Research Article

# Microbial Quality of Unregulated Herbal Medicinal Products in Kenya

Meshack O. Onyambu [a], Hezekiah K. Chepkwony [b], Grace N. Thoithi [c], Godfrey. O. Ouya [b], and George O. Osanjo [a],*

[a] *Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi, Kenya*
[b] *National Quality Control Laboratory for Drugs and Medical Devices, Nairobi, Kenya*
[c] *Department of Pharmaceutical Chemistry, School of Pharmacy, University of Nairobi*
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* **Corresponding author:** Department of Pharmacology and Pharmacognosy, University of Nairobi, P.O. Box 19676-00202 KNH, Nairobi, Kenya; **Tel**: +254-72-1794666; **Email**: gosanjo@uonbi.ac.ke

**Background:** The use of herbal medicines is global, with the majority of the world's population depending on traditional medicines, particularly herbal remedies for their primary healthcare needs. In Kenya, it is estimated that conventional healthcare system provides for approximately 30 % of the population, while nearly 70 % rely on herbal remedies. Herbal medicines, however, present safety concerns as they carry a relatively high risk of contamination by pathogenic microbes, organic and inorganic pollutants including toxic metals and non-metals, organic, mycotoxins, endotoxins, and agrochemical residues.

**Objective:** This study was designed to assess the microbial quality of regulated and unregulated herbal medicinal products in diverse Kenyan markets, such as the supermarkets, roadside vendors, retail pharmacies and herbal clinics, for levels of microbial contaminants.

**Materials and Methods:** Thirty samples of registered and unregistered herbal medicinal products were collected by purposive sampling from five Kenyan provinces.  Microbial load analysis was performed in accordance to pharmacopoeial methods (BP and USP). Microorganisms were further isolated and characterized using differential and selective media and by biochemical analyses.

**Results:** All registered products had microbial load below 100 cfu/ml, and complied with BP and USP requirements. However, none of the unregistered samples complied with pharmacopoeial limits for either or both bacterial and fungal load limits. Most of the unregistered samples had microbial loads ranging from $3.00 \times 10^6$ to $1.56 \times 10^{10}$ cfu/ml, thus exceeding by far BP or USP standards. The microbial isolates belonged to fifteen (15) different bacterial genera and seven (7) fungal genera.  *Escherichia coli* was the most frequently isolated bacteria from 75 % of the unregistered product samples while *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Staphylococcus aureus* followed in 70 %, 60 % and 45 % of the samples, respectively. *Salmonella* spp was isolated in 40 % of the samples while *Shigella* spp was found in 20 % of the samples.

**Conclusion:** Unregulated herbal medicinal products that are available in diverse Kenyan markets show poor microbial quality and exhibit contamination by pathogenic microorganisms.  There is need to extend regulatory control by the drug authorities to herbal medicinal products to enhance microbial quality and safety.

**Keywords:** Microbial contaminants, herbal medicines, microbial load

# 1. Introduction

The majority of the world's population depends on traditional medicines, particularly herbal remedies for their primary healthcare needs (WHO, 2008; Akerele, 1993). In Kenya, it is estimated that conventional healthcare system provides for approximately 30 % of the population, while nearly 70 % rely on herbal remedies (NCPAD, 2007). Furthermore, medical pluralism is widespread in Kenya, with patients seeking both conventional and alternative medicine therapies (Kigen *et al.*, 2013). Indeed the complementary medicine practitioner: patient ratio is much higher than the conventional doctor: patient ratio (Mwangi et al, 2005). Such widespread use of herbal medicines, calls for the assurance of sustainable availability of quality and safe herbal medicines to ensure continued access especially for rural communities, without compromising patient safety (Kigen et al, 2013).

Herbal medicines present safety concerns as they carry a relatively high risk of contamination by pathogenic microbes, organic and inorganic residues including toxic metals and non-metals, organic pollutants, mycotoxins, endotoxins, and agrochemical residues (Okunlola et al, 2007; Abba et al, 2009; de Freitas Araújo and and Bauab, 2012).

In Kenya, the competent authority responsible for regulating the safety and efficacy of pharmaceutical products is the Pharmacy and Poisons Board (PPB). Products for which any medicinal claims are made must be granted marketing authorization by the PPB, which ensures that registered products are manufactured in compliance with Good Manufacturing Practices (GMP), amongst other criteria. The majority of herbal products marketed to members of the public in Kenya are not regulated by the PPB as they are categorized as traditional or cultural remedies whose mode of utilization predate modern regulation, and they thus enjoy regulatory exemption. The few manufacturers of herbal remedies, who register their products with the PPB, mostly do so to access the conventional medicine market through doctors' prescribing and the retail pharmacies.

This study was therefore designed to assess the microbial quality of regulated and unregulated herbal medicinal products in diverse Kenyan markets, such as the supermarkets, roadside vendors, retail pharmacies and herbal clinics, for levels of microbial contaminants.

# 2. Materials and Methods

## 2.1 Collection of Samples

A list of the herbal products that are registered by the Pharmacy and Poisons Board was obtained from the regulatory authority. The total number of registered products was fifty-four, out of which 10 were readily available in retail pharmacies or supermarkets. All 10 available products were purchased. Twenty samples of unregistered herbal products were collected by a purposive sampling technique from five Kenyan provinces, namely: Nairobi, Central, Rift Valley, Nyanza and Western provinces. The samples were obtained from herbal clinics, supermarkets and road side vendors. The non-packaged samples were collected in sterile polythene containers. Thus a total 30 samples, in both liquid and powder formulations were subjected to analysis for this study.

## 2.2 Microbiological Analyses

### Media preparation

All samples were analyzed at the National Quality Control Laboratory for Drugs and Medical Devices, Nairobi, Kenya.

Bacteriological and fungal media were supplied by Himedia Laboratories (India). Media in powder form were weighed, reconstituted with distilled water, heated to completely dissolve the medium, and sterilized by autoclaving at 115 kPa, 121 °C for 15 min. The following media were used: Nutrient Agar, Sabouraud Dextrose Agar (SDA), MacConkey Agar, Eosin-Methylene Blue (EMB) Agar, Salmonella Shigella Agar (SSA), Xylose-Lysine Deoxycholate (XLD) Agar and Mannitol Salt Agar.

All media were tested for viability using *Bacillus pumilus* NC08241, *Staphylococcus aureus* NC07447, *Pseudomonas aeruginosa* NC12924, *Saccharomyces cerevisiae* NC010716, *Candida albicans* NC10400, and *Aspergillus niger* NCPF3179. All standard strains of microorganisms were obtained from National Collection of Type Cultures (NCTC), United Kingdom.

### Sample preparation

For water soluble samples, 1 g or 1 ml of the herbal product was dissolved in 10 ml buffered peptone water, pH 7.0, followed by tenfold serial dilution. For lipophillic products, 1 g or 1 ml of sample was homogenized with 0.5 ml of polysorbate 20 and heated to 40 °C in a water bath followed by addition of 10 ml of buffered peptone water, mixing for 15 min, and then followed by $10^{-1}$ serial dilutions.

### Isolation of pure colonies

Colonies were isolated based on morphologic differences from their axenic cultures. Colony form, elevation, pigmentation and size were the major distinguishing characteristics. Petri-dishes were divided to quadrants and sub-culturing done by the streak plate technique (Holt et al, 1994).

### Microscopy

One loopful of bacteria culture was applied on a clean labeled microscope slide. The smear was heat fixed on the slide by passing it over a flame of the Bunsen burner, and Gram-stained according to Preston and Morel (1962). The smear was blot dried before viewing under the oil immersion objective (X1000) lens.

## 2.3 Test for specific microbes using differential and selective media

Specific microorganisms were tested using differential and selective media as follows:

***Staphylococcus aureus.*** A pure culture from Nutrient Agar was sub-cultured on a plate of mannitol salt agar freshly prepared as per the manufacturer's details. It was incubated at 33 °C for 72 h. Growth of white

colonies surrounded by yellow zones indicated the presence of *Staphylococcus aureus* and was confirmed by Gram staining and biochemical tests.

***Escherichia coli.*** A pure culture from Nutrient Agar was inoculated into Eosin Methylene Blue agar. The innoculum was incubated at 33 °C for 72 h. Colonies with dark centers and greenish metallic sheen indicated the presence of lactose fermenters. *E. coli* was confirmed by biochemical tests.

***Salmonella* spp*.*** A pure culture from Nutrient Agar was inoculated into Xylose Lysine Desoxycholate agar. It was then incubated at 33 °C for 48 h. Growth of well developed red colonies with black centers was considered to be the presence of *salmonella* spp. They were further subjected to biochemical tests for confirmation.

***Klebsiella pneumonia.*** A pure culture from Nutrient Agar was inoculated into MacConkey agar. It was then incubated at 33 °C for 72 h. growth of round mucoid colonies with colorless edges was considered to be the presence of *Klebsiella pneumonia* which were further subjected to biochemical tests for confirmation.

***Enterobacter aerogenes.*** A pure culture from Nutrient Agar was inoculated into Eosin Methyhlene Blue. It was then incubated at 33 °C for 72 h. Growth of pinkish large mucoid colonies was considered to be the presence of *Enterobacter aerogene*. Further biochemical tests were done for confirmation.

***Shigella* spp.** A pure culture from Nutrient Agar was inoculated into *Shigella Salmonella* agar. It was incubated at 33 °C for 72 h. growth of mucoid colonies with black centers was considered to be the presence of *Shigella* spp. A further subjection to biochemical tests was done for confirmation.

***Candida spp.*** A pure culture isolated in Sabouraud Dextrose Broth was inoculated into Sabouraud Dextrose Agar. It was incubated at 25 °C for 5 days. Growth of small round moist, white to colorless colonies with even edges was considered to be the presence of *Candida* spp. Further microscopic studies were done for confirmation.

## 2.4 Biochemical Analyses

Biochemical analyses were performed with the API 20 E system (bioMérieux, France) which tests nitrate reduction, indole production from tryptophan, presence of urease, arginine dihydrolase, β-galactosidase, β-glucosidase, cytochrome oxidase, protease, glucose fermentation and assimilation of L-tryptophan, sodium pyruvate, gelatin, D-glucose, D-mannitol, Inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, Amygdalin, and L-arabinose.

Analyses with the API 20 E system were performed according to the manufacturer's instructions. Briefly the API 20 E strip was prepared by distributing 1 ml of distilled water into the honeycombed wells of the tray to create a humid atmosphere. The inoculum was suspended in 5 ml of 0.85 % Nacl. Anaerobiosis was created in the tests for arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase

(ODC), $H_2S$ production and urea hydrolysis (URE) by overlaying with mineral oil. Samples were incubated at 37 °C for 24 h. The strips were read using a color chart provided by the manufacturer.

The oxidase test was done by using oxidase discs (Himedia laboratories, India) for detection of oxidase production. Isolated colonies were applied on oxidase discs and observed within 10 sec. at room temperature (25 °C) for change in colour.

The microorganisms were identified based on a numerical taxonomy profile according to the manufacturer's chart (bioMérieux, France, available at http://www.biomeriux.com).

## 2.5 Molecular Analyses and Identification

Bacterial genomic deoxyribonucleic acid (DNA) was isolated from plate cultures using a modified alkaline lysis method as described in Osanjo et al (2009). The 16S rDNA was amplified using the primer pair F27 (5'-AGAGTTTGATCCTGGCTCAG-3') and R1492 (5'-TACGGYTACCTTACGACTT-3') that amplifies bacterial 16S rDNA from position 27 to 1492 in the *E. coli* numbering system. The primers were supplied by MWG Biotech (Germany). Polymerase chain reaction (PCR) amplifications were performed in a thermal cycler (GeneAmp PCR systems 9700, Applied Biosystems USA) under the conditions described previously (Osanjo et al, 2009). The amplification products were purified from agarose gel using the QIAXEX II extraction kit (Qiagen, USA) according to the manufacturer's method.

Purified 16S rDNA was sequenced with the PCR primers (F27 and R1492) in the forward and reverse directions, in a Perkin-Elmer fluorescent dye-labelled dideoxynucleotides (Perkin-Elmer, USA) by the Sequencing Unit (Segolip) of the International Livestock Research Institute (ILRI, Kenya).

## 3. Results

The results of the microbiological analyses are summarized in **Table 1**.

Two samples, KM and MB, had an average total aerobic microbial count of $6.0 \times 10^5$ and $6.50 \times 10^5$ cfu/ ml respectivel, thus complying with the BP and USP limits for bacterial loads (BP, 2007 and USP-NF 2009). However even though these samples complied with bacterial load limits, their fungal loads were nonetheless higher than pharmacopoeial limits. The rest of the unregistered samples had a microbial loads ranging from $3.00 \times 10^6$ to $1.56 \times 10^{10}$ cfu/ml, and did not comply with BP or USP standards. All registered products had microbial load below 100 cfu/ml, and therefore complied to BP and USP requirements.

The microbial isolates belonged to fifteen (15) different bacterial genera and seven (7) fungal genera. *Escherichia coli* was the most frequently isolated bacteria from 75 % of the unregistered product samples while *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Staphylococcus aureus* followed in 70 %, 60 % and 45 % of the samples respectively. *Salmonella* spp was isolated in 40 % of the samples while *Shigella* spp was

in 20 %. The rest of the bacteria were isolated from less than 10 % of the samples.

*Candida* spp formed the major fungal isolate from 50 % of the samples followed by *Fusarium* and *Aspergillus*

species which were in 35 and 25 % of the samples, respectively. *Rhizopium* spp was isolated from 20 % of the samples while the rest of the fungal species were isolated from less than 10 % of the samples.

**Table 1:** Microbial Counts for Unregistered Herbal medicinal samples obtained from Kenya

| Sample Code | Aerobic Microbial Count (cfu/ml) | Fungal Count (cfu/ml) | Microorganism identified |
|---|---|---|---|
| AD | $3.00 \times 10^6$ | $5.20 \times 10^6$ | *Klebsiella pneumonia, Chryseomonas luteola, Aspergillus sp.* |
| BP | $2.61 \times 10^7$ | $2.04 \times 10^7$ | *Klebsiella oxytoca, Enterobacter cloacae, Candida sp.* |
| CS | $1.21 \times 10^7$ | $1.00 \times 10^7$ | *Staphylococcus aureus, *E. coli, Rhizopium sp. Fusarium sp., B. subtillis* |
| DF | $2.50 \times 10^7$ | $1.84 \times 10^6$ | *S. aureus, K. pneumonia, E. coli, Enterobacter aerogenes, Salmonella sp* |
| GI | $2.08 \times 10^7$ | $2.23 \times 10^7$ | *Enterobacter aerogenes, K. pneumonia, E. coli, Aspergillus Sp. Fusarium* |
| GS | $1.56 \times 10^{10}$ | $1.07 \times 10^7$ | *E. coli, K. pneumonia, †E. aerogenes Fusarium sp. Aspergillus sp. Rhizopus sp., Baccillus safensis* |
| HB | $1.03 \times 10^{10}$ | $8.1 \times 10^8$ | *S. aureus, K. pneumonia, E. coli, E. aerogenes, Salmonella Sp, Candida sp. Aspergillus sp, Torula sp, Bacillus flexus* |
| KC | $1.01 \times 10^7$ | $2.05 \times 10^7$ | *Salmonella sp. Enterobacter aerogenes, Candida sp.* |
| KD | $1.56 \times 10^9$ | $1.48 \times 10^7$ | *Staphylococcus aureus, E. coli, Candida sp., Fusarium sp.* |
| KH | $2.14 \times 10^7$ | $1.14 \times 10^7$ | *K. pneumonia, Enterobacter aerogenes, E. coli, Candida sp. Torula sp* |
| KM | $6.00 \times 10^5$ | $5.30 \times 10^4$ | *S. aureus, K. pneumonia, E. coli, E. aerogenes, Salmonella sp. Shigella sp. Candida sp. Serratia marsenses, Kocuria rosea* |
| MB | $6.50 \times 10^5$ | $5.40 \times 10^5$ | *Klebsiella pneumonia, E. coli, Fusarium sp.* |
| MC | $2.50 \times 10^7$ | $6.80 \times 10^7$ | *K. pneumonia, E. coli, Rhizopus sp. Candida sp. Penicillium sp, Torula sp.* |
| MH | $1.50 \times 10^{10}$ | $5.40 \times 10^6$ | *K. pneumonia, E. coli, E. aerogenes, salmonella sp, Shigella sp, Candida sp, Enterobacter agglomerurans, Flavobacterium sp, Enterobacter cloacae* |
| MS | $2.50 \times 10^7$ | $7.10 \times 10^6$ | *S. aureus, K. pneumonia, E. aerogenes, Salmonella sp., Penicillium sp.* |
| MW | $2.94 \times 10^7$ | $2.63 \times 10^7$ | *K. pneumonia, E. coli, Enterobacter aerogenes, Fusarium sp.* |
| NE | $1.80 \times 10^9$ | $1.56 \times 10^9$ | *S. aureus, K. pneumonia, E. coli, E. aerogenes, Salmonella sp, Shigella sp, Candida sp. Pseudomonas aeruginosa., Bacillus pumillis* |
| NP | $3.6 \times 10^6$ | $5.00 \times 10^5$ | *Staphylococcus aureus, Salmonella, Aspergillus sp. Fusarium sp.* |
| ST | $2.73 \times 10^7$ | $2.07 \times 10^7$ | *E. coli, Candida sp. Penicillium sp.* |
| US | $1.66 \times 10^7$ | $1.86 \times 10^7$ | *S. aureus, K. pneumonia, E. coli, E. aerogenes, Salmonella sp, Shigella sp* |

**Key**: * *E. Coli - Escherichia coli*; † *E. aerogenes - Enterobacter aerogenes*

## 4. Discussion

All unregulated herbal medicinal products did not comply with the BP standards for microbial quality. The limits for microbial contamination by the BP for aerobic microbial and fungal counts for herbal products to which boiling water is added before use are not more than $10^7$ and $10^5$ cfu/g, respectively. *Salmonella* and *Escherichia coli* and should also be absent according to BP specifications. The unregulated herbal medicinal products therefore did not meet these pharmacopoeial specifications.

The majority of the samples contained pathogenic bacterial contaminants. *Escherichia coli* which contaminated 75 % of the samples is a well known

enteropathogen, and is the most common causative agent of childhood diarrhea of bacterial origin (Bonkoungou et al, 2013). *E. coli* is also used as a marker of fecal contamination in standard assays of water and food (APHA, 1992; Jay, 1997).

The high levels of microbial contamination observed in this study may be attributed to the methods of their preparation. Personnel could introduce microorganisms to the products during handling of the raw materials and packaging of products. Abba et al (2009) have also suggested that unhygienic equipment and materials could also be a source of microbial contaminants. Good Manufacturing Practices are therefore required throughout the process of

harvesting, drying, storage, handling and preparation of the herbal medicinal product.

Other potential pathogenic microorganisms isolated from the samples included: *Salmonella* spp, *Klebsiella pneumoniae*, *Pseudomomas aeuroginosa*, *Enterobacter aerogenes*, and *Staphylococcus aureus. Staphylococcus aureas*, for example has been associated with a number of complications especially to immune-compromised individuals. It can produce proteins that disable the immune system and damage tissues. It may also release exotoxins which cause gastroenteritis (Lowy, 1998).

The finding of other potential pathogens may also be significant.

*Klebsiella pneumonia*, a Gram negative lactose fermenting bacterium found in the normal flora of the mouth is ranked second from *E. coli* for urinary tract infections in older people and is also an opportunistic infection of people with weakened immunity. It also commonly causes pneumonia (Nordmann and Naas, 2009). *Pseudomonas aeruginosa* is a notorious cause of nosocomical infections, and may cause life threatening illness in immune compromised individuals (Van Eldere, 2003).

*Salmonella spp* is clinically relevant as it may cause typhoid, a disease that is a public health concern in Kenya (Kariuki, 2010).

The presence of moulds such as *Penicillium* spp, *Candida* spp and *Aspergillus* spp are of additional concern. Both *Penicillium* spp and *Aspergillus* spp are associated with food poisoning and may be responsible for infections particularly in immunocompromised individuals (Lin, 2001; Bateman, 2002). *Penicillium* spp has been isolated from solids and liquid herbal products in a study by Esimone *et al.* (2007). In addition *Penicillium* spp produces the mycotoxin, ochratoxin A, which is both nephrotoxic and carcinogenic (Burge 1989).

## 5. Conclusion

This study has showed that unregulated herbal medicines marketed in Kenya are highly contaminated with microorganisms, some of which are pathogenic. Such unregulated medicinal products may facilitate transmission of communicable diseases in the population and therefore present a public health problem.

There is consequently need to extend government regulation to herbal medicinal products to ensure that their processing, preparation or manufacture comply with Good Manufacturing Practices, and thus lessen risks to consumers and patients.

## Conflict of Interest declaration

The authors declare no conflict of interest

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