MINAR International Journal of Applied Sciences and Technology
ISSN: 2717-8234
Article type: Research Article

Received: 13/04/2022 **Accepted:** 12/05/2022 **Published:** 01/06/2022

ASSESSMENT OF FUNGI AND MYCOTOXINS CONTAMINATING ANTIDIABETIC HERBS

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Abstract

This study was conducted to detect the contamination of some mixture of antidiabetic herbs with fungi and mycotoxins producing fungi. 30 of the most popular antidiabetic herbs mixture samples in Diyala Governorate, Iraq were collected from herbal selling stores. The results revealed that 100 % of the examined samples were contaminated with fungi. The total number of colony fungi was in the range between (5.66×103 and 12.33×103) CFU/gm . 252 fungal isolates were isolated belong to genera Aspergillus , Penicilium , Rhizopus , the species were belong to Aspergillus niger, A. terrus, A. verscolor, A. flavus, A. ochraceus, Penicillum expansum, Rhizopus stonifer. HPLC analysis showed that 100% of the samples contained mycotoxins which included Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 and Ochratoxin A in different concentration. Aflatoxin B1 was found in all samples at a rate ranging (from 1.8 to 6.483) ng/gm, as well as aflatoxin G2 which appeared in all the studied samples and its quantity ranged from (from 2.088 to 3.857) ng/gm, and the study also recorded the presence of toxins aflatoxin B2, G1 and Ochratoxin A in varying proportions between samples and their presence in some samples.

Keywords: Mycotoxins, Diabetic, Herbs mixture, Contamination, Aspergillus.

http://dx.doi.org/10.47832/2717-8234.11.19

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Introduction

Diabetes is a group of diseases characterized by high blood glucose levels that result from defects in the body's ability to produce or use insulin,this increases the risk of developing large blood vessels and its complications such as ischemic heart disease, stroke, and peripheral vascular disease (Khazeem, 2011). The treatment of diabetes requires the use of different drugs according to the stage of disease, and the treatment lasts almost for life, so we find that many patients resort to alternatives based on medicinal plants, which in some developing countries is the only option for treatment (Lal *et al.*, 2011). There are many medicinal plants that have shown their efficacy as anti-diabetes, more than 1200 types of plants are used in the treatment of diabetes around the world (American Diabetes Association, 2014).

There are many herbs that studies have indicated to be effective as a treatment for diabetes, such as *Ferula assafoetida, Annona squamosal, Zingiber officinale, Gymnema sylvestre, Tamarindus indica, Azadirachta indica, Trigonella foenumgraecum, Moringa oleifera, Aegle marmelos, Cajanus cajan, Cinnamomum tamala, Cinnamomum verum, Urtica, Allium sativum, Carthamus tinctorius, Bauhinia, Swertia, Combretum, Sarcopoterium, Liriope, Caesalpinia bonduc, Coccinia grandis, Syzygium cumini, Mangifera indica, Momordica charantia, Ocimum tenuiflorum, Pterocarpus, Tinospora cordifoli, Salvia officinalis, Panax, Abelmoschus moschatus, Vachellia nilotica, Achyranthes, Fabaceae, Mentha, Asphodelaceae, Andrographis paniculata, Artemisia herba-alba, Artemisia dracunculus, Caesalpinioideae, Pachira aquatic, Gongronema latifolium, Nigella Sativa, Tinospora cordifolia, Chrysanthemum morifolium, Symphytum, Cactaceae, Symplocos, Perilla frutescens, Terminalia chebula*, *Aloe vera* and other that effective to control and treat diabetes (Al-Asadi and Salih, 2012;Panda *et al.*, 2013;Damnjanovic *et al.*, 2015; Kamau *et al.*, 2017; Moradi *et al.*, 2018; Njoroge ,2020). But it should be noted that many of the herbal mixtures used to treat diseases that are sold in local stores and markets are contaminated with many sources of danger, such as Mycotoxigenic Fungi and Mycotoxins (Abed *et al.*, 2020 ; Ikeagwulonu *et al.*, 2020), As well as bacteria and heavy metals (Zamir *et al.*, 2015; Abualhasan *et al.*, 2019). The aim of the current study is to detect the presence of fungal contamination and mycotoxins in some of selected antidiabetic herbs mixtures are widely spread among Iraqis in Diyala Governorate population.

2. Materials and methods

2.1 Study Area and Sample Collection:

Samples of thirty antidiabetic herbal mixtures were purchased randomly from different herbalists' shops of Diyala Governorate-Iraq, All samples were preserved in the fungi laboratory in the College of Education for Pure Sciences - University of Diyala to conduct biological examination. As for the tests for the detection of mycotoxins, they were carried out in the Department of Environment and Water in the Ministry of Science and Technology, Iraq. Six samples were collected from different places in Diyala Governorate, which included Baqubah, Bani Saad, Muqdadiya, Khalis and Baladrooz as shown in Figure (1).

2.2 Screening samples for fungal contamination :

The 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 *Sabouraud dextrose agar* (SDA) pH was 5.6, Petri dishes were incubated for 7 days at 28 C°, After incubation the following measurements were taken:

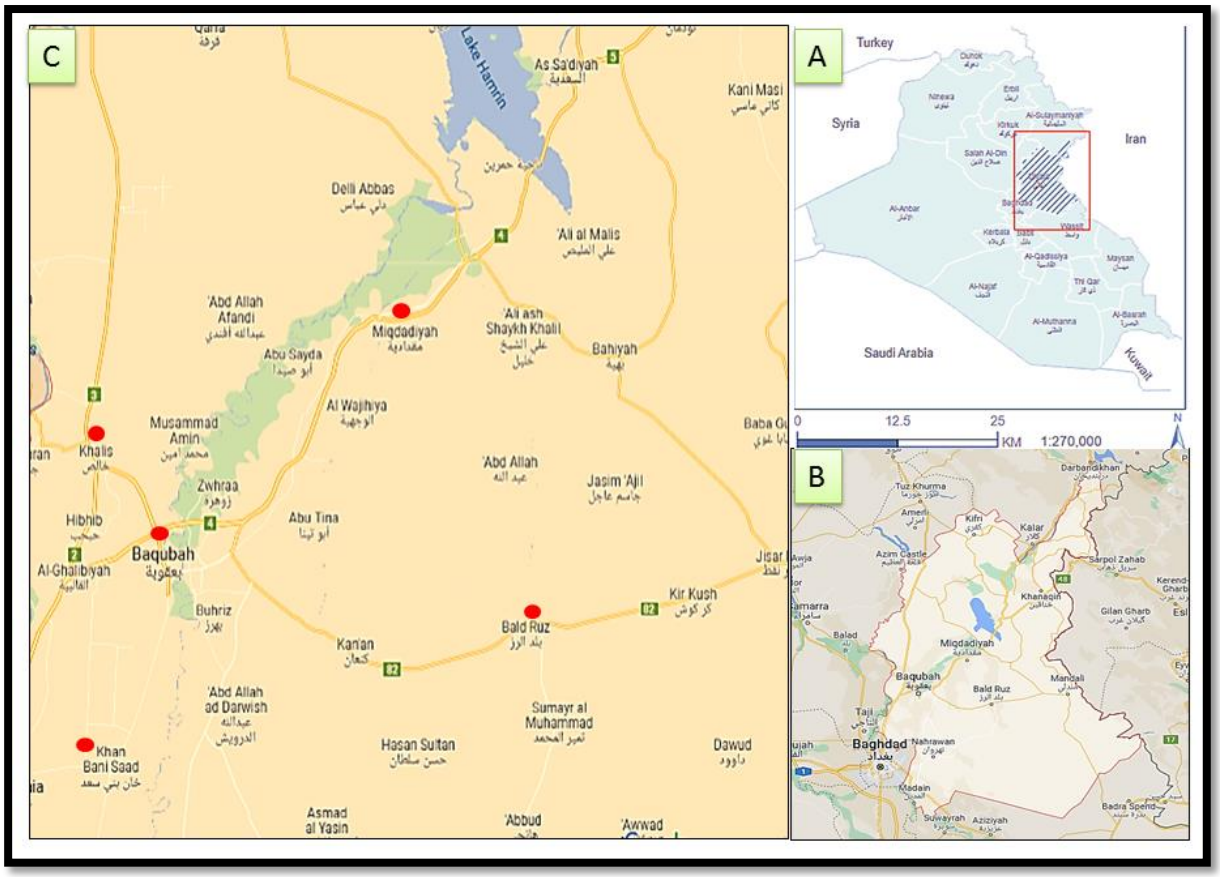

Figure 1: Map of Diyala governorate showing sampling sites marked with red circles (A: location of Diyala Governorate in Iraq, B: map of Diyala Governorate, C: sites from samples.

● The average of the colony forming units (CFU) has been done using the following formula based on International Commission on Microbiological Safety for Foods (ICSMF) protocol.

$$\text{CFU/gm} = \text{numberof colonies} \times \text{inverted dilution}$$

Fungal isolates were cultured on SDA with the aim of obtaining pure cultures, and the isolates were identified by their morphological and microscopic characteristics according to (Raper and Fennell, 1965 , Ellis *et al.*, 2007).

2.3 Screening samples for mycotoxins contamination : According to (Kumar *et al.* 2010) five grams of the grounded samples were extracted with 20 ml chloroform, The extract was filtered through Whatman paper, evaporated to dryness, and redissolved in 1 ml chloroform. The chloroform extract was evaporated to dryness and redissolved in deionizedwater for detection of mycotoxins Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 and Ochratoxin using High Performance Liquid Chromatography (HPLC) Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A shimadzu. 100 µm was injection into HPLC sampler and deionizedwater: methanol (60:40) was used as mobile phase at a flow rate of 1 mL/ minutes, Mycotoxin were detected with UV detector at a wavelength of 365 nm (Akiyama, 1999; Stroka *et al.*, 2000), Table (1) showed Retention time for each one .

Table 1: Analysis of Mycotoxins by High Performance Liquid Chromatography (HPLC) .

Mycotoxins	Retention time (min)	Area µ volt
Aflatoxin B1	6.88	317579
Aflatoxin B2	5.79	279232
Aflatoxin G1	3.75	277484
Aflatoxin G2	2.65	305633
Ochratoxin A	4.96	254978

The amount of mycotoxins were calculated according the following equation

$$\text{concentration of mycotoxin} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{concentration of standard}$$

3. Results and Discussion

3.1 Fungal contamination

The results obtained showed that all of the 30 samples analyzed were contaminated with fungi in 100 % percentage, The highest total number of fungal colonies was (12.33×10^3) cfu/gm in the Al-Khalis site , followed by samples from Al-Muqdadiya, Baqubah, Baladrooz and Beni Saad which were (10.83×10^3, 7×10^3, 6.16×10^3, 5.66×10^3) CFU/gm as shown in Table (2) . The results also showed that 252 fungal isolates were obtained from a total of 30 samples of herbal mixtures used to treat diabetes. The results also showed Table (3) that 252 fungal isolates were obtained from 30 samples of herbal mixtures used to treat diabetes, they belong to 3 fungal genera that *Aspergillus*, *Penicillium*, *Rhizopus* which included the 7 species *Aspergillus niger, A. terrus, A. verscolor, A. flavus, A. ochraceus, Penicillum expansum, Rhizopus stonifer. The fungus Aspergillus niger recorded the highest percentage was among the fungal isolates, which was 30.16 % followed by the fungus Rhizopus stonifer which was 16.27 %, As for the rest of the fungal isolates Aspergillus flavus, Aspergillus terrus, Penicillum expansum, Aspergillus ochraceus, Aspergillus verscolor they were (14.29, 11.5, 11.11, 9.92, 6.75)% respectively.*

Table 2: Fungal contamination in some antidiabetic herbal mixtures collected from different herbalists shops of Diyala Governorate- Iraq

Sites	colony forming units (CFU/gm)	Number of fungal isolates
Baqubah	7×10^3	42
Beni Saad	5.66×10^3	34
Al-Muqdadiya	10.83×10^3	65
Al-Kalis	12.33×10^3	74
Baladrooz	6.16×10^3	37
Totsl		252

* Each number represents an average of 6 samples per site

Table 3: fungi isolated from some antidiabetic herbal mixtures collected from different herbalists shops of Diyala Governorate- Iraq.

Fungal isolate	Funfal isolates number	%
Aspergillus niger	76	30.16
Aspergillus terrus	29	11.50
Aspergillus verscolor	17	6.75
Aspergillus flavus	36	14.29
Aspergillus ochraceus	25	9.92
Penicillum expansum	28	11.11
Rhizopus stonifer	41	16.27
Total number of fungal isolates	252	100

The result showed that 100% of the samples have a fungal colony units above the permissible limit of the World Health Organization, A contamination limit of yeast and molds in medicinal plant was 1×10^3 CFU/gm (WHO 2005). These results are consistence with the study conducted by (Pereira *et al.*, 2015; Shakhenib *et al.*, 2011; Abed *et al.*, 2020). In this study *Aspergillus* fungi recorded the highest level in fungal isolates, this is attributed to its widespread in the environment by ability to produce huge numbers of asexual reproductive units and its ability to grow in all environments and endure bad conditions (Pitt & Hocking, 1997). The results are consistent with (Idu *et al.*, 2011; Ikeagwulonu *et al.*, 2020)

3.2 Mycotoxins contamination

The HPLC analysis for antidiabetic herbal mixtures samples showed the presence of mycotoxins. In samples from Baqubah and Al-Kalis recorded presence all Mycotoxins Tested Aflatoxin B1,B2,G1,G2 and Ochratoxin A, as for the samples belonging to Al-Muqdadiya and Baladrooz 4 types of mycotoxins have been identified, As for the samples belonging to Beni Saad, it was determined that there are 3 types of mycotoxins, which are Aflatoxin B1,G1, G2 only. The results show that all samples are contaminated with at least a variety of mycotoxins, which have different concentrations, as shown in the Table (4).

Table 4: HPLC analyzes for mycotoxins contamination in some antidiabetic herbal mixtures collected from different herbalists shops of Diyala Governorate- Iraq

Sites	Mycotoxins concentration (ng/gm)				
	Aflatoxin B1	**Aflatoxin B2**	**Aflatoxin G1**	**Aflatoxin G2**	**Ochratoxin A**
Baqubah	3.74	0.661	0.598	3.857	3.98
Beni Saad	1.8	_	0.652	2.89	_
Al-Muqdadiya	3.883	_	0.758	2.194	1.749
Al-Kalis	4.985	3.089	2.967	2.088	3.152
Baladrooz	6.483	2.344	_	2.667	2.557

* Each number represents an average of 6 samples per site

The results of HPLC analysis showed the presence of mycotoxins in 100 % 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 & Qattan, 2001). This result in conforming with what has been concluded by (Zheng *et al.*,2017; Ali, 2017)

In this study was recorded the presence of some mycotoxins such as Aflatoxin B1, B2, G1,G2 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. 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, Abed *et al.*, 2020).

4. Conclusions

In this work, we can conclude that despite the great importance of medicinal herbs and plants, they may be a source of danger to human health and patients, especially when they are stored in bad conditions or not used for long periods, which provides a greater opportunity for contamination with fungi and thus mycotoxins for these fungi

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