Mycotoxin contamination of herbal medications on sale in Ebonyi State, Nigeria


ABSTRACT

The practice of herbal medication is as old as the culture of the people and despite the advent of modern medication, many people of south eastern Nigeria, still patronizes herbal medication. Herbal medications are consumed directly and could be contaminated with mycotoxins which are detrimental to human and animal health. This study was therefore, designed to determine the extent of mycotoxin contamination of herbal medications on sale in Ebonyi State, South-Eastern Nigeria. In this regard, a multistage random sampling technique was used to select 19 herbal medication samples from stores and markets in Ebonyi State, Nigeria and evaluated for occurrence of three major mycotoxins - aflatoxins (AFs), ochratoxin A (OTA) and fumonisins (FB). Employing wet extraction procedure, mycotoxin occurrence and levels were determined via lateral flow immunoassay technique. Results showed high prevalence of all three mycotoxins in the samples in the order OTA (89.47%), FB (82.46%) and AF (82.21%). Ochratoxin A was highest in Goodswill herbal (23.66 ± 3.51 ppb) and lowest in Goko mixture (0.00 ± 0.00) while fumonisin was highest in Ukwara (634.33 ± 8.00 ppb) and lowest in Iketo-2 mixture (0.00 ± 0.10). Aflatoxin B1 was highest in African Iba (20.00 ± 2.00 ppb) and lowest in Dunamis and Divine roots herbals (0.00 ± 0.00). Data from the analysis of herbal medication samples showed varying concentrations of mycotoxins AFs (0 – 20 ppb); OTA (0 – 23 ppb); FB (0 – 634 ppb) respectively. In conclusion, mycotoxin concentrations determined in the herbal samples were above Nigerian and European Union (EU) set limits for OTA only. The co-occurrence of these mycotoxins in herbal samples analyzed in this study raises further awareness to the health risks consumers of these herbal commodities.

Keywords: Mycotoxin, herbal medicine, quality, Nigeria.

INTRODUCTION

Owing to their preharvest, postharvest, and storage conditions, herbs can be contaminated with mycotoxins. Report has shown 5–10% moulds contamination of all agricultural products in the world which contribute enormously to some ailments that occurs in humans and animals (Tosun and Arslan, 2013). Nigeria is among the tropics, with high range of temperature, humidity, and rainfall all through the year. These have greatly contributed to large production of herbs both in
Nigeria and in other related countries (Darwish et al., 2014), thereby, promoting proliferation of mycotoxin and increased the growth of filamentous fungi and moulds in food commodities consumed in this environment. Improper storage, extended drying times, and elevated moisture contents have also contributed to proliferation of mycotoxins in herbs and food commodities. Aflatoxins, Ochratoxins, Citrinin, Ergotamine, Fumonisin B₁, Patulin, Trichothecenes and Zearalenone among others have been reported as most prevalent groups of mycotoxin contaminating food and herbal products in this region (Cho et al., 2008; Jalili and Jinap 2012). These toxins have been attributed to weather, which results under favourable conditions from secondary metabolism of filamentous species of moulds.

According to previous reports, Aflatoxins, Ochratoxin and Fumonisin are classified among teratogenic, mutagenic and carcinogenic molecules in humans and animals at higher than recommended concentration (Bayman and Cotty 1993; I.A.R.C, 1993). Traditional belief, high cost of medical care, good quality drugs and consequent proliferation of faked cheaper drugs among others have also contributed to increased usage of herbal medication in Nigeria. Poor handling methods and non standardization of herbal medicines have resulted in poor quality and unsafe herbal products in Nigeria (Ezekwesili-Ofili, 2014). The present study therefore, aimed to investigate the mycotoxin levels and quality of most herbal medications sold in Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study area and population

Ebonyi State is one of the 36 states of the federal republic of Nigeria located in the south eastern part of the country. Ebonyi State as shown in figure 3.1 and 3.2 was carved out from the old Abia and Enugu State in October 1, 1996 with its capital as Abakaliki. It occupies a land mass of 5,935 square kilometers and is situated between latitudes 5º40’ and 6º54’N and longitudes 7º30’ and 8º30’E. The State is divided into 13 Local Government Areas (LGAs) grouped into three senatorial zones namely, Ebonyi North comprising Abakaliki, Ebonyi, Ohaukwu and Izi LGAs; Ebonyi central made up of Ishielu, Ikwo, Ezza North and Ezza South LGAs; and Ebonyi South made up of Afikpo North, Afikpo South, Ivo, Ohaozara and Onicha LGAs. Ebonyi State has one tertiary and many secondary and primary health institutions spread across the state which can either be public, private, mission or owned by nonprofit nongovernmental organization. The tertiary health institution is Federal Teaching Hospital in the Abakaliki capital city. The study was conducted in 6 out of these 13 local government areas of Ebonyi State. They are: Abakaliki, Ikwo, Afikpo North, Ohaukwu, Ezza North and Ebonyi Local Government Areas (LGAs). Each of the LGAs has one or more local markets where goods like Herbal medications are sold in the open market, in the bus, car and shops.

Sample collection

Three samples were collected each for twenty (20) different herbal medications shown in Table 3.0. The sixty (60) herbal medications were purchased from various locations in Ebonyi State. The samples in liquid formulation were contained in plastic or bottle containers and contained such information like herbal product name, manufacturers name and address, production and expiration dates, NAFDAC enlisting and batch numbers, etc. All herbal medications studied were produced and processed in Nigeria. Parameters analyzed were mycotoxins (total aflatoxin, ochratoxin A and Fumonisin) and trace elements (zinc, copper and manganese).

Sampling procedure/technique

A questionnaire was designed to ascertain the total number of herbal medications (liquid herbal formulations) sold in Ebonyi State and provide such information like the name of the herbal product, manufacturers name and address, production and expiration dates, NAFDAC enlisting and batch numbers, etc. All herbal medications studied were produced and processed in Nigeria. Parameters analyzed were mycotoxins (total aflatoxin, ochratoxin A and Fumonisin) and trace elements (zinc, copper and manganese).
cover, village and market name and visiting date. A total of 44 herbal medications were sighted in all at the end of the survey which lasted from 1st to 26th June 2016 and the location of sales ranged from big stores/shops, chemist shops, buses, cars and vendors (table and hand to hand sellers) in open markets. 21 out of the 44 medications had frequencies of fifty percent sales and below while 22 medications were above fifty percent. One medication was inconclusive and was dropped. Simple random sampling without replacement technique was used to select a total of 20 herbal medications (10 from those below 50 % and 10 from above 50% of sales) used for this study and are shown in Table 3.0. One sample (Katoka) could not be digested thus 19 different herbal medications were finally utilized for the study. The herbal samples were purchased directly from dealers/or hawkers from 2nd to 18th September, 2018.

**Ethical Consideration**

The ethical approval for this study was obtained from the Faculty of Health Sciences and Technology Ethics Committee, College of Health Sciences Nnamdi Azikiwe University, Nnewi Campus.

**Inclusion criteria**

The herbal medications used for this study were those produced and packaged in Nigeria.

**Exclusion criteria**

All herbal mixtures imported into the country were excluded in this study because the source of the raw materials for their manufacture was obtained outside the country.

**Method of analysis**

**Mycotoxins determination as described by Charm Sciences Incorporation (2012)**

The procedure for mycotoxin determination used for this study was based on Charm EZ-M Rapid One Step mycotoxin assay as described by Charm Sciences Incorporation, 2012. The method is a lateral flow immunosorbent assay, high performance liquid chromatography and liquid chromatography with tandem mass spectrophotometry (Meulenberg, 2012). Results obtained from this method are in conformity with the European regulations (Europroxima Netherlands, Donnelly, 2014) and is similar to that described by Vicam corporations (Vicam, 2014) for the analysis of milk.

**Principle**

Mycotoxin in the sample interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the Charm EZ®-M system and displayed as ppb (parts per billion) for aflatoxin and ochratoxin or ppm (parts per million) fumonisin.

**Sample preparation (extraction, filtration and dilution)**

In the extraction stage, 50 g of the sample was weighed after mixing using a chemical weighing balance and poured into a beaker. A wet extraction powder (1 Packet for 50 g sample) was added into the liquid sample in the beaker and mixed for 2 minutes using a mixer to obtain homogeneity. The mixture was then filtered using Whatman No. 1 filter paper to obtain a filtrate which was used for the analysis of the total Aflatoxin. Again, 100 µl of the filtrate was mixed with 900 µl of Aflatoxin buffer (1:10 dilution) to obtain a diluted extract. For ochratoxin A and fumonisin, the extraction and filtration processes were omitted. Finally, 100 µl and 300 µl of the sample were picked directly from the weighed sample in the beaker and mixed with 900 µl and 300 µl of ochratoxin and fumonisin buffer respectively to obtain a diluted extract used for the analysis.

**Test procedure**

The test strip for the mycotoxins was placed in Charm EZ®-M system. Appropriate test, commodity and dilution were carefully selected. The tape was peeled and 300 µL of the Diluted Extract was pipetted into sample compartment and the tape resealed. The result was read with Charm EZ-M system after 5
minutes incubation for fumonisin, 10 minutes for ochratoxin A, and 5 minutes for aflatoxin total. The quantitative range using the Charm EZ-M system is 0 – 150 ppb for total aflatoxin, 0 – 30 ppb for ochratoxin A and 0 – 6 ppm (0 to 6000 ppb) for fumonisin. The detection limits of the technique are quite sensitive enough to meet the standards of both national and international regulatory agencies and therefore validates the results obtained. Prior to the above procedure, the negative Control and positive control extract was tested to verify performance of equipment and test strips. Control values obtained were valid within the specified ranges (Negative Control: Less than 100 ppb (0.1 ppm) and Positive Control Extract: 1400 to 2600 ppb (1.4 to 2.6 ppm) fumonisin). All analyses were run in triplicates.

**Statistical analysis**

Data were expressed as percentage, mean and standard deviation. One sample t-test and descriptive statistics were used for data analysis. The statistical package used was SPSS 23 for windows and Microsoft Excel 2007 (for graphs). Statistical significance was set at P ≤ 0.05.

**Table 1:** Names of herbal medications used with the local governments where they were purchased in Ebonyi State, Nigeria.

<table>
<thead>
<tr>
<th>S/N</th>
<th>SAMPLE NAME</th>
<th>FUNCTION</th>
<th>LOCATION (LGA)</th>
<th>FREQUENCY OF SALE % per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Goko mixture</td>
<td>Anti-oxidant Immune booster</td>
<td>Abakaliki</td>
<td>≥50</td>
</tr>
<tr>
<td>2</td>
<td>Goodwills</td>
<td>Treats Infections</td>
<td>Abakaliki</td>
<td>≥50</td>
</tr>
<tr>
<td>3</td>
<td>Dunamis</td>
<td>Treats Infections</td>
<td>Afikpo North</td>
<td>≥50</td>
</tr>
<tr>
<td>4</td>
<td>Divine roots</td>
<td>Treats Infections Immune Booster</td>
<td>Afikpo North</td>
<td>&lt;50</td>
</tr>
<tr>
<td>5</td>
<td>Bitter extra</td>
<td>Treats Infections Anti-Oxidant</td>
<td>Ikwo</td>
<td>&lt;50</td>
</tr>
<tr>
<td>6</td>
<td>Zaram pile</td>
<td>Reliefs and heals Pile</td>
<td>Afikpo North</td>
<td>&lt;50</td>
</tr>
<tr>
<td>7</td>
<td>Deep roots</td>
<td>Treats Infections and Male infertility</td>
<td>Abakaliki</td>
<td>≥50</td>
</tr>
<tr>
<td>8</td>
<td>Blood purifier</td>
<td>Treats infections</td>
<td>Abakaliki</td>
<td>≥50</td>
</tr>
<tr>
<td>9</td>
<td>Ezinne herbal</td>
<td>Enhance female fertility and maintenance of pregnancy</td>
<td>Afikpo North</td>
<td>&lt;50</td>
</tr>
<tr>
<td>10</td>
<td>Cordel silver</td>
<td>Treats infections (anti-viral, anti-bacterial)</td>
<td>Afikpo North</td>
<td>≥50</td>
</tr>
<tr>
<td>11</td>
<td>Iketo</td>
<td>Treats infections and a purgative</td>
<td>Ohaukwu</td>
<td>&lt;50</td>
</tr>
<tr>
<td>12</td>
<td>African iba</td>
<td>A purgative and anti-infectious agent</td>
<td>Ezza North</td>
<td>&lt;50</td>
</tr>
<tr>
<td>13</td>
<td>Restorative tonic</td>
<td>Male fertility</td>
<td>Ebonyi</td>
<td>≥50</td>
</tr>
<tr>
<td>14</td>
<td>Akwasa</td>
<td>Immune booster anti-infectious agent (anti-viral and anti-bacterial)</td>
<td>Afikpo North</td>
<td>≥50</td>
</tr>
</tbody>
</table>
RESULTS

Mycotoxins concentration in herbal medications studied

The result shows the contaminant profiles of herbal medications found in various locations in Ebonyi State, Nigeria. It was observed that out of the 19 herbal medications studied, 15 (78.95%) had a mixture of three (3) contaminants which are aflatoxins, fumonisin and ochratoxin A while 4 (21.05%) of the herbal medications contained only two mycotoxins. However, none was found to be totally free of mycotoxin contamination.

The highest concentration of aflatoxins was observed in African Iba (20.00±2.00 ppb), followed by Elcocyn Ds (18.00±1.73 ppb). There was absence of aflatoxins in Dun amis and Divine roots herbal medications. One sample t-test was computed to compare the various concentrations of aflatoxins found in the studied herbal medications with a test value of 20 ppb (the maximum tolerance level of aflatoxins in consumable foodstuffs). The result showed a significant decrease (P < 0.05) from a test value of 20 ppb for all the herbal medications with the exceptions of African Iba, Zaram pile, Deep roots, Iketo 2, and Elcocyn Ds which was not significant (P > 0.05).

The result of the fumonisin in the herbal medications showed that the highest concentration of fumonisin was found in Ukwara (634.33±8.00 ppb), followed by Divine roots (353.67±50.40 ppb) and Cordel silver (281.33±27.30 ppb). There was absence of fumonisin in Iketo-2 mixture. One sample t-test was computed to compare the various concentrations of fumonisin-B1 found in the herbal medications with a test value of 1000 ppb (the maximum tolerance level of fumonisin in consumable foodstuffs). The result showed a significant decrease (P < 0.05) from a test value of 1000 ppb for all the herbal medications studied.

The highest concentration of Ochratoxin A was found in Goodswill (23.66±3.51 ppb). It was followed by Restorative Tonic (22.67±2.52 ppb). There was absence of Ochratoxin A in Goko mixture. One sample t-test was computed to compare the various concentrations of Ochratoxin A found in the herbal medications with a test value of 5 ppb (the maximum tolerance level of Ochratoxin A in consumable foodstuffs). The result showed a significant increase (P < 0.05) from a test value of 5 ppb for all the herbal medications. The concentrations of Goodswill, Divine roots, Zaram pile, African Iba, Akwasa and Restorative Tonic herbal medications were significantly higher when compared to 5 ppb (Table 2 and Figure 1, 2 and 3).

Frequency and concentration of mycotoxins in herbal medications studied

Table 3 shows the frequency and concentration data of the mycotoxin contaminants in the herbal medications. It was observed that the samples were contaminated with mycotoxins with the highest contaminant seen with Ochratoxin A (89.47%), followed by Aflatoxin (84.21%) and Fumonisin B1 (82.46%).
### Table 2: Concentration of mycotoxins in commonly used herbal medications in Ebonyi State, Nigeria.

<table>
<thead>
<tr>
<th>Herbal Medications</th>
<th>Ochratoxin A (Part per Billion) N=57 (Mean ± SD)</th>
<th>Total Aflatoxin (Part per Billion) N=57 (Mean ± SD)</th>
<th>Fumonisin B1 (Part per Billion) N=57 (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goko mixture</td>
<td>0.00±0.00</td>
<td>6.67±3.06**</td>
<td>0.33±0.58**</td>
</tr>
<tr>
<td>Goodwills</td>
<td>23.66±3.51**</td>
<td>3.67±1.15**</td>
<td>6.67±11.55**</td>
</tr>
<tr>
<td>Dunamis</td>
<td>2.67±1.15</td>
<td>0.00±0.00</td>
<td>250.00±2.00**</td>
</tr>
<tr>
<td>Divine roots</td>
<td>12.00±1.73**</td>
<td>0.00±0.00</td>
<td>353.67±50.40**</td>
</tr>
<tr>
<td>Bitter extra</td>
<td>10.00±2.00</td>
<td>8.33±2.52**</td>
<td>3.33±5.77**</td>
</tr>
<tr>
<td>Zaram pile</td>
<td>12.33±2.52**</td>
<td>16.00±5.00</td>
<td>109.67±10.02**</td>
</tr>
<tr>
<td>Deep roots</td>
<td>1.33±1.53**</td>
<td>15.67±3.51</td>
<td>156.67±20.82**</td>
</tr>
<tr>
<td>Blood purifier</td>
<td>7.33±2.52</td>
<td>5.67±2.52**</td>
<td>76.67±25.17**</td>
</tr>
<tr>
<td>Ezinne herbal</td>
<td>7.67±3.21</td>
<td>9.00±1.73**</td>
<td>55.33±21.50**</td>
</tr>
<tr>
<td>Cordel silver</td>
<td>2.67±1.15</td>
<td>5.33±2.52**</td>
<td>281.33±27.30**</td>
</tr>
<tr>
<td>Iketo</td>
<td>2.50±0.707</td>
<td>13.50±6.36</td>
<td>0.00±0.00**</td>
</tr>
<tr>
<td>African iba</td>
<td>14.33±2.08**</td>
<td>20.00±2.00</td>
<td>76.67±25.17**</td>
</tr>
<tr>
<td>Restorative tonic</td>
<td>22.67±2.52**</td>
<td>6.33±2.89**</td>
<td>170.00±51.96**</td>
</tr>
<tr>
<td>Akwasa</td>
<td>12.67±2.08**</td>
<td>2.33±3.21**</td>
<td>140.00±10.00**</td>
</tr>
<tr>
<td>Chindus</td>
<td>12.00±4.58</td>
<td>6.00±3.00**</td>
<td>49.00±4.58**</td>
</tr>
<tr>
<td>Ukwara</td>
<td>10.00±3.61</td>
<td>6.33±3.06**</td>
<td>634.33±8.23**</td>
</tr>
<tr>
<td>Asheitu adams</td>
<td>2.50±0.707</td>
<td>4.00±1.41**</td>
<td>165.00±21.21**</td>
</tr>
<tr>
<td>Elcocyn-Ds</td>
<td>2.67±1.53</td>
<td>18.00±1.73</td>
<td>107.33±6.43**</td>
</tr>
<tr>
<td>Golden seed</td>
<td>1.33±0.577**</td>
<td>6.67±3.06**</td>
<td>133.00±58.03**</td>
</tr>
</tbody>
</table>

Where ** Values are significant at p < 0.05
Table 3: frequency and concentration of the mycotoxins in herbal medications from Ebonyi State.

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Number of samples analyzed</th>
<th>Frequency of positive samples</th>
<th>Concentration (ppb) in herbal medications (Mean ± SD)</th>
<th>Range (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Aflatoxin</td>
<td>57</td>
<td>48(84.21%)</td>
<td>7.35 ± 1.86</td>
<td>0 – 20</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>57</td>
<td>51(89.47%)</td>
<td>6.25 ± 0.26</td>
<td>0 – 23</td>
</tr>
<tr>
<td>Total Fumonisin</td>
<td>57</td>
<td>47(82.46%)</td>
<td>116.88 ± 56.79</td>
<td>0 – 634</td>
</tr>
</tbody>
</table>

Figure 1: Concentration of Fumonisin B1 in commonly used herbal medications in Ebonyi State, Nigeria, as determined by lateral flow immunoassay technique.
Figure 2: Concentration of Total Aflatoxin in commonly used herbal medications in Ebonyi State, Nigeria, as determined by lateral flow immunoassay technique.

Figure 3: Concentration of Ochratoxin A in commonly used herbal medications in Ebonyi State, Nigeria, as determined by lateral flow immunoassay technique.
DISCUSSION

The present study provides for the first time the mycotoxins concentrations of herbal medications intended for human consumption in Ebonyi State with respect to three of the most important mycotoxins worldwide, namely aflatoxins (AFs), ochratoxin A (OTA), fumonisin (FB). Since these medications normally called herbal drugs or herbal medicines were made in Nigeria and transported to the state for sale, they equally represent a fraction of herbal medications sold or consumed in Nigeria.

Data obtained from the analysis of various herbal medicines studied showed varying concentrations of mycotoxins. High frequencies of OTA 51/57 (89.47%), AF 48/57 (82.21%) and FB 47/57 (82.46%) were observed. Out of the 19 herbal medications studied, 15 (78.95%) had a mixture of three (3) mycotoxin contaminants while 4 (21.05%) of the herbal medications contained only two mycotoxins. However, none was found to be totally free of mycotoxin contamination.

The occurrence of these mycotoxins could be attributed to the raw materials used in the preparation of these medications which are not under the scope of this work but need to be further studied. It can also be due to poor handling and contamination of the samples or the raw materials used by mycotoxigenic fungi Aspergillus and Fusarium (Matasyoh et al., 2013; Egbuta et al., 2015).

From the result, 48 out of the 57 herbal medications (84.21%) was positive for aflatoxin with the highest concentration of aflatoxin seen in African Iba medication, (20.00±2.00 ppb) followed by Elcocyn Ds (18.00±1.73 ppb). There was absence of aflatoxins in Dunamis and Divine roots herbal medications. Some researchers have reported varying concentrations of aflatoxins in some organic herbs samples in different places in Nigeria (Tosun and Arslan, 2013; Ezekwesili-Ofili, 2014). It has to be emphasized that the 82.21% prevalence rate of aflatoxin is very significant and even though the samples had AF levels significantly below the acceptable limits (20 ppb) set by the 77 countries that regulate AFs, including the European Union (CAST, 2003; EC, 2006). The presence of aflatoxin in almost all the herbal medications studied is very appalling and alarming. This calls for restraints because even relatively low concentration of aflatoxin can cause illness (Carlson and Ensley, 2003; Owaga et al., 2011). However, presence of aflatoxin contamination of food products sold in various markets has been recently reported (Aristil, 2019).

Ochratoxin A was detected in 89.47% of the herbal medications. Like AFs, 8 (42.00%) and 11 (57.89%) of the 19 herbal medications contain ochratoxin A respectively lower and higher than maximum tolerated levels (5 ppb) set by the aforementioned international regulatory bodies for human consumption. The highest concentration of Ochratoxin A was found in Goodswill (23.66±3.51 ppb), followed by Restorative Tonic (22.67±2.52 ppb). There was absence of Ochratoxin A in Goko mixture.

Fumonisn occurred in 82.46% of the herbal medications and were found at levels significantly below the acceptable limit by mycotoxin regulatory agencies. The acceptable limits for FBs is <1,000 ppb (CAST, 2003; EC, 2006). The results showed the highest concentration of fumonisn in Ukwara (634.33±8.00 ppb), followed by Divine roots (353.67±50.40 ppb) and Cordel silver (281.33±27.30 ppb). Presence of Zealenone and FBs have been reported previously in Nigerian feeds and foods (Bankole and Adebanjo, 2003; Makun et al., 2007). However, the present study seems to be the first to report the occurrence of FBs in liquid herbal medications in Nigeria though the concentrations were all below common maximum levels, including the European Union acceptable limit.

The observation of various concentrations of mycotoxins in Nigerian herbal medicines revealed the quality of the herbal drugs with regards to its acceptability for human and animal consumption. The demonstrated presence of Ochratoxin A at concentrations above the limits acceptable to world mycotoxin regulatory agencies and the co-occurrences of toxins with possible toxic
The differences in concentrations of various mycotoxins studied with respect to the individual herbal medications could be attributed to the raw materials used in their preparation. This is in line with previous studies (Egner et al., 2001; Makun et al., 2010; Egbuta et al., 2015). It is therefore, advised that presence and levels of mycotoxins in various raw materials be investigated before its use in preparations of herbal medications even though it may be difficult controlling such contamination especially in non-developed world, including Nigeria.

In the present study, the mycotoxin profile showed that the contaminants did not singly occurred but in combinations of twos and threes. Dual co-contamination with two types of mycotoxins occurred in a few samples only, with AFs and OTA only found in one sample, OTA and FB occurred together in two samples, whereas AF and FB occurred together in only one sample. Simultaneous contamination with three mycotoxins (AFs/OTA/FB) in one sample was found in fifteen samples. No sample was seen to be mycotoxins free. Similar finding has been documented elsewhere (Creppy et al., 2004). The implications of such toxin “cocktails” on human health are presently unknown (Makun et al., 2011). However, the interactive effects of mycotoxins in these natural combinations could be synergistic, additive or antagonist in host organisms (Miller, 2008). Interaction between AF and FB, though not one of the combinations observed in this study, had an additive effect in mice, causing increased injuries to liver and kidneys of the experimental animals (Gelderblom et al., 2002). The present study observed combinations of FB and OTA in some medications. This has been shown to exhibit synergistic interaction to each other (Creppy et al., 2004), while exposure of OTA and AFB1 to rabbits and humans have been reported to show antagonistic interactions with evidence of teratogenic and mutagenic effects (Wangikar et al., 2005). This pilot study show that AFs, OTA and FBs are toxins found in most herbal medications studied in Ebonyi State, Nigeria.
Similar studies have documented same in food products (Ayejuyo et al., 2008).

Conclusion
Herbal medications sold in Nigeria contained varying degrees of mycotoxins with prevalence rate of 89.47% for ochratoxin A, 84.21% for aflatoxin and 82.46% for fumonisin B1. 78.95% dual contamination (AF, OTA and FB) were observed in 15 of the 19 herbal medications studied. Regrettably, no medication was found free of contaminant. The result consequently, revealed the quality of the herbal drugs with regards to its acceptability to human and animal consumption since the synergistic effect of these mycotoxins increases their toxicity and effect. Finally, the high frequency and levels of mycotoxins, ochratoxin A in particular, found in some of these herbal medications in Nigerian markets clearly shows that they are seriously contaminated and unsafe for human consumption and this necessitate for increased surveillance.

COMPETING INTERESTS
The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS
Conceptualization, CCO; Methodology, RCI; Software, RCI and NRU; Validation, CCO, NRU; Formal analysis, RCI, IPE, CGI and AFE ; Investigation, RCI, IPE, CGI and AFE; Resources, RCI, AFE, IPE and CGI; Data curation, RCI, IPE, CGI; Writing – Original Draft Preparation, RCI, CCO and NRU; Writing – Review & Editing, CCO, RCI, NRU, IPE, CGI and AFE; Visualization, CCO, RCI, NRU; Supervision, CCO; Project Administration, RCI.

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