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MICROBIAL QUALITY OF SOME LOCALLY CONSUMED HERBAL CONCOCTIONS IN ABEOKUTA METROPOLIS, NIGERIA

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ABSTRACT

Some herbal samples usually consumed in Abeokuta metropolis, Ogun State were collected for microbiological analysis. The total bacteria count in the samples tested ranged from 0.84 x 10^4 CFU/ml in herbal sample for fistula purchased at Sapon in Abeokuta South Local Government Authority (LGA) to 8.5 x 10^4CFU/ml in the product for skin rash (naarun) treatment purchased from Adatan in Odeda LGA. Some of the bacteria isolates include *Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis* and *Proteus vulgaris*. The fungi isolated and identified also include *Penicillium notatum*, *Rhizopus stolonifer, Aspergillus niger* and *A. flavus*. Antibiotics sensitivity tests performed on some of the bacteria isolates revealed the sensitivities of some of the isolates to such as Ciprofloxacin, Gentamycin etc. Health implications of these microorganisms in consumers were discussed.

Keywords: Herbs, quality, bacteria, fungi, Nigeria

INTRODUCTION

Plants have been used in the prevention, treatment and cure of disorders and disease since ancient times. In spite of their origin, natural drugs should not be viewed as simple tools for folk medicine since they are class of pharmaceutical products and should meet the requirements of quality, safety, and efficacy (Aziz *et al.*, 1998). In the last years, there was a progressive increase in the demand of herbs and preparation of botanical origin as alternative or complementary medicine due to economical, social and cultural factors (Calixto, 2000). The increasing population of natural drugs made their use a public health problem due to lack of effective surveillance in the use, efficacy, toxicity and quality of these natural products.

Indeed, the adverse effect on long term herbal use, adulteration with toxic compounds and contamination by pathogenic microorganisms or natural toxins such as mycotoxins have been reported for herbal products and medicinal plants (Abou-Arab *et al.*, 1999). The concern over quality of the products is mainly due to their potential contamination, considering their natural origin. Practices used in harvesting, handling, storage, production and distribution make medicinal plants subject to contamination by various fungi, yeast and bacteria which may be responsible for spoilage and production of mycotoxins (Roy and Kumari, 1999).

Traditional herbalists in Nigeria use various preparations to treat various types of ailments including diarrhea, cough, neonatal fistula, convulsions, skin diseases etc (Sofowora, 1982). Most of these prepara-

tions are used in the form of concoctions (soup or drink made usually from ingredients after boiling) or infusions (soaking the plant material and allowing it to dry for varying length of time) (Adeleke and Opiah, 2003). Medicinal plant materials normally carry a large number of microbes originating from the soil. Microorganisms of various kinds are normally adhered to leaves, stems, flowers, seeds and root. Additional contaminants may also be introduced during harvesting since no conscious efforts are made to decontaminate the herbs other than washing (Adenike *et al.*, 2007).

Level of herbal concoction in Nigeria is on increase as there have been beliefs of their abilities to cure many diseases. Since preparation of these herbal medicines is not strictly under high hygiene condition, hence the purpose of this research to assess the microbial quality of some of these locally consumed herbal medicines at two locations in Abeokuta metropolis.

MATERIALS AND METHODS

Three types of herbal samples which have been compounded into powder form by the sellers for the treatment of malaria (agbo iba), fistula (jedijedi) and skin rash (agbo 'narun) were purchased from six herbal sellers located at three local government authorities (LGAs) of Abeokuta. The locations were Camp and Adatan, both in Odeda LGA; Itoku and Sapon in Abeokuta South LGA and Lafenwa and Omida in Abeokuta North LGA. In all, eighteen herbal samples in powder form were collected and tested. During purchase, some questions were poised to the sellers, regarding the plant source of the herbs and the dosages with the ideal food good for the dosage (Table 1).

Samples were brought to the laboratory and weights of the normal dosage level were taken, including the pH of the representative samples dissolved in distilled water (Table 1).

Serial dilution and plating of herbal samples

One gram of each herbal sample was introduced into 9 ml sterile distilled water aseptically in a test tube and the content mixed thoroughly.

Ten fold serial dilution of this mixture was prepared by transferring aseptically 1ml of this mixture, using a sterile pipette into a second test tube containing 9 ml sterile distilled water. This procedure was further repeated until higher dilutions were obtained.

One ml of each of these diluents was pippetted into a sterile petri dish and mixed with molten autoclaved nutrient agar at about 45° C that was poured aseptically into the petri dishes. This process was also repeated for potato dextrose agar (PDA) for the isolation of fungi. Plates were incubated at 37°C for 24 h bacteria isolates and at the same temperature for 4-5 days for fungi isolates.

Pure cultures of each of the isolates were obtained through subsequent streakings on nutrient agar and PDA respectively and these were later subcultured on appropriate agar slant in MacCartney bottles. From these, inocula for subsequent studies were obtained.

Bacterial and fungi isolates were characterized based on colonial and cultural morphology and subjection to various biochemical tests which were later confirmed using findings of Sneath *et al*. (1986) The biochemical characteristics performed include Gram's staining, catalase, coagulase, urease, motility, citrate tests and sugar fermentation tests.

Table 1: Sources of herbal samples and methods of application as directed by the sellers

Location	Herbal sample for:	Part & type of plant used	Dosage	Laboratory weight	pH	Ideal food for usage
Camp	Malaria	Stem, tree bark, roots of Hipocratea indica, Nuclea latifolia,Enantia sp., Citrus medicarva and Mangifera indica	Two levelled tablespoons in the morning and night	0.7	5.2	Custard, oat, boiled maize paste (ogi)
	Fistula	Stem, bark, roots of Tetrapleura tetraptera, Ancistrophyllum secundiflorum, Eugenia caryophyllus and Parinari sp.	One levelled spoon in the morning	0.8	6.7	Seven up drink
	Skin infection	Leaves, roots and barks of Lophira alata, Ceiba petandra and Pergu laria daemia	One levelled tablespoon once daily	1.3	4.4	Boiled maize paste
Adatan	Malaria	Stem, tree bark, roots of Hipocratea indica, Nuclea latifolia,Enantia sp., Citrus medicarva and Mangifera indica	Two tablespoonfuls in the morning and night	1.3	5.6	Custard, boiled maize paste
	Fistula	Stem, bark, roots of Tetrapleura tetraptera, Ancistrophyllum secundiflorum, Eugenia caryophyllus and Parinari sp.	One full tablespoon in the morning and at night	0.7	5.3	Boiled maize paste and seven up drink
	Skin infection	Twigs, roots and barks of Lophira alata, Ceiba petandra and Pergu laria daemia	One level table spoon in the morning and night	1.0	5.7	Boiled, maize paste or guinea corn paste

Antibiotics sensitivity tests were performed on two of the opportunistic pathogens isolated during the course of study using disc diffusion method according to CLSI (2011). In all, nine antibiotics namely; Nitrofurantoin, Amoxicillin, Ciprofloxacin, Pefloxacin, Tetracyline, Augmentin, Gentamycin, Ofloxacin and Cotrimoxazole were used.

RESULTS

Herbal samples tested were found to be contaminated with various microorganisms, which after biochemical characterization were confirmed to be *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Micrococcus luteus* etc. Total bacteria count ranged from 0.84 x 10^4 in powder fistula herbal concoction purchased at Sapon in Abeokuta South LGA to 8.5 x 10^4 in herbal sample meant for skin rash (naarun) bought at Adatan in Odeda LGA of Abeokuta.

When grouped into various types of infection they are meant for, the herbal concoction applied for the treatment of Malaria from Adatan, had the highest bacteria count of 8.4 x 10^4 as compared to the concoction obtained from Itoku at Abeokuta South LGA which had 0.94 x 10^4 (Table 2). In herbal samples compounded for Fistula and obtained at different locations of Abeokuta, herbal sample from sapon had the lowest total bacteria count (0.84 x 10^4), while sample purchased at Camp in Odeda LGA had the highest bacteria count (5.2 x 10^4) (Table 3). Microbial load obtained from herbal samples compounded for skin rash and purchased from Adatan in Odeda LGA was highest with total bacteria count of 8.5 x 10^4. Similar samples bought at Sapon in Abeokuta South LGA has count of 0.91 x 10^4 (Table 4). In all the samples tested, the total fungal count was lower than the total bacteria count.

Microbial characterization revealed the contamination of the herb concoctions with various types of microorganisms such as *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Micrococcus luteus* etc.(Figure 1). Prevalence of various bacteria in the total number of herbal samples tested ranged from 66.7 % for *Escherichia coli* to 5.6 % for Salmonella sp., (Figure 1) while that of fungi was 44.4 % for *Penicillium notatum* and 5.6 % for *Aspergillus oryzae* (Figure 2). Other fungi isolated were *A. flavus*, *A. niger* and *Rhizopus stolonifer.*

Table 2: Total Bacterial and Fungal counts from herbal samples meant for Malaria (agbo iba)

Sample tag	Location of purchase	Bacteria count (x 104 CFU / ml)	Fungal count (x 104 CFU / ml)
A	Itoku	0.94	0.30
D	Lafenwa	1.06	0.40
G	Sapon	1.31	0.50
J	Omida	1.35	0.30
M	Camp	6.00	Nt
P	Adatan	8.40	Nt

Table 3: Total Bacterial and Fungal counts from herbal samples meant for Fistula (Jedijedi)

Sample tag	Location of purchase	Bacteria count (x 104 CFU / ml)	Fungal count (x 104 CFU / ml)
B	Itoku	1.05	0.2
E	Lafenwa	1.34	0.5
H	Sapon	0.84	0.1
K	Omida	1.01	0.1
N	Camp	5.2	Nt
Q	Adatan	3.9	Nt

Table 4: Total Bacterial and Fungal counts from herbal samples meant for Skin rash (Naarun)

Sample tag	Location of purchase	Bacteria count (x 104 CFU / ml)	Fungal count (x 104 CFU / ml)
C	Itoku	1.01	0.4
F	Lafenwa	0.96	0.1
I	Sapon	0.91	0.1
L	Omida	0.93	0.2
O	Camp	6.8	Nt
R	Adatan	8.5	Nt

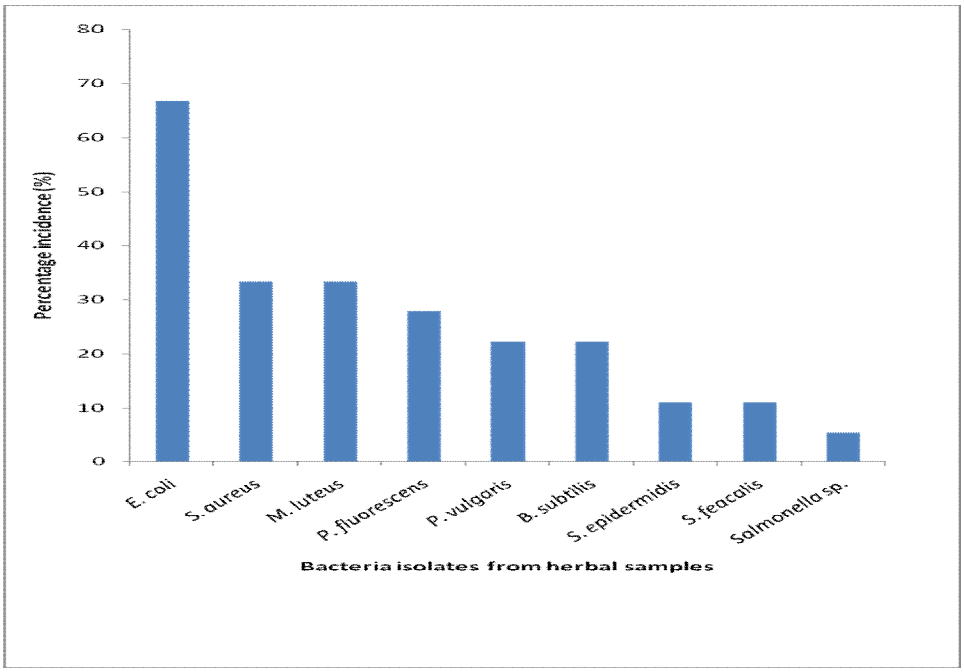

Figure 1: Prevalence of Bacteria isolates detected in the herbal samples

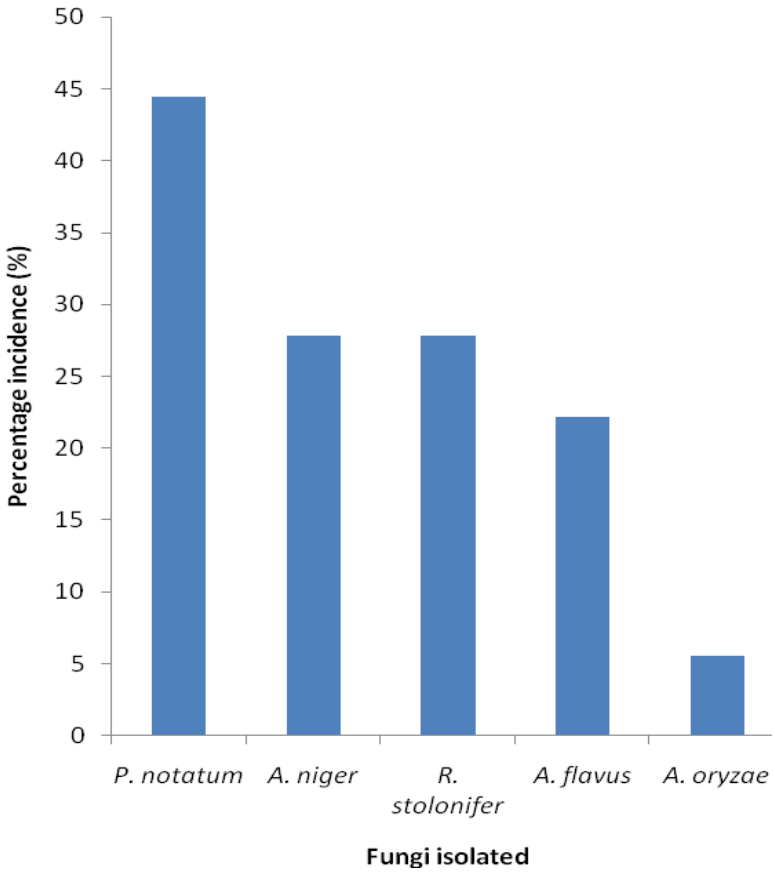

Figure 2: Prevalence of fungi isolates detected in the herbal samples

The antibiotics sensitivity tests performed on isolates of *Bacillus subtilis* and *E. coli* showed all the antibiotics to resist the growth of *B. subtilis* with the exception of Augmentin while *E.coli* was sensitive to the inhibition properties of all the antibiotics applied with exception of Amoxicillin and Augmentin (Table 5).

Table 5: Antibiotics sensitivity tests on the isolated opportunistic pathogens

Antibiotic (conc: µg/disc)	Microorganism tested: Bacillus subtilis	Escherichia coli
	Zone of inhibition (mm)*:	
Nitrofuratoin (30)	28	25
Amoxicillin (10)	24	7
Ciprofloxacin (10)	31	28
Pefloxacin (10)	30	28
Tetracycline (30)	29	27
Augmentin (5/25)	9	10
Gentamycin (10)	21	24
Ofloxacin (10)	29	27
Cotrimoxazole (5/25)	23	23

Key: *16mm and above = Sensitive; 0-12 mm = Resistant ; 13-16mm Intermediate

DISCUSSION

There were indications that the herbal concoctions being consumed by most dwellers in three local government areas of Abeokuta in Ogun State, Nigeria are contaminated, hence might have health implications on the consumers.

Herbal medicines harbour various hazardous microorganisms. This is because herbs are made from trees and plants which have microorganisms adhered to their stems, barks, leaves, flowers, fruits and roots. Though these microorganisms exist in their natural environment, and are normal floras of the tree, they could be source of infection, when in contact with human body.

The microbiological background of herbal medicines depends on several environmental factors and exerts an important impact on the overall quality of the herbal products and preparations (Akerele, 1993). Kolajo (2000) isolated similar bacteria as found in this study and remarked that the presence of these microorganisms in herbal medicines signifies the sub standard production processing of the herbs. Poor hygiene conditions during the production of herbs could introduce non -existing microorganisms into the herbs.

Such microorganisms might originate from the dust in the atmosphere, from tools used in herbal preparation, or from herbal seller himself especially when not physically healthy. Since these herbs do not go through proper sterilization technique, these microorganisms might have been able to survive all the production processes of the herbs, thus the high microbial counts observed in all the herb samples tested.

The ability of some of the antibiotics applied in the sensitivity tests to resist the growth of the two opportunistic pathogens such as *B. subtilis* and *E. coli* indicated the potency of these orthodox medicine against such bacteria and might be resulted to by herbal consumer in case of probable infection after herbal consumption.

Since applications of herbal medicines for curative purposes is on increase, there is a need for risk assessment of microbial load of the medicinal plants at critical control points during processing. Nigerian government also need to introduce some standards that must be met by every herbal processor and seller. There is a need for good sanitation training of all herbal processors so as to safeguard the health of herbal consumers.

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