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ISOLATION AND CHARACTERISATION OF MICROORGANISMS CONTAMINATING HERBAL INFUSION SOLD IN MINNA, NIGERIA

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Abstract

The microbiological assessment of ten herbal infusion samples from ten different locations in Minna, Niger State was investigated. The assessment of the microbial contamination on the herbal products was carried out, using standard methods. Pour plate method was used to cultivate serially diluted portions of the medicinal plant infusion samples. The results revealed that all the herbal preparations had the presence of microbial contaminants. The total heterotrophic counts of the different herbal samples ranged from 0 cfu/mL to 25.0×10^{8}cfu/mL while the total fungal counts ranged from 3.0×10^{6}cfu/mL to 3.5×10^{8}cfu/mL. The total viable bacteria counts showed that the highest counts of 25.0×10^{8}cfu/mL was recorded in the sample from Bosso and the least counts of 0 cfu/mL from Kasuwan-Gwari while the total fungal counts showed that the highest count of 3.5×10^{8}cfu/mL was found in the sample obtained from FUT campus and the least counts of 3.0×10^{6}cfu/mL in the sample from Mai-Kunkele. One way analysis of variance (ANOVA) showed that there was significant difference ($p < 0.05$) in the microbial load of the herbal infusions from each location. The microbial isolates identified were E. coli, Staphylococcus aureus, Shigella sp, Klebsiella sp, Pseudomonas sp, Micrococcus sp, Salmonella sp, Aspergillus sp, Penicillium sp and Saccharomyces cerevisiae. Members of the genus Aspergillus were found to be predominant. This suggests that the herbal infusion harbors microorganisms that could be hazardous to human health and hence producers should maintain the highest possible level of hygiene during the processing and packaging of the products in order to ensure safety of the products.

Keywords: *Contamination; Herbal infusion; Isolation; Microorganisms*

Introduction

Herbal medicine, a form of complementary and alternative medicine, is described as the total combination of knowledge and practices used in preventing or eliminating physical, mental or social disease and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing (Sofowora, 1993). Herbal drugs are crude preparations of various kinds of medicinal plants or any part thereof, such as leaf, stem, root, flower or seed (Hitokotoet al., 1978). The World Health Organization also defined herbal medicine as finished labeled medicinal products that contain as active ingredients aerial or underground parts of plants or other plant materials or combinations thereof whether in the crude state or as plant preparations (WHO, 1996). Plant materials include juices, gums, fatty oils and any other substances of this nature. The use of herbal medicine has always been part of human culture, as some plants possess important

therapeutic properties, which can be used to cure human and other animal diseases (Oyetayo, 2008). Herbal medicine is a primordial healthcare known to mankind. It has been practiced by all cultures throughout history and has become an integral parts of development of modern civilization (Girish*et al.,* 2007).

In the past, medicinal plants was the first line of treatment known to man and traditional medicinal practice remain an important part of the primary healthcare delivery system in most of the developing world (Akerele, 1998).Research had it that about 70-80% of the world population especially in developing countries depend on non-convectional medicines mainly of herbal origins for their primary health care because of its easy accessibility and cheapness (WHO, 1998; Sofowora, 2008). The synthetic or convectional medications used in the recent age are derived from plants, for example, the antimalarial drug quinine from *Cinchona* sp (Lamikanra, 1999). Tapsell reported that about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant materials (Tapsell *et al.*, 2006). Most of the herbal preparations are used in different forms. Generally, plants constitute a major source of orthodox medicines and the presence of plant secondary metabolites has been attributed for most plants' therapeutic activities (Sofowora, 2008; Lamikanra, 1994). In Nigeria, there appears to be an overwhelming increase in public awareness and usage of herbal medicinal products in the treatment and/or prevention of diseases. The quality and safety of herbal preparations are of great concern. The WHO (2007) explained that quality is the basis of reproducible efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparations is of utmost importance. Bauer (1998) showed that the quality criteria for herbal drugs are based on a clear scientific definition of the raw materials.

It is difficult to establish comprehensive quality criteria for herbal drugs due to 'professional secrecy' of herbalists, but in order to improve the purity and safety of the products, observation of basic hygiene during preparation, standardization of some physical characteristics such as moisture content, pH and microbiological contamination levels are desirable. The present study was therefore aimed at isolating, characterizing and identifying microbial contaminants in herbal infusion and to determine the microbial load of these herbal infusions.

Materials and Method
Study Area
Minna is a city (estimated population 304,113 in 2007) in West Central Nigeria. It's the capital of Niger State, one of Nigeria's 36 Federal State, and is the Headquarters of Chanchaga Local Government Area. The areas surveyed were Mai-Kunkele, Chanchaga, Maitunbi, Kpakungu, Bosso, Kure market, Mobil, FUT campus, Keteregwari and Kasuwan-Gwari.

Collection of Samples
Ten different samples of locally prepared herbal infusions were bought from ten different locations and designated as A to J. The plant species origins of the herbal drugs were not ascertained, but the infusions were classified based on the diseases cured as claimed by the vendors. Such diseases include malaria, rheumatism, worm, gonorrhoea, staphylococcal infection typhoid fever and pile. The infusions were collected in sterile containers and transported to the laboratory for analyses.

Preparation and Sterilization of Media
The media used were nutrient agar (NA) and Sabouraud dextrose agar (SDA) for enumeration of bacteria and fungi, respectively. They were prepared according to the

manufacturers' guide. They were mixed with distilled water and dissolved by gentle heat to boil. The media were sterilized in an autoclave at 121°C for 15 minutes.

Methodology and Preparation of Samples
The pour plate method as described by Abba *et al*. (2009) was used where 1 mL of the serially diluted sample was transferred into well labelled clean sterile Petri dish and molten agar medium (20 mL) poured. Poured plates were swirled gently to allow for proper distribution of colonies.

Bacteriological Analysis
Total viable count: The method of Abba *et al*. (2009) was used where a tenfold serial dilution of the samples were carried out. Aliquots of 1 mL of the sample were pipetted from the 10^{-6} and 10^{-8} dilution tube into well labeled Petri dishes. Then 20 mL of molten nutrient agar was added into each plate and swirled gently to allow for proper mixing. The plates were incubated for 24 hrs at 37°C. Then the colonies which developed on the plates were counted using a colony counter and expressed as colony forming unit per millilitre (cfu/mL). The samples from each hawker were analyzed in triplicates and the average was recorded. The colonies differing in size, shape and colour were selected from the different plates on nutrient agar and sub-cultured repeatedly to obtain pure isolates. The pure isolates were maintained on agar slant for further characterization and identification.

Mycological Analysis
The fungal count was determined by pipetting 1 mL of the serially diluted herbal infusion on Sabouraud Dextrose Agar (SDA) containing 0.01% chloramphenicol. The plates were incubated for 3 days at ambient temperature.

Characterization and Identification of Isolates
Bacterial Isolates
The characterization and identification of the bacterial isolates were carried out based on cell morphology, Gram's reaction and biochemical tests according to methods described by Oyeleke and Manga, (2008). The isolates were identified by comparing with those of known taxa using the schemes of Cowan and Steel (1973).

Fungal Isolates
The mold isolates were characterized based on the colour of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spores, presence of foot cell, sporangiophore, conidiophores, and the characteristics of the spore head. A small portion of the mycelia growth was carefully picked with the aid of a sterile inoculating needle and placed in a drop of lactophenol cotton blue on a microscopic slide and covered with a cover slip. The slide was examined under the microscope, first with (x10) and then with (x40) objective lens to detect the spores and some special structures of the fungi. The isolates were identified by comparing their characteristics with those of known taxa using the schemes of Domsch and Gams (1970).

Results
Total viable bacterial and fungal counts
The total viable bacterial and fungal counts in herbal infusions from different locations in Minna are shown in Table 1. The total counts in the herbal infusion samples ranged from 0 cfu/mL to 25.0×10^8cfu/mL and 3.0×10^6cfu/mL to 3.5×10^8cfu/mL for bacteria and fungi respectively. The highest counts of 25.0×10^8cfu/mL was recorded in the sample obtained from Bosso and the least count of 0 cfu/mL was recorded in the sample from Kasuwan-Gwari while the highest count of 3.5×10^8cfu/mL was recorded in sample from FUT campus

and the least counts of 3.0×10^6 cfu/mL was recorded in the sample from Maikunkele for bacteria and fungi respectively. There was significant difference in the microbial load from all the locations sampled, except Maikunkele and Mobil.

Table 1: Total viable bacterial and fungal counts obtained in herbal infusion samples from ten different locations

SAMPLE CODE	SAMPLE LOCATION	TVBC (cfu/mL)	TVFC (cfu/mL)
A	Mai-kunkele	5.0×10^{8e}	3.0×10^{6d}
B	Chanchaga	10.0×10^{8d}	5.5×10^{6c}
C	Maitunbi	2.0×10^{6f}	2.2×10^{7f}
D	Kpakungun	15.0×10^{8b}	6.0×10^{6b}
E	Bosso	25.0×10^{8a}	9.0×10^{6a}
F	Kure market	12.5×10^{8c}	3.3×10^{8i}
G	Mobil	5.0×10^{8e}	3.2×10^{8j}
H	FUT	12.5×10^{6g}	3.5×10^{8h}
I	Keteregwari	7.5×10^{6h}	3.9×10^{7e}
J	Kasuwan-Gwari	0^i	1.1×10^{7g}

Keys: TVBC- Total viable bacterial counts, **TVFC**- Total viable fungal counts. Values (a, b, c, d, e, f, g, h, i, j) on the same column with different superscript are significantly different ($p<0.05$) while those with the same superscript are not significantly different ($p>0.05$).

Frequency of occurrence of bacterial isolates in the herbal infusion samples
The frequency of occurrence of bacterial isolates is shown in Table 2 where *E. coli* and *S. aureus* had the highest occurrence of 28.6% while *Pseudomonas* sp and *Shigella* sp had the least occurrence of 4.8% each.

Table 2: Frequency of occurrence of bacterial isolates

S/N	Organism	No	Frequency (%)
1	*E. coli*	6	28.6
2	*S. aureus*	6	28.6
3	*Salmonella* sp	2	9.5
4	*Micrococcus* sp	3	14.3
5	*Klebsiella* sp	2	9.5
6	*Pseudomonas* sp	1	4.8
7	*Shigella* sp	1	4.8
	Total	21	100

Frequency of occurrence of fungal isolates in the herbal infusion samples
The frequency of occurrence of fungal isolates is shown in Table 3 where *Aspergillus niger* had the highest occurrence of 33.3% while *Aspergillus fumigatus* and *Saccharomyces cerevisiae* had the least occurrence of 13.3% each.

Table 3: Frequency of occurrence of fungal isolates

S/N	Organism	No	Frequency (%)
1	*Aspergillus niger*	5	33.3
2	*Aspergillus flavus*	3	20.0
3	*Aspergillus fumigates*	2	13.3
4	*Saccharomyces cerevisiae*	2	13.3
5	*Penicillium* sp	3	20.0
	Total		**100**

Discussion

The results of the present study reveal that there was a remarkable bacterial and fungal contamination of the different herbal infusions sampled. The samples were contaminated to varying degrees with pathogenic bacteria and fungi. One of the ten (10) herbal infusion samples was however free from bacterial contamination, while all the samples showed fungal contaminants. The bacterial counts ranged from 0 cfu/mL to 25.0×10^8cfu/mL and the fungal counts ranged from 3.0×10^6cfu/mL to 3.5×10^8cfu/mL. These results differ from the work of Abba *et al.* (2009) who reported average bacterial counts that ranged between 1.0 x 10^7cfu/g and 1.8 x 10^8cfu/g in powdered herbal preparations sold in Kaduna metropolis. The high counts of bacteria detected in the herbal infusion in this present study may be due to poor hygiene, use of contaminated water for washing and preparation, use of contaminated equipment and contaminated packaging materials. Other possible sources of contaminants are the personnel that could introduce the microbes when handling the raw materials during processing.

Therefore, the process of harvesting, drying, storage, handling and the soil, influence the bacteriological quality of raw materials which in turn affect the entire quality of the herbal infusions. The herbal infusion samples contained high level of bacteria and the counts were beyond the European Pharmacopoeia standard, and also carried pathogenic Gram negative bacteria such as *Salmonella, Shigella and E. coli* that are expected to be absent as reported by Okunlola *et al.* (2007). The predominant fungal isolates obtained in the present study are the members of *Aspergillus* sp. The presence of these fungi in herbal medicines has been reported (Pitt, 2000; Adeleye *et al.*, 2005; Okunlola *et al.*, 2007). The predominance of *Aspergillus* sp in herbal drugs have been reported by Elshafie *et al.*, (2002) and Mandeel (2005).The contamination of herbal preparations by fungi has been attributed to neutral extraneous contamination by dust following storage in humid conditions (Domsch *et al.*, 1981). Some of the identified contaminants have been reported to have the ability to produce mycotoxins (Bugno *et al.*, 2006). For instance, Pohland and Wood (1987) reported that 70 − 80% of penicillia are potential mycotoxin producers. The results of the present study reveal that the herbal infusions preparations were all contaminated by potentially pathogenic microbes. The herbal infusions were, therefore, not sterile and could be a vehicle for transmission of pathogens to the end users. There is, therefore, the need for constant monitoring and quality control of herbal medicinal products sold in Minna metropolis.

Conclusion

The results of the present study reveal that the herbal infusions were all contaminated by pathogenic microbes. It could be concluded from this study that most traditionally prepared herbal medications in Minna metropolis are likely to be contaminated with a wide variety of pathogenic microorganisms. Manufacturers should ensure the highest possible level of hygiene during manufacturing in order to ensure safety of the products so as to maintain correct quality, safety and efficacy of the final herbal preparations.

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