxins detected in plant-based supplements and medicines. Prevalence of each mycotoxin in the 35 report published since 2000.

Finally, the risk of mycotoxin contamination in natural products, infected or not with fungi, associated with increased consumption of this type of products, impose the need of discuss appropriate standards for toxigenic moulds and mycotoxins in crude herbal drugs, medicinal plants and plant-based supplements in order to reduce the risks for consumers.

Conflict of interest statement

Nothing declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.cofs.2018.08.003>.

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