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RESEARCH ARTICLE

A preliminary study of multi-mycotoxins contamination in some selected South Africa medicinal plants

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ABSTRACT

The use of medicinal plants in folklore remedies and as sources of raw materials for pharmaceutical industries is extensively increasing. The problem surrounding the use of such plants rests with the manner in which such plants like other agricultural commodities are contaminated with fungi, some of which are toxigenic, with possible production of mycotoxins in such plants. This study was aimed at investigating the degree of mycotoxin contamination of 36 South African medicinal plants. A multi-mycotoxin extraction method was followed and mycotoxins so extracted were quantified by high performance liquid chromatography (HPLC). High performance liquid chromatographic data revealed the presence of aflatoxins (AFs), ochratoxin A (OTA) and zearalenone (ZEA) 0.03 to 31.46 µg/kg, 0.2 to 10.09 µg/kg and 0.1 to 23.35 µg/kg, respectively. Most of the plants were found to be contaminated with one or two mycotoxins tested for. The use of such contaminated medicinal plants may lead to high risk of mycotoxins consumption which might result to adverse human health problems and therefore represents a special hazard. In view of this, it is crucial to establish and implement fungal and mycotoxin control programmes so as to limit quality loss and exposure of consumers of these products to these hazardous substances that could be accompanied by ill-health.

Keywords: Fungi; Health; High-performance liquid chromatography; Medicinal plants and Mycotoxins

INTRODUCTION

Fungal contamination of medicinal plants during handling process and storage does not only reduce the quality of the plants but also results in mycotoxin production (Rai and Mehrotra, 2005; Truckesses and Scott, 2008). Mycotoxins are mainly produced as secondary metabolites by toxigenic fungal species belonging mainly to genera such as *Aspergillus*, *Penicillum*, and *Fusarium*. These toxins have severe effects on health and the economy (Stoev et al., 2010). Presently, more than 400 mycotoxins are recognized (Binder, 2007), however the most noticeable based on their socio-economic importance include the aflatoxins (AFs), ochratoxins (OTs), deoxynivalenol (DON), fumonisins (FBs), zearalenone (ZEA) and patulin (van der Gaag et al., 2003). Among these mycotoxins, aflatoxin B_1 (AFB_1), ochratoxin A (OTA) and fumonisins (FB_1) are the most toxic to both humans and animals, causing various toxicities being carcinogenic, mutagenic, hepatotoxic, teratogenic and immunosuppressive (Berek et al., 2001; Speijers and Speijers, 2004).

Medicinal plants may be defined as a group of plants with natural compounds, which enable them to be used as sources of therapeutic agents for numerous pharmaceutical drugs and folklore medicine (Yuan et al., 2016). South Africa has a very long and strong history of herbal and traditional healing (Street and Prinsloo, 2013), with about 3,000 herbal plant species existing (van Vuuren, 2008). The presence of mycotoxins is linked to certain aspects related to the environmental, storage and ecological conditions of food, feed and medicinal plants. Mycotoxin contamination can therefore occur at various stages of the food chain (e. g during storage, in the field and processing) (Bennett and Klich, 2003; Musto, 2015). This is potentiated by inadequate storage facilities used, damage from insects and rodents, conducive environmental conditions for fungal proliferation and poor handling (Iqbal et al., 2011). Nowadays, increased intake of medicinal plants has immensely become public health problem because medicinal plants might not meet the desires of safety, worth and efficacy as recently reviewed by Ashiq et al. (2014).

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According to Ashiq et al. (2014), the European Union (EU) in variety of foodstuffs, has set regulatory limits for AFs, OTA, FBs, ZEA and DON, but despite their wide use both traditionally and as a source for new drugs, no official regulation has been established for all mycotoxins in medicinal plants. Nevertheless, in some herbs, there is an establishment of official regulation for only AF and OTA. Medicinal plants contaminated with fungi and attendant mycotoxins can confer some serious health threats to the general public especially those within traditional settings with increased intake of such commodities. Several reports on mycotoxins like AFs, OTA, ZEA and various trichothecenes in herbal products worldwide have been published in the literature (Santos et al., 2013), but not really in South Africa. The incidence of mycotoxins in some medicinal plants of South African origin was investigated considering the lack of information on the subject from the South African perspective'. Therefore, this study aimed at detecting and quantifying the levels of natural contamination of mycotoxins in South Africa medicinal plants and to establish their safety.

MATERIALS AND METHODS

Sampling and sample preparation

A total of 36 dried medicinal plant samples of different types were randomly purchased from different points within a well-known location, Faraday Muthi market, in the Johannesburg metropolis. These medicinal plants were selected on the basis of their availability and popularly in terms of their uses in folklore medicine. The samples were finely ground into powder using a sterile laboratory miller and then stored at -4 °C until further analysis. A list of the medicinal plants used in this study is provided in Table 1.

Materials and reagents

All chemicals and reagents were of HPLC grade unless otherwise stated. All solvents and standards were purchased from Sigma, Aldrich unless otherwise specified. The materials included dialysis tubing, amber vials, saturated sodium hydrogen bicarbonate ($NAHCO_3$), 4% potassium chloride (KCl), sodium sulphate (Na_2SO_4), Whatman no. 1 filter paper, and rotary vacuum evaporator. The solvents include acetone, dichloromethane (DCM), distilled water, methanol, acetonitrile and acetic acid. Mycotoxins standards: The following mycotoxin standards used as reference: AFB_1, AFB_2, AFG_1 and AFG_2, ZEA and OTA.

Equipment

The equipment included a Shimadzu HPLC: The system consists of LC-20AB degasser, HPLC Spectra Physics SCM400 SYSTEM (Waters, Milford, MA, USA), SIL-20A auto sampler, RF-10AxL fluorescence detector, Nova-Pak 4 mm C18 reversed phase analytical column (250×4.6 mm, 5μm), CTO-20A column oven, RF-10AxL fluorescence detector, SPD-M20A photodiode array detector linked to LC solutions version 1.22 Software Release and Shimadzu Corporation (Kyoto, Japan) LC-20AB liquid chromatograph equipped with CBM-20A communication bus module.

Analysis of mycotoxins in medicinal plants samples

Multi-mycotoxins screening method for the extraction of AF, ZEA and OTA was adapted with some modifications from (Patterson and Roberts, 1979). Twenty-five (25 g) of milled medicinal plant samples was weighed in 100 ml of acetonitrile/4% potassium chloride (KCL) (90/10 v/v) and shaken for 1 hr. The extract were defatted thrice with iso-octane. The content was then evaporated using a rotary evaporator, the extract were transferred into the dialysis tube and the placed on as shaker overnight. The overnight dialysis extracts were extracted three times with 25 ml DCM, and the extract were dried using a rotary evaporator. The dried extracts (neutral and acid) were reconstituted with 3 ml of DCM and transferred into a screw-capped amber HPLC vials evaporated under a stream of nitrogen gas at 60 °C, stored at 4 °C for HPLC analysis.

HPLC parameter

HPLC determination of the mycotoxins was achieved using the Shimadzu HPLC (Shimadzu Corporation, Kyoto, Japan), equipped with liquid chromatograph LC 20A fitted to degasser (DGU 20A3), auto sampler SIL 20A, communications bus module (CBM 20A), column oven (CTO 20A), PDA detector (SPD M20A) with column Waters Symmetry C18 5 μm, 4.6 x 250 mm, (Waters, Milford, MA, USA) all connected to a Dell computer with Intel Core DUO with Microsoft XP. For the analysis of AFs, the mobile phase consisted of H2O/MeOH/ACN (60:20:20, v/v/v) pumped at a flow rate of 1 mL/min. Two (2) μL of the extracts were injected into the HPLC and the separated analytes detected and quantified at a wavelength of 333-800 nm. Ochratoxins A was analysed with a mobile phase: ACN/H2O/AA (50:48:2, v/v/v), pumped at 1 mL/min flow rate and an injection volume of 2 μL. Detection of the toxin was done at a wavelength range set between 333 and 443 nm. Zearalenone was detected by with a mobile phase: ACN/H_2O/MeOH (46/46/8, v/v/v) pumped into the instrument at a flow rate of 1 mL/min. Two microlitres (μL) each of analytes and ZEA standard was injected into the system and the toxin detected at excitation and emission wavelengths of 274 and 455 nm, respectively, using a fluorescence detector ($10A_{XI}$).

Method validation

To check the effectiveness of the analytical method used, a number of method performance parameters were

Table 1: Medicinal plants collected from Faraday muthi market Johannesburg

Medicinal plant	Scientific name*	Part uses	Medicinal uses	Province (Zulu name)
Black iron wood	*Oleacampesis (AAT)*	Leaves, stem & root	To treat stomach ailments, diarrhea & as a blood purifier and aphrodisiac.	Northern Cape *(Umozana)*
Wild garlic	*Tulbaghia Violacea (WG)*	Bulb, leaves & root	To treat high cough, diabetes, colds, cholesterol, fever, high blood pressure, tuberculosis, cancer, blood & asthma.	Eastern Cape *(Isweli lezinyoka)*
Cancer bush	*Sutherlandia frutescens (MA)*	Whole plant	To treat cancer, epilepsy, diabetes, fever, high blood pressure, immune system booster, backache & blood purifier.	Western Cape (*Unwele*)
Pineapple Flower	*Eucomis Autumnalis (PT)*	Bulb & leaves	To treats fever, urinary complaints, syphilis and used to assist in post-operative recovery.	Eastern Cape (*Umathuga*)
African potato	*Hypoxis hemerocallidea (AP)*	Corm, root & bulbs	To treat, cancer, stroke, diabetes, bladder and kidney problems, high blood pressure, stomach complaints, body cleansing, appetizer, anti-poison and fatigue.	Kwazulu-Natal, Eastern Cape (Ilabatheka)
Sidvana	*Gladious aurantiacus (INZ)*	Leaves	To treat stomach complaints, hypertension, stress & also used to treat skin irritation.	Western Cape (*Isinlazi*)
Wild cock scomb	*Harmbstaedti aodorata (UBH)*	Leaves & stem	To treat heart problem, arthritis, headache, stomach complaints, heart problems, low blood pressure, fatigue, blood purifier & skin problem.	Western Cape (*Ubuphuphu*)
Honey flower	*Melianthus major (HF)*	Leaves & stem	To treat wounds, blood purifier & chest complaints.	Western cape (Ibonya)
Lucky bean	*Putranjiva roxburghii. (LB)*	Bark & root	To treat leg problems, stomach complaints, increase sperm count & protect pregnancy.	Eastern Cape (*Umdlavusa*)
Honey flower	*Melianthus major (HF)*	Leaves & stem	To treat wounds, blood purifier & chest complaints.	Western Cape (*Ibonya*)
River pumpkin	*Gunnera perpensa (GOB)*	Root	To treat bladder and kidney problems, infertility in women & body cleansing.	Limpopo (*Ughobo*)
Warthy jackal food	*Hydrnoraaficana(MGA)*	Leaves & root	To treat various diseases like fever, blood poison & common cold	Eastern Cape (*Mavumbuka*)
Horse wood	*Hippobromusp auciflorus (RP)*	Leaves, stem & root	To treat colds, influenza, headache, toothache, bladder and kidney problems & blood purifier.	Western Cape (*Umfaziothethayo*)
Speta	*Merwilla linearis (SE)*	Leaves & bark	To treat gastrointestinal ailment, sprains and fractures, and infertility	Eastern Cape (*Inguduza*)
Everlasting	*Helichrysum spp. (AAL)*	Leaves, root & stem	To treat lung cleaning, stomach complaints, lightening, wounds, vomiting, heart problems, headache, fever, backache, blood purifier, chest complaints, stroke & high blood pressure.	Eastern Cape (*Imphepho*)
Red storm	*Bulbine frutescens (INT)*	Whole part	To treat body cleansing, stomach complaints, swelling legs, chase vomiting & chase away evil acne.	Eastern Cape (IBhucu)
Generic Viagra	*Silidenafil citrate (GIB)*	Leaves & stem	To treat dysfunction in man	Western Cape (*Gibidir*)
African worm wood	*Artemisia afra (AWW)*	Leaves, stem, root	To treat cough, colds, fever &loss of appetite.	Northern Cape (*Mhlonyane*)
Bush lily	*Clivia miniata (BL)*	Whole plant	To treat fever, snake bite and relieve pains, also help with child birth.	Western Cape (Umayime)
Pepper bark tree	*Warburgia salutaris (PBT)*	Whole plant	To treat cancer, stomach ulcers, abdominal pain & constipation.	Limpopo (*Isibhaha*)
Milk Bush	*Xysmlobium undulatum. (ISH)*	Root	To treat wounds, indigestion, fever, typhoid & malaria.	All the provinces (*Ishongwe*)
Pale yellow eriosema	*Eriosema kraussianum (BAN)*	Root	To treat erectile dysfunction or as a sexual performance enhancer.	Kwazulu-Natal (*Bangalala*)
Wild verbena, broad-leaved Pentanisia	*Pentanisia prunelloides (CHI)*	Leaves &root	To treat heartburn, snakebite, toothache, haemorrhoids, rheumatism, vomiting, tuberculosis and fever also taken by pregnant women to ensure an easy childbirth and leaf poultices are applied for a retained placenta.	Eastern Cape (*Icimamlilo*)
Nut grass	*Cyperus rotundus (MAT)*	Bark & root	To treat ulcers and sores, fevers, dyspepsia & urinary concretions.	Eastern Cape (*Umathuga*)
Sweet Thorn	*Acacia karoo (IMY)*	Leaves, stem & root	To treat dysentery, diarrhoea, colds, haemorrhage, oral thrush and conjunctivitis.	Western Cape (*Inyathelo*)

(Contd...)

Table 1: ***(Continued)***

Medicinal plant	Scientific name*	Part uses	Medicinal uses	Province (Zulu name)
Mafula	*Sclerocarya birrea (UMG)*	Bark	To treat diarrhoea, stomach problems, dysentery, diabetes, indigestion & fever	Northern part of South Africa (*Umganu*)
Elephant root	*Elephantorriza elephantine (UMD)*	Root	To treat heart ailments, diarrhea, dysentery, stopping bleeding, treating intestinal disorders, haemorrhoids & syphilis.	All provinces except Western Cape (*Umdabu*)
Devil fuge, Cape misttloe	*Viscum album (IPH)*	Whole plants	To treat diarrhoea, bronchitis, asthma, menstruation problems, bleeding.	Eastern & Western Cape (*Iphakama*)
Sorghum	*Sorghum bicolor (AMA)*	Leaves	To treat cancer including lung cancer, anemia, high cholesterol, pains in body, constipation, diabetes, osteoporosis, headache, cataract, inflammation & sickle cell.	Limpopo (*Amabele*)
Fever tree	*Acacia xanthophloea (IHI)*	Bark	To treat heart fever, eye complaints and sleeping sickness.	KwaZulu-Natal (*UmHlosinga*)
Weeping boer-bean or African walnut	*Schotia brachypetata (UMX)*	Bark & root	To treat diarrhea, facial saunas, heart bum, nervous heart conditions and to strengthen the body and purify the blood.	Eastern Cape, Kwazulu-Natal & Mpumalanga (*Umgxamu*)
Hottentot tea	*Hellichyscnm nudifolium (MPI)*	Root & leaves	To treat colds, headaches, colic in children, chest complaints, coughs, internal sores, fever & for dressing wounds	Eastern Cape *(Isidwaba-Somkhovu*)
Wild cat	*Albizia adianthifolia (KA)*	Bark	To treat tapeworm, headaches & sinusitis	Eastern Cape (*Umgadankawu*)
Fennel	*Foenicilum vulgare (IMB)*	Root & leaves	To treat backache, cough, intestinal gas, bronchitis, cholera, colic in infants, heartburn, bloating & loss of appetite.	Eastern Cape & KwaZulu-Natal (*Imboziza*)
Cheesewood	*Pittosporum viridiflorum (WS)*	Bark	To treat stomach complaints, easing pain, fever & having a generally calming effect	Western Cape (*Umvusamvu*)

*Proposed acronyms of medicinal plants analysed in bracket: *Eriosema kraussianum, Warburgia salutaris, Albizia adianthifolia, Foenicilum vulgare, Sclerocarya birrea, Viscum Album, Merwilla linearis, Gunnera perpensa, Silidenafil citrate, Cyperus rotundus, Acacia Karoo, Olea campesis, Pittosporum viridiflorum, Bulbine frutescens, Eucomis Autumnalis, Hydrnora aficana, Melianthus major, Harmbstaedti aodorata, Xysmlobium undulatum, Schotia brachypetata, Acacia xanthophloea, Sutherlandia frutescens, Putranjiva roxburghii, Hippobromusp auciflorus, Pentanisia prunelloides, Tulbaghia Violacea, Artemisia afra, Clifforti aodorata, Eriocephalus africanus, Hellichyscnm nudifolium, Sorghum bicolor, Helichrysum spp. , Maesa lanceolata, Gladious aurantiacus, Hypoxis hemerocallidea, Elephantorriza elephantine, Aloe ferox, Clivia miniata, Adansonia digiata, Garcinia Living*

examined including recovery, LOD and LOQ. For the recovery determination, negative samples were spiked with known concentrations of mycotoxin standards mixed thoroughly and the solvent allowed to evaporate, then multi-mycotoxin extraction methods was used. The recovery for each mycotoxins was calculated as:

$$\text{Percentage Recovery} = \frac{A - B}{C} \times 100$$

Where A and B are the concentrations of the toxin in the spiked and non-spiked sample, respectively, while C is the concentration of the toxin spiked. The limits of detection (LOD) and quantification (LOQ) were calculated based on a signal-to-noise ratio (S/N) of 3/1 and of 10/1, respectively

Statistical analysis

All medicinal plant samples were replicated three times, and the data was statistically analyzed using IBM SPSS Statistics software, version 20. The calibration curves used for quantification were determined using the method of least-squares. Samples with mycotoxin concentration tested higher than the detection limit were considered positive, whereas samples with concentration were considered positive.

RESULTS

A total of 36 medicinal plant samples were analyzed to evaluate the contamination level of total aflatoxins, ochratoxin A and Zeralenone by HPLC. The mean recovery %, LOD and LOQ values at different spike levels are calculated and summarized in Table 2. The studied compounds were recognized by using retention time that matches against those of the calibration standards while the quantification was achieved by means of peak area that match the standards. The HPLC chromatograms of AFs, OTA and ZEA standards were displayed in Figs. 1-3.

The LOD and LOQ values ranged from 0.01-0.05 and 0.03-0.2, respectively (Table 2). The LOQ values were all below the maximum recommended values for the mycotoxins stipulated by the EU, 2010 and the recoveries shows a good value ranging from 88 – 97%.

The results of sample analysis revealed that all the medicinal plants were found to be contaminated with one or two mycotoxins Table 3. There were varying incidences and levels of the different mycotoxins in the medicinal samples. Accordingly, AFs were found in 86% (n=36) of samples analyzed at concentrations that ranged

Table 2: The limit of detection and limit of quantification obtained for the tested toxins

Mycotoxin	LOD (µg/kg)	LOQ (µg/kg)	Recovery %
AFB_1	0.03	0.1	88
AFB_2	0.01	0.03	92
AFG_1	0.05	0.2	98
AFG_2	0.02	0.1	95
OTA	0.05	0.2	86
ZEA	0.03	0.1	97

% - Percentage, LOD- Limit of detection, LOQ-Limit of quantification. Aflatoxin B_1 (AFB_1), Aflatoxin B_2 (AFB_2), AflatoxinG_1 (AFG_1), Aflatoxin G_2 (AFG_2), Ochratoxin A (OTA) and Zearalelone (ZEA).

Table 3: Quantitative detection of mycotoxins in South African medicinal plants by high performance liquid chromatography

Mycotoxin	% Incidence	Range (µg/kg)	Mean*
AFB_1	36	0.1 – 4.38	1.28±1.32
AF B_2	8.3	0.06 – 0.08	0.07±0.01
AF G_1	36	0.2 – 31.46	3.97±8.75
AF G_2	5.5	0.1 – 0.20	0.23±0.16
Total AFs	86	0.03 -31.46	1.26±2.11
OTA	61	0.2 – 10.09	1.84±2.09
ZEA	39	0.1 – 23.35	4.45±7.15

*Mean- mean of positive samples, * - standard deviation. Concentrations of AFB_1, AFB_2, AFG_1, AFG_2, OTA and ZEA in samples are in µg/kg.

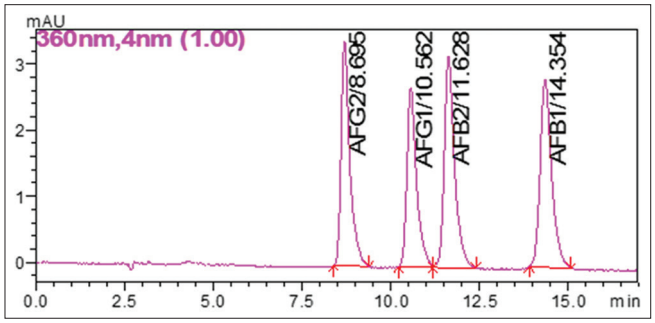

Fig 1. HPL chromatograph for AFs

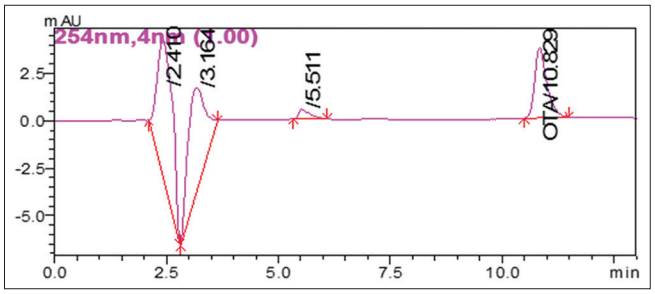

Fig 2. HPL chromatograph for OTA standard

from to 0.03 – 31.46 µg/kg with AFG_1 having the highest contamination level of 31.46 µg/kg which was recorded in *Harmbstaedti aodorata*. Out of 36 samples analysed, 36% of the samples were contaminated with AFB_1 and the highest concentration level of this toxin (4.38 µg/kg) was obtained from *Gunnera perpensal* Table 4. AFB_2 had and incidence rate of 8.3% (n=36) while AFG_2 was the least contaminant of the AFs chemotypes. There was less difference in which OTA and ZEA were present in these samples. For example, OTA occurred in 61% of samples within the range of 0.20 -10.09 µg/kg, whereas 39% (n=36) of the samples contained ZEA at levels that ranged from 0.1 to 23.35 µg/kg see Table 3. Sixty-one percent (61%, n=36) of samples were contaminated with OTA, with the highest level of contamination found in *Foenicilum vulgare* at concentration of 10.09 µg/kg, while 39% of the samples contained ZEA with highest level recovered from *Helichrysum* see Table 4. The following table represent the incidence percentage, range and means of the analysed samples.

DISCUSSION

From the present study, the incidence of fungal contamination in the samples may be due to poor handling and storage practices. This was clearly evident during sampling when it was observed that some of the plant materials were exposed to environmental conditions that favour microbial contamination. Fungal contamination of stored medicinal plants does not only result in reduced quality, decreased market value and healing potential, but equally may present a health hazard if the fungi are themselves toxigenic in producing mycotoxins i.e., AFs, DON, FBs, CIT, OTA and ZEA (Pereira et al., 2015; Aiko and Mehta, 2016). It is likely that similar samples analysed may be contaminated with mycotoxins such as AFs, OTA and ZEA, where applicable that are produced by some of the fungi, i.e., *A. flavus*, *A. niger* and *F. graminearum* recovered from these plants under study. For that reason, there is thus the need to investigate mycotoxins contamination in the same samples. This category of microorganisms is of extreme significance especially in sub-Saharan Africa, where favourable conditions prevail for their proliferation (Makun et al., 2012) accompanied by mycotoxins development in these analysed materials. Mycotoxins are a threat to human health and the economy of any given country (WHO, 2006), especially within the sub-Saharan region with prevailing favourable environmental conditions for their proliferation. Medicinal plants whose quality, safety and health benefits can be compromised by these toxicants (Ashiq et al., 2014), have made their use worldwide in the treatment of many diseases, and also to improve human health for many centuries. Increased consumption of medicinal plants has contributed immensely to public health problem due to improper investigation on quality and safety of such commodities.

Mycotoxins including all four chemotypes of AFs, OTA and ZEA were studied because they are reported

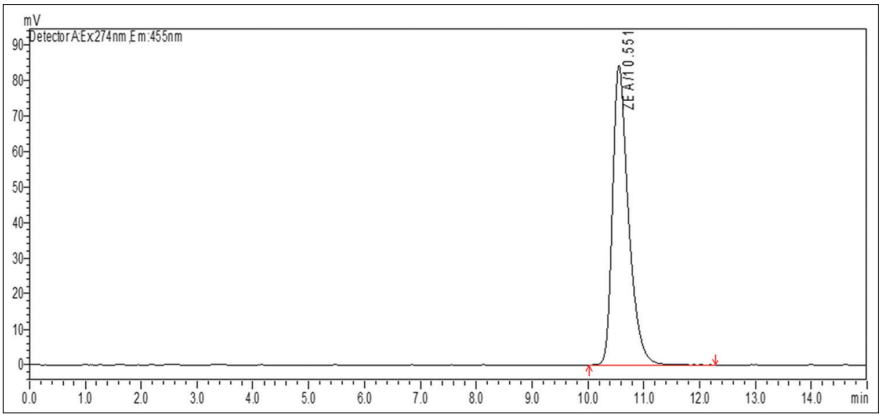

Fig 3. HPLC chromatograph for ZEA standard

Table 4: Incidence of mycotoxin contamination of some selected medicinal plants in South Africa (µg/kg)

Sample ID	Scientific name	AFB_1	AFB_2	AFG_1	AFG_2	OTA	ZEA
RP	*Hippobromuspauciflorus*	**4.38**	ND	0.63	0.34	1.74	0.37
UMX	*Schotia brachypetata*	ND	0.08	ND	ND	0.37	0.33
UBH	*Harmbstaedti aodorata*	0.46	ND	**31.46**	ND	1.15	0.11
IMB	*Foenicilum vulgare*	ND	ND	10.7	ND	1.13	ND
AAL	*Helichrysum*	ND	ND	ND	ND	ND	**23.35**
IMY	*Acacia Karoo*	2.02	ND	ND	ND	**10.09**	0.49
SE	*Merwilla linearis*	ND	ND	ND	ND	4.25	ND
IHI	*Acacia xanthophloea*	ND	ND	0.57	ND	2.42	ND
AP	*Hypoxis Hemerocallidea*	ND	0.06	ND	ND	ND	8.75
MPI	*Hellichyscnm nudifolium*	0.24	ND	1.42	ND	ND	0.81
UMD	*Elephantorriza elephantine*	ND	ND	ND	ND	5.81	0.14
MATT	*Cyperus rotundus*	ND	ND	ND	ND	2.75	14.23
AMA	*Sorghum bicolor(L) moench*	3.41	ND	0.81	ND	7.68	ND
UMG	*Sclerocarya birrea Subsp*	ND	ND	ND	ND	ND	6.12
AWW	*Artemisia afra*	ND	ND	0.29	ND	0.82	2.98
CM	*Clifforti aodorata*	0.26	ND	ND	ND	ND	0.72
MAH	*Maesa lanceolata*	0.23	ND	4.19	ND	ND	0.42
BAN	*Eriosema kraussianum*	ND	ND	ND	ND	ND	0.36
HF	*Melianthus major L*	0.78	ND	0.37	ND	1.08	0.29
IPH	*Viscum Album.L*	ND	ND	ND	ND	0.76	0.17
PT	*Eucomis undulate*	ND	ND	0.21	ND	ND	0.12
WS	*Pittosporum viridiflo-rum Sims*	ND	ND	ND	ND	ND	ND
MGA	*Hydrnora aficana*	ND	ND	ND	ND	ND	ND
KA	*Albizia adianthifolia*	ND	ND	ND	ND	ND	ND
CHI	*Pentanisia prunelloides*	0.62	ND	0.31	ND	2.57	ND
MA	*Sutherlandia frutescens*	ND	ND	0.49	ND	0.35	ND
WG	*Tulbaghia Violacea*	0.32	ND	ND	ND	ND	ND
ISH	*Xysmlobium undula-tum.VAR*	ND	ND	ND	ND	0.31	ND
GOB	*Gunnera perpensaL.*	ND	ND	ND	ND	ND	ND
LB	*Afzelia quanzensis*	ND	ND	ND	0.11	3.64	ND
BL	*Clivia miniata*	ND	ND	ND	ND	3.15	ND
INZ	*Gladious aurantiacus*	1.35	0.06	ND	ND	2.07	ND
AAT	*Olea campesis*	ND	ND	ND	ND	ND	ND
PBT	*Warburgia salutaris*	0.22	ND	0.23	ND	ND	ND
GIB	*Silidenafil citrate*	ND	ND	ND	ND	ND	ND
INT	*Bulbine frutescens*	2.45	ND	ND	ND	ND	ND

ND- Not detected, *Acronyms of medicinal plants analysed AAL; Helichrysum spp, AAT;Olea campesis, AMA; Sorghum bicolor, AP; Hypoxis hemerocallidea, AWW; Artemisia afra BAN; Eriosema kraussianum, BL; Clivia miniata, CHI; Pentanisia prunelloides, CM; Clifforti aodorata, GIB; Silidenafil citrate, GOB; Gunnera perpensa, HF; Melianthus major, IHI; Acacia xanthophloea, IMB; Foenicilum vulgare, IMY; Acacia Karoo, INT; Bulbine frutescens, INZ; Gladious aurantiacus, IPH; Viscum Album, ISH; Xysmlobium undulatum, KA; Albizia adianthifolia, LB; Putranjiva roxburghii, MA; Sutherlandia frutescens, MAH; Maesa lanceolata, MAT; Cyperus rotundus, MGA; Hydrnora africana, MPI; Hellichyscnm nudifolium, PBT; Warburgia salutaris, PT; Eucomis Autumnalis, RP; Hippobromusp auciflorus, SE; Merwilla linearis, UBH; Harmbstaedti aodorata, UMD; Elephantorriza elephantine, UMG; Sclerocarya birrea, UMX; Schotia brachypetata, WG; Tulbaghia Violacea and WS;Pittosporum viridiflorum.*

in the literature to cause severe health problems as being hepatotoxic, teratogenic, nephrotoxic, genotoxic, carcinogenic, neurotoxic and mutagenic in humans (Pfohl-Leszkowicz and Manderville, 2007). As found via HPLC analysis, data revealed the presence of these mycotoxins either singly or in combination with concentrations of AFs, OTA and ZEA respectively, ranging between 0.06 - 31.46 μg/kg, 0.2 - 10.09 μg/kg and 0.1 - 23.35 μg/kg Table 3. It is important to note that AFB_1 was found in the highest level of 4.38 μg/kg, a level above the official permissible limit of herbal drug stated by European Union (EU, 2010) and total AFs levels in 4 samples out of the 36 samples tested exceeded EU permissible limit of 4 μg/kg.

Liu et al. (2012) reported aflatoxins contamination from Chinese medicinal plants. Accordingly, the study result revealed that out of 174 Chinese herb samples, only 27 were contaminated with AFs, indicating a 15.5% incidence rate. In China, AFB_1 was detected in platycladi seeds at a maximum level of 52.0 μg/kg, higher when compared with our data as well as the maximum permitted levels in most medicinal herbs, placed at 5 μg/kg for AFB_1 and 10 μg/kg for total AFs (Chinese Pharmacopoeia Council, 2010). Similar studies were conducted in the Southern part of Nigeria whereby, commonly used indigenous crude herbal preparations were found to be contaminated with AFs ranging from of 0.004 – 0.345 μg/kg (Oyero and Oyefolu, 2009) although these levels were extremely low to effect any mark health effects among humans. This group of fungal secondary metabolites are classified amongst the most powerful mutagenic and carcinogenic substances known so far (FAO, 1997). In fact, AFB_1, the most toxic of all AFs is the most potent naturally occurring carcinogen (Makun et al., 2012). This is why the International Agency for Research on Cancer (IARC, 1993) classified it as a Group 1 human carcinogen. Considering the data obtained in this study, levels recorded may illicit some health effects in humans especially those consuming such herbs on a daily basis and with hepatitis B viral (HBV) infection that synergistically acts with AFs in developing hepatocellular carcinoma as reviewed by Ashiq et al. (2014).

Aside from AFs, OTA is also amongst the most important mycotoxins being the most toxic member within the ochratoxin group. Analysis of OTA in this study revealed that 56 % of samples tested were found to be contaminated by this toxin. The levels at which the plants tested in this studies contained OTA were within the permissive limit proposed for some herbs when compared with those reported elsewhere on medicinal plants. For example, in a study by Bresch et al. (2000), liquorice roots were examined for OTA and contamination levels ranged from 0.3 to 216 μg/kg. This is extremely high. OTA is known to cause some nephrotoxic and immunotoxic effects amongst humans and animals alike (Pfohl-Leszkowicz and Manderville, 2007; Janette and Bradley, 2012). Another study on OTA occurrence in traditional Chinese medicinal plants was reported wherein 44% of the 57 samples analysed and Mongolian milk-vetch root showed the highest concentration of 158.7 μg/kg with 92% of the positive samples clearly mouldy (Yang et al., 2010). The health consequence of OTA have been associated with a serious human kidney disease called Balkan endemic nephropathy (BEN) (Pena et al., 2006) widely encountered in the Balkan states. Also, ZEA analysis by HPLC revealed a highest contamination level of 23.35 μg/kg. Almost all the samples were contaminated with ZEA, only 8 samples were free. The range at which this samples were contaminated were from 0.1 to 23.35 μg/kg with the highest level of contamination observed in *Helichrysum*. Furthermore, the levels of ZEA in this study were also generally low compared to those reported in China with levels that ranged from 18.7 – 211.4 μg/kg (Zhang et al., 2011). The International Agency for Research on Cancer (IARC, 1993) considered the carcinogenicity of ZEA and found to be possibly carcinogenic to humans.

As earlier mentioned, mycotoxins are produced by invasion of fungi on plants during agricultural practice and storage processing. It is obvious that most users of medicinal plants purchase them from markets without checking for visible signs of fungal contamination and with poor perception and lack of knowledge about mycotoxin contamination and associated adverse effect on human health. This is definitely a matter of great concern because, such contaminated herbs can seriously compromise the health benefits that can be derived from them and instead cause more health complications than one would expect.

CONCLUSION

Regardless of the importance of medicinal plants worldwide, limited studies have been done to establish the incidence of mycotoxins in South African herbal plants. The results generated herein has established the contamination levels of AFs, OTA and ZEA in some South African medicinal plants. The degree of intake of these medicinal plants by many consumers are vary depending on their health conditions and personal interest, as well as the degree with which they are exposed to mycotoxins via consuming these herbs. In as much as mycotoxins contamination are not eradicated in the field, before and after harvest stages, dynamic approaches for decreasing fungal growth and mycotoxins production are imperative for reducing thereafter, the exposure and toxicity among humans. Before the distribution of any plant material for medical purposes at any market outlet, there should be proper analysis of mycotoxins to ensure these plants are safe in order to

guarantee the consumers' well-being. Therefore, mycotoxins control is recommended in these medicinal plants in order to minimize possible health hazards that could be associated with these toxic compounds.

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Authors' contributions

Oluwaseun Mary Areo was involved in planning the experiment, acquisition of data, analysis and interpretation of data and drafting the manuscript; Judith Zanele Phoku help with the instrumentation and planning of the experiment; Sefater Gbashi involved in planning the experiments and interpretation of data; Partick Berka Njobeh supervised the work. All authors read and approved the final manuscript.

REFERRENCE

Aiko, V. and A. Mehta. 2016. Prevalence of toxigenic fungi in common medicinal herbs and spices in India. 3 Biotech. 6: 159.

Ashiq, S., M. Hussain and B. Ahmad. 2014. Natural occurrence of mycotoxins in medicinal plants: A review. Fungal Genet. Biol. 66: 1-10.

Bennett, J. W. and M. Klich. 2003. Mycotoxins. Clin. Microbiol. Rev. 16: 497-516.

Berek, L.., I. B. Petri, A. Mesterhazy, J. Teren and J. Molnar. 2001. Effects of mycotoxin on human immune functions *in vitro*. Toxicolo. *In Vitro*. 15: 25-30.

Binder, E. M., L. M. Tan, L. J. Chin, J. Handl and J. Richard. 2007. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Tech. 3: 265-282.

Bresch, H., M. Urbanek and M. Nusser. 2000. Ochratoxin A in food containing liquorice. Nahrung. 44: 276-278.

Chinese Pharmacopoeia Council. 2010. Chinese Pharmacopoeia. Vol. 1. Chemical Industry Press, Beijing, China, pp. 1-361.

EU. 2010. European Union Commission Regulation No. 165/2010 of 26 February amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs as Regards Aflatoxins. Official J. Eur. Union. 50: 8-12.

FAO. 1997. Legislative Maximum Tolerated Levels of Aflatoxin and Regulatory Guidelines of other Mycotoxin in Some Foodstuffs, Feedstuffs and Dairy Products, from Worldwide Regulations for Mycotoxins. FAO Food Nutr. Paper. 64: 20-25.

Iqbal, S. Z., A. I. Bhatti, M. R. Asi, H. N. Bhatti and M. A. Sheikh. 2011. Aflatoxin contamination in chilies from Punjab Pakistan with reference to climate change. Int. J. Agric. Biol. 13: 261-265.

Janette, H. H. and E. H. Bradley. 2012. A Review of the diagnosis and treatment of ochratoxin a inhalational exposure associated with human illness and kidney disease including focal segmental glomerulosclerosis. J. Environ. Public Health. 2012: 835059.

Liu, L., H. Jin, L. Sun, S. Ma and R. Lin. 2012. Determination of aflatoxins in medicinal herbs by high-performance liquid chromatography-tandem mass spectrometry. Phytochem. Anal. 23: 469-476.

Makun, H. A., M. F. Dutton, P. B. Njobeh, T. A. Gbodi and G. H. Ogadu. 2012. Aflatoxin contamination in foods and feeds: A special focus on Africa. In: A. E. Ayman (Ed.), Trends in Vital Food and Control Engineering. IntechOpen, London, pp. 187-234.

Musto, M. 2015. Preliminary report on antifungal activity of a *Solanum nigrum* extract against five mycotoxin-producing fungi. Emir. J. Food Agric. 27: 825-830.

Oyero, O. G. and A. O. Oyefolu. 2009. Fungal contamination of crude herbal remedies as a possible source of mycotoxin exposure in man. Asian Pac. J. Trop. Med. 2: 38-43.

Patterson, D. S. P. and B. A. Roberts. 1979. Mycotoxins in animal feedstuffs: Sensitive thin layer chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone and T-2 toxin. J. Assoc. Off. Anal. Chem. Int. 62: 1265-1267.

Pena, A., M. Selfritova, C. Linn, L. Silevera and P. Solich. 2006. Estimation of ochratoxin a in Portuguese population: New data on the occurrence in human urine by high performance liquid chromatography with florescence detection. Food Chem. Toxicol. 44: 1449-1454.

Pereira, C. G., J. R. O. Silva and L. R. Batista. 2015. Isolation and Identification of toxigenic and non-toxigenic fungi in samples of medicinal plants from the market. Rev. Bras. Planta Med. 17: 262-266.

Pfohl-Leszkowicz, A. and R. A. Manderville. 2007. Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans. Mol. Nutr. Food Res. 51: 61-99.

Rai, V. and S. Mehrotra. 2005. Toxic contaminants in herbal drugs. Environ. News. 11: 1-3.

Santos, L., S. Marin, V. Sanchis and A. J. Ramos. 2013. Mycotoxin in medicinal/aromatic herbs a review. Bol. Latinoam. Caribe Plant. Med. 12: 119-142.

Speijers, G. J. A. and M. H. M. Speijers. 2004. Combined toxic effects of mycotoxins. Toxicol. Lett. 153: 91-98.

Stoev, S. D., S. Denev, M. Dutton, P. Njobeh, J. Mononik and P. Steenkamp. 2010. Complex etiology and pathology of mycotoxic nephropathy in South African pigs. Mycotoxin. Res. 26: 31-46.

Street, R. A. and G. Prinsloo. 2013. Commercially important medicinal plants of South Africa: A review. J. Chem. 2013: 205048.

Truckesses, M. W. and P. M. Scott. 2008. Mycotoxins in botanicals and dried fruits: A review. Food Addit. Contam. 25: 181-192.

van der Gaag, B., S. Spath, H. Dietrich, E. Stigter, G. Boonzaaijer, T. van Osenbruggen and K. Koopal. 2003. Biosensors and multiple mycotoxin analysis. Food Cont. 24: 252-254.

van Vuuren, S. F. 2008. Antimicrobial activity of South African medicinal plants. J. Ethnopharmacol. 119: 462-472.

World Health Organization. 2006. Mycotoxins in African Foods: Implications to Food Safety and Health (AFRO Food Safety (FOS). World Health Organization, Geneva.

Yuan, H., Q. Ma, L. Ye and G. Piao. 2016. The traditional medicine and modern medicine from natural products. Molecules. 21: 559-577.

Zhang, X., W. Liu, A. F. Logrieco, M. Yang, Z. Ou-yang, X. Wang and Q. Guo. 2011. Determination of zearalenone in traditional Chinese medicinal plants and related products. Food Addit. Contam. 28: 885-893.