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MICROBIOLOGICAL CHARACTERISTICS AND PHYTOCHEMICAL SCREENING OF SOME HERBAL TEAS IN NIGERIA

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Abstract

Aims: The present study was undertaken to investigate the bacterial and fungal contamination of herbal teas in the Nigerian market with special reference to Benin City.

Methodology And Results: Twenty-six (26) samples of five different types of herbal teas: antidiarrhoeal, antimalarial, antiobesity (slimming), antihypertensive and antidiabetic teas were analysed using standard microbiological procedures. All samples of the herbal teas were contaminated with both bacteria and fungi. The bacteria count ranged from $1.1x10^1$ to $4.8x10^2$cfu/g. The fungal count ranged from $1.1x10^2$ to $4.5x10^5$cfu/g. Bacterial isolates from herbal teas include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomanas flourecens*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcenscens*, *Salmonella typhimurium*, and *Escherichia coli.* Fungal isolates were *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Rhizopus stolonifer* and *Fusarium solanii.* Of all the bacterial isolates, *Bacillus subtilis* had the highest occurrence (100%) and the least was *Salmonella typhimurium* (3%). Among the fungi, *Aspergillus niger* had the highest occurrence (100%) and the least was *Rhizopus stolonifer* (10%). The bacteria isolates showed multiple resistace pattern to the antibiotics with all gram-negative exhibiting resistance to ampicillin. Phytochemical screening of the herbal teas revealed the presence of flavonoids, alkaloids, phenols, tannin and saponins.

Conclusion, Significance And Impact Of Study: The presence of secondary metabolites justify the use of herbal teas.Good manufacturing practice and proper quality control is however needed for the continued use of the products to curtail antibiotic resistance.

Keywords: Herbal Products, Quality Issues, Microbial Contamination, Safety

Introduction

A herb in phytomedicine denote a plant or plant part that is employed in medicine-making to aid the healing process in time of illness or disease. Herbal teas consist of exclusively of one or more plant part such as leaves, flower, bark or seeds prepared by means of decoction or infusion. Herbal teas are wonderful, low calorie and relaxing drinks. They have beautiful fragrance and are very appealing. When brewed for 5-10 minutes are immediately taken. Herbal teas are usually supplied in wholesale quantity or in retail sachets (Lai and Roy, 2004). Many herbal teas are found around the world. Bush tea (*Athrixia phylicodes*) and lemon grass are popular beverages used as herbal teas and as a medicinal plant by traditional African people (Roberts, 1990). Green tea is a herbal tea of great health benefit that it is called the wonder herb. This product has high concentration of total polyphenols which are known to exert a wide range of beneficial biochemical and physiological properties (Hirasawa *et al*., 2002). Consumption of green tea lowers the risk of cancer, lowers cholesterol and triglyceride levels and also prevents dental diseases owing to its polyphenol (a potent antioxidant) content. A major polyphenol antioxidant reported in green tea is epigallocatechin-gallate (EGCG) (Katiyara and Mukhtar, 1996). Bush tea leaves contain a flavonoid called 5-hydroxy-6,7,3',4', 5' – hexamethoxy flavon 3-ol, a compound with biological activity as reported by Mashimbye *et al*. (2006). Dried hibiscus flowers made into tea is known to reduce high blood pressure and cholesterol as well as strenghten the immune system because its rich in vitamin C. This tea which is rich in antioxidants is widely consumed as zobo drink in Nigeria. McGaw *et al*. (2007) reported that bush tea leaves do not contain caffeine or pyrrolizidine alkaloids, thus justifying its medical potential. Ivanova *et al*. (2005) reported that the roles of herbal tea in disease prevention and cure have been partly attributed to the antioxidant properties of phenolic compounds present in their extracts. The presence of total phenols in tea leaves are the main potential indicators for medicinal usage due to their antioxidant activities (Hirasawa *et al*., 2002; Mogotlane *et al*., 2007).

Herbal teas have many health benefits and they taste great too. The widespread popularity of herbal teas such as green tea, rooibos and chamomille is due to their perceived therapeutic effects against some chronic diseases (Joubert *et al.*, 2008). When administered, chamomille has been found to sooth the stomach, relieves indigestion, fight insomnia, calms the body and mind thus relieving stress. Decoctions of spearmint (*Mentha spicata*), marjoram (*Origanum marjorana*), thyme (*Thymus vulgaris*) and

papaya-mint tea are primarily consumed to treat digestive ailments, to reduce fever and for their anti-inflammatory, anti-oxidant and antimicrobial properties (Abe *et al*., 2004; Dorman *et al*., 2004). Despite the significant role of herbal teas in improving nutrition and health, there have been reports of microbial contamination and adverse effects resulting from their consumption. These include neurological, cardiovascular and haematological hazards (Palmer *et al*., 2003). Toxin-producing microbial contaminants are often the cause of these adverse effects. Therefore, it is important to identify the microbial contaminants of herbal tea products as indicators of safety and quality (Schweiggert *et al*., 2005).

A few reports demonstrating microbial contamination of medicinal herbs from various parts of the world exist in the literature. Rizzo *et al*. (2004) indicated that medicinal plants in Argentina harbored toxigenic fungi such as *A. flavus*, *A. parasiticus* and several members of the Genus *Fusarium*. Efuntoye (1999) showed that dried medicinal plants from Nigeria herb markets contained *A. flavus*, *A. parasiticus* and *A.ochraceus*. He added that the above fungal isolates were capable of producing mycotoxins when grown on semi-synthetic media. Martins *et al*. (2001), after evaluating several medicinal herbs obtained from Portugese markets, reported that the commodities were infested with a variety of moulds such as *Aspergillus* and *Fusarium spp*. Halt (1998) isolated a wide spectrum of fungi (including *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Rhizopus* and *Mucor* species) from Croatian herbal teas and medicinal plants. Czech *et al*. (2001) reported bacterial and fungal contamination of medicinal herbs in Austria, while Skorska *et al*. (2005) showed that the air in chamomile and peppermint processing farms in Poland contained high levels of *Pantoea agglomerans* and other gram negative bacteria. Such reports indicate the existence of a ubiquitous problem. In Benin City, Nigeria, there are many retail outlets for a host of herbal teas with promising cure for many ailments. A large number of people rely on these teas for cure, howbeit without thinking of the microbiological characteristics. This research was designed to determine the bacteriological and fungal contamination of herbal teas in the Nigerian market with special reference to Benin City.

Materials And Methods

Sources Of Samples

Twenty-six (26) samples of five different types of herbal teas: antidiarrheal, antimalarial and antiobesity (slimming), antihypertensive and antidiabetic teas were obtained from herbal vendors in Benin City. They were then transported to the laboratory for microbiological analysis.

Mycological Analysis

One gram (1g) of each sample was aseptically removed from the sachet and transferred to a sterile mortar. Subsequently each sample was

blended in 10ml of 0.1% peptone water for 1minute. Serial dilutions of the homogenate (in 0.1% peptone water) were surface plated in duplicate in potatoe dextrose agar (PDA) (DIFCO, Detroit, MI, USA) containing 0.01% chloramphenicol (0.1%ml/plate) for the inhibition of bacteria contaminants. The agar plates were incubated for 3-5 days at 28± 2^0C. Then, colonies were counted after incubation and counts were expressed as colony forming units per gram (cfu/g). Fungal isolates were purified on PDA and further sub-cultured on malt extract agar (MEA) for microscopic examination and identification. Identification was performed according to the methods and keys described by Pitt and Hocking (1997).

Bacteriological Analysis

The total aerobic bacteria count was determined as follows: 1g sample portion was aseptically transferred into sterile mortar with pestle and 9ml of peptone water was added. The sample was then beaten into a homogenate. Serial dilutions were surfaced plated in duplicate nutrient agar plates and incubated at 37^0C for 24-48 hours. Plate reading and colony count were reported as colony forming unit per gram (cfu/g) (Collins *et al.,* 2004).

Identification Of Microorganisms

Bacteria isolates from herbal teas were examined for their pigmentation/color, elevation and growth pattern and inspected by light microscopy. The bacteria were Gram-stained (Collins *et al.,* 2004). Phenotypic profiling of both gram-positive and gram-negative bacteria was undertaken using API 50CHB and API 20E strips (BioMerieux, Marsielle, France). Additional tests of spore stain and catalase were also performed. Fungi were characterized according to the methods of Barnett and Hunter (1998).

Antibiotic sensitivity test.

Susceptibility tests of bacteria isolates was performed with Muller-Hinton agar using the disc diffusion method (Bauer *et al*., 1966). A cocktail of five distinct colonies of the pure culture of the isolate was transferred to Tryptone Soy Broth (TSB) and incubated for 3h at 37^0C for growth to the 0.5 McFarland BaSO4 turbidity. From this growth medium a 1ml inoculum was seedeed onto the Mueller-Hinton agar plates. Commercially prepared antibiotic discs (Abtek Biologicals ltd., England and AB-Biodisc, Sweden) containing ciprofloxacin (5µg), perfloxacin (5µg), ofloxacin (5µg), norfloxacin (10µg), nitrofurantoin (300µg), amoxicillin (10µg), gentamicin (10µg), nalidixic acid (30µg), tetracycline 10µg), erythromycin (5µg), penicillin (10µg), augmentin (30µg), cloxacillin (5µg), cotrimoxazole (25µg), chloramphenicol (30µg) were used. The discs were placed on Muller-Hinton agar plates which were seeded with the broth culture of the test organisms. The plates were inverted and left on the work bench for 30min to allow for diffusion of antibiotics into the agar. This was followed

by incubation at 37^0C for 48h after which zones of inhibition were examined and interpreted accordingly using standard chart (NCCLS, 2003). Earlier, the potencies of all the antibiotics used were confirmed using susceptible *E coli* strains.

Phytochemical Screening

Herbal teas were tested for alkaloids, flavonoids, saponins, volatile oils, tannins and phenols. according to the methods of Trease and Evans (1996).

Results

Microbial Burden Of Herbal Teas

Bacterial and fungal isolates from various herbal teas are shown in Table 1. Bacterial isolate B_1, B_2, B_3, B_4, B_5 and B_6, B_7, B_8, and B_9 were identified as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas flourescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcencens*, *Salmonella typhimurium* and *Escherichia coli* respectively. Fungal isolates F_1, F_2, F_3, F_4 and F_5 were identified as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium expansum*, *Rhizopus* stolonifer, and *Fusarium* solanii respectively.

The microbial burden of herbal teas in this study is presented in Table 2 while the frequencies of microbial isolates are shown in Table 3. Bacteria count in antidiarrhoea tea (AD) ranged from 1.1×10^1- 2.6×10^2 cfu/g. Fungal count in this product ranged from $3.2 \times 10^2 - 4.5 \times 10^5$ cfu/g. The bacteria burden in antimalarial (AM) tea ranged from 2.5×10^1- 1.7×10^2 cfu/g while fungal count in this product ranged between $1.3 \times 10^2 - 2.5 \times 10^4$ cfu/g. Antihypertensive tea had bacterial count of 4.8×10^1- 3.6×10^2 cfu/g. The fungal count in this tea was $4.0 \times 10^2 - 3.8 \times 10^5$ cfu/g. The bacteria in antiobesity (AO) or slimming teas was low compared to the rest and ranged between 1.1×10^1- 2.5×10^2 cfu/g. The fungal count in this herbal product was between $2.4 \times 10^2 - 5.6 \times 10^3$ cfu/g. Antidiabetic tea had a bacteria count of between 7.6×10^1-6.1×10^2 cfu/g. Fungal count in this product was low ranging from $0.24 \times 10^2 - 1.1 \times 10^2$ cfu/g.

On the whole the highest bacteria count was found in antidiabetic tea (SAMPLE ADi3) with a total count of 5.00×10^2 cfu/g. The highest fungal count was recorded for antidiarrhoea tea (AD) with a total count of 4.5×10^5 cfu/g. Among the fungal isolates *Aspergillus niger* was more frequently isolated from the herbal teas (100%) while the least was *Rhizopus stolonifer* (10%).

Bacillus subtilis was more predominant (100%) compared to the rest bacterial isolates in the herbal teas. In Table 3 is shown the antibiotic resistance patterns of bacterial isolates from herbal teas. The bacteria isolated in this study were sensitive to fluoroquinolones (ciprofloxacin) however demonstrated resistance to ampicillin, augmentin, amoxicillin, erythromycin

and cotrimoxazole. The two strains of *Staphylococcus aureus* isolated from the herbal teas were resistant to augmentin, amoxicillin, trimethoprim, erythromycin, tetracycline, cloxacillin and penicillin. All *Escherichia coli* strains were resistant to ampicillin. Infact most of the gram-negative organisms isolated were resistant to the β-lactam antibiotics such as ampicillin. This is an indication that they could be producers of β-lactamase. The result of this study showed that the coagulase negative Staphylococci *(Staphyloccus epidermidis*) had resistance to the antibiotics against which it was tested. This include tetracycline, erythromycin, fusidic acid, norfloxacin and cotrimoxazole. On the whole, all the isolates in this study exhibited multiple antibiotic resistance patterns ranging from three to six (Table 3).

Phytochemical analysis of the herbal teas showed that they contain tannins, flavonoids, alkaloids and saponins and volatile oils and phenols (Table 4). Of these secondary metabolites, antidiarrhoea herbal tea contained tannin, saponin, flavonoid and phenols. Saponins and volatile oils where not found in antimalarial and antihypertensive herbal teas. Antidiabetic herbal tea was found to contain alkaloids,volatile oils, flavonoids and phenols. The slimming tea (slimming tea) had alkaloids, saponins, flavonoids and phenols but was devoid of volatile oils and tannins.

Discussion

The presence of microorganisms in medicinal plants is common but medically unfavourable phenomenon. The results of the present study demonstrated the presence of both saprophytic and pathogenic microbial flora. The aerobic plate counts from herbal teas showed the contamination of 100% of the samples analyzed. The presence and numbers of bacteria could be explained by the fact that some of these organisms like *Bacillus* produce spores which are resistant to harsh processing elevated heat and dry conditions. Therefore they can survive for a long time in the product in a dormant state. This result is corroborated by the report of (Martins, 2001), who isolated *Bacillus cereus* and *Clostridium perfrigens* from Chamomile and other herbs. Also, part of the bacterial bio-burden may have originated from the personnel handling the tea materials after processing, especially if strict good manufacturing practices (GMPs) and hygienic conditions were not followed. *Staphylococus aureus* and *Staphylococcus epidermidis* are organisms which can be transferred from humans to teas during processing. Microbial loads of the herbal products analyzed may have also originated from processing plants, the environment and air. Settled dust in herbs processing plants are often highly contaminated with bacteria which could add to the microbial burden already present in the commodity brought in from the field. Dutkiewiez *et al*. (2001) reported the presence of *Bacillus cereus, Alkaligenes faecalis,* various fungi, and *actinomycetes* from herb such as mint, sage and marjoran processing plants. Some of these organisms

are capable of causing human infections allergies and/or producing endotoxins which makes them health risks (Dohmae *et al.*, 2008). Thus care should be taken to reduce such contaminants mainly by following strict GMPs at all stages of processing.

The fungi isolated from the herbal teas in this study as shown in table 2 includes *Aspergillus niger, Aspergillus flavus, Penicillium expansum* and *Fusarium solanii*. *Aspergillus niger* was the most frequently encountered mould found in 100% of the samples tested. The presence of these fungi in herbal teas in this study is corroborated by the reports of Lee and Jo (2006). These authors noted that *Aspergillus niger, A. flavus, Rhizopus sp*. and *Alternaria alternata* are common air contaminants probably present in the drying and packing areas. At such low numbers seen in the present study, there is no indication that these organisms were growing on the product. However if the storage conditions were to change, that is, if the moisture of the product was to increase they could proliferate and spoil the product, and possibly produce mycotoxins (Zhang *et al*., 2005). The isolation of various Aspergilli in this study especially *Aspergillus flavus* is of the highest concern because it is known to produce aflatoxin and can grow at low water activities (Riba *et al*., 2008). Thus in order to avoid such growth and possible production of toxic metabolites, care should be taken to dry the product quickly before these moulds have the chance of establishing any significant growth. The frequency of isolation of *Fusarium solanii* though low (20%) in this study is also worrisome. This plant pathogen of papaya in the fields produce spores which can survive drying conditions and remain dormant for several months possibly years on the dried herb (Tournas and Katsoudas, 2008). During that time, if the moisture of the product increase to levels allowing spore germination, significant mould growth and possibly mycotoxin production could occur. Omurtag and Yazigoglu, (2004) reported the production of 160ppb fumomisin B 1 by *Fusarium* in mint herbal tea. *Penicillium expansum*. and *Rhizopus stolonifer* isolates in this study may have originated as contaminants from the herb itself used in preparing the herbal teas. Although they are saprophytic, they can cause skin and mucous membrane infections.

The relatively high level of resistance to antimicrobial agents is a reflection of possible misuse or abuse of these agents in the environment (Malik and Ahmad, 1994). Antibiotics prescriptions in some hospitals are given without clear evidence of infection or adequate medical indication. Furthermore some sick folks obtain drugs over the counter without medical advice. The resistance of *Staphylococcus epidermidis* to tetracycline and cotrimoxazole is not surprising because these antibiotics are seriously abused by over-use in the study area. This is corroborated by the reports of Okeke *et al.* (1999). Multiple drug resistance evidenced from this study poses a

serious public health problem as it can lead to an outbreak of major epidemic (Prescott *et al.*, 2002).

The presence of secondary metabolites in the herbal teas may attribute to their use by traditional medicine practioners in the treatment of different ailments (Omogbai and Eze, 2011). However it is important for regulatory agencies like NAFDAC (National Agency for Food, Drug Administration and Control, Nigeria) to monitor the quality of these herbal teas since microbial contamination can lead not only to deterioration but decrease or loss of efficacy of the product.

Conclusion

The results of this study has shown that microorganisms associated with herbal teas belong to either saprophytic or pathogen flora. High bacterial or fungal contamination could constitute a health hazard if the organisms present are pathogenic or capable of producing toxin under favourable conditions. Thus good manufacturing practices (GMPs) and appropriate hygiene should be adhered to for high quality herbal teas.

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Table 1: Microbial Isolates from Herbal teas

Microorganism	**% Occurrence**
Fungi	
Aspergillus niger	100
Aspergillus flavus	45
Penicillium expansum	60
Rhizopus stolonifer	10
Fusarium solanii	20
Bacteria	
Staphylococcus aureus	90
Staphylococcus epidermidis	60
Bacillus subtilis	100
Pseudomonas aeruginosa	40
Escherichia coli	10
Klebsiella pneumoniae	8
Serratia marcencens	5
Salmonella typhimurium	3
Pseudomonas fluorescens	15

Table 2: Microbial Burden Of Herbal Teas

PRODUCT	SAMPLE TESTED	BACTERIAL COUNT (cfu/g)	FUNGAL COUNT (cfu/g)
Antidiarrhoea (AD)	AD1	1.8×10^{2}	4.5×10^{5}
	AD2	1.4×10^{2}	3.2×10^{2}
	AD3	8.3×10^{1}	4.1×10^{4}
	AD4	2.6×10^{2}	2.9×10^{3}
	AD5	1.1×10^{1}	6.3×10^{2}
Antimalaria (AM)	AM1	5.5×10^{1}	2.5×10^{4}
	AM2	9.6×10^{1}	2.2×10^{4}
	AM3	1.7×10^{2}	1.92×10^{4}
	AM4	7.9×10^{1}	1.6×10^{2}
	AM5	6.8×10^{1}	1.46×10^{3}
	AM6	2.5×10^{1}	1.3×10^{2}
Antihypertensive (AH)	AH1	2.7×10^{2}	3.8×10^{5}
	AH2	3.6×10^{2}	4.7×10^{4}
	AH3	4.8×10^{1}	4.0×10^{2}
	AH4	7.9×10^{1}	3.6×10^{3}
Antiobesity (AO) (Slimming tea)	AO1	1.1×10^{1}	2.4×10^{2}
	AO2	2.5×10^{2}	7.4×10^{2}
	AO3	4.7×10^{1}	3.1×10^{2}
	AO4	3.2×10^{1}	4.8×10^{3}
	AO5	4.0×10^{1}	5.6×10^{3}
Antidiabetic (ADi)	ADi1	2.3×10^{2}	0.48×10^{2}
	ADi2	4.8×10^{2}	0.36×10^{2}

ADi3	5.0×10^{2}	0.73×10^{2}
ADi4	7.6×10^{1}	0.24×10^{2}
ADi5	8.9×10^{1}	1.1×10^{2}
ADi6	6.1×10^{2}	1.1×10^{2}

Table 3: Antibiotic Resistance Patterns of Bacteria Isolates from Herbal Teas.

BACTERIAL ISOLATES	ISOLATE CODE	MULTIPLE ANTIBIOTIC RESISTOTYPES	NO OF ANTIBIOTICS
Staphyloccus aureus	SA001	AMP.PEN,ERY,TET,CXC	5
Staphylococcus aureus	SA002	AMP,AMX,TRM	3
Staphylococcus epidermidis	SE001	COT,TET,ERY,FUS,NOF	5
Escherichia coli	ES001	AMP,COT,TET,NAL,GEN	5
Escherichia coli	ES002	AMP,AUG,AMX	3
Escherichia coli	ES003	AMP,AUG,AMX,ERY,CXC	5
Escherichia coli	ES004	AMP,AUG,ERY,TET,CXC,GEN	6
Pseudomonas aeruginosa	PA001	AMP,COT,GEN,STR	4
Pseudomonas aeruginosa	PA002	AMP,COT,GEN,NAL,NIT	5
Bacillus subtilis	BS001	AUG,AMX,TET,CXC	4
Bacillus subtilis	BS002	AUG,AMX,CXC,COT	4
Klebsiella pneumoniae	KP001	AMP,NIT,COT	3
Klebsiella pneumoniae	KP002	AMP,ERY,COT,NIT	4
Serratia marcencens	SM001	AMP,TET,ERY,GEN,NAL	5
Salmonella typhimurium	ST001	AMP,ERY,TET,GEN	4

Table 4: Phytochemical Composition of Herbal Teas.

Herbal Tea	**Phytochemical constituent**					
	Alkaloids	**Tannins**	**Saponins**	**Volatile oil**	**Flavonoids**	**Phenols**
Antidiarrhoea	-	+	+	-	+	+
Antimalaria	+	+	-	-	+	+
Antihypertensive	+	+	-	-	+	+
Antidiabetic	+	-	-	+	+	+
Antiobesity (Slimming Tea)	+	-	+	-	+	+

+ = Present, - = Absent