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Assessment of the microbial quality of some oral liquid herbal medicines marketed in Ile-Ife, South-western Nigeria

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The extensive use of herbal medicinal products in the treatment and management of disease states within communities in Ile-Ife, south western Nigeria has made it imperative to investigate the microbial quality of a sample of these products in the light of the standards prescribed by regulatory bodies. This study was therefore carried out to assess the microbial quality of locally prepared and unregistered herbal oral liquid medicines available in Ile-Ife. A total of 50 herbal medicine samples were procured from various randomly selected markets in Ile-Ife. The microbial load of each sample was determined and the contaminants associated with each sample were identified. Samples that did not yield either bacterial or fungal growth were tested for their ability to elicit antimicrobial activity using the agar cup diffusion method. Results obtained in the course of the study showed that 90% of the samples carried microbial loads beyond officially permissible limits with *Escherichia coli* and *Salmonella* species being found in 2 and 6% of the samples, respectively. Since it was suspected that antibiotics were sometimes added to herbal products by some herb sellers to prolong the shelf life of their products, the screening exercise showed that the sample that did not yield bacterial growth exercised marked antimicrobial activity against both Gram positive and Gram negative organisms. The results of this study suggest that the herbal oral liquid products available to consumers in Ile-Ife are of unacceptable quality.

Key words: Microbial quality; herbal preparations; *Escherichia coli*; *Salmonella* species; antibiotics.

INTRODUCTION

Although oral pharmaceutical preparations are not required to be sterile, they are not supposed to be heavily contaminated by microorganisms or potentially pathogenic organisms including *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* (USP, 2013). This is because apart from the safety of consumers, the presence of high microbial count in any preparation may lead to the proliferation of such organisms within the preparation leading to

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spoilage (Bloomfield, 2007). This is why pharmaceutical companies are required to adhere to the principles of Current Good Manufacturing Practice (FDA, 2015) and their products must be subjected to total quality control measures, with the overall drug manufac-turing process being made to undergo quality assurance tests at every level. It has to be said however that, only the big pharmaceutical companies have the capacity to adhere to the principles of Current Good Manufacturing Practice. Okeke and Lamikanra (2001) noted that small pharmaceutical companies involved in the production of orthodox drugs, many of which are found in countries with challenged economies, are not able to invest in machinery, controls for production environments and the employment of qualified staff to see that their products are of consistently high quality. The situation is worsened in Nigeria by the existence of a large informal sector which is responsible for small scale production of a large number of unregistered and usually unstandardised medicines using rudimentary equipment and raw materials of plant derivation which are highly susceptible to extensive microbial contamination. In addition, the packaging of these products is often rudimentary with final products being packed in recycled plastic bottles which are frequently unlabelled. These unregistered herbal medicines are outside the control of the relevant regulatory bodies. However, they cannot be ignored as they are available virtually everywhere including and especially in rural areas, which are short of modern pharmaceutical cover.

The widespread use and availability of herbal medicines has been reported to be due to perceived efficacy, safety and absence of side effects from herbal products when compared to orthodox medicines (Kennedy, 2005; Clement et al., 2007). The use of herbal medicines is also likely to have increased because of emerging infections such as AIDS and drug resistant malaria (Gyasi et al., 2013; Lorenc and Robinson, 2013). The high cost of hospital consultation and orthodox drugs is an additional reason why herbal therapy may be attracting greater patronage (Gyasi et al., 2011). The use of improved packaging materials and increased public awareness through the organization of trade fairs on traditional medicine and the presence of NAFDAC registration numbers on registered herbal products are also likely factors that have increased the use of herbal products in Nigeria.

The issue of the safety of herbal products is however of great concern to many regulatory bodies who have therefore set out specifications on the quality of herbal products. Some regulatory bodies have given specifications on the microbial load and presence of specific organisms in herbal products (WHO, 2007a; European Pharmacopoeia, 2007). Many studies carried out over the years and in some parts of Nigeria have documented the different types of contaminants present in herbal products (Onawunmi and Lamikanra, 1987; Arias et al., 1999; Erich et al., 2001; Wolfgang et al., 2002; Adeleye et al., 2005; Okunlola et al., 2007; Abba et al., 2009). This study was carried out to determine the levels and identity of microbial contaminants of unregistered oral herbal liquid preparations available to consumers in Ile-Ife, south western Nigeria.

MATERIALS AND METHODS

Collection of herbal samples

Fifty (50) unregistered herbal oral liquid preparations produced and hawked by herb sellers were procured from major markets located in Ile-Ife. They were mostly aqueous decoctions produced from mixtures of several plant parts such as leaves, stems, roots and barks. The producers were found to be men and women, usually with no formal education. The markets included Ife New Market, Mayfair market, Sabo Market, Ede Road Market and Obafemi Awolowo University (O.A.U.) Central Market. Information concerning the uses and dosage of each preparation was obtained from the peddlers and documented. The samples were purchased as packaged by the herb-sellers and transported to the laboratory. Ile-Ife is a semi-urban city in south western Nigeria which lies on latitude 7°28' 0" N and longitude 4° 34' 0" E. It has a total area of 1,791 km^2 (692 sq mi) and as at 2006, the population was 509, 035. It is home to two universities; the Obafemi Awolowo University and the Oduduwa University as well as a teaching hospital, the Obafemi Awolowo University Teaching Hospitals Complex and several Public Health Centers and private clinics.

Determination of bacterial and fungal counts

In the laboratory, each of the samples was shaken properly to ensure a uniform distribution of the contents. Serial 10 fold dilutions in sterile water were then carried out and duplicate, 1ml portions of each dilution was aseptically placed into sterile petri-dishes. Twenty ml of molten nutrient agar (Oxoid, England) sterilized at 121°C for 15 min and 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 at room temperature and the plates incubated at 37°C for 24 h for bacterial and 25°C for seven days for fungal populations. Plates containing 30 to 300 colonies were observed and the number of colonies that grew on each plate was recorded. Microbial load was expressed as colony forming units per ml of sample.

Isolation and storage of bacterial contaminants

Each colony having distinct colonial characteristics such as colour, shape, consistency and elevation, growing on the Nutrient agar count plate was picked and streaked onto freshly prepared Nutrient agar plates and incubated at 37°C for 24 h. The isolated colonies were each stored in an appropriately labeled cryovial and nutrient agar stabs stored at -4°C in a freezer and 4°C in a refrigerator respectively.

Identification of bacterial isolates

The Gram stain procedure was carried out on each isolate to

Table 1. Indications specified for samples of herbal oral liquid products.

Indication	Number percentage (%)
Body and joint pain	4 (8)
Blood infection	8 (16)
Candidiasis	6 (12)
Cough	6 (12)
Diabetes	8 (16)
Erectile dysfunction	2 (4)
Gonorrohea	2 (4)
Hypertension	6 (12)
Infection	2 (4)
Malaria	20(40)
Malaria and typhoid	4(8)
Mouth thrush	4(8)
Pile	12 (24)
Stomach ache	8 (16)
Tonsil infection	2 (4)

determine the shapes and class of the isolates. All Gram positive cocci were streaked onto the surface of over-dried Mannitol Salt agar and the colonial characteristics were noted. Further tests for identification of the Gram positive cocci included catalase and coagulase tests. All Gram negative rods were streaked onto MacConkey agar, Eosine Methylene Blue agar, Salmonella-Shigella agar and Triple Sugar Iron agar. The colonial characteristics were noted and biochemical test which included Indole, Urease, Citrate utilization, M.R.V.P. and oxidase tests were carried out on the isolates which were identified based on the interpretation of results of the biochemical tests (Barrow and Feltham, 1993; Farmer 1999).

Determination of anti-microbial activity of herbal product samples with no microbial contaminants

One of the herbal samples did not show any bacterial growth on Nutrient agar and another did not yield fungal contaminants. Both of them were further examined for antimicrobial activity against selected bacterial and fungal isolates respectively. The sample which did not show any bacterial contamination was tested against reference bacterial strains namely *Bacillus subtilis* NCTC 8236, *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 10145 while the sample not yielding fungal contaminants was tested against *Candida albicans* ATCC 24433. A volume of 0.2 ml of overnight broth culture of each test bacterium was seeded into 20ml of sterile molten Nutrient agar at 45°C. The plates were allowed to set and harden before incubating at 37°C for 20 minutes for acclimatization and growth of the inocula. Two holes of 8 mm diameter and equidistant to each other was 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. Four drops of each of the test samples were placed in each of the holes and the plates were left to stand for 1 h to allow adequate diffusion of the samples. The plates were thereafter incubated at 37°C for 24h. The diameters of the zones of inhibition around each hole in the plates were measured in millimeters. The same procedure was repeated for the test against *C. albicans* ATCC 24433 but using Sarbouraud Dextrose Agar as the test medium and incubation at 25°C for 48 h.

RESULTS

The 50 preparations used in this study were presented by the herb sellers for the treatment of various ailments (Table 1). A total of 48 (96%) of the herbal products were packaged and sold in plastic bottles of which 79.2% were discarded with bottles previously used for packaged water and 20.8% were discarded with alcoholic drinks' containers (Plate 1).

The mean bacterial load of the samples ranged from zero cfu/ml to 2.94×10^{12} cfu/ml while the mean fungal count ranged from zero cfu/ml to a maximum of 3.54×10^{12} cfu/ml. According to the World Health Organization (2007a) and the European Pharmacopoeia (2007), for herbal medicinal products to which boiling water is not added before use, the limits specified for total viable aerobic count are 10^5 bacteria and 10^3 fungi per gram or per millilitre. Only 10% of the samples were therefore of acceptable quality in terms of microbial loads (Table 2).

Identity of contaminants

A total of 85 bacterial isolates were recovered from 49 of the 50 samples and included 36 Gram-positive and 49 Gram-negative organisms. The most frequently isolated contaminants in the tested sample were *Bacillus* species (40%), followed by *Klebsiella* species (31.8%) as shown in Table 3. Other contaminants included *Escherichia coli*, *Staphylococcus* species, *Salmonella* species and *Pseudomonas aeruginosa.* A total of 52% of the samples had one bacterial contaminant each, 26% of the samples had two while 20% had three contaminants. Four contaminants were recovered from one sample.

Plate 1. Picture showing some samples in their packaging (samples were labeled by authors after purchase).

Table 2. Acceptability status of samples.

Quality	Frequency (%)	Remark
Only bacterial load within permissible limit	18	Unacceptable
Only fungal load within permissible limit	4	Unacceptable
Both bacterial and fungal load within permissible limit	10	Acceptable
Bacterial and fungal load beyond permissible limit	68	Unacceptable

One of the samples from which only fungal contaminants were recovered showed marked anti-microbial activity against reference strains of bacteria (Table 4). The other sample from which only bacterial contaminants were recovered exhibited an appreciable antifungal but weak antibacterial activity.

DISCUSSION

According to the WHO report, there is widespread availability and usage of herbal preparations by a large percentage of persons in many developing countries (Robinson and Zhang, 2011). Some reasons for this have been documented by several authors and these include perceived efficacy, safety and absence of side effects (Kennedy, 2005; Clement et al., 2007; Gyasi et al., 2013; Lorenc and Robinson, 2013). One observation made by the authors of this study suggests that the high patronage of herbal medicine peddlers in Ile-Ife may actually be the appearance of a ready capacity for the treatment or management of all manner of communicable and non-communicable diseases. An example is that many of the producers of herbal medicine claim to have products for curing AIDS, a condition yet to have a specific orthodox cure (Gyasi et al., 2013; Lorenc and Robinson, 2013). The analysis of the indications for which the preparations in this study were produced showed that over 80% of the preparations were claimed to be for infectious diseases. This is not surprising as there is a high incidence of infectious disease in developing countries (Krämer et al., 2010) associated with many conditions such as

Table 3. Identity of contaminants present in herbal preparations.

Organism	Number of samples contaminated percentage (%)
Bacillus cereus	7 (14)
Bacillus spp. other than *B. Cereus*	27 (54)
Citrobacter spp.	1 (2)
Enterobacter spp.	5 (10)
Escherichia coli	1(2)
Klebsiella spp.	27 (54)
Pantoea agglomerans	3 (6)
Proteus spp.	6 (12)
Pseudomonas fluorescens	1 (2)
Other *Pseudomonas* spp.	2 (4)
Salmonella species	3 (6)
Coagulase negative *Staphylococcus*	1 (2)
Staphylococcus epidermidis	1(2)

environmental and sanitary conditions that favor the proliferation of infectious disease causing agents (Krämer et al., 2010). Some of these ailments, for example malaria, typhoid, blood infection (septicaemia) and candidiasis are quite serious and may be life-threatening.

Only 10% of the samples assessed in this study were of acceptable microbial quality. As judged by the absence of any form of labels on this class of herbal samples, it was difficult to determine why these were better than the other 90%. The acceptable samples were similar in appearance and packaging and the vendors were located in the same areas as the unacceptable ones. The absence of a label which is one of the characteristics of these unregistered herbal drugs makes it difficult to compare the samples in terms of concentration of herbs or identity of the components of each preparation. The microbial loads of 90% of the samples assessed in this study were beyond the limits stipulated by the regulatory bodies (WHO 2007; European Pharmacopoeia, 2007). Apart from the heavy microbial loads, the presence of unacceptable organisms or pathogens was demonstrated in the herbal samples. The unacceptable organisms recovered included the Gram negative organisms *E. coli* and *Salmonella* species. These are organisms associated with the gastrointestinal tract and indicate the likelihood of faecal contamination (Edberg, 2000). These contaminants could be acquired from the use of water of poor quality for the preparation of the samples and rinsing of containers. Other likely sources are the use of inadequately washed or disinfected plant parts previously exposed to manure. Plant materials such as vegetables have been reported as reservoirs of a wide range of bacteria including enteric pathogens (Holden et al., 2009).The presence of *Escherichia coli* and *Salmonella* spp. has also been stated to be an indication of poor quality of production and harvesting practices (WHO 2007). The recovery of a high number of *Bacillus* species, the frequently predominant aerobic spore-forming bacteria naturally occurring microflora of medicinal plants, supports the fact that vegetative plant parts and roots that have been in contact with the soil or dust are among the components of the preparations. The presence of organisms such as *S. aureus* suggest that contamination could also have occurred through handling by personnel who carry pathogenic bacteria or normal commensals during harvest/collection, post-harvest processing and the manufacturing process. The presence of several contaminants in a single preparation as observed in this study is expected since the preparations usually contain more than one plant or plants parts that have been obtained from multiple harvest sites. The practices of transportation and storage may also cause additional contamination and microbial growth. Proliferation of microorganisms also results from failure to control the temperatures of liquid forms and finished herbal products (WHO, 2007a). Other possible sources of contaminants are the environment and utensils in which the preparation were made as well as the containers used for packaging the preparations. An ideal package should be such that it does not adversely affect the microbial quality of intended preparations. Traditional packaging containers for herbal medicines were small gourds, earthenware pots, tortoise shells, horse hooves, horns of various animals, brass pots, as well as hollow tin rods plugged at both ends or sealed at one end into a conical shape (Sofowora, 2008). These containers were not air tight, so contamination through atmospheric microorganisms was inevitable. One plausible explanation for the absence of these older packaging materials among the samples obtained in this study is that herb sellers may be deliberately packaging their product in a way to improve acceptance by the general public. In addition, the older packaging materials

Table 4. The antimicrobial activity of samples from which either bacteria or fungi was not recovered.

Herbal sample	Test organism / Average zone of inhibition (mm)				
	C. albicans ATCC 24433	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 10145	*B. subtilis* NCTC 8236	*S. aureus* ATCC 29213
Minus bacteria	ND	17	20	18	24
Minus fungi	20	0	0	11	0
Ciprofloxacin 2 mg/ml	ND	32	28	20	28
Ketoconazone 1 mg/ml	30	ND	ND	ND	ND

are now less likely to be available than they were in the past. A total of 48 (96%) of the containers of the samples in this study were plastic bottles of packaged water or carbonated drinks that had been discarded after use. The remaining were bottles of alcoholic beverages and polythene bags. In the environment where this study was carried out, such containers are usually picked up from dump sites or from sites of outdoor parties. Other sources of these discarded bottles are peddlers who purchase these used bottles from bulk sellers. In a case where these containers were usually not washed or simply rinsed with a small quantity of water or water of poor quality, they are potential sources of heavy microbial contamination. The WHO already has specifications for the ideal presentation of herbal products (Patel et al., 2011).

The identity of the contaminants recovered in this study which suggests that possible sources of the contaminants include the environment, raw materials, hands of the producers and the water for production is an indication of a high level of non-adherence to the requirements of Good Manufacturing Practice. This needs to be addressed and producers should be made aware of the benefits of implementing best practice guidelines such as GACP and GMP. In these guidelines, requirements have been described for the raw materials, water for production, preparation utensils and items of equipment, the environment and personnel. The need for hygiene and sanitation of material and environment and the personal hygiene of personnel has been well spelt out to ensure the production of micro-biologically safe preparations (WHO, 2007b)

The instruction given by one of the peddlers to the author to include the antibiotic chloramphenicol in some of the products in order to prolong the shelf life of the products is an indication that the particular peddler knew that the presence of gross contamination of the preparations could lead to the spoilage of the preparation. The presence of antibiotics may partly explain the inability to recover bacterial contaminants from one of the samples which was also later on found to possess strong antibacterial activity against the Gram negative and Gram positive organisms tested in this study. This finding supports an observation made years ago at a workshop organized for traditional herbal practitioners (Ogungbamila and Ogundaini, 1993) and it indicates that the practice is still on. This practice is unethical as it exposes the users' commensal flora or pathogens to sub-inhibitory concentrations of antibiotics, further compounding the already existing problem of antimicrobial resistance in the community.

In conclusion, this study has shown that most of the unregistered herbal oral liquid products available to consumers in Ile-Ife contain unacceptable levels of microbial contamination. In order to benefit from the use of these products, there is the need to ensure that the persons involved with the production and distribution have adequate knowledge. There is an urgent need to implement proper herbal medicines monitoring and quality control for producers and the products. Subjection of raw materials for herbal medicines to appropriate processing will reduce the microbial load and potentially the inclusion of preservatives will help keep the microbial load of the products within standard specification, providing safe medicines to the users.

Conflict of interests

The authors have not declared any conflict of interests.

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