



Isolation and Characterization of *Mycoflora* Associated with Herbal Concoction sold for human consumption in Lapai Market, Niger State Nigeria.

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Abstract

The occurrence of mycoflora contamination of some herbal concoctions sold in Lapai Nigeria were mycologically evaluated. A total of 12 samples of herbal concoction were collected from herbal vendors from central Market. Fungal contaminants of each of the concoctions were serially diluted and isolated on Potato Dextrose Agar using 1ml of each of 10^{-1} and 10^{-2} dilutions. Fungi Identification was done on the basis of morphological characteristics of the colony, conidia and conidiogenous cells and mycological monographs. Some physico-chemical parameters were also determined using standard methods. A total of six different fungal species including; *Microsporium audouinii*, *Mucor species*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium notatum* were isolated. *Penicillium notatum* had the highest frequency occurrence in 29.26% followed by *Aspergillus niger* at (26.82%), *Aspergillus fumigatus* (21.95%), *Aspergillus flavus* (9.75%), *Microsporium audouinii* (7.31%) while *Mucor hiemalis* had the least occurrence of (4.87%). The herbal mixtures did not have manufacturing nor expiring dates. The colour of the samples varied between brown, green, yellow, yellowish brown, reddish brown, yellowish green and cloudy white. The pH ranged between 3.22 and 6.82, the turbidity ranged between 122.5 and 908 while suspended solids (mg/L) ranged between 340 and 4105. All the concoctions had the mean score below 3 (on a 1-4 hedonic scale) for all the sensory attribute analyzed except for miscarriage and tooth pain concoctions which had taste scores of 3.21 and 3.30 respectively. The results of this study revealed that herbal concoctions sold in Lapai were mostly contaminated with fungi, having poor sensory attributes, inadequate pH, no expiry dates, Batch number and NAFDAC number. Based on this, adequate control measures and distribution practices should be adopted. This would help check the fungal density of the concoction especially *Aspergillus* spp. and *Penicillium* spp. which are of public health importance and so improve the safety.

Keywords: Concoction, Contamination, Herbal, Mycoflora, Sensory.

Introduction

Medicinal plants have been used as source of medicine in many indigenous communities in Nigeria and throughout the world. According to WHO, (2006) herbal medicines serve the health needs of about 80% of the world's population, especially for thousands and hundreds of thousands of people in rural areas of a developing country like Nigeria (Ebo, 2012). A herbal concoction is the combination of various ingredients,

usually herbs, spices, condiments, powdery substances, or minerals, mixed up together or dissolved into a liquid and consumed as drug (Shamsudeen *et al.*, 2008). Herbal plants are plants valued for their medicinal aromatic properties and are intended to prevent, alleviate, mitigate or cure a mental or physical state of wellbeing in humans, or animals or alter the structure or function of the body (Ebo *et al.*, 2012; Akande *et al.*, 2013). Herbal concoctions in their natural state vary in



potency and may contain multiple pharmacological substances which can cause undesirable effects. Some of the plants that are commonly used to prepare concoction includes, among others; lemon grass, neem, pawpaw, guava, mango and bitter leaves (Adeleye *et al.*, 2010).

Despite its enormous benefits it also poses serious public consequences as a result of improper or unhygienic preparations, excessive quantities of a specific herb concoction, substitution of one herb for another that is readily available, the addition of other pharmacologic agents or heavy metals, and the effects of using herbal medications in combination with other pharmacologic products (Sofowora, 2010). Another problem with herbal products is that no records are kept on batches of products that would help manufacturers to identify and track after distribution (Lai and Roy, 2004).

Pathogenic fungi and some species isolated from herb concoction produces mycotoxin that causes food poisoning (Adeleye *et al.*, 2010). This study was conducted to identify fungi contaminants that are present in the herbal concoctions sold in Lapai market.

MATERIALS AND METHODS

Collection of Samples

A total of 12 samples of concoction advertised for the treatment of ailments such as Malaria, Ulcer, Diabetes, Menstruation problem, Tooth pain, Body weakness, Fibroid, Hypertension, pile, Rheumatism, Back ache, Miscarriage, were collected between July and August 2015 on weekly basis. Samples were collected in empty 20ml plastic bottles from the Main Market and Badeggi market and labeled accordingly (with names, dates of collection and location) and taken to the laboratory of the Department of Biological Science, Ibrahim Badamasi Babangida University, Lapai, Niger State.

Physico-chemical Parameters

Herbal vendors were asked questions on the date of formulation and expiration of the concoction. Other information such as the

Batch number and NAFDAC number were also collected.

PH

pH of each collected sample was measured by placing the electrode in 20ml of the liquid at room temperature using Jenway 3310 digital pH meter (Shamsuddeen *et al.*, 2008).

Turbidity

The turbidity of each of the samples was determined using Hach DR/2010 spectrophotometer (Daniel, 2007; Ebo *et al.*, 2012).

Total Suspended Solids

The sample mixtures were thoroughly shaken to provide a uniform mixture. 10ml of the mixture was pipetted into a tarred nickel dish and heated on a water-bath until the residues were apparently dried. They were then transferred to an oven and dried to a constant weight at 105°C and then cooled in desiccators. The percentages were calculated in grams per 100ml of the liquid preparation (Handa *et al.*, 2008; Das *et al.*, 2010).

Organoleptic Test

The organoleptic evaluations were carried out by 10 judges. All the judges formed the panel and were conversant with the factors governing the quality. The panelists were asked to rate the samples for colour, taste, aroma, texture and over all acceptability on a 1-4 hedonic scale where 4=like extremely, 3=like well, 2=dislike, 1=extremely dislike. (Feng, 2012).

Isolation of Fungi

Serial dilution technique was used. 10grams of each sample was aseptically transferred into 9ml of sterile distilled water in test tubes. It was shaken properly to allow even distribution of microorganisms present in the sample. A dilution factor 10^{-1} and 10^{-2} were used as stock solutions. 1ml of each dilution was aseptically taken from the suspension and transferred into sterile Petri dish. 10ml Potato Dextrose Agar (PDA) was

poured into the Petri dish with 1ml chlorophenicol. The plates was swirled gently to allow even distribution of the sample. Incubation was done at room temperature ($28 \pm 2^\circ\text{C}$, for 24hrs). Subcultures were made from the mix culture and fungi identification was done on the bases of morphological characteristics of the colony, conidia and conidiogenous cells and mycological monographs (Amadi and Adebola, 2008; Cannon and Kirk, 2001).

RESULTS

Physical Properties of Herbal Preparation

None of the herbal concoction had Batch or NAFDAC number. The samples had

various colours such as brown, green, yellow, yellowish brown, reddish brown, yellowish green, cloudy ash. The pH of the herbal concoction ranged between 3.22 and 6.82. The turbidity (FAU) ranged between 122.5 and 908.0 being highest in rheumatism concoction and lowest in concoction for menstruation problem, while the suspended solids (mg/L) ranged between 340 and 4105 being highest in diabetics concoction and lowest in concoction for menstruation problem (Table 1).

Table 1: The physiochemical features of sampled herbal concoction sold in Lapai market

| Physical parameters | | | | | | | |
|---------------------|-----------------|-----------|-----------------|------------------------|-------------------------|-------------------|------------|
| Concoction Sample | Uses : To treat | pH values | Turbidity (FAU) | Colour | Suspended Solids (mg/L) | Batch δ No | NAFDAC /No |
| A | Hypertension | 3.78 | 228 | Brown | 71.5 | Nil | Nil |
| B | Ulcer | 4.51 | 453.5 | Dirty green | 905 | Nil | Nil |
| C | Malaria | 3.64 | 182.5 | Dirty green | 540 | Nil | Nil |
| D | Tooth pain | 6.82 | 167.5 | Yellow | 595 | Nil | Nil |
| E | Fibroid | 4.15 | 750 | Yellowish | 875 | Nil | Nil |
| F | Miscarriage | 3.22 | 238.5 | Brown Reddish brown | 685 | Nil | Nil |
| G | Menstruation | 4.95 | 122.5 | Brown | 340 | Nil | Nil |
| H | Diabetics | 4.30 | 1930 | Yellowish green | 4105 | Nil | Nil |
| I | Body Weakness | 4.61 | 461.5 | Yellow | 655 | Nil | Nil |
| J | Pile | 4.53 | 229.5 | Dirty green | 935 | Nil | Nil |
| K | Rheumatism | 6.41 | 908.0 | Cloudy white | 565 | Nil | Nil |
| L | Backache | 3.68 | 169.0 | Yellowish brown | 430 | Nil | Nil |

Mean of triplicates

Organoleptic Assessment

The result of the organoleptic assessment of herbal concoction sold in Lapai Niger state are presented in Table 2: on a hedonic scale of 1-4, all the herbal concoctions have their mean score below 3 for all the sensory attribute analyzed except for miscarriage and tooth pain concoction which had taste scores of 3.13 and 3.09 respectively,

weakness and tooth pain concoction which had the colour score of 3.21 and 3.00 respectively and backache concoction which had texture score of 3.11. All the herbal concoction analyzed had the score range of 1.98 to 3.21 for colour. Taste score ranged between 1.20 to 3.13, while 1.34 to 3.11 was for texture range and 1.91 to 2.98 for overall acceptance.

Table 2: Organoleptic assessment of herbal concoction sold in Lapai Niger

| Concoction Sample | Uses : To treat | Aroma | Colour | Texture | Taste | Overall acceptance |
|-------------------|-----------------|-----------|-----------|------------|------------|--------------------|
| A | hypertension | 2.00±0.32 | 2.00±0.32 | 2.00±0.32 | 2.00±0.32 | 1.91 ±0.32 |
| B | ulcer | 2.50±0.10 | 1.98±0.10 | 2.50±0.10 | 2.50±0.10 | 2.20±0.10 |
| C | malaria | 1.90±0.01 | 2.00±0.01 | 2.00±0.01 | 2.00±0.01 | 2.00±0.01 |
| D | tooth pain | 2.10±0.02 | 3.00±0.02 | 2.30±0.02 | 3.09±0.02 | 2.98±0.02 |
| E | fibroid | 2.10±0.11 | 2.00±0.11 | 1.34 ±0.11 | 2.00±0.11 | 2.00±0.11 |
| F | miscarriage | 1.09±0.32 | 2.00±0.32 | 2.00±0.32 | 3.13±0.32 | 2.00±0.32 |
| G | menstruation | 2.00±0.32 | 2.00±0.32 | 2.00±0.32 | 1.20 ±0.32 | 2.00±0.32 |
| H | diabetics | 1.29±0.32 | 2.50±0.10 | 2.50±0.10 | 2.50±0.10 | 2.50±0.10 |
| I | body Weakness | 3.21±0.10 | 3.21.01 | 2.00±0.01 | 2.00±0.01 | 1.09±0.01 |
| J | pile | 2.00±0.01 | 2.30±0.02 | 2.30±0.02 | 2.30±0.02 | 2.00±0.32 |
| K | rheumatism | 2.30±0.02 | 2.00±0.11 | 2.00±0.11 | 2.00±0.11 | 2.10±0.10 |
| L | backache | 2.00±0.11 | 2.00±0.32 | 3.11±0.32 | 2.00±0.32 | 2.00±0.01 |

Data are mean±SEM from 10 judges

The results obtained from the microbiological analysis of the different types of herbal concoction sold in Lapai, Niger state are shown in Table 2 and 3. A total of six different fungi species including *Microsporum audouinii*, *Mucor hiemalis*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus* belonging to 4 genera

(*Aspergillus* sp, *Penicillium* sp. *Mucor* sp. *Microsporum* sp.) were isolated from all the concoctions sampled (malaria, ulcer, typhoid, backache, diabetes, fibroid, menstruation problem, tooth pain, hypertension, miscarriage, pile, rheumatism, body weakness).

Table 3: Fungal isolated from herbal preparation sold in Lapai, Niger state

| Sample | Uses: To treat | Fungal isolates |
|--------|----------------|--|
| A | hypertension | <i>A. niger</i> , <i>A. fumigatus</i> , <i>P. notatum</i> and <i>M. audouinii</i> . |
| B | Ulcer | <i>A. niger</i> , <i>A. fumigatus</i> and <i>P. notatum</i> . |
| C | malaria | <i>A. niger</i> , <i>A. fumigatus</i> , <i>M. audouinii</i> , <i>M. hiemalis</i> , and <i>P. notatum</i> . |
| D | tooth pain | <i>A. niger</i> , <i>A. fumigatus</i> and <i>P. notatum</i> . |
| E | Fibroid | <i>A. niger</i> , <i>A. flavus</i> and <i>P. notatum</i> |
| F | miscarriage | <i>A. niger</i> , <i>A. fumigatus</i> and <i>P. Notatum</i> |
| G | menstruation | <i>A. niger</i> , <i>A. flavus</i> and <i>P. notatum</i> |
| H | diabetes | <i>A. niger</i> , <i>A. flavus</i> and <i>P. notatum</i> |
| I | weakness. | <i>A. niger</i> , <i>A. fumigatus</i> , <i>A. flavus</i> and <i>P. notatum</i> , |
| J | Pile | <i>A. fumigatus</i> , <i>P. notatum</i> and <i>M. hiemalis</i> . |
| K | rheumatism | <i>A. niger</i> , <i>A. flavus</i> and <i>P. notatum</i> |
| L | backache | <i>A. niger</i> , <i>A. fumigatus</i> , <i>P. notatum</i> and <i>M. audouinii</i> . |

Percentage Occurrences of Fungal Isolates

Percentage occurrences of fungi species in herbal concoction sold in Lapai Niger state are presented in Table 4. *Penicillium notatum* was found to be the most prevalent mycoflora isolated from herbal concoction. It had the highest percentage occurrence of 29.26% , followed by *Aspergillus niger* with percentage

occurrence of 26.82%, *Aspergillus fumigatus* with percentage occurrence of 21.95%, *Aspergillus flavus* with percentage occurrence of 9.75%, *Microsporum audouinii* with percentage occurrence of 7.35% while *Mucor hiemalis* had the lowest percentage occurrence of 4.87%.

Table 4: Percentage occurrences of fungal isolates in herbal preparation sold in Lapai, Niger state Nigeria

| Fungal isolates | Number of Samples examined | Positive samples | %occurrence |
|------------------------------|----------------------------|------------------|-------------|
| <i>Aspergillus niger</i> | 12 | 11 | 26.82 |
| <i>Aspergillus fumigatus</i> | 12 | 9 | 21.95 |
| <i>Aspergillus flavus</i> | 12 | 4 | 9.75 |
| <i>Penicillium notatum.</i> | 12 | 2 | 29.26 |
| <i>Mucor hiemalis</i> | 12 | 2 | 4.87 |
| <i>Microsporum audouinii</i> | 12 | 3 | 7.35 |

Discussion

Four different genera of fungi isolated from herbal concoctions in this research represented by six species including *Microsporum audouinii*, *Mucor hiemalis*, *Penicillium notatum*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus* confirmed the fact that fungal spores and hyphae are ubiquitous in the environment and their ability to grow on different substrates under a wide range of environmental conditions has enabled some of them to colonize virtually any substance (Adebanjo and Bankole, 2003). Among them, *Penicillium notatum* was the most frequent representative accounting for 29.26% and *Aspergillus niger* accounting for 26.82% of the total mycoflora isolate. These findings was in agreement with the report of Serra *et al.*, (2005) who also found that *Penicillium notatum*(31%) and *Aspergillus species* (10%) were the most often encountered genera out of 11 samples of grapes from Southern France.

The source of fungi contamination of herbal concoction sold in Lapai could be attributed to soil and water. This is probably due to the methods of their preparation or the equipment used in preparing the herbal

concoction as also reported by Adenike *et al.*, (2006). In addition, the presence of *Aspergillus* sp, *Mucor* sp. and *Penicillium* sp. were not surprising as they are common spoilage organisms of carbohydrates containing foods (Shamsuddeen *et al.*; 2008). The herbal concoctions particularly those with high carbohydrate contents might therefore be prone to increased fungi growth (Barnes *et al.*, 2002).

Physico-chemical indices of the herbal concoction sampled showed that they were all water-based and majority of the samples appeared not to have been produced without following good manufacturing practices. The pH values (the hydrogen ion concentration of a solution and the measure of the acidity of the solution) of some of the samples including; hypertension (3.78), malaria (3.64), Miscarriage (3.22) Backache (3.68) were below the WHO acceptable limits of 4.0 – 7.0 (WHO, 1980), thus consumption of this herbal concoction can cause serious health problems like acidosis which is a condition of decreased alkalinity of the blood tissues, it can also damage the kidneys and the liver, can irritate the skin or tissues, and can cause inflammation of skin and tissues. It can also penetrate the

skin to cause deep sores (Ghana National Drug Programme, 2004).

The organoleptic properties of the samples in this study were below the acceptable range for herbal concoction as previously documented by Daniel, *et al.*, (2007).

All the herbal concoctions sampled in this study had no expiry dates, Batch number and NAFDAC number despite all efforts put in place by National Agency for Food and Drug Administration and Control (NAFDAC). In ensuring quality, safety, registration and advertisement on herbal medicines. Probably NAFDAC has no control over marketed herbal preparation or that policy and laws are not yet promulgated as earlier reported by Ebo *et al.* (2012).

Reference

- Adebanjo, A. and Bankole, S. A. (2003). Mycotoxins in Food in West Africa Control and Possibilities of Controlling It. *African Journal of Biotechnology*; 2(9): 254-263
- Adenike, A.O., Oyushe, Taiye, R., and Egunyomi, F. A. (2006). Bacterial profiles and consumer preference of some indigenous orally consumed herbal medications in Nigeria. *Journal of Rural and Tropical Public Health* 5: 27-33
- Adeloye, L.A., Okogi, G., and Ojo, E.O. (2010). Microbial contamination of herbal preparation in Lagos, Nigeria. *Food Chemistry*, 101: 1012-18.
- Akande, T., Agbulu, C.O., and Oche, M. (2013). Microbial Contamination of Herbal Mixtures (Local Concoctions) Used in the Treatment of Typhoid Fever, Malaria Fever, and Dysentery in Makurdi Metropolis. *Journal of Medical and Applied Biosciences*, 5(1): 31-45.
- Amadi, J.E and Adebola, M.O. (2008). Effects of moisture content and storage conditions on the storability of garri. *African journal of Biotechnology* 7(24): 4591-4594.
- Barnes, J., Anderson, L.A. and Phillipson J.D. (2002). *Herbal Medicine: A guide for healthcare professionals*, 2nd Edition, Pharmaceutical Press, Great Britain. pp 54-56.
- Daniel, O. B. (2007). The Quality Assessment Of Some Herbal Preparations Produced In Ghana, Thesis Submitted to the Department of Chemistry, Kwame Nkrumah University of Science And Technology.
- Das, K., Tiwari, R.K.S. and Shrivastava, D.K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4(2): 104-111.
- Ebo, P. U., Mukhtar, M.D. and Taura, D.W. (2012). Evaluation of microbiological safety of herbal concoctions sold in some markets in Gabasawa and Nassarawa Local Government Areas In Kano State. *Bayero Journal of Pure and Applied Sciences*, 5(1): 127-131.
- Feng, Y. (2012). A critical appraisal: the 9-point hedonic scale and hedonic ranking as an alternative. MS thesis, University of California, Davis.
- Ghana National Drug Programme, (2004). A Manual of Harmonized Procedures for assessing the Safety, Efficacy and Quality of Plant-Medicines in Ghana, *Ministry of Health, Ghana*, 1: 316-320.
- Handa, S.S., Khanuja, S.P.S., Longo, G. and Rakesh, D.D. (2008). Extraction Technologies for Medicinal and Aromatic Plants. *International centre for science and high technology, Trieste*, 21-25.
- Lai P.K and Roy J (2004) "Antimicrobial and chem-opreventive properties of Herbs and Spices. *Current Medical Chemistry*, 11(11): 1451-1460.
- Serra, R., Braga, A. and Venâncio, A. (2005). Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. *Research in Microbiology*; 156: 515-521.
- Shamsuddeen, U, Mukhtar, M.D. and Rakiya

A; (2008):A survey on the microbiological quality of traditional medicine herbs concoctions vended in Kano, Nigeria. *Journal of Medicinal and Pharmaceutical sciences*,(4)1:1597 – 1599.

Sofowora: In Adeleye, I.A., Okogi, G., Ojo, E.O. (2010).Microbial contamination of herbal preparation in Lagos, Nigeria. *Journal of Health population and Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. Food Chemistry*, 101: 1012-18.

World Health Organization (WHO), (1980) Expert committee on diabetes mellitus second report technical report series 646 Geneva, 12-15.