Fungal Contamination of Some Medicinal Plant Materials Sold at Omdurman Local Market, Khartoum State, Central Sudan

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Abstract

Plants have long been used as herbal medicines in many countries. However, microbial contamination of these medicines may affect human health. In the present study fifteen medicinal plants namely, *Acacia nilotica ssp. nilotica*, *Trigonella foenum-greacum*, *Nigella sativa*, *Hyphaene thebaica*, *Nauclea latifolia*, *Cyperus rotundus*, *Cymbopogon schoenanthus* spp, *proximus*, *Artemisia herba-alb*, *Cassia acutifolia*, *Solenostemma argel*, *Tamarindus indica*, *Ziziphus spina-christi*, *Lepidium sativum*, *Foeniculum vulgare* and *Coriandrum sativum* were evaluated for their fungal contamination. The pour plate method was used to cultivate serially diluted portions of the medicinal plant material investigated. The colonies of fungi were identified morphologically according to form and pigment. They were observed microscopically through Lactophenol cotton bluestaining. The identified fungal isolates were consist of three fungal species, the most dominant fungi were *Aspergillus nigar* and *Aspergillus flavus*. *Penicillium ssp* is least one. The total fungal counts ranged from 00×10^1 cfu/g (*Cymbopogon schoenanthus* spp, *proximus*, *Trigonella foenum-greacum*, *Artemisia herba alb*, *Lepidium satinum*, *Nigella sativa* *Cassia acutifolia* and *Naucle latifolia*) to 11×10^5 cfu/g (*Cyperus rotundus*).. The highest total fungal load was found in *Cyperus rotundus* The levels of contamination varied greatly between the commercially available plant samples investigated.

Key words:

Fungal, Contamination, *Aspergillus nigar*, *Aspergillus flavus* *Penicillium ssp*

Introduction

Microbial contamination of medicinal herbal can be influenced by environmental factor such a temperature, humidity and extent of rainfall during pre-harvesting and post-harvesting periods, handling practices and the storage condition of cured and processed medicinal-plant materials. The presence of lipolytic mould such as *Penicillium ssp.* and *Aspergillus nigar* have been implicated in food poisoning (Bampton, 1963). Although bacterial endospores and fungal spores can be regarded as the two dominating groups of contaminants associated with medicinal plants, a broad diversity of bacterial, fungal cells and viruses can be found either in or on the plant material (Kneifel and Kopp, 2002)..

It is known that, under favorable conditions, some fungi can synthesize toxic metabolites – mycotoxins. Among the known mycotoxins , the most toxic one is aflatoxin synthesized by species of *A. flavus* and *A. parasiticus*, and a minor number of other fungi (Kulshrestha *et al*, 2008). Contamination by *A. flavus*, the most famous aflatoxin producer , is common in medicinal plants and herbal tea (Halt, 1998). In study of 91 medicinal herb samples in Brazil Bugno *et al* (2005) found that 50% of aerial part samples were contaminated with fungi. Moulds are responsible for bio-deterioration of raw materials of some medicinal plants .These moulds reduce raw herbal drug shelf life and market value. The fungal deterioration adversely affects the chemical composition of the raw materials and therapy decreases the medicinal potency of herbal drug Kumar *et al*, (2009). There is a need for consistent quality assessment of crude herbal drugs for safe therapeutic products suitable for human beings (Chanda *et al.* 2019). The present study aimed to throw light on the fungal load of some medicinal plants of widely uses in Sudan.

Materials and Methods**Plant Materials**

A total number of fifteen different medicinal plant parts (Table 1) were purchased from a local market in Omdurman, Khartoum State, central Sudan (during the period September–October, 2016). The Plant parts were collected aseptically with gloves in to sterile pouches.

The plants were identified at the Botany Department, Faculty of Science and Technology, Omdurman Islamic University.

The Media

Malt extract agar and Potato dextrose agar were used to enumerate the total fungi population.

Sample Preparation:

The dried plant samples were cleaned to remove adhering polyethylene and other unwanted materials. The samples were stored at 4°C till further analysis

Procedure for Determination:

The determination of fungi contamination was established for fungal load, total fungi count and specific pathogen in the studied medicinal plant samples. For calculation of fungal contents, the plant materials were tested to determine the number of microorganisms per gram samples. The standard plate count method was used for detecting and determining the number of microorganism in plant materials. One gram of each of the studied plant parts were infused in 9ml of distilled water. Serial dilutions were made; viability assessed using Pour Plate method. The plates were incubated at 25°C for 10 days. The plates were placed on a colony counter and the number of Colony Forming Units was taken. Then all isolates were identified by using colonies morphological characters: form and pigment (Cheesbrough, 1984).

Table 1: The studied plants

No	Plant Species	Plant Part	Family	Medicinal Uses
1	<i>Acacia nilotica ssp. nilotica</i>	Pods	Fabaceae	anti-hyperglycemic, antiparasmodial, anti inflammatory, analgesic and antipyretic
2	<i>Artemisia herba alb</i>	Aerial Parts	Asteraceae	diuretic, anthelmintic, intestinal troubles, relieves gastro-intestinal cramps
3	<i>Cassia acutifolia</i>	Fruits	Fabaceae	flavoring agent and a traditional medicinal herb
4	<i>Coriandrum sativum</i>	Fruits	Apiaceae	diabetes, an anti-fungal, hypolipidemic, antimicrobial, hypo cholesterolemic and anticonvulsant
5	<i>Cymbopogon schoenanthus spp , proximus</i>	Aerial Parts	Poaceae	treat fever, cold, cough , stomach upset, diuretic, and water retention
6	<i>Cyperus rotundus</i>	Roots	Cyperaceae	spasms, stomach disorder and inflammatory diseases.
7	<i>Foeniculum vulgare</i>	Fruits	Apiaceae	Diuretic, analgesic and antipyretic
8	<i>Hyphaene thebaica</i>	Fruits	Palmae	<i>bilharziasis, haematuria</i> , Bleeding , and as haematinic agent
9	<i>Lepidium sativum</i>	Seeds	Brassicaceae	in asthma, coughs and bleeding piles.
10	<i>Nauclea latifolia</i>	Fruits	Naucleae	stomach pain, constipation, fever, and diarrhea
11	<i>Nigella arvensis</i>	Seeds	Ranunculaceae	headache, nasal congestion, toothache, intestinal worms and diuretic
12	<i>Solenostemma argel</i>	Leaves	Apocynaceae	stomach colic, cold , urinary tract infections and as an anti-syphilitic

13	<i>Tamarindus indica</i>	Fruits	Fabaceae	cold, fever, stomach disorder, diarrhea and jaundice and as skin cleanser
14	<i>Trigonella foenum-graecum</i>	Seeds	Fabaceae	aiding digestion,. Hypoglycemic, anti hyperlipidemic
15	<i>Ziziphus spina-christi</i>	Fruits	Rhamnaceae	healing of fresh wounds, for dysentery, bronchitis, cough and tuberculosis

Results :

A total number of three fungal strains were isolated from the fifteen studied medicinal plant parts , then they were investigated and identified. They were *Aspergillus niger*, *Aspergillus flavus* and *Penicillium spp.* From the fifteen investigated plants eight plants were found to be contaminated and seven plants not contaminated. Table 2 shows the distribution of the fungal load from the studied plant parts. The dominant fungi in most of the tested samples belong to *Aspergillus niger*. where as the least one was *Penicillium spp.* Morphologically, the colonies of fungi were identified according to form and pigment. They were observed microscopically through Lactophenol cotton bluestaining. Plate (1) shows the results of culture characteristic .

Table 2: Total fungal Count of Selected Medicinal Plant materials obtained From Omdurman Local Market/Sudan

Plant species	Count of fungi	Predominant fungal species isolated
<i>Acacia nilotica ssp.nilotica</i>	1×10^1	<i>Penicillium spp</i>
<i>Artemisia herba alba</i>	NIL	No growth
<i>Cassia acutifolia</i>	NIL	No growth
<i>Coriandrum sativum</i>	5×10^2	<i>Aspergillus nigar.</i>
<i>Cymbopogon schoenanthus spp.proximus</i>	NIL	No growth
<i>Cyperus rotundus</i>	11×10^5	<i>Aspergillus nigar</i>
<i>Foeniculum vulgare</i>	6×10^2	<i>Aspergillus nigar</i> and <i>Aspergillus flavus</i>
<i>Hyphaene thebaica</i>	9×10^3	<i>Aspergillus nigar</i>
<i>Lepidium sativum</i>	NIL	No growth

<i>Nauclea latifolia</i>	NIL	No growth
<i>Nigella sativa L.</i>	NIL	No growth
<i>Solenostemma argel</i>	3×10^2	<i>Aspergillus nigar.</i>
<i>Tamarindus indica</i>	4×10^1	<i>Aspergillus nigar</i> , <i>Aspergillus flavus</i> and <i>Penicillium spp</i>
<i>Trigonella foenum-graecum</i>	NIL	No growth
<i>Ziziphus spina-christi</i>	3×10^1	<i>Aspergillus nigar.</i>

a

b

c

Plate (1): Morphological Appearance of the fungal colonies a. *Aspergillus niger*, b. *A. flavus*, c. *Penicillium spp.*

Discussion:

The present study revealed that commercially available medicinal herbs might be high-risk materials, as it contained pathogenic fungal load. Among the studied plants, seven plants are not contaminated and may be because they are dry, all the three seeds investigated are free of fungal contamination, six of eight fruits are contaminated because the fruits are delicate and so acceptable to infection. *A. niger* was recorded as most abundant whereas *Penicillium spp* was found to the least abundant fungal specie. *Aspergillus* species has the potential for toxin production in the products. Raw materials of good microbial quality should be used in the production of these medicinal herbs.

Another investigation reported that *Aspergillus spp.* was recorded as one of most abundant fungi in the samples of prepared powdered herbal medicine while, the herbs were collected from certain areas of Nigeria. (Anyanwu, 2010).. Medicinal plants can be dried in a number of ways: in the open air (shaded from direct sunlight) by direct sunlight, in rooms, in the case of achieve uniform drying of medicinal plant materials to avoid mould formation (WHO, 2003). To prevent from these contaminants special hygienic, care must be taken like soak during boiling of water and kept in air tight tumbler Ara *et al*, (2015). Rizzo *et al.* (2004) indicated that medicinal plants in Argentina harbored toxigenic fungi such as *A. flavus*, *A.*

parasiticus and several members of the Genus *Fusarium*. The incidence of toxigenic fungi producing aflatoxins, ochratoxin A and fumonisin on medicinal herbs was reported from Argentina . Aflatoxins, ochratoxin A and citrinin producing *Aspergillus* and *Penicillium* were isolated from medicinal herbs in Brazil Bugno, *et al* (2006).

Conclusion:

The results of this study revealed that the use of these herbs as home remedy could be prolong the infections and ultimately cause other disease since it was observed that herbal remedies were not sterile and herbs are available in market in open air, so aerial contamination risk is very high. The delicate and soft organs like fruits and leaves are more acceptable to fungal contamination than the hard seeds and roots.

Acknowledgments:

Thanks to Dr Mutaz Nassir for help and to microbiology commission for Biotechnology and Genetic Engineering Khartoum.

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