Bacteriological Evaluation of Non-Regulated Herbal Remedies Sold in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

An essential mandate of food and drug regulatory agencies is to ensure that products offered for public consumption are free from such level of microbial contamination as to endanger the health of consumers. A number of herbal remedies offered to the public were found not to be regulated as evidenced by the absence of regulation numbers on the labels. Thus, this study sought to determine the level of bacterial contamination of packaged, labeled, non-regulated herbal remedies sold in Port Harcourt. Seventy two samples of twelve different locally produced, liquid, packaged, labeled, orally administered, non-regulated herbal remedies were purchased randomly from retail outlets within Port Harcourt metropolis. They were assessed for total heterotrophic bacterial counts (THBC) and total coliform counts (TCC). One hundred and sixty four bacterial strains obtained were characterized and identified by standard techniques employing Gram staining and biochemical methods. The mean THBC was 3.77 ± 0.77 Log_{10}cfu/ml ranging from 3.20 ± 0.99 to $4.37\pm0.91Log_{10}$cfu/ml. The mean TCC was 3.17 ± 1.02 Log_{10}cfu/ml; with the range between 2.32 ± 1.81 and 3.98 ± 0.47 Log_{10}cfu/ml. All 164 bacterial isolates belong to eleven genera, and 22 species namely *Staphylococcus aureus* (59;36.0%), *Enterobacter cloacae* (13; 7.9%), *Enterobacter pyrinus* (10; 6.1%)*, Klebsiella pneumoniae* (10; 6.1%), *Pseudomonas aeruginosa* (10; 6.1%), *Bacillus subtilis,* (8; 4.9%) *Enterobacter aerogenes* (7;4.3%)*, Serratia rubidaea* (7;4.3%)*, Proteus (Cosenza) myxofaciens* (6;3.7%)*, Staphylococcus epidimidis* (6;3.7%) *Serratia marcescens* (4;2.4%) *Bacillus cereus* (3;1.8%)*, Citrobacter rodentium* (3;1.8%)*, Enterobacter hormaechei,* (3;1.8%) *Klebsiella oxytoca,* (3;1.8%) *Proteus mirabilis* (3;1.8%). *Hafnia alvei* (2;1.2%)*, Salmonella pullorum* (2;1.2%)*, Streptococcus pyogenes* (2;1.2%) *Enterobacter cancerogenus* (1; 0.6%)

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Salmonella enterica (1; 0.6%)*, Salmonella typhi* (1; 0.6%)*.* Given that these products were processed, packaged, labeled and offered for sale to the public without regulatory numbers, it is suggested that regulatory agencies should ensure that all such products are brought within the ambits of the regulatory laws.

Keywords: Herbal remedies; non-regulated; bacterial contamination; coliforms.

1. INTRODUCTION

Herbal medicines include industrially manufactured preparations in which the active ingredients are purely and naturally original plant substances, chemically unaltered and responsible for the overall therapeutic effect of the product [1]. There had been reported surge and rising interest across the globe in the use of herbal medicinal products in the treatment and prevention of diseases. This may be partly attributable to a widely held belief that herbal medicines are safe and devoid of adverse reactions, as natural products [2,3,4,5]. However, deficient or nonexistent regulatory control of herbal medicines in most countries have raised concerns and awareness on the need to monitor safety and deepen understanding of possible harmful as well as potential benefits associated with the use of herbal medicines [6]. Most conventional medicines contain known active ingredients whose identification are required as evidence of adherence to Good Manufacturing Practice, quality control and all such necessary regulatory protocols [7,8,9]. These are more clear-cut than for a single herbal remedy, which may contain hundreds of natural constituents, what more for polyherbal products containing mixtures of various herbs containing several times more. Thus producers of herbal products and regulatory agencies are confronted with challenges in terms of standardization, as well as technical, financial and ethical constraints [7,8,9]. Notwithstanding the constraints hampering the regulation of herbal medicines, efforts should be intensified to bring all drugs for human consumption under the ambits of regulation.

Studies have also revealed that not only are herbal medicines intrinsically not free from toxicities and adverse reactions but are also exposed to extraneous factors through contamination or adulteration which may render them unsafe for human consumption. These extraneous materials include microorganisms, metals, various toxins,, pesticides, and agrochemicals organic and inorganic contaminants; [10,3,4,11,12] as well as adulterations with several approved and unapproved conventional drugs [13]. It therefore becomes imperative that studies are conducted on herbal remedies to ascertain the levels of contaminants which are likely to endanger the health of consumers impair the physicochemical properties of the remedies. The intent of this study is to determine the heterotrophic bacterial load and unravel the relevant bacterial contaminants found in herbal remedies and proffer solutions towards safer products.

2. MATERIALS AND METHODS

2.1 Collection and Handling of Herbal Remedy Samples

Seventy two samples of twelve different locally produced, liquid, packaged, labeled, orally administered non-regulated herbal remedies were purchased randomly from various herbal shops and retail outlets in within Port Harcourt metropolis. The remedies include Action STD, Ash-Bitters, Energi 003, GHH Cleanser, Green-herbs, Moringa WG, Perfect Herbs, Power-Herbs, Pro Mal, Sima Herbs, Pro-Diabetes and Pro-Eyes. The sample labels were examined to ascertain that they were within their shelf lives and to confirm the absence of regulatory numbers. They were kept at room temperature in line with the makers' directives and were analyzed within two weeks of collection. None of the samples used in this study were regulated by the statutory agency as evidenced by the absence of regulatory numbers on the labels.

2.2 Enumeration and Isolation of Heterotrophic Bacteria

The isolation and enumeration of total heterotrophic bacteria was performed as described by Ejukonemu & Isiosio [14]. The samples were serially diluted by aseptically transferring 1.0 ml amount of sample using a sterile automatic pipette into 9.0 ml amount of sterile distilled water and well mixed to obtain a 1 in 10 dilution. Then, 1.0 ml aliquot of each aliquot was serially diluted in ten folds serial dilution up to 10^{-8}. Following careful, but vigorous shaking, 0.1ml of each dilution was inoculated in triplicates by spread plating on each of the different relevant media. The media include

Nutrient agar as a general purpose medium, Blood agar for the more demanding bacteria, Macconkey agar and Eosin methylene blue agar as selective and differential media on the basis of lactose fermentation particularly for coliforms. The colony counts were taken from apposite dilutions of each of the samples with countable plates having between 25 and 250 colonies. The counts were multiplied with the appropriate final dilution factors to obtain the bacterial counts for each sample [15]. The colonial appearances of the isolates were examined carefully and recorded while isolates were broadly characterized on the basis of colonial features and Gram's reactions. Pure cultures were obtained by aseptically streaking presumptively identified isolates on nutrient agar plates and incubating at the appropriate temperatures overnight [14].

2.3 Characterization and Identification of Bacterial Isolates

The characterization and identification of the bacterial strains were carried out in accordance with the morphological and biochemical scheme stated in Benson's Microbiological Applications Laboratory Manual [16,17]. The morphological, biochemical, and physiological parameters analyzed include Colony Size, Colony Elevation, Colony Texture, Gram Reaction, Cellular Morphology, Cellular Arrangement, Haemolysis, Spores formation, Pigments production, Motility, Catalase, Oxidase, ,Indole, Methyl red, Voges prauskuer, Citrate, Urease, H2S , Nitrates, Gelatin, Starch, Glucose, Lactose, Maltose, Manitol, Succrose, Gas and Oxidation/ Fermentation. The data were inputted into the ABIS online bacterial identification software and the best matching results were compared with those of standard organisms (Costin & Ionut, 2017; [17]).

3. RESULTS

The results of the mean total heterotrophic bacterial counts (THBC) and total coliform counts (TCC) are presented in Figs. 1 and 2, respectively. The mean THBC for the herbal remedies was found to range from 3.77±0.77 Log10cfu/ml. The most contaminated remedy was Action STD with THBC of 4.37±0.91 Log10cfu/ml, while the least contaminated was Ash-bitters with THBC of 3.202±0.99 Log10 cfu/ml. The mean TCC was 3.17±1.02 Log10cfu/ml; the remedy with highest coliform load was Action STD with TCC of 3.98±0.47 Log10cfu/ml, while that with the least coliform

contamination was Ash-bitters with 2.32±1.81 Log10cfu/ml.

The results of the frequency and distributions of bacterial species among Non-regulated herbal remedies were shown in Table 1 and Fig. 3. A total of 164 bacterial strains belonging to eleven genera and twenty two were obtained from the herbal remedies. The frequency and occurrence pattern of the contaminants among the remedies revealed the predominant contaminant, *Staphylococcus aureus* (59;36.0%) as being most widely distributed, with occurrence in all the twelve (100%) unregulated remedies; the next isolates with respect to occurrence amongst the remedies were *Enterobacter aerogenes* (7;4.3%) and *Enterobacter cloacae* (13; 7.3%), each found in four (33.3%) remedies. Four isolates, namely *Bacillus subtilis,* (8; 4.9%) *Enterobacter pyrinus* (10; 6.1%)*, Klebsiella pneumoniae* (10; 6.1%) and *Pseudomonas aeruginosa* (10; 6.1%) were each isolated from three (25%) remedies, six contaminants, *Enterobacter hormaechei,* (3; 1.8%) *Klebsiella oxytoca,* (3; 1.8%) *Proteus (Cosenza) myxofaciens* (6;3.7%)*, Serratia rubidaea* (7;4.3%)*, Staphylococcus epidimidis* (6;3.7%) and *Streptococcus pyogenes* (2;1.2%) were each recovered from two (16.7%) remedies, while nine of the contaminants, were each found in one (8.3%) remedy, they include *Bacillus cereus* (3;1.8%)*, Citrobacter rodentium* (3;1.8%)*, Enterobacter cancerogenus* (1; 0.6%) *Hafnia alvei* (2;1.2%)*, Salmonella enterica*(1; 0.6%)*, Salmonella pullorum* (2;1.2%)*, Salmonella typhi*(1; 0.6%)*, Serratia marcescens* (4;2.4)*,* and *Proteus mirabilis* (3;1.8%).

4. DISCUSSION

Herbs and herbal materials normally carry a large number of bacteria, including naturally occurring microflora of the medicinal plants, and those originating from soil or derived from manure, handling by personnel during harvest/collection, post-harvest processing and the manufacturing ; process; [18] and also from water used in washing, preparation or processing, as well as processing equipment and fomites. Various practices of harvesting, production, transportation and storage may promote contamination and microbial growth, just as failure to control the moisture levels of herbal medicines during transportation and storage, and inadequate control of the temperatures of liquid forms of finished and unfinished products [18].
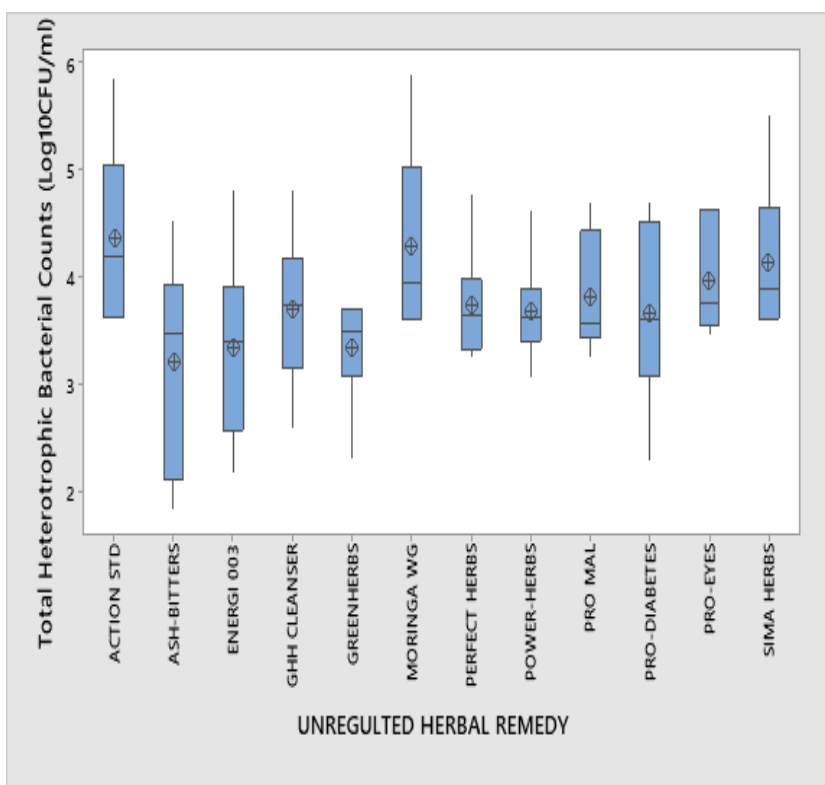
Fig. 1. The levels of Total heterotrophic bacterial counts (THBC) among the Non-regulated Herbal Remedy Samples
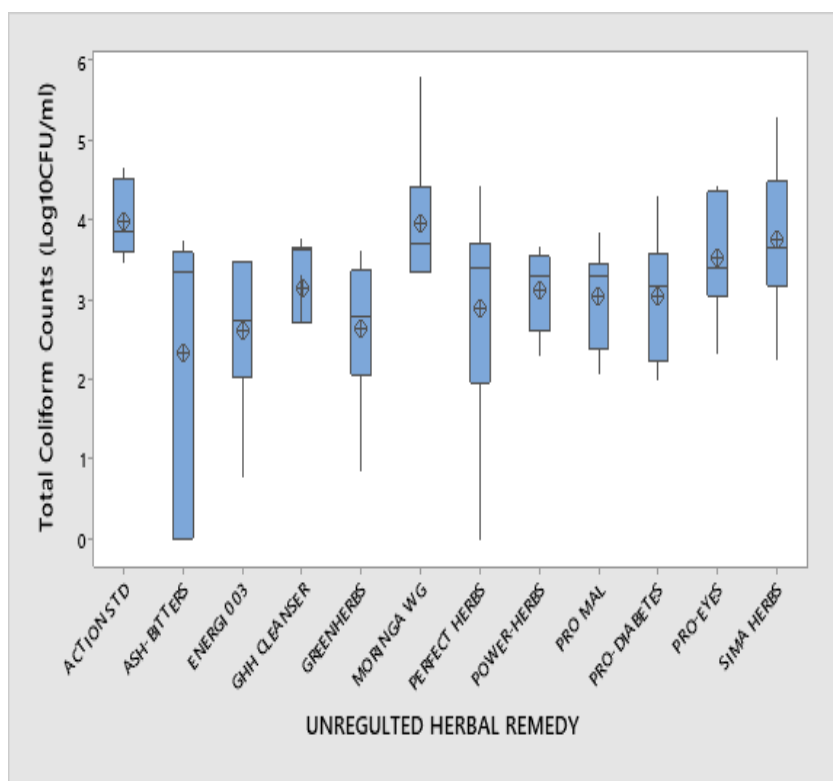

Fig. 2. The levels of Total Coliform Counts (TCC) among the Non-regulated Herbal Remedy Samples

Table 1. Frequency and Distribution of bacterial species among Non-regulated herbal remedies

Bacterial species	ACTION-STD	ASH-BITTERS	ENERGI-003	GHH CLEANSER	GREENHERBS	MORINGA-WG	PERFECT-HERBS	POWER-HERBS	PRO-MAL	PRO-DIABETES	PRO-EYES	SIMA-HERBS	Frequency (%)
Staphylococcus aureus	3	4	6	5	4	6	6	5	4	5	5	6	59 (36.0)
Enterobacter cloacae	0	4	3	0	0	0	0	0	4	0	0	2	13 (7.3)
Enterobacter pyrinus	0	0	3	5	0	0	0	0	0	0	0	2	10 (6.1)
Klebsiella pneumoniae	0	0	0	0	4	3	0	0	0	0	3	0	10 (6.1)
Pseudomonas aeruginosa	5	0	0	0	2	0	0	0	0	3	0	0	10 (6.1)
Bacillus subtilis	3	0	0	0	0	0	4	0	0	1	0	0	8 (4.9)
Enterobacter aerogenes	2	0	0	1	0	0	0	2	0	0	2	0	7 (4.3)
Serratia rubidaea	0	0	0	0	0	5	0	2	0	0	0	0	7 (4.3)
Staphylococcus epidermidis	0	0	0	0	0	3	0	0	3	0	0	0	6 (3.7)
Proteus(Cosenza) myxofaciens	0	0	2	0	4	0	0	0	0	0	0	0	6 (3.7)
Serratia marcescens	4	0	0	0	0	0	0	0	0	0	0	0	4 (2.4)
Bacillus cereus	0	0	0	0	3	0	0	0	0	0	0	0	3 (1.8)
Citrobacter rodentium	0	3	0	0	0	0	0	0	0	0	0	0	3 (1.8)
Enterobacter hormaechei	0	0	1	0	0	0	2	0	0	0	0	0	3 (1.8)
Klebsiella oxytoca	0	1	0	0	0	0	0	0	2	0	0	0	3 (1.8)
Proteus mirabilis	0	0	0	0	0	0	0	0	0	0	0	3	3 (1.8)
Hafnia alvei	0	0	0	0	2	0	0	0	0	0	0	0	2 (1.2)
Salmonella pullorum	0	0	0	0	0	0	0	2	0	0	0	0	2 (1.2)
Streptococcus pyogenes	0	0	1	0	0	0	0	0	0	0	0	1	2 (1.2)
Enterobacter cancerogenus	0	0	0	0	0	0	1	0	0	0	0	0	1 (0.6)
Salmonella enterica	0	0	0	1	0	0	0	0	0	0	0	0	1 (0.6)
Salmonella typhi	0	0	0	1	0	0	0	0	0	0	0	0	1 (0.6)
Total	**17**	**12**	**16**	**13**	**19**	**17**	**13**	**11**	**13**	**9**	**10**	**14**	**164 (100)**
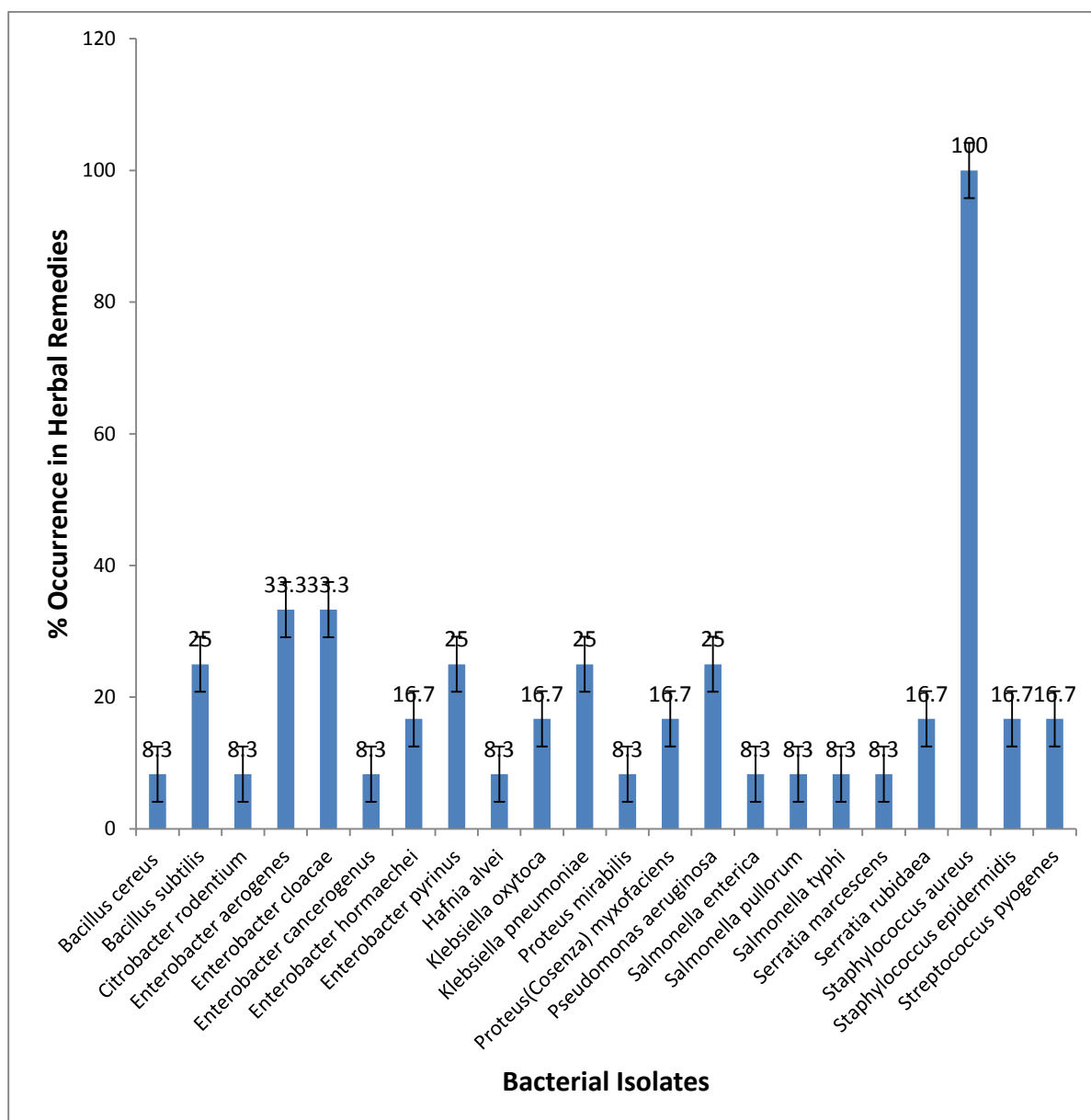
Fig. 3. Percentage occurrence of the bacterial isolates across the twelve unregulated herbal remedies

All the herbal remedies involved in this study had some levels of aerobic bacterial contamination which includes coliform bacteria. It is heartening however that all were below the WHO maximum limits of 10^5 for total aerobic bacteria, however *Salmonella* spp were found in two of the remedies in violation of the WHO guidelines which prescribes an absence of *Salmonella* [18]. Coliforms are bacteria of major public health concern and one of the largest groups of bacteria, associated with faecal pollution, [19] thus the preponderance of coliform bacteria constituting 38.4% of contaminants found in the remedies may be attributable to contaminated water used in washing, boiling and extraction of the phytochemicals. Coliform bacteria are known to flourish in the gut of endothermic animals, and indefinitely discharged into the environment through the feces. Their presence is thus amply suited as circumstantially indicative of the likely prevalence of potential pathogens, a factor that has sustained their choice for more than a century as indicators of fecal pollution [19]. Several coliform bacteria including those reported in the instant study have been shown to be regular contaminants of herbal remedies.

Their substantial presence in the remedies could thus been seen as indicative of poor microbial quality [12,11,14].

Staphylococcus is one of the commonest opportunistic bacteria colonizing the skin and mucous membranes of humans and animals [20]. *Staphylococcus aureus* as common inhabitants of the skin and mucous membranes, have humans are their major reservoir [21]. The high prevalence of *Staphylococcus* contaminants as observed in this and many previous studies have been ascribed to handlers and probably pet animals and rodents [12,11]. The genus *Bacillus* is one of the predominant genera of bacteria found in soil, having several species isolated from diverse ecological niches, [22] including herbal remedies [23,11,14]. The most distinguishing features of *these* Gram positive spore-forming rods are the ability to form endospores within cells that provide high resistance to radiation, desiccation [24].

Streptococcus pyogenes is a major human-specific bacterial pathogen transmitted through airborne droplets, direct skin contact with nasal discharge or with formites, with contaminated lesions, or contaminated food sources., and herbal remedies [25,26,27,28].

Salmonella species are Gram-negative facultative anaerobe *Enterobacteria* representing the most common foodborne pathogens frequently isolated from food-producing animals that is responsible for zoonotic infections in humans and animal species including birds; and is a major souce of global public health concern [29]. Salmonella contamination is common in herbal products and could be from handlers or livestock [12,11].

Proteus is abundant in the soil and water, and form part of the human intestinal flora. They are known to be causative agents of human infections including urinary tracts infections with their reported presence in this and other studies on herbal remedies is hardly surprising [14,30]. The *Proteus* contaminants were likely to have originated from water like the coliforms, though it is difficult to avoid exposure to these opportunistic non-fermentative gram negative rods due to their ubiquity [31].

Pseudomonas aeruginosa are ubiquitous, non-fastidious, non fermentative, Gram negative rods. They have little nutritional requirements,

and are able to survive in diverse environments including fomites like door handles, hospital room sinks, toilets seats, showers, respiratory ventilators and various healthcare equipments.. They have been reported as common contaminants in herbal remedies [12,11,32].

5. CONCLUSION

This study identified worrisome bacterial contamination of the unregulated herbal remedies. There was presence of potentially pathogenic organisms such as *Salmonella* and *Staphylococcus;* as well as indicators of poor quality products such as coliforms. Given that all the products in this study were processed, packaged, labeled and offered for sale to the public without regulatory numbers, the statutory regulatory agencies are enjoined to ensure that all such products are brought within the ambits of the regulatory statutes.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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