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THE ORGANOLEPTIC AND MICROBIAL QUALITY OF SOME HERBAL MEDICINAL PRODUCTS MARKETED IN FREETOWN, SIERRA LEONE.

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Abstract

Background: The WHO has advocated for the integration of herbal medicinal products into the primary health care system of developing countries. Safety, however, is a concern to the drug regulatory bodies. This study was carried out to determine the organoleptic properties and the microbial quality of herbal products available to consumers in the Freetown metropolis of Sierra Leone.
Materials and Methods: Twenty herbal preparations purchased within the Freetown metropolis were assessed for taste, colour, odour, and pH. The microbial load and the presence of pathogens were determined. The residual antibacterial activity of two of the preparations that showed no microbial contamination was determined using the cup plate method. The ability of a combination of methyl- and propyl-parabens to prevent growth in some of the herbal products was studied.
Results: It was found that 80% of the samples contained mean bacterial and fungal counts ranging from 1.47 x 10^8 to 9.375 x 10^8cfu/ml and 3.45x10^8 to 1.6x10^9cfu/ml, respectively. The bacterial contaminants were predominantly Gram-positive organisms of the genera Bacillus and Staphylococcus. *Escherichia coli*, *Salmonell*a spp. and *Shigella* spp. were among the isolated pathogens. *Aspergillus* spp., *Trichoderma harzianum*, *Candida albicans* and *Cryptococcus neoformans* were the predominant fungal contaminants. Two of the herbal samples from which no contaminants were recovered inhibited test organisms while the tested preservative system consisting of a mixture of methyl- and propyl-para hydroxyl benzoic acid in the ratio 2:1 and a use concentration of 0.2%w/v completely inhibited growth in tested samples.
Conclusion: The findings of the study suggest that many of the herbal medicinal preparations marketed in Freetown are likely to be contaminated with potentially pathogenic microorganisms. The microbial quality of these herbal products may however be improved by the incorporation of appropriate preservatives.

Key words: Herbal products, Sierra Leone, organoleptic properties, microbial quality, preservatives

Introduction

According to the definition by the World Health Organization (WHO), herbal medicines are raw or dried medicinal plants or any part thereof, such as the leaves, stems, roots, flowers, fruits or seeds used for medicinal purposes (WHO, 1998). Herbal medicines have a long history of use in therapy and they are still an important component of traditional medicine. The WHO estimates that four billion people (about 65 to 80 percent) of the world population, particularly in developing countries, use herbal medicines for some aspect of primary health care (WHO, 1998). This is because herbal medicines are accessible and cheap, often providing first line treatment to people living in remote and inaccessible areas where it is the only available alternative medicine remedy (Sofowora, 1993). The continued use of the herbal medicinal products made the WHO to advocate for the integration of herbal medicinal products into the primary health care system of developing countries (WHO, 1998). The people of Sierra Leone, like in most other countries, recognize the use of herbal medicines for the treatment and or prevention of certain diseases. A great number of residents in Freetown and other parts of the country are believed to depend on traditional herbalists for their medical needs. With the ever increasing use of these herbal medicines and the global expansion of the herbal medicine market generally, safety has become a concern for both the health authorities and the general public in many countries and studies carried out in some other locations have shown the presence of potential contaminants in herbal preparations (Arias et al., 1999; Erich et al., 2001; Wolfgang et al., 2002; Adeleye et al., 2005; Okunlola et al., 2007). There is, however, no documented data on the microbiological quality of herbal medicines in Sierra Leone.

This study was, therefore, carried out to assess the microbial quality of herbal medicines marketed in Freetown, Sierra Leone with a view to making the results obtained available to the relevant regulatory body for necessary steps in ensuring the quality of herbal medicines on sale in the country.

Methodology

Collection of Samples

A total of 20 (10 powders and 10 liquids), different, packaged herbal preparations were purchased randomly from identified herbal shops and retail outlets in different parts of the Freetown metropolis. The herbal preparations were kept at room temperature and analyzed microbiologically at the Microbiology Laboratory of the Pharmacy Board of Sierra Leone, Freetown.
The 20 herbal samples were documented and coded as shown in Table 1.

Determination of the pH of Samples

The pH of each of the liquid samples was measured directly using the pH meter (Hannah Instruments, UK). For the powdered samples, a 10% solution of each was prepared by weighing 10g of the sample into 100ml distilled water in a 200ml beaker and then shaken. Thereafter, the pH was read on the Hannah pH meter.

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Determination of the Organoleptic Properties of Samples

The colour, odour and taste of each sample were determined by visual inspection, perception of odour and tasting, respectively.

Microbiological Studies
Determination of Bacterial and Fungal Counts

Each of the 10 liquid herbal samples was shaken properly to ensure a uniform distribution of contents in each sample. Serial 10 fold dilutions in sterile water were carried out and 1ml of each dilution was aseptically placed into sterile petri-dishes in duplicates. 20ml of molten Nutrient agar (Oxoid, England) cooled to 45°C for bacterial count or Sabouraud Dextrose agar (Oxoid, England) for fungi was later added to each of the plates and gently mixed. The mixture was allowed to solidify and the plates incubated at 37°C for 24h for bacterial and 25°C for 7 days for fungal populations.

Table 1: Code numbers, trade names and dosage forms of herbal samples

Code number	Trade name of product sample	Dosage form
1.	Sielco	Liquid
2.	Axis	Liquid
3.	Smilax	Liquid
4.	Agbo	Liquid
5.	Broomstone	Liquid
6.	Egbeshi	Liquid
7.	Niruri	Liquid
8.	SCC	Liquid
9.	'Common' root	Liquid
10.	Popa	Liquid
11.	King songo	Powder
12.	Moringa	Powder
13.	Kushiment	Powder
14.	Gbangba	Powder
15.	Ojologbo	Powder
16.	Wojo	Powder
17.	Mopia	Powder
18.	Binthai	Powder
19.	Gambia tea bush	Powder
20.	Songo lemon grass tea	Powder

For each of the 10 powdered herbal samples, the method of Colle and Miles (1989) was employed with some modifications. A stock solution of the sample was prepared by weighing 1g of the solid sample into 25 ml of sterile water and shaking properly. Thereafter, 10 fold serial dilutions, count plates and incubation as done for the liquid samples were carried out. All the procedures were performed under the laminar airflow cabinet using aseptic techniques. The colonies obtained on each plate after incubation were counted using the Stuart Digital Colony counter (Hannah instruments, UK) and expressed as colony forming units per milliliter (cfu / ml) of liquid samples and colony forming units per gramme (cfu / g) of the solid samples .

Isolation of Bacterial Contaminants

Each colony having distinct colonial characteristics such as colour, shape, consistency and elevation, growing on each plate was picked and streaked onto freshly prepared Nutrient agar plates and incubated at 37 °C for 24 h. The colonial morphology of the bacteria on each plate was documented and each was then streaked onto Nutrient agar slope, incubated for 24 h and stored in the refrigerator for further identification by Gram-staining and biochemical tests which included citrate utilization test, Triple Sugar Iron agar test and urease test.

Isolation and Identification of Specific Pathogenic Bacteria
Pre-enrichment

To facilitate the quick recovery of contaminants, 1 g of each powdered sample and 1ml of each liquid sample was added to respective bottles of 9 ml peptone water and incubated for 18 h at 37 °C.

Isolation and Identification of *Staphylococcus aureus*

Isolation of *S. aureus* was done by spreading 0.1 ml of the pre-enriched 18 h old peptone water culture of each of the samples onto freshly prepared sterile Mannitol Salt agar (MSA) plates. The plates were then incubated at 37 °C for 48 h and observed for growth and colour change. Confirmation of the identity of the isolated organism was done by carrying out Gram-staining and biochemical tests which included the catalase and coagulase tests.

Isolation and Identification of *Escherichia coli*

Isolation of *E. coli* was done by spreading 0.1 ml of a pre-enriched 18 h old peptone water culture of each of the samples onto freshly prepared sterile MacConkey agar plates. The plates were incubated at 37 °C for 24 h and observed for growth. Colonies from the MacConkey

agar were then streaked onto the surface of Eosin Methylene Blue (EMB) agar plates and incubated for 24 h at 37 ^{o}C. The plates were observed for growth and colour change. Confirmation of identity of the isolated organism was done by carrying out Gram-staining and biochemical tests which included the Methyl Red-Voges Proskauer, citrate utilization, urease and indole production tests.

Isolation and Identification of *Salmonella* spp. and *Shigella* spp.

Enrichment of the bacteria was achieved by introducing 1g (powdered) or 1 ml (liquid) of sample into 9 ml of sterile Nutrient broth and incubating for 18 h at 37 °C. 0.1ml of the pre-enriched culture from the Nutrient broth was then streaked onto the surface of freshly prepared Salmonella-Shigella agar plates and incubated at 37 °C for 48 h. Suspected colonies were identified by culturing on Triple Sugar Iron (TSI) agar slopes. The slopes were observed for growth and colour change including the production of gas.

Isolation and Identification of Fungi

The isolated fungal colonies on each of the Sabouraud Dextrose Agar (SDA) count plates were sub cultured onto fresh SDA plates and incubated at 25 ^{o}C for 7 days. The identity of the fungal contaminants on each plate was determined using their cultural and morphological characteristics and microscopy with lactophenol plus cotton blue as the mounting fluid.

Effect of Preservatives on Selected Herbal Product Samples

The ability of preservatives to prevent microbial growth in herbal product samples with different levels of microbial load was determined. Three different herbal samples: sample number 10 (Popa) which was heavily contaminated with microorganisms and sample numbers 9 ('Common' Root) and 4 (Agbo) which had medium and low levels of contaminants, respectively, were selected for the test. The preservative system used consisted of a mixture of methyl- and propyl-para hydroxyl benzoic acid (paraben) in the ratio of 2:1 and a use concentration of 0.2%w/v (Lund, 1994).

0.0333 g of methyl-paraben was triturated thoroughly with 0.0167 g of propyl-paraben in a mortar. 25 ml of product sample number 10 was slowly added with trituration. The mixture was poured into a McCartney bottle, labeled and then stored on the shelf for 3 days at 37 ^{o}C. The same procedure was carried out for product samples 9 and 4, respectively.

Duplicate 0.1 ml from each of the 3 mixtures was diluted with 10 ml of molten and cooled sterile Nutrient agar for bacteria and Sabouraud Dextrose agar for fungi. The mixture was poured into duplicate plates and allowed to set before incubating at 37 ^{o}C for 24 h for bacteria and 25 ^{o}C for 7 days for fungal contaminants.

Duplicate 0.1 ml of each of the same 3 herbal samples (that is, samples10, 9 and 4), but without the selected preservative system were also inoculated and incubated similarly as the preserved samples. These served as the controls. Both sets of plates, that is, those inoculated with the preserved samples and those without the preservatives (controls) were observed for the presence or absence of growth.

Determination of Anti-microbial Activity of Herbal Product Samples with no Microbial Contamination

The herbal samples which did not show any microbial growth were examined for their possible residual antimicrobial activity on selected bacterial isolates. The product samples selected for this test were samples 2(Axis) and 3(Smilax) while the isolated bacteria selected for the test were *S. aureus, S. epidermidis, Shigella* spp. and *E. coli.* 0.1ml of overnight broth culture of each test organism was swabbed on the surface of each of two plates containing 20 ml of sterile and solidified Nutrient agar. The plates were incubated at 37 ^{o}C for 20 minutes for acclimatization and growth of the inocula. Two round holes of 8mm diameter and equidistant to each other were bored into the plates using a sterile glass cork borer. The bottom of each hole was then sealed with one drop of molten Nutrient agar. Five drops of each of the test samples (samples 2 and 3) were placed in each of the holes and the plates refrigerated at 4^{o}C for 30 minutes to allow adequate diffusion of the samples. The plates were thereafter incubated at 37 ^{o}C for 24 h. The diameters of the zones of inhibition around each hole in the plates were measured in millimeters.

Determination of the Alcohol Content of the Liquid Herbal Samples

To 2 ml of each of the liquid herbal samples in a test tube was added 1.0 ml of concentrated sulfuric acid (1760 g/l) and potassium dichromate (100 g/l), respectively, and then observed for change in colour and odour.

Results

Table 2 shows the organoleptic properties, including the pH of the herbal preparations. The pH analyses of the samples indicated that 8 (40%) of the samples were within the pH range of 4 to 6, 6 (30%) were at pH 7 and 5 (25%) above pH 7. Only 1 (5%) sample had pH below 4. The product samples with pH range of 1-6 were considered acidic; pH 7 as neutral and pH above 7 to be alkaline or basic. As shown in the Table, 6 (30%) of the samples with astringent taste were within the pH range of 1-6.

As observed in Table 3, a total of 16 (80%) samples contained average bacterial counts of between 1.47 x 10^{8} and 9.375 x 10^{8} cfu/ml while 2 (10%) samples contained less than 30cfu/ml. Two (10%) samples (samples 2 and 3) did not show any bacterial growth. Fungal growths were observed in 16 (80%) samples with fungal counts ranging between 3.35x10^{8} and1.60 x10^{9}cfu/ml while 2 (10%) samples did not have any fungal growth. Two (10%) samples had the highest level of fungal growth ranging between 1.27 x10^{9} and 1.6 x 10^{9} cfu/ml.

As shown in Table 4, *S. aureus* and *Bacillus* species were the most frequently isolated bacterial contaminants. They were isolated in 10 (50%) and 9 (45%) of the samples, respectively. *S. epidermidis* and *E. coli*, respectively, were detected in 4 (20%) of the samples. The fungal contaminants isolated from the tested samples included *Candida albicans, Cryptococcus neoformans, Aspergillus flavus, Aspergillus nidulans, Aspergillus niger* and *Trichoderma harzianum. Aspergillus* species was isolated from 9 (45%) of the 20 samples while 5 (25%) and 3 (15%) of

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the samples contained *Candida albicans* and *Cryptococcus neoformans*, respectively. Two (10%) samples were contaminated with *Trichoderma harzianum*. As shown in Table 5, some of the contaminants co-occurred in the herbal preparations.

Table 2: pH and organoleptic properties of herbal preparations.

Sample*	pH	Colour	Odour	Taste
1.	4.32	Dark brown	Odourless	Mildly astringent
2.	4.44	Dark brown	Odourless	Mildly astringent
3.	4.65	Light brown	Pungent	Mildly astringent
4.	5.75	Brown	Odourless	Bitter
5.	7.80	Light brown	Odourless	Very bitter
6.	7.18	Yellow	Odourless	Tasteless
7.	7.32	Brown	Odourless	Slightly bitter
8.	6.12	Muddy	Odourless	Sweet, mildly astringent
9.	7.01	Milky	Pungent	Tasteless
10.	7.32	Yellow	Odourless	Tasteless
11.	5.40	Green	Odourless	Mildly astringent
12.	7.91	Green	Odourless	Bitter
13.	7.32	White	Odourless	Tasteless
14.	5.64	Yellow	Odourless	Bitter
15.	2.65	Dark brown	Odourless	Astringent
16.	7.75	White	Odourless	Tasteless
17.	7.97	Black	Odourless	Bitter
18.	6.58	Brown	Odourless	Tasteless
19.	6.89	Green	Odourless	Bitter
20.	7.57	Green	Odourless	Tasteless

*1-10 liquids; 11 – 20 powders.

Table 3: Mean bacterial and fungal counts and presence of alcohol in herbal preparations
*NA = Not applicable

Sample	Mean bacterial count cfu/ml	Mean fungal count cfu/ml	*Presence of alcohol
1	$4.8X10^8$	$4.15x10^8$	Present
2	0	0	Present
3	0	0	Present
4	<30	<30	Present
5	$5.25x10^8$	$7.04x10^8$	Present
6	$7.75x10^8$	$3.45x10^8$	Present
7	$3.325X10^8$	$4.5x10^8$	Present
8	$3.2X10^8$	$3.47x10^8$	Present
9	$8.3x10^8$	$4.17x10^8$	Absent
10	$9.375x10^8$	$1.27x10^9$	Absent
11	$2.4x10^8$	$4.7x10^8$	NA
12	$3.55X10^8$	$1.6x10^9$	NA
13	$6.75x10^8$	$3.85x10^8$	NA
14	$1.47x10^8$	$9.95x10^8$	NA
15	<30	$4.925X10^8$	NA
16	$4.0X10^8$	$3.47x10^8$	NA
17	$3.3x10^8$	<30	NA
18	$4.63x10^8$	$3.35x10^8$	NA
19	$4.25x10^8$	$7.04x10^8$	NA
20	$6.8x10^8$	$3.5x10^8$	NA

The two samples from which microbial contaminants were not recovered demonstrated antimicrobial activity against the tested organisms as shown in Table 6. A 0.2%w/v mixture of methyl- and propyl-paraben (1:2) inhibited bacterial growth in the selected herbal preparations as shown in Table 7. The results obtained from the determination of the presence of alcohol in the liquid herbal samples indicated that 8 out of the 10 liquid samples contained alcohol while 2 of the samples were alcohol-free.

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Table 4: Occurrence of contaminants in samples

Organism	**Number of samples (%)**
S. aureus	10 (50)
Bacillus spp.	9 (45)
E. coli	4 (20)
S. epidermidis	4 (20)
Salmonella spp.	3 (15)
Shigella spp.	2 (10)
C. albicans	5 (20)
A. flavus	4 (20)
A. niger	3 (15)
C. neoformans	3 (15)
T. harzanium	2 (10)
A. nidulans	2 (10)

Table 5: Co- occurrence of contaminants in herbal preparations

Sample	Organisms
1	*Bacillus* spp., *S. epidermidis, C. neoformans*
2	No contaminants recovered
3	No contaminants recovered
4	*S. aureus, C. albicans*
5	*S. aureus, E. coli, A. nidulans, T. harzanium, Bacillus* spp.
6	*S. aureus, A. niger, Salmonella* spp., *Bacillus* spp.
7	*S. aureus, A. flavus, E. coli, Bacillus* spp.
8	*C. albicans, S. epidermidis, E. coli*
9	*C. albicans, Shigella* spp., *Bacillus* spp.
10	*S. aureus, A. flavus*
11	*T. harzanium, Shigella* spp., *Bacillus* spp.
12	*S. aureus, C. neoformans, Bacillus* spp.
13	*S. aureus, C. neoformans*
14	*S. epidermidis, C. albicans*
15	*E. coli, A. nidulans*
16	*A. niger, Bacillus* spp.
17	*S. epidermidis, A. niger, Salmonella* spp.
18	*S. aureus, A. flavus, Salmonella* spp.
19	*S. aureus, A. flavus*
20	*S. aureus, C. albicans, Bacillus* spp.

Table 6: The antimicrobial activity of herbal product samples that showed no microbial contamination.

Organism	Average zone of inhibition (mm)	
	Sample 2 (Axis)	Sample 3 (Smilax)
Staphylococcus aureus	14	14
S. epidermidis	14	13
Shigella spp.	13	15
E. coli	11	12

Table 7: Effect of preservatives (methyl- and propyl-paraben) on bacterial and fungal growth in selected herbal product samples

Sample	Mean Bacterial Count (cfu/ml)	Mean Fungal Count (cfu/ml)	With preservative	Without preservative
Sample 10 with high microbial load	$9.375x10^8$	$1.27x10^9$	-	+
Sample 9	$8.3x10^8$	$4.17x10^8$	-	+
Sample 4 with low microbial load	<30	< 30	-	+

Discussion

In orthodox medicine, the treatment of many human and animal diseases is effected through the use of active pharmaceutical ingredients which could be formulated into various dosage forms. Similarly, many herbal preparations currently available for use in the treatment of many human and animal diseases are formulated into several dosage forms which include solids, semi-solids and liquids. When formulated for oral use, the acceptability of pharmaceuticals, which is a function of the compliance of the consumer to the dosage regimen, is determined by the organoleptic properties of the formulation. Such properties include the colour, the taste and the odour of the preparation. This should also be applicable to herbal preparations as their organoleptic properties are important parameters in their identification and acceptability. However, the traditional herbal medicine producers are generally not concerned about the aesthetics and taste of their products. The samples assessed in this study were of their natural or acquired (due to processing and storage) colour, odour and taste which in most cases were neither appealing nor attractive.

An important determinant of the taste of oral preparations is the pH. Shallenberger (1996) reported the existence of a positive correlation between pH and sour taste / astringency. Astringency was described as a mouth-feel perception that commonly occurs with acid solutions in addition to the sour taste perception (Rubico et al., 1992). Sourness was considered as the simplest taste that is associated with acidity because the hydrogen ion is the only chemical species that causes a sour taste (Shallenberger 1996). In addition to the effect of pH on taste, the pH value also influences the quality of medicinal products. While its effect on the stability of the therapeutically active components in medicinal products cannot be over looked, pH determines to a great extent the type of microorganisms that grow in products of different pH values (Araújo and Bauab, 2012). Thus, in samples with low pH values, the bacterial counts were observed to be lower but with high fungal counts. On the other hand, the neutral or alkaline samples had higher level of bacterial contamination with lower fungal loads. This is in line with the observation that a neutral or alkaline pH favors high bacterial growth or contamination levels of herbal preparations since bacterial growth is optimal at more or less neutral pH, around pH 5.0 to 8.5 (Lamikanra, 2010) while fungal growth is optimal at acidic pH (Rousk et al., 2009).

Apart from the consumers' acceptability of the herbal preparations, the compliance to standards specified by various regulatory bodies is very important. In most cases, herbal preparations are intended for oral and external uses. They are, therefore, not expected to be sterile or completely devoid of living microorganisms. There is, however, a limit to the number and types of organisms permissible in an oral herbal preparation. The European Pharmacopoeia (2007) specified that oral herbal preparations should not contain more than 1.0×10^5 total aerobic bacteria per millilitre or gramme while yeasts and moulds should not exceed 1.0×10^3. According to the WHO guidelines (WHO, 2007), *Pseudomonas aeruginosa*, *S. aureus*, *E. coli*, *Salmonella* spp. and *Shigella* spp. must not be present in herbal medicines intended for internal use at any stage and the presence of any of these organisms is an indication of the poor quality of herbal medicinal products. The results of this study showed that almost all the herbal preparations assessed did not meet these specifications.

Apart from the unacceptable microbial loads observed in about 85% of the samples, the presence of contaminants considered to be completely unacceptable in oral preparations was demonstrated. The most common contaminants in the tested samples were Gram-positive organisms belonging to the genus Staphylococcus. Although some *Staphylococcus* species belong to the normal epiphytic flora of plants, the species recovered from the tested herbal samples were mostly of human and animal origin. *Staphylococcus aureus* and *Staphylococcus epidermidis* are normal commensals of the mammalian skin, hands and mucous membranes. Upon the consideration of the extent of human contact involved in the preparation of herbal medicinal samples, it is obvious that the most likely sources of the contaminating *Staphylococci* are the producers of the samples. This suggests that the level of hygiene of persons involved in the preparation of the tested samples is low resulting in unacceptable products. Similar studies as this which have been carried out on herbal samples marketed in other countries including Thailand (Chomnawang et al., 2003) and Nigeria (Adeleye et al., 2005; Okunlola et al., 2007; Abba et al., 2009; Oluyege et al., 2010) have reported that the human pathogen isolated the most in the herbal products was *S. aureus*.

The presence of *Bacillus* spp. in about half of the samples is not really surprising since the raw materials involved in the preparation of the samples are organic in nature and *Bacillus* spp. are normal environmental spore forming contaminants. Their presence, however, suggests the possibility of inadequate heat processing, unhygienic handling of products and the use of contaminated processing equipment during the production of the samples (Adams and Moss 2004). Oyetayo (2008) reported that the presence of *Bacillus* spp. in herbal preparations is an indication that the water used in the preparation of the products is not from a good source or the plant parts used are the roots which may contain soil.

The isolation of organisms belonging to the family Enterobacteriaceae especially *E. coli* from one out of every five of the tested herbal samples is an indication of recent faecal contamination of one or more of the materials, at one or more steps during the production process. The coliform organism, *E. coli*, is an acceptable indicator of recent faecal contamination of samples (Edberg, 2000). Furthermore, the presence of feacal coliforms in a sample or product is suggestive of the presence of other enteric pathogens in the sample or product (APHA, 1992; Jay, 1997). It is, however, surprising that the other enteric pathogens, *Salmonella* spp. and *Shigella* spp., isolated in this study were each recovered from samples other than those from which the *E. coli* strains were recovered. This observation rules out the possibility that the three organisms originated from the same source of contamination. It may, therefore, be inferred that the herbal medicinal products available to consumers in the Freetown metropolis of Serra Leone are liable to bacterial contamination from a number of different sources.

Fungi are common environmental contaminants of herbs and contamination chiefly occurs due to a slow drying process, inadequate drying or during post-harvest storage if relative humidity is high and temperatures are favourable (Sharma, 1990). The most predominant contaminating moulds in the studied samples were *Aspergillus* spp. and *Trichoderma harzianum* while the predominant yeasts were *Candida albicans* and *Cryptococcus neoformans*. The high fungal count observed in the majority of the samples rendered the products unacceptable for human consumption.

The implication of the high microbial load and presence of specific pathogens in the tested samples cannot be dismissed. There are serious clinical as well as pharmaceutical implications of the findings of this study. Clinically, consumers of any of these unacceptable products are at risk of contacting infections by the different pathogens which may be of great consequences if not identified and treated appropriately. Even in the absence of the specific pathogens, the intake of a high concentration of accumulated toxins produced by organisms such as *Bacillus cereus*, *Staphylococcus aureus* (Le Loir et al., 2003) and *Aspergillus flavus* (Kneifel et al., 2001) may lead to undesirable reactions in consumers. The risk is greater if the consumer is a young child with undeveloped immunity, an elderly with diminished immunity or the immunocompromised patients. Incidentally, these groups of consumers are the most in need of herbal or orthodox medicines for the treatment of many diseases to which they are susceptible. Pharmaceutically, the presence of microbial contaminants may lead to the spoilage of the products, thus reducing their shelf life. The organoleptic properties of the product may be adversely affected such as the taste becoming unpalatable, the

odour becoming offensive and foul from the metabolic waste produced by contaminants while the colour may become unsightly. The production of gas by certain organisms can also lead to some sort of explosion when the bottles containing the herbal products are opened. The natural pH of the product may be completely altered rendering the product more acidic or basic depending on the contaminants. A more serious effect is the possible inactivation of the therapeutic activity of the products and the potential to cause adverse reactions in the consumers of the products (Araújo and Bauab, 2012).

The inclusion of alcohol in some liquid herbal products may be an attempt to reduce the presence and effects of contaminants in the preparations by a very few number of producers of herbal medicinal products. With a larger percentage of producers, however, the use of alcohol in herbal preparations is primarily as an extraction medium for the plant parts used. Where the product is undiluted with water, the alcohol may be present at such a concentration that is effective to prevent microbial growth. Only 20% of the tested liquid samples contained inhibitory concentrations of alcohol and no microbial cells were recovered from the samples. Moreover, these were shown to exhibit antimicrobial activity against tested microorganism. The other 80% of the liquid herbal preparations in this study contained alcohol, but at such concentrations that was insufficient to function as a preservative. Data available on the activity of ethanol showed that the bactericidal effect of ethanol is a function of concentration and contact time. Ethanol is reported to be bactericidal in aqueous mixtures at concentrations between 60-95% V/V (Rowe et al., 2006) and the optimum concentration is generally considered to be 70% V/V (Hugo and Russell, 2004). Ethanol is however ineffective against bacterial spores and has a poor penetration of organic matter (EMA 2014). Furthermore, an alcoholic content of 15% by weight in acid solutions and 18% by weight in alkaline solutions is sufficient to prevent microbial growth. For this reason, most alcohol containing preparations such as elixirs, spirits and tinctures are self preserving and do not require preservation (Troy and Beringer, 2006).

Considering the nature of the raw materials used in the preparation of liquid and powdered herbal products, it will be necessary to include preservatives to prevent the growth and proliferation of contaminating microbes. Normally, the type(s) and concentration(s) of preservative(s) to be employed should be determined for each product using the standard preservative testing procedures. However, the level of education and the facilities available to traditional herbal product manufacturers are inadequate and they, therefore, cannot effectively determine the effective preservation of their products. Notwithstanding, the results obtained in this study indicated that inclusion of preservatives, such as a 0.2%w/v mixture of methyl- and propyl - paraben, was very effective in inhibiting the growth of both the bacterial and fungal contaminants that were present in the samples. Inclusion of chemical preservatives may, therefore, enhance the microbial quality of herbal products.

Conclusion

It may be concluded from this study that many herbal medicinal preparations marketed in Freetown in particular, and in Sierra Leone generally, are likely to be contaminated with a wide variety of potentially pathogenic bacterial and fungal cells. The microbial quality may however be improved by the inclusion of preservatives. The quality of herbal products offered for sale should, therefore, be monitored and enforced from the stages of collection, handling and preparation to storage, distribution, marketing and use of the final products.

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