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Investigation of fungi and mycotoxins contamination in some herbal slimming mixtures in Baquba city- Iraq

Abed RM[1*], Al-Khazreje AA[1], Ibrahim NS[2], Abbas RA[1] and Hussein RA[3]

[1] *Department of Biology, College of Education and Pure Science, University of Diyala, Iraq.*
[2] *Department of Sciences, College of Basic Education, University of Diyala, Iraq.*
[3] *Internal Medicine, College of Veterinary Medicine, University of Diyala, Iraq.*

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ABSTRACT

This study was conducted to detect the contamination of some herbal slimming mixtures with mycotoxins producing fungi. Five of the most popular herbal slimming mixture samples in Baquba city were collected and investigated. The results revealed that 80% of the examined samples were contaminated with fungi. 40 fungal isolates were recorded with a total count ranging from 0.66×10^{-3} to 6.66×10^{-3} belonging to 4 different genera *Aspergillus*, *Rhizopus*, *Mucor*, *Alternaria* in addition to four imperceptible fungal isolates. *Aspergillus niger* recorded the highest percentage of frequency and appearance with 50% and 75% respectively. HPLC analysis showed that about 80% of the samples showed the presence of mycotoxins such as Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Ochratoxin A in different concentration. First sample recorded 3.50 ng/gm of Aflatoxin B2 and 6.79 ng/gm (Aflatoxin G1), sample (2) recorded Ochratoxin A and Aflatoxin B1 in concentration 2.892 ng/gm and 2.877 ng/gm, sample (3) recorded Ochratoxin A in concentration 5.424 ng/gm, sample (5) showed presence Ochratoxin A and Aflatoxin B1 with concentrations 4.121 ng/gm and 3.061 ng/gm respectively. The results showed that Fumonisins, Aflatoxin G2 and Patulin were not present in all samples.

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Introduction

Herbal medication lately has grabbed a considerable attention and has become as important as modern medicine. Nowadays, wide varieties of diseases are treated with medicinal herbs that are sold as medicines in markets and special stores, also they are sold in some shops that are devoted for selling spices and other similar products (Al-Shatti 1970).

The increase in patient's interest in herbal therapy or what we sometimes call alternative medicine, is attributed to many religious, social, cultural and economic reasons which may be summarized as follows: The current political and security situation in Iraq has led to a great migration of Iraqi doctors abroad, and the high cost of some chemical drugs has forced patients to search for a drug at a lower price, and there is a prevailing idea among medical herbal users that if they do not succeed in treatment, they will not harm (Mahmood 2009). The prevailing belief among community members is "medication with herbs that do not benefit you, do not harm you" this belief is considered wrong due to the numerous health risks associated with the herbal treatments. These risks are summarized as lack of effectiveness, and the side effects associated interaction with the chemical drugs (Cheij 1984). Many studies have reported a contamination of the medicinal herbs with many fungal genera, such as the study presented by Idu et al. (2010) This study considered 60 samples of the medicinal plants available in the local market of five different regions in Nigeria. The results showed that all samples were contaminated with fungi belong to six fungal genera which were *Aspergillus, Absidia, Mucor,*

*** Corresponding author**
E-mail address: **rabab.majead81@gmail.com (R. Abed)**

Penicillium, *Saccharomyces*, *Rhizopus* and the total count ranged from 0.0×10^{-1} to 6.1×10^{-6} CFU/g. In another study conducted by Shakhenib et al. (2011) were isolated from 30 samples of medicinal herbs included thyme, bantam, and fenugreek were collected from the Attarin market in the governorates of Basra, Baghdad and Nasiriyah, 71 species of fungi that belong to (43) genera, The genera of *Aspergillus* recorded highest percentage of emergence which was 100% and 90 % for *Alternaria* and *Cladosporium*, *Emericella*, *Ulocladium*, *Mucor* and *Chaetomium* recorded 76.66%, 73.33%, 53.33%, 46.66% and 40% respectively. The study presented by Zheng et al. (2017) concluded that medicinal herbs collected from the Chinese domestic market were contaminated with fungi and their toxins. During this study, 15-samples were collected from different places, and 126 fungal isolates were isolated, these isolates were identified *Penicillium* and *Aspergillus* fungi.

Mycotoxins are toxic secondary metabolites produced by certain mold species belonging to the genus of *Aspergillus*, *Penicillium*, and *Fusarium*, *Aspergillus* and *Penicillium* are commonly known as storage molds which can grow post-harvest and during the drying and storage stages, especially when insufficient drying and unsuitable storage condition favor their proliferation (Scott 1984).

These contaminants have a broad range of toxic effects, including acute toxicity, carcinogenicity, immunotoxicity, neurotoxicity, and reproducibility and developmental toxicity (Chu 1997).

Medicinal plants and herbs available on the market do not meet the quality and safety standards and are considered a dangerous source of contamination with contamination and mycotoxins. The results showed that 89.9% of the isolates corresponded to genera *Aspergillus* and *Penicillium*, which are extremely important from the mycotoxicological standpoint, 21.97 % of the *Aspergillus* and *Penicillium* isolates proved to have the ability for producing aflatoxins 42.9 %, ochratoxin A 22.4 % and citrinine 34.7%. A study conducted by Keter et al. (2017) confirmed that some medicinal herbs mixtures in the local Kenyan markets were found to be contaminated with fungi and their toxins. In the study of Chien et al. (2018) It was found that most medicinal herbs in Taiwan contained aflatoxin toxins. mycotoxins, especially aflatoxin and ochratoxin A is one of the most common mycotoxins in herbal products and has been reported to have concentrations that exceed European Union regulatory levels (Altyn and Twaruzek 2020).

The increasing demand from people to using herbal mixtures for lose weight because they believe it is safe and healthy and does not cause side effects, Therefore, the present study aimed to : investigation fungi contamination in some herbal slimming mixtures taken from herbal stores in Baquba City – Iraq and detecting the presence of some mycotoxins in them.

Materials and Methods

Sampling

Herbal slimming mixtures were collected from herbs selling shops in local market of Baqhuba city, Iraq. The samples which chosen based on their efficiency in losing weight and being the bestselling. Five different samples were collected, and each sample were divided into 3 replicas all become 15 (Table 1) shows the components of each samples under study.

Screening samples for fungal contamination

Dilution method was used to determine total fungal counts in samples (Al-Shtayeh et al. 1998). One ml of 10^{-3} dilution was used to inoculate petri dishes each containing Potato Dextrose Agar (PDA) pH (5.6), Petri dishes were incubated for 7 days at 28 C°. Three replicates plates per medium were used for each sample and the developing fungi were counted. Recorded taxa were purified using PDA medium and identified according to their cultural and morphological characteristics according to (Raper and Fennell 1965, Ellis et al. 2007).

Screening samples for mycotoxins contamination

Five grams of the grounded samples were extracted with 20 ml chloroform. The extract was filtered through Whatman paper, evaporated to dryness, and re-dissolved in 1 ml chloroform (Kumar et al. 2010). The chloroform extract was evaporated to dryness and re-dissolved in deionized water for detection of mycotoxins (Ochratoxin A, Fumonisins, Patulin and Aflatoxin B1, B2, G1, G2) using high performance liquid chromatography (HPLC) Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu. 100 µm was injected into HPLC sampler and deionized water: methanol (60 :40) was used as mobile phase at a flow rate of 1 ml / min, Mycotoxin were detected with UV detector at a wavelength of 365 nm, (Table 2) showed Retention time for each one (Akiyama 1999; Stroka et al. 2000).

Table 1 Studied sliming herbal mixtures

Sample	Sample composition
1	*Nigella sativa, Theaceae, Cuminum cyminum, Pimpinella anisum, Cinnamomum cassia*
2	*Nigella sativa, Cuminum cyminum, Pimpinella anisum*
3	Unknown herb mixture
4	*Nigella sativa,* Theaceae, *Cuminum cyminum, Pimpinella anisum, Cinnamomum cassia*
5	Unknown herb mixture

Table 2 Analysis of mycotoxin by HPLC

Mycotoxins	Retention time (Min)	Area µ volt
Fumonisins	1.75	296776
Aflatoxin G2	2.65	305633
Aflatoxin G1	3.75	277484
Ochratoxin A	4.96	254978
Aflatoxin B2	5.79	279232
Aflatoxin B1	6.88	317579
Patulin	8.14	298773

Results

Overview of recorded taxa

The results obtained showed that out of the 5 samples analyzed, 4 were positive for fungal contamination in 80 % percentage while 1 free from any fungus in 20 % percentage, Sample 5 had the highest fungal contamination (6.66×10^{-3}) CFU/g followed by samples 3,1 and 2 which were (4.66×10^{-3}, 1.33×10^{-3}, 0.66×10^{-3}) CFU/g respectively and no fungal contamination has been recognized in number 4 as shown in (Table 3). The result also shown isolated 40 fungal isolates, the highest number of fungal isolates was in sample 5 which was 20 followed by samples 3,1 and 2 which were (14, 4, 2) respectively, while sample 4 no fungi was reported (Table 3). A total of 40 fungal isolates were isolated that belong to four different genera of fungi which were *Aspergillus*, *Rhizopus*, *Mucor*, and *Alternaria*. Among the genera and species *Aspergillus niger* was found to be the most commonly occurring contamination which was isolated from 3 sample and recorded highest number of isolates was 20, for *A. parasiticus* found in 3 samples in 7 isolates, 4 isolates for *Rhizopus* spp. Which appeared in 2 samples, the fungi *Aspergillus* spp, *Mucor* spp. and *Alternaria* spp. recorded (1, 3, 1) fungal isolates respectively which were appeared in 1 sample, the results shown isolated 2 unknown isolates as shown in table (4).

In this study the fungal domination was for *A. niger* which has a frequency percentage that 50 % other fungi showed lower frequency percentage which were (17.5, 10 and 7.5) % for *A. parasiticus*, *Rhizopus* spp., and *Mucor* spp. respectively. Moreover, the frequency percentage of the *Alternaria* spp. And *Aspergillus* spp. fungi was found to be 2.5% as shown in (Table 4). In the same Table the percentage of appearance for fungi *A. niger, A. parasiticus*, *Rhizopus* spp., *Mucor* spp., *Alternaria* spp. and *Aspergillus* spp. were (75, 75, 50, 25, 25, 25) % respectively.

Mycotoxins contamination

The HPLC analysis for herbal slimming mixtures samples showed the presence of mycotoxins. In sample 1 recorded presence Aflatoxin B2 and Aflatoxin G1 in concentration 3.50 ng/g and 6.79 ng/g for each one , In sample 2 Ochratoxin A and Aflatoxin B1 were recorded in concentration 2.892 ng/g and 2.877 ng/g for each one, Sample 3 recorded presence of Ochratoxin A in concentration 5.424 ng/g, Sample 5 showed presence Ochratoxin A and Aflatoxin B1 in concentration 4.121 ng/g and 3.061 ng/g respectively, While sample 4 not record presence of any mycotoxins.

The HPLC analyzes also shown absence of Fumonisins, Aflatoxin G2 and Patulin in all samples as shown in table (5).

Table3 Total count of recorded fungal taxa

Sample	Total count (CFU)	Number of fungal isolates
1	1.33×10^{-3}	4
2	0.66×10^{-3}	2
3	4.66×10^{-3}	14
4	0	0
5	6.66×10^{-3}	20

Table 4 Recorded fungal taxa from collected local herbal slimming mixtures from Baquba city- Iraq

Fungi	Total count (CFU)	NCI*	Frequency %	Appearance %
Aspergillus niger	20	3	50	75
A. parasiticus	**7**	3	17.5	75
Aspergillus spp.	**1**	1	2.5	25
Rhizopus spp.	**4**	2	10	50
Mucor spp.	**3**	1	7.5	25
Alternaria spp.	**1**	1	2.5	25
Unknown	**4**	2	10	50

*Number of cases of isolation in 5 samples

Table 5 HPLC analyzes for mycotoxins contamination some herbal slimming mixtures

Sample	Mycotoxins conc. (ng/g)						
	Fumonisins	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A	Patulin
1	0	0	6.79	3.50	0	0	0
2	0	2.88	0	0	0	2.89	0
3	0	0	0	0	0	5.42	0
4	0	0	0	0	0	0	0
5	0	3.06	0	0	0	4.12	0

Discussion

The result showed that 60 % of the samples have a fungal colony unit above the permissible limit of the World Health Organization (WHO 2005). A contamination limit of yeast and molds in medicinal plant was 1×10^3 CFU/g. These results are consistence with the study conducted by Pereira et al. (2015) which reported fungus infections in the all 12-samples collected from the local markets.

The total number of fungi ranged between (1.3×10^{-3} to 6.5×10^{-3}) CFU/g. The result also showed that the fungal contamination found in 80 % of samples this observation is conforming with what has been reported by Shakhenib et al. (2011) where all medicinal herbs samples collected from the local Iraqi market were found to be contaminated with fungi. In this study *Aspergillus* recorded highest level in fungal isolates, percentage of frequency and appearance this is attributed to its widespread in the environment by ability to produce huge numbers of asexual reproductive units known as conidia. These reproductive units able to grow in all environments and endure bad conditions (Pitt and Hocking 1997). The results are consistent with Idu et al. (2010) which found that that all samples were contaminated with fungi belong to six fungal genera which were *Aspergillus, Absidia, Mucor, Penicillium, Saccharomyces, Rhizopus*. In another study recorded highest percentage of emergence for genera *Aspergillus* which was 100% and 90 % for *Alternaria* and *Cladosporium*, *Emericella*, *Ulocladium*, *Mucor* and *Chaetomium* recorded 76.66%, 73.33%, 53.33% ,46.66% and 40% respectively (Shakhenib et al. 2011) this partially consistent with the results of the current study. The results of HPLC analysis showed the presence of mycotoxins in 80 % of samples. Fungi toxins

productivity is highly influenced by the storage conditions which play a vital role in stimulating fungi to produce the toxins, these conditions include temperature, acidity of the medium, humidity; and light and darkness, especially the storage of foodstuffs in darkness (Kuchari and Qattan 2001). This result in conforming with what has been concluded by Zheng et al. (2017) which reported that all testing medicinal herbs were contaminated with mycotoxins. In another study conducted by Ali (2017) a conclusion has been achieved that 60.52% of the *Aspergillus flavus* isolates had the ability to produce mycotoxins.

In this study was recorded presence of some mycotoxins such as Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Ochratoxin A, this confirms that the presence of fungi means contamination of mycotoxins in herbs, especially with the presence of fungi known to produce mycotoxins like *Aspergillus* spp. In Pietri et al. (2010) study showed that Ochratoxin A occurred in all samples of dried liquorice extract and Aflatoxin B1 was detected only in 15.8% of samples. Aiko and Mehta (2016) found that from 187 fungi were isolated from some medicinal herbs 28 were toxigenic which included 19 Aflatoxin-producing *Aspergillus flavus* and 9 citrinin producing *Penicillium citrinum*, while the natural contamination with Aflatoxin B1 was detected only in one sample. The results of this study showed that medicinal herbs are susceptible to fungi and mycotoxins contamination and this not consistent with some studies that indicated that medicinal herbs are considered an inappropriate medium for fungi growth and toxin production (Al-Rahmah et al. 2011, Prakash et al. 2011).

The results of the current study confirmed the incorrectness of the idea prevalent in Iraqi society, that "using medicinal herbs that may not benefit you will not harm you. Because there is a risk of contamination with fungi and their toxin.

Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript**.**

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