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Mycological Quality of Powdered Herbal Medicinal Preparations Packaged for Human Consumption in North Western Nigeria

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Abstract.
The increase in the consumption of natural drugs have made their use a public health problem due to its poor quality, presence of fungal contamination and the risk of the presence of mycotoxins. This investigation was designed to throw light on the mycological and aflatoxigenic status of powdered herbal medicinal products marketed in North Western Nigeria. A total of four hundred and thirty two(432) powdered herbal medicinal preparations consisting of twelve(12) each from six(6) localities in each of the six(6) states' metropolis of the North Western Nigeria were obtained. These samples were subjected to various analyses. The parameters measured were grouped as follows: level of fungal contaminations of fungi; frequency of distribution of fungi and mycoflora type present in the herbal preparations. Results indicated that all the four hundred and thirty two herbal medicinal preparations assessed did not comply with the maximum acceptable limit of $2x10^2$ cfu/g for fungal load. The study showed that samples from Kaduna and Kebbi had a significantly higher mean fungal count ($1.09x10^5$cfu/g and $1.05x10^5$cfu/g respectively) that were not significantly different ($p\leq0.05$), hence suggesting higher contamination with fungi. The least was observed in Zamfara state with the lowest mean value of fungal load. The statistical analysis showed that fungal load in Katsina, Sokoto, Kano and Zamfara states were not the same but not significantly different ($p\leq0.05$). This suggested low level of contamination with fungi when compared with samples from Kaduna and Kebbi state. In terms of fungal distribution in herbal medicinal preparations, this study indicated that fungi of the genus *Aspergillus* spp and *Penicillus* spp were the most frequently isolated and were found to be higher in frequency of occurrence. Out of one thousand and ninety five (1095) total frequency of occurrence of fungi in the herbal medicinal preparations, the total frequency of occurrence of *Aspergillus spp* in this study is seven hundred and seventy five(70.77%), *Penicillium* spp (n=190;17.35%); *Fusarium* spp (n=86;7.85%) and *Rhizopus* spp (n=44;4.02%). In this finding it could be suggested that *Aspergillus* spp and *Penicillium* spp are the major contaminant of herbal drugs. In all the samples screened from the six states, higher level of contamination with *Aspergillus* spp were found. Among the *Aspergillus* spp observed, *A. flavus, A. paraceticus, A. niger* were the most frequently occurred fungi in the herbal medicinal samples suggesting that these type of fungi are the major contaminant of the herbal medicinal products in all the six states of the North West of Nigeria. The highest frequency of occurrence of fungi observed in samples from Kaduna 151(69.59%), Kano139 (72.02%),sokoto135 (73.37%), Kebbi127 (66.49%), Kastina112 (70.89%) and Zamfara111 (68.52%) may be as a result of poor harvesting, processing and storage practice of the handler of the herbal products. The means of frequency of occurrence of fungal isolates in herbal preparations from the North Western Nigeria also showed that incidence of *Aspergillus flavus, Aspergillus paraceticus, Aspergillus niger* and *Penicillium* spp were not significantly different at $p<0.05$ but significantly higher than *Aspergillus ochraceus* and *Aspergillus versicolor*. The result obtained also indicates that *Fusarium* spp and *Rhizopus* spp were significantly the lowest. This result suggests that the samples of herbal medicines obtained in the North Western Nigeria is heavily contaminated with the fungal species of *Aspergillus* spp and *Penicillium* spp.
Keywords: herbal medicines, fungal contamination, North Western Nigeria

Introduction

Herbal remedies are perhaps the most common form of alternative medicine. Almost every nation or people have at one time used herbs and preparations of various sorts to treat illnesses and diseases (Barrett, 2003). However, despite the use of herbs in medicine throughout the centuries, only a relatively small number of plant species and their extracts have been carefully studied and there is a minimal amount of information available on their safety and efficacy. Although, reports of investigations of their clinical efficacy or otherwise have been published in prestigious national and international journals. For instance, many plant extracts have been shown to have a variety of pharmacological effects and anti-inflammatory effects (Chainani, 2003).

Sofowora (1993) emphasized that herbal drugs, apart from being unstandadized, they are cheap, accessible and also enjoy a wider acceptability among the people of developing countries. Furthermore, World Health Organization survey indicated that about 70-80% of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary healthcare (WHO, 1998).

In Africa, up to 80% of the population uses traditional medicine for primary health care and the global market for herbal medicines currently stands at over US $ 60 billion annually and is growing steadily (WHO,

2003). The need to get access to cheap drugs such as herbal medicines outweighs other considerations such as sources, standard and health hazards associated with consumable herbal drugs, and as such, this has made herbal drugs-borne intoxications to be a serious problem in many parts of the continent. Regrettably, many of the people in the region are not even aware of the effect of consuming moldy products. Due to the poor education levels and other socio-economic factors, even if steps are taken to make herbal products safe, the consumers will be unwilling to pay the extra costs, and will still prefer to buy the cheaper ones. In Nigeria, it was reported that not less than 70% of the population patronizes herbal medicinal preparations (Maiwada,2004).

The quality of the herbal preparations is of great concern and still remains a contentious issue. Studies have been carried out and confirmed the presence of potential contaminants such as pathogenic fungi that present serious health hazards (Martins *et al.,*2001). Unfortunately, several cases of adverse effects of herbal medicinal preparation have been reported worldwide during the last few years, which are allegedly caused by taking herbal medicinal products prescribed by the practitioners of indigenous systems of medicine (Vartika and Shanta, 2005). These products may be contaminated with excessive microbial contaminants such as pathogenic fungal species that produce toxins especially aflatoxin in herbal drugs which have been determined to be potent toxic, mutagenic, and carcinogenic compounds in humans (IARC, 2002), even if they are absorbed in minute amounts.

Aim of the study

the aim of the present study is to determine the mycological quality of powdered herbal medicinal preparations packaged for human consumption in North Western Nigeria.

Objectives of the studies are:

1. To determine the level of fungal contamination of herbal medicinal preparations in the North Western Nigeria.
2. To isolate and identify the fungi present in the herbal medicinal preparations.

Methodology

Study area and sampling

A total of Four hundred and thirty two (432) samples of powdered medicinal herbal preparations were purchased randomly from identified herbal shops and retail outlets in different parts of Sokoto, Kebbi, Zamfara, Katsina, Kano and Kaduna metropolis. Six (6) samples were collected from each named state at regular intervals of two weeks of every month for one year. Packaged herbal samples were collected and taken to the laboratory, while those that were not packaged (such as herbal preparations sold by the local herbalist) were collected in sterile polythene bags (Pearce *et al.*,2004). All samples collected from the sites were classified based on their usage, and analyzed in the laboratories of Department of Microbiology, Ahmadu Bello University, Zaria and NAFDAC Area Laboratory, Kaduna.

Preparation of dehydrated media

All dehydrated media were prepared according to manufacturer instructions. They were mixed with distilled water and dissolved by gentle heat to boil. The media were sterilized in an autoclave (LTE J7090 Model, LTE Scientific Ltd, England) at 121°C for 15 minutes. About 20ml of the sterile media were dispensed into each of many sterile Petri plates and allowed to cool. The sterility of the prepared media was checked by incubation of the plates at 37°C for 24 hrs. The sterile agar plates were stored in the refrigerator at 4°C before use.

Fungal analyses

Evaluation of fungal contamination

Ten gram of each sample was mechanically homogenized in 90.0 mL of buffered peptone water and shaken vigorously for 2 minutes. Tenfold serial dilution was performed up to 10^{-4} in buffered peptone water and 0.1 mL of the dilution was transferred aseptically to sterilized petri plates containing freshly prepared Potato Dextrose Agar (PDA) with 0.01% chloramphenicol (DIFCO) to inhibit bacterial growth. Plates were incubated upside down at 26 ± 1ºC for five days. After incubation, enumeration of fungi was performed by pour plating method (USP, 2005). the fungal colonies were counted, recorded and the number of colony-forming units (CFU) per gram were calculated. Samples were analyzed using standard aseptic techniques to insure that no outside contaminants were introduced into the samples.

Isolation and identification of fungi in herbal medicinal preparations.

Mould colonies representative of all morphologically different types present were inoculated onto Potato Dextrose Agar (PDA) and incubated for 6-8 days at room temperature as described by Harley and Prescott (1993). The pure isolates were identified microscopically by putting 2-3 drops of cotton lactophenol on the clean microscopic slide and spores of the isolates were teared and placed on the slide and covered with cover slip (Davise,2002; Pepper and Gerba, 2004). It was mounted on the microscope and observed using x10 and x40

objectives. Macroscopic(cultural) features like colony color and margins as well as microscopic such as conidia and conidiophores arrangements were examined for species differentiation (Gilman, 2001; You *et al.,*2007).

Cultural and Microscopic identification of fungi

The identification of filamentous fungi for species differentiation were done based on the Macroscopic(cultural) features like colony color and margins as well as microscopic such as conidia and conidiophores arrangements as described by John, (1986); Larone(1995);); Gilman.(2001); Klinch, (2002); Gabriel, (2005); You *et al*.,(2007)

Cultural and microscopic identification of *Aspergillus* spp

Culturally and microscopic identified of *Aspergillus niger, Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus versicolor* were carried out as described by John (1986); Larone (1995); Gilman (2001); You *et al.* (2007).

Identification of *Aspergillus flavus*

Culturally, colonies identified with yellow or green surface often with black sclerotia and the reverse side of the plate golden at first then later turned red brown, and microscopically the conidiophores was observed to be colorless, rough with biserated phialides, round and radiated vesicles were suspected to be *Aspergillus flavus* (Larone, 1995) .

Identification of *Aspergillus niger*

Culturally, colonies identified with black surface and white to yellow side and microscopically, the conidiophores were observed to be long, smooth, colourless or brown with biserated philialides and round but columnar head were confirmed to be *Aspergillus niger* (Collier *et al.,*1998).

Identification of *Aspergillus parasiticus.*

Culturally, the colonies observed as bright green but without sclerotia. Condiophore had only one set of sterigmata and stalk was spiny only at the top. Globose spiny conidia are produced., the mould is confirmed to be *Aspergillus parasiticus*(Philip, 1989)

Identification of *Aspergillus ochraceus.*

Culturally on PDA at room temperature the colonies consistently observed to remain bright yellow and sometimes produced purple sclerotia, Conidiophores had two sets of sterigmata, the stalk was spiny with definite yellow pigment in the walls. Conidia were usually smooth walled and globose to slightly ellipitical. This mould was confirmed to be *Aspergillus ochraceus*(Philip, 1989).

Identification of *Aspergillus versicolor.*

Culturally on PDA at room temperature the colonies observed grew rapidly reaching 19-22mm in diameter with dark green or blue green and reddish, pale yellowish to uncolored with sometimes centrally rising, velvety to rather floccose and with purple reverse. The heads were fragmentary and like penicillum Conidiophores monoverticillate or biverticillate, Hyaline, smooth-walled, Phialides, flask-shaped and some phialides directly bored on mycelia. These type of mould were confirmed to be *Aspergillus versicolor* (You *et al.,* 2007).

Identification of *Penicillium* spp.

Culturally, colonies identified with Greenish blue color, septate hyphae under microscope with conidiophores bearing brush-like conidia were confirmed to be (Gillman,1957; Burnet, 1976)

Identification of *Fusarium* spp.

Culturally, colonies identified with Pink Centered and periphery colony, septate multinucleated hyphae with sickle or canoe shaped conidia in cluster under microscope were confirmed to be *Fusarium* spp (Burnet,1976; Booth,1997).

Identification of *Rhizopus spp.*

Culturally colonies observed to be Dark-green possessing septate multinucleated hyphae with root-like structure rhizoids under microscope were confirmed to be *Rhizopus* spp. (Gillman,1957 ; Raper and Fennel,1965; and Klinch,2002).

Result

Table 1 shows the distribution of fungi in herbal preparations. At a fungal count range of $0.1x10^5$cfu^{-}g to $0.55x10^5$cfu^{-}g, the fungal count observed in all the states were remarkable; Samples obtained from Zamfara being the highest with 28(38.89%), Katsina 21(29.17%), kebbi and Kano had 18(25.00%) each, while Kaduna the lowest with 5(6.94%). At a fungal count range of $0.6x10^5$(cfu^{-}g) to$1.05x10^5$(cfu^{-}g), Sokoto with 49(68.05%) had the highest number of samples followed by Kano with 38(52.78%), Kaduna 31(43.06%), Kastina 28(38.89%) , Zamfara and Kebbi with 20(27.78%) each.

Table 2 shows the ranges and Means of average fungal count analysed in six states. The average fungal count in this study ranged from $0.1x10^5$cfu^{-}g - $2.15x10^5$cfu^{-}g. The highest average fungal count was observed in sample from Kano with $2.15x10^5$cfu^{-}g, and the lowest fungal count was in Zamfara with $1.0x10^5$cfu^{-}g. The means with the same superscripts were not significantly different ($P<0.05$), while those mean values with different superscripts were significantly different($P<0.05$). Samples from Kaduna and Kebbi were observed to have a very high fungal count that were not significantly different($P<0.05$). The least fungal load was observed

in Zamfara. The statistic showed that fungal load in Katsina, Sokoto, Kano and Zamfara were not the same but not significantly different($P<0.05$). This suggested low level of contamination with fungi when compared with samples from Kaduna and Kebbi.

Table 1: Distribution of the herbal drugs samples according to average plate fungal count.

Average aerobic fungal plate count of sample (10^5cfu/gm)	Number and percentage (%) of samples in each State					
	Zamfara	Kaduna	Kano	Katsina	Sokoto	Kebbi
0.1 – 0.55	28(38.89)	5(6.94)	18(25.00)	21(29.17)	10(13.89)	18(25.00)
0.6 – 1.05	20(27.78)	31(43.06)	38(52.78)	28(38.89)	49(68.05)	20(27.78)
1.1 – 1.55	21(29.17)	32(44.44)	15(20.83)	20(27.78)	13(18.06)	26(36.11)
1.6 – 2.05	3(4.17)	4(5.56)	0(0)	2(2.78)	0(0)	8(11.11)
2.1– 2.05	0(0)	0(0)	1(1.39)	1(1.39)	0(0)	0(0)

Table 2: Ranges and Means of Average fungal count of herbal medicinal preparations of six states of the North Western Nigeria.

States	Number (n)	Parameters	
		Ranges (cfu$^-$g)	Mean ± SEM
Zamfara	72	$0.1x10^5$-$1.85x10^5$	$7.79 \times 10^{4\ b} \pm 5.73 \times 10^3$
Kastina	72	$0.2x10^5$-$2.10x10^5$	$8.57 \times 10^{4\ b} \pm 5.16 \times 10^3$
Kebbi	72	$0.2x10^5$-$1.95x10^5$	$10.90 \times 10^{4\ a} \pm 8.71 \times 10^3$
Kaduna	72	$0.2x10^5$-$1.70x10^5$	$10.57 \times 10^{4\ a} \pm 3.92 \times 10^3$
Sokoto	72	$0.45x10^5$-$1.25x10^5$	$8.28 \times 10^{4\ b} \pm 2.61 \times 10^3$
Kano	72	$0.25x10^5$-$2.15x10^5$	$8.28 \times 10^{4\ b} \pm 4.13 \times 10^3$

Mean values in the same column with same superscripts are not significantly different at $p \leq 0.05$.

The result presented in table 3 indicates the frequency occurrence of fungi in the herbal preparations in six states of the North Western Nigeria.

Aspergillus flavus occurred in all the samples obtained from the six states. The highest frequency was observed in samples from kano(n=33;45.83%), followed by Zamfara (n=32;44.44%), Kaduna (n=31;43.06%), Kebbi (n=30;41.67%), while Sokoto states and Kastina state had the frequency occurrence of (n= 29;40.28%) and (n=26;36.11%) respectively.

Aspergillus parasiticus was present in all the samples of herbal preparations from the six states under study. The highest frequency was observed in samples obtained from Kaduna(n=40;55.56%), followed by Sokoto and Kebbi (n=33;45.83%), then Kano and Katsina (n= 31;43.06%) each. The least occurrence was observed in Zamfara (n=25;34.72%).

The frequency of occurrence of *Aspergillus niger* was observed to be highest in samples obtained from Kaduna (n=47;65.27%) followed by those from Kano and Sokoto (n=32;44.44%) each. Kebbi (n=28;38.89%), Katsina(n=24;33.33%) and Zamfara had least(n= 18;25.72%).

Aspergillus ochraceus occurred more frequently in samples obtained from Sokoto(n=23;31.94%) followed by those from Kebbi (n=19;26.39%) and Kano(n=18;25.00%). Zamfara and Katsina indicated (n=16;22.22%) each. The lowest occurrence was observed in Kaduna (n=15;20.83%).

The frequency of occurance of *Aspergillus versicolor* showed samples from Kano had the highest (n=25;34.72%) followed by Zamfara (n=20;27.78%). while Kaduna and Sokoto (n=18;25.00%) each. The least occurrence was observed in Katsina (n=15;20.83%).

Penicillium spp had the highest frequency in samples from Kaduna(n=37;51.39%) followed by Katsina (n=35;48.61%). Samples obtained from Sokoto indicated (n=34;47.22%), Kano(n=29;40.28%), Zamfara(n=28;38.89%) and Kebbi showed the least of 37.50%(n=37).

Fusarium spp occurred less frequently with the highest occurance in herbal preparations obtained from Kebbi (n=27;37.50%) followed by those from Kaduna (n=19;26.39%). Samples from Zamfara showed (n=13;18.06%), Kano (n=10;13.89%), Sokoto and Katsina showed the least occurance with (n=9;12.50%) and (n=8;11.11%) respectively.

Rhizopus spp was least in occurrence in all the states. The highest frequency was observed in Kebbi, Zamfara and Kaduna(n=10;13.89%) followed by Sokoto with (n=6;8.33%). Samples obtained from Kano and

Katsina had the least frequency with (n=5;6.94%) and (n=3;4.17%) respectively.

Table 4 is the result indicating the mean frequency of occurrence of fungi in the herbal medicinal preparations in the North Western Nigeria. The result showed that *Aspergillus parasiticus* had the highest mean frequency of occurance in the entire North Western Nigeria followed by *Aspergillus flavus*. The least frequency was observed with *Fusarium sp* and *Rhizopus sp.* Statistically, the frequency of occurrence of *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger and Penicillium sp* were observed not to be significantly different (P≤0.05). *Fusarium spp* occurred frequently but less than *Aspergillus versicolor* and *Aspergillus ochraceus,* even though statistically their mean values were not different. *Rhizopus sp* had the least mean frequency of occurrence in this study.

Table 3: Percentage frequency occurrence of fungal isolates on herbal preparations in six States of the North-West of Nigeria

Fungi	Number and percentage(%) frequency					
	Kano	Kebbi	Katsina	Sokoto	Zamfara	Kaduna
Asp. Flavus	33(45.83)	30(41.67)	26(36.11)	29(40.28)	32(44.44)	31(43.06)
Asp. paraciticus	31(43.06)	33(45.83)	31(43.06)	33(45.83)	25(34.72)	40(55.56)
Asp. Niger	32(44.44)	28(38.89)	24(33.33)	32(44.44)	18(25.00)	47(65.27)
Asp. ochreceus	18(25.00)	19(26.39)	16(22.22)	23(31.94)	16(22.22)	15(20.83)
Asp. versicolor	25(34.72)	17(23.61)	15(20.83)	18(25.72)	20(27.78)	18(25.00)
Pen. spp	29(40.28)	27(37.50)	35(48.61)	34(47.22)	28(38.89)	37(51.39)
Fusarium spp	10(13.89)	27(37.50)	8(11.11)	9(12.50)	13(18.06)	19(26.39)
Rhizopus spp	5(6.94)	10(13.89)	3(4.17)	6(8.33)	10(13.89)	10(13.89)
Total (1095)	**183(16.72)**	**191(17.44)**	**158(14.433)**	**184(16.80)**	**162(14.80)**	**217(19.82)**

Table 4: Mean of frequency of occurrence of fungal isolates in herbal preparations in six states of the North Western Nigeria

Fungi	Mean ± SEM
Aspergillus flavus	30.17 ± 1.014^{a}
Aspergillus paraciticus	32.17 ± 1.973^{a}
Aspergillus niger	30.17 ± 4.003^{a}
Aspergillus ochraceus	17.83 ± 1.195^{b}
Aspergillus versicolor	18.83 ± 1.400^{b}
Penicillium spp	31.67 ± 1.706^{a}
Fusarium spp	14.33 ± 3.007^{b}
Rhizopus spp	7.33 ± 1.256^{c}
Total	22.81 ±1.473

Mean values with the same superscripts are not significantly different at $p \leq 0.05$.

Discussion

Herbal medicinal preparations are known as complex mixtures which originate from biological sources and most of the preparations are used in different forms (such as powders) and they normally carry a large number of various kind of microbes originating from soil, and are normally adhered to parts of plants (Adeleye *et al.,* 2005). In this result it is showed that all the four hundred and thirty two (432) herbal medicinal preparation assessed did not comply with the maximum acceptable limit of $2x10^2$ cfu/g as specified by United State Pharmacopoeia(2005). However, the distribution of the herbal medicines according to fungal count vary from state to another but none of the samples in each state conform to the stated limit by US pharmacopoeia. Considering the stated limit, the results are in agreement with those of previous studies (Roy and Kumari,1991; Pitt, 2000; Tassaneeyakul *et al.,*2004; Bugno *et al.,*2006). The result in table 2 showed that samples from Kaduna and Kebbi were observed to have a significantly higher mean fungal count (1.09×10^5 cfu/g and $1.0.6x10^5$ respectively) that were not significantly different (p≤0.05), hence suggesting higher contamination with fungi. This finding is in agreement with that of Bugno *et al.,*2006 and Okunlola *et al.,* 2007. The least fungal count was observed in Zamfara with the lowest mean value of fungal load. The statistic showed that fungal load in Katsina, Sokoto, Kano and Zamfara were not the same but not significantly different(p≤0.05). This suggested low level of contamination with fungi when compared with samples from Kaduna and Kebbi. The samples were contaminated to varying degrees with fungi may be due to poor practice used in harvesting, handling, storage, production and distribution process(Mandeel, 2005).

In this study, fungi of the genus *Aspergillus* and *Penicillium* were the most frequently isolated, and the finding conform with the work of Bugno *et al.*(2006) who showed that the most commonly observed fungi genera in herbal drugs were *Aspergillus* and *Penicillium.* Although a number of these fungi were identified as common storage fungi that contaminate herbal drugs(Agrios,1978). In all the samples screened from the six states, higher level of contamination with *Aspergillus* spp were found, and this suggest that *Aspergillus* spp are considered as the most common contaminant of herbal medicinal products as reported by Roy and Kumari (1990) and Martins *et al*(2001). The highest frequency of occurrence of fungi observed in samples from Kaduna 151 (69.59%), Kano139 (72.02%), Sokoto135 (73.37%), kebbi127 (66.49%), Kastina112(70.89%) and Zamfara111(68.52%) may be as a result of poor harvesting, processing and storage practice of the handler of the herbal products as reported by Zhang *et al.* (2005). Among the *Aspergillus* spp observed, *A. flavus, A. paraciticus, A. niger* were the most frequently occurred fungi in the herbal medicinal samples suggesting that these type of fungi are the major contaminant of the herbal medicinal products in all the six states of the North West of Nigeria. The finding of Abou-Arab *et al.* (1999) and Elshafie *et al.* (1999) supported this discovery in the North West of Nigeria. The Constant presence of these fungi in the herbal medicine around these areas perhaps due to fact that their spores are the common air contaminants probably present in the processing and packing area as reported by Lee and Jo(2006) and Zhang *et al*(2005). *Aspergillus* spp and *Penicillium* spp were found to be higher in frequency of occurrence. Out of one thousand and ninety five (1095) total frequency of occurrence of fungi in the herbal medicinal preparations, the total frequency of occurrence of *Aspergillus* spp in this study is 775(70.77%), *Penicillium* spp (n=190;17.35%); *Fusarium* spp (n=86;7.85%) and *Rhizopus* spp (n=44;4.02%). In this finding it could be suggested that *Aspergillus* spp and *Penicillium* spp are the major contaminant of herbal drugs, and the result is in agreement with that of Halt(1998) who indicated the contamination of herbal drugs with *peniciilium* spp. The variation in the level of contamination of the products with *Penicillium* spp could be due to some growth factors like moisture as reported by Matsushima *et al.*(1958). *Fusarium* spp and *Rhizopus* spp were found in a few samples at low level as showed in this finding(table 7).The presence of these fungi in herbal preparations is reported by Halt (1998) in Croatia; Efuntoye (1996) in Nigeria; Martins *et al* (2001) in Portuguese; Czech *et al* (2001) in Australia and Rizzo *et al* (2004) in Argentina. The low level of contamination discovered may be associated with the fact that their spores could survive drying conditions and remain dormant for several months possibly years on the dried herbs within with if the moisture of the products improve to levels allowing their germination(Zhang *et al*.,2005).

The means of frequency of occurrence of fungal isolates in herbal preparations in six states of the North Western Nigeria shown in table 8 is a clear indication that in this study the incident of *Aspergillus flavus, Aspergillus paraciticus, Aspergillus niger* and *Penicillium species* were not significantly different at $p<0.05$ but significantly higher than *Aspergillus ochraceus* and *Aspergillus versicolor.* The result obtained also indicates that *Fusarium* spp and *Rhizopus* spp were significantly the lowest. This result suggest that the samples of herbal medicines obtained in the North Western Nigeria is heavily contaminated with the fungal species of *Aspergillus* spp and *Penicillium* spp . These result fairly accurate with previous reports that showed *Aspergillus* spp and *penicillium* spps were the main contaminant and frequently isolated fungi in herbal medicines (Roy and Chourasia, 1990; Kumar and Roy, 1993; Rizzo *et al.,* 2004; Bugno *et al.,* 2006) . Herbal medicines could also be very hazardous due presence of fungi that are reported to be the major producer of mycotoxins (Halt, 1998 and Martin *et al.,*2001). The incidence of *Fusarium* spp and *Rhizopus* spp were obtained to be significantly lower than the other fungi obtained, this may be as a result of the fact that both *Fusarium* spp and *Rhizopus* spp require higher moisture content for their spores to germinate (Zhang *et al.,* 2005; Lee and Jo, 2006). The finding suggest that the samples from the North west of Nigeria are not significantly contaminated with *Fusarium* spp and *Rhizopus* spp, but those samples that are contaminated with *Fusarium* spp could present hazard due to the ability of *Fusarium* spp to produce fumonisin B_1 of to 20-70ppb (Martins *et al.,* 2001).

Conclusion

The study observed that herbalists from the North western Nigeria sale herbal medicinal preparations that were claimed to cure different type of ailments without undergoing proper or modern training in the business. Over a decades, studies have been carried out on herbal medicinal preparations in different part of the world with a view to ascertain the hygienic nature of the products. The presence of a wide range of storage and field fungi that had been proven to be toxigenic in herbal medicinal preparations indicates that considerable could be made during post- harvest period. Although the presence of fungi in the herbal preparations below the stated limit did not imply the unsafe nature of the product, but their presence represents a potential risk of contaminations with aflatoxins. Considering the worldwide increased in use of herbal products as alternative medicines and the risk of purchase and use of natural products contaminated with moulds suspected to be pathogenic, it is necessary setting appropriate standards for toxigenic fungi in herbal medicinal preparations and all medicinal plants in order to reduce the risks for consumer health in Nigeria.

REFERENCE

Abou-Arab,A.A.K.; Kawther, M.S.; Tantaway, M.E.; Badeaa, R.I. and Khayria, N.(1999). Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food Chemistry*. 69:357-363

Adeleye, I.A.; Okogi, G. and Ojo, E.O.(2005). Microbial contamination of herbal preparations in Lagos. *Journal of Health, Population and Nutrition.* 23(3): 296-297.

Agrios N.G.(1978). Plant pathology. Academic Press, New York: 703.

Barrett, B. (2003) Medicinal properties of Echinacea: a critical review. *Phytomedicine.* 10(1):66-86.

Booth, C. (1997). Fusarium laboratory guide to the identification of the major species. Common wealth mycological institute (C M I). Kew, Surry, England.

Bugno, A; Adriana A.B.A.; Tatiana, C.P; Terzinha de Jesus, A.P and Myrna, S. (2006). Occurrence of toxigenic fungi in herbal drugs. *Brazilian Journal of Microbiology*. 37: 47-51

Burnet, J.H. (1976). Fundamentals of mycology. Second edition William Clowes and Sons, Limited. Pp. 221-281.

Chainani, W.N. (2003) Safety and anti-inflammatory activity of Curcumin: a component of tumeric *(Curcuma longa). Journal of Alternative and complementary Medicine*. 9(1) 161-168.

Collier, L., A. Balows, and M. Sussman. (1998). Topley & Wilson's Microbiology and Microbial Infections, 9th ed, vol. 4. Arnold, London, Sydney, Auckland, New York.

Czech,E.; Kneifel, W. and Kopp, B.(2001). Microbiological status of commercially available medicinal herbal drugs- a screening study. *Planta Medicinal*. 67:263-269.

Davise, H.L. (2002). A guide to identification of fungi. 4th edition. ASM Press, Washington D. C. U.S.A.

Dutta, B.K.; Rahman, I.; Dass,T.K.(1998). Antifungal activity of Indian plant extracts. *Mycoses.* 41:535- 536.

Efuntoye, M.O.(1996). Fungi associated with herbal drugs during storage. *Mycopathologia*. 136:115-118.

Efuntoye, M.O.(1999).Mycotoxins of fungal strains from stored herbal plants and mycotoxin contents of Nigerian crude herbal drugs. *Mycopathologia.* 147: 43-48.

El-shafie, A.E.; Al-Lawatia,T. and Al-Bahry, S.(1999). Fungi associated with black tea and tea quality in Sultanate of Oman. *Mycopathologia*. 145:89-93.

Gabriel, B.G.(2005). Taxonomical atlas for identification of fungi. 3rd edition, John Wiley publication, Florida, U.S.A.

Gilman, J.C., (2001). A Manual of Soil Fungi. Oxford and IBH Publishing Corporation, New Delhi, India.

Gillman, J. K. (1957). A manual of soil fungi. Iowa State University Press, Ames, Iowa. U.S.A.

Halt, M. (1998). Moulds and mycotoxins in herb tea and medicinal plants. *European Journal of Epidemiology.* 14: 269-274.

Harley, J.P. and Prescott, L.M. (1993). Basic laboratory and culture techniques, 2nd edition.W.C.Brown Publisher,pp. 14-46.

International Agency for Research and Cancer (IARC) (2002) Aflatoxins. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monograph No. 82. Lyon, France.

John, W. (1986). Introduction to fungi. Secound edition, Cambridge University Press, London. Pp.269-282.

Klinch, M.A. (2002). Identification of common Aspergillus species, Utrecht, Netherlands: Central Bereauvoor. Netherland.

Kumar, S. and Roy, A.K. (1993). Occurrence of aflatoxin in some liver curative herbal medicines. *Letters in Applied Microbiology*. 17: 112-114.

Lee, J.H. and Jo, W.K.(2006). Characteristics of indoor and outdoor bioaerosols at Korean high-rise apartment buildings. Environmental Research. 101(1):11-17.

Larone, D. H. (1995). Medically Important Fungi - A Guide to Identification, 3rd ed. ASM Press, Washington, D.C. 1-10.

Maiwada J.(2004). Presidential address at the opening ceremony of a 2- day zonal herbal fair/workshop organized by association of lady pharmacists of Nigeria in Abuja. Nasarawa state weekly news paper of August 11, 2004: Newsday@ nasarawastate.org.

Mandeel, Q.A.(2005) Fungal contamination of some imported spices. *Mycopathologia.* 159:291-298.

Martins, H.M.; Martins, H.L.; Dias, M.L. and Bernardo, F. (2001). Evaluation of microbiological quality of medicinal plants used in natural infusions. *International Journal of Food Microbiology.* 68: 149-153.

Okunlola, A.; Adewoyin, A.B. and Odeku, A.O. (2007). Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in South Western Nigeria. *Tropical Journal of Pharmaceutical Research.* 6(1): 661-670.

Pearce, M.C., Fenlon, D., Low, J.C., Smith, A.W., Knight, H.I., Evan, J., Foster, G., Synge, B.A. and Gunn, G.J. (2004). Distribution of Escherichia coli o157 in Bovine faecal parts and its impact on estimates of the prevalence of faecal shedding. *Applied and Environmental Microbiology*. 70: 5737-5743.

Pepper, I. L. and Gerba, C. P. (2004). Environmental microbiology. A laboratory manual, 2nd Plant nutrients. May 24-26th, 1992. Islamabad, Pakistan. pp. 607-610. pp: 666-681.

Philip, B. M.(1989). Training in mould isolation, identification, handling and evaluation of conditions leading to mycotoxin production: UNDP/FAO/THA/82/004. Reductionof post harvest losses in stored grains. Retrieved June 15, 2002 from http://www.fao.org/docrep/x5036E/x5036E00.htm.

Pitt, J.I. (2000). Toxigenic Fungi and Mycotoxins. *British Medical Bulletin*. 56:184-192. Retrieved May 15, 2009, from http://bmb.oxfordjournals.org/cgi/reprint/56/1/184.pdf.

Rizzo,I.; Vedoya,G. and Maurutto, S. (2004). Assessment of toxigenic fungi on Argentinean medicinal plants. *Microbio Research.*159: 113-120.

Raper, K. B. and Fennel, D.I. (1965). The genus Aspergillus. William and Wilkins Company Company. Baltore, U. S. A.

Roy, A.K. and Chourasia, H.K.(1990). Mycoflora, mycotoxin producibility and mycotoxin in traditional herbal drugs in India. *Journal of General and Applied Microbiology*. 36: 295-302.

Roy, A.K. and Kumari, V. (1991) Aflatoxin and citrinin in seeds of some medicinal plants under storage. *International. Journal of Pharmacognosy*: 29, 62-65.

Sofowora, A. (1993) Medicinal plants and Traditional medicine in Africa. Spectrum books Ltd (pub) Ibadan: 50-195.

Tassaneeyakul, W.; Razzazi-Fazeli, E.; Porasuphatana, S.; Bohm, J.(2004). Contamination of aflatoxins in herbal medicinal products in Thailand. *Mycopathologia*. 158: 239-244.

United States Pharmacopeia(USP)(2005) 28. ed. Rockville: United States Pharmacopeial Convention, 2005.

Vartika, Rai and Shanta mehrotra (2005). Toxic contamination in herbal drugs. International Society of Environmental Botanists. Environews: *News letter of ISEB India.* 11(4).

WHO (1998) Regulatory situation of herbal medicines: A world wide Review, World Health Organization, Geneva.

WHO (2003). Traditional medicine. Media centre world Health Organization. http://www.who.int/mediacentre/factsheets/fs134/en/

You, S., Huimin, W., Xiaonan, M., Jun, G., Li, Y., and Hong, G.(2007). Morphological and molecular identification of Aspergillus Versiculor D-1 with selective reduction ability. *Journal of Traditional Medicines*. 2(1): 39-44.

Zhang, Y.; Chen, J. and Chen, Y.(2005). Enviromental mycological study and allergic respiratory disease among tobacco processing workers. *Journal of Occupational Health.* 47(2):181-187

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