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Evaluation of the microbial quality of commercial liquid herbal preparations on the Ghanaian market

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Abstract

Conventionally, the microorganisms in liquid herbal mixtures are curtailed due to the fresh preparation before the administration to patients. Prolonged storage of liquid herbal preparations (due to commercialization) coupled with primeval routine production processes may increase the potential of microbial contamination in liquid herbal preparations. This study aims to analyze the microbial quality of 15 selected commercial liquid herbal preparations on the Ghanaian market. The samples were obtained from accredited pharmacies and herbal outlets in the Greater Accra region of Ghana, specifically Central Accra, between November 2019 and January 2020. The selected samples were coded HM1 to HM15. The effectiveness of the primary package of all samples was determined using the seal integrity test. The presence of microorganisms in the sampled brands was determined using nutrient agar. Isolated microorganisms from the sampled herbal mixtures were then identified using various selective media. All 15 samples (100%) passed the seal integrity test. Ten (67%) out of the 15 samples were contaminated with various microorganisms, whereas the remaining 5 samples (33%) were devoid of microorganisms. Eight (53%) out of the 15 samples were contaminated with fungi, with 3 (20%) being above the pharmacopeial limit. Six (40%) out of the 15 samples showed the presence of *Escherichia coli*. Out of the 15 sampled products, only HM11 contained *Staphylococcus aureus*. Similarly, only one sampled product (HM15) contained *Salmonella* typhi. None of the sampled products was contaminated with *Pseudomonas aeruginosa*. Ultimately, this study revealed that commercialized liquid herbal preparations in Ghana are likely to be contaminated with pathogenic microorganisms. Good manufacturing practices must therefore be strictly adhered to bring out the best in local herbal manufacturing industries.

Keywords: Herbal medicines; Microbial quality; Good manufacturing practices

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1. Introduction

Traditional medicine is described by the World Health Organization (WHO) as the total knowledge, skills and practices based on the theories, beliefs, and experience indigenous to different cultures, whether explicable or not, that are used to maintain health as well as to prevent, diagnose, improve, or treat physical and mental illness[1]. Thus, African traditional medicine is indigenous to African culture and well patronized by the general populace. According to the WHO, the use of traditional medicine has increased exponentially over the past decade[1,2].

Traditional medicine plays a crucial role in healthcare all over the world, especially in developing countries. In Ghana, traditional medicines form an integral component of the health care system and due to cultural diversity in Ghana; herbal medicines are also highly diverse. Herbal medicines may contain either single or multiple herbs in a single drug preparation. Due to this reason, herbal medicines contain many chemical compounds that have therapeutic benefits in a wide variety of diseases[3]. The perception that herbal medicines are relatively safe and harmless is one of the key reasons for the recent increase in its usage among Ghanaians. However, the quality and safety of herbal products have become a matter of public interest due to their commercialization, global market expansion, and universality[2,4]. Studies carried out by Abba *et al.* in 2009 indicated that 46.67% of sampled herbal products in Kaduna were contaminated with *Salmonella* typhi, while 58.67% and 65.33% were contaminated with *E. coli* and *Staphylococcus aureus*, respectively[5]. Concomitantly, studies carried out by Enayatifard *et al.* in 2010 revealed that all the sampled herbal products (n = 20) in Sari were contaminated with *Salmonella* spp.[6]. These studies have revealed the potential serious health implications of herbal products and raise a serious public health concern about the paradoxical notion of the safety of herbal medicinal products in general.

In Ghana, approximately 65% of the population relies on herbal medicine alone to meet their basic health-care needs[7,8]. The majority of these herbal products, which have been licensed by the Food and Drugs Authority (FDA) of Ghana for sale on the Ghanaian market, are mostly in liquid dosage forms. Studies carried out by Agyeman-Duah *et al.* in 2017 indicated that herbal powders marketed in Ghana were contaminated with microorganisms[7]. Herbal liquid preparations are known to be more susceptible to microbial growth due to varied reasons, such as presence of water or moisture, contamination in raw materials, and primeval routine production processes. These microorganisms can make the product esthetically unpleasant, change the color of the preparation, and if they are pathogenic, cause serious infections in the patient with a resultant decrease in patronage and usage by clinicians and patients. It is therefore very crucial to perform assessment of the level of microbial contamination in herbal liquid mixtures after preparation as a routine post market surveillance activity[9,10]. In view of this, our study sought to evaluate the microbial quality of 15 selected commercial liquid herbal preparations on the Ghanaian market.

2. Materials and methods

2.1. Materials

All culture media, such as nutrient agar, MacConkey Agar, cetrimide nutrient Agar, Sabouraud dextrose agar, mannitol salt agar, and bismuth sulfite agar, were provided by Central University Microbiology Laboratory stores and sourced from Merck chemical company limited.

2.2. Methods

2.2.1. Sample collection

The samples were obtained from the accredited pharmacies and herbal medicine outlets in the Greater Accra region of Ghana, specifically Central Accra, between November 2019 and January 2020. The shelf life of all samples was longer than a year from the date of purchase.

2.2.2. Seal integrity test

The seal integrity test was done by inverting and clamping each sampled herbal product onto a ring stand. The herbal product was then immersed into a glass beaker full of water containing dye with the cap completely submerged. For prevention of any leakage, the setup was monitored for 30 min[11-13].

2.2.3. Preparation of media for microbial analysis

The various media, such as nutrient agar, cetrimide agar, bismuth sulphite agar, mannitol salt agar, MacConkey agar, and Sabouraud dextrose agar, were prepared according to the International Pharmacopeia 2019 and British Pharmacopeia 2018 standards[14,15].

2.2.4. Microbial analysis

2.2.4.1. Presence of microorganisms

Nutrient agar was used in ascertaining the presence of microorganisms in all the 15 samples (HM1 to HM15) using the method described by Esimone *et al.* and Okunlola *et al.*[16,17]. The procedure was carried out in triplicates for each sample.

2.2.4.2. Test for fungi (yeast or molds)

One milliliter of each sample was pipetted and subjected to a 10-fold serial dilution to 10^{-6}. An inoculum of 0.1 mL

of the 10^{-6} dilution was spread-plated on the well-dried surface of Sabouraud dextrose agar and incubated at 25°C (room temperature) for 4 days to allow for possible growth of fungi[2,15,18,19]. Each sample was inoculated in triplicates.

2.2.4.3. Test for E. coli

One milliliter of each sample was pipetted and subjected to a 10-fold serial dilution to 10^{-6}. An inoculum of 0.1 mL of the 10^{-6} dilution was spread-plated on the well-dried surface of MacConkey agar. The Petri dishes containing the spread-plated MacConkey agar were inverted and incubated at 37°C for 24 h to allow for possible growth of *E. coli*[2,15,18,19]. Each sample was inoculated in triplicates.

2.2.4.4. Test for S. typhi

One milliliter of each sample was pipetted and subjected to a 10-fold serial dilution to 10^{-6}. An inoculum of 0.1 mL of the 10^{-6} dilution was spread-plated on the well-dried surface of bismuth sulfite agar in a petri dish. The petri dishes containing the spread-plated bismuth sulfite agar were inverted and incubated at 37°C for 24 h to allow for possible growth of *S.* typhi[2,15,18,19]. Each sample was inoculated in triplicates.

2.2.4.5. Test for Pseudomonas aeruginosa

One milliliter of each sample was pipetted and subjected to a 10-fold serial dilution to 10^{-6}. An inoculum of 0.1 mL of the 10^{-6} dilution was spread-plated on the well-dried surface of cetrimide agar. The petri dishes containing the spread-plated cetrimide agar were inverted and incubated at 37°C for 24 h to allow for possible growth of *P. aeruginosa*[2,15,18,19]. Each sample was inoculated in triplicates.

2.2.4.6. Test for S. aureus

One milliliter of each sample was pipetted and subjected to a 10-fold serial dilution to 10^{-6}. An inoculum of 0.1 mL of the 10^{-6} dilution was spread-plated on the well-dried surface of mannitol salt agar. The petri dishes containing the spread-plated mannitol salt agar were inverted and incubated at 37°C for 24 h to allow for possible growth of *S. aureus*[2,15,18,19]. Each sample was inoculated in triplicates.

3. Result

Manufacturing dates, expiry dates, and batch numbers obtained from the sampled liquid herbal products together with their designated codes are summarized in Table 1. The shelf life of all sampled products was longer than 1 year from the date of purchase.

Results of the seal integrity test on the primary package of the sampled herbal liquid preparations are summarized in Table 2. None of the sampled herbal liquid preparations failed the sealed integrity test.

Table 1. Information of selected samples

Code	Manufacturing date (month/year)	Expiry date (month/year)	Batch number
HM1	06/19	06/21	LVCM 000290 LHC
HM2	05/19	05/22	HL-0015V
HM3	06/18	06/21	001
HM4	06/19	06/22	00G05
HM5	03/19	03/22	0319
HM6	08/18	08/22	FPVL0018
HM7	11/19	11/21	TX97
HM8	10/19	10/21	00342
HM9	02/19	02/21	MAMI 01/2019
HM10	10/19	10/21	0052
HM11	01/19	01/24	004
HM12	11/17	11/22	004
HM13	08/19	7/21	MC/08/19
HM14	03/19	03/21	ADT/1116
HM15	10/19	10/21	589

Table 2. Seal integrity test results for sampled herbal preparations

Sample code	Observation
HM1	No leak observed
HM2	No leak observed
HM3	No leak observed
HM4	No leak observed
HM5	No leak observed
HM6	No leak observed
HM7	No leak observed
HM8	No leak observed
HM9	No leak observed
HM10	No leak observed
HM11	No leak observed
HM12	No leak observed
HM13	No leak observed
HM14	No leak observed
HM15	No leak observed

The results of the presence or otherwise of specific microorganisms in the sampled liquid herbal preparations are shown in Table 3. HM1, HM2, HM3, HM8, and HM10 were not contaminated with microorganisms based on the nutrient agar results, and these results were confirmed by the absence of specific microorganisms in the various selective media used. Samples HM4, HM5, and HM9 were contaminated with both fungi and *E. coli*, while samples

HM6, HM7, and H13 contained only fungi. HM11 was contaminated with both fungi and *S. aureus*, while HM12 and HM15 were contaminated with *E. coli*. Fungi, *E. coli,* and *S.* typhi were found to be present in HM14.

The level of microbial contamination in the sampled herbal preparations is recorded in Table 4. Samples HM4, HM5, and HM12 had their *Escherichia coli* counts beyond pharmacopeial limits whiles HM9, HM14, and HM15 had *Escherichia coli* levels within pharmacopeial limits[20]. Samples HM 7, HM11, and HM14 had fungi counts beyond pharmacopeial limits whiles HM4, HM5, HM6, HM9, and HM13 had their counts being within pharmacopeial limits[15,19-21]. *S.* typhi counts in HM 14 were above pharmacopeial limits[15,19-21]. None of the sampled herbal liquid preparations contained *P. aeruginosa*. HM11

Table 3. Results of microbial analysis

Sample	Nutrient agar	Fungi	*E. coli*	*S.* typhi	*P. aeruginosa*	*S. aureus*
HM1	No growth	No growth	No growth	No growth	No growth	No growth
HM2	No growth	No growth	No growth	No growth	No growth	No growth
HM3	No growth	No growth	No growth	No growth	No growth	No growth
HM4	Growth	Growth	Growth	No growth	No growth	No growth
HM5	Growth	Growth	Growth	No growth	No growth	No growth
HM6	Growth	Growth	No growth	No growth	No growth	No growth
HM7	Growth	Growth	No growth	No growth	No growth	No growth
HM8	No growth	No growth	No growth	No growth	No growth	No growth
HM9	Growth	Growth	Growth	No growth	No growth	No growth
HM10	No growth	No growth	No growth	No growth	No growth	No growth
HM11	Growth	Growth	No growth	No growth	No growth	Growth
HM12	Growth	No growth	Growth	No growth	No growth	No growth
HM13	Growth	Growth	No growth	No growth	No growth	No growth
HM14	Growth	Growth	Growth	Growth	No growth	No growth
HM15	Growth	No growth	Growth	No growth	No growth	No growth

Abbreivations: *E. coli: Escherichia coli*; *S.* typhi: *Salmonella* typhi; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa.*

Table 4. Level of microbial contamination in sampled herbal preparations

Sample	*E. coli* (cfu/mL)	Fungi (cfu/mL)	*S.* typhi (cfu/mL)	*S. aureus* (cfu/mL)	*P. aeruginosa* (cfu/mL)
HM1	-	-	-	-	-
HM2	-	-	-	-	-
HM3	-	-	-	-	-
HM4	TNC	$<1\times10^1$	-	-	-
HM5	TNC	$<1\times10^1$	-	-	-
HM6	-	$<1\times10^1$	-	-	-
HM7	-	TNC	-	-	-
HM8	-	-	-	-	-
HM9	$<1\times10^1$	$<1\times10^1$	-	-	-
HM10	-	-	-	-	-
HM11	-	TNC	-	TNC	-
HM12	TNC	-	-	-	-
HM13	-	$<1\times10^1$	-	-	-
HM14	$<1\times10^1$	TNC	$<1\times10^1$	-	-
HM15	$<1\times10^1$	-	-	-	-

Abbreviations: TNC: Too numerous to count; *E. coli*: *Escherichia coli*; *S.* typhi: *Salmonella* typhi; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa.*

was contaminated with *S. aureus* above pharmacopeial limits[15,19-21].

4. Discussion

The safety of herbal medicinal products has been a significant concern for health agencies, pharmaceutical industries, and the general public[20]. More frequently than not, primary sources of raw materials for herbal products are tainted by several microorganisms from soils and the environment. Harvesting, handling and production activities also frequently contribute to additional microbial contamination. Commonly reported microorganism that may be present in liquid herbal preparations includes *E. coli*, fungi, *S.* typhi, *S. aureus,* and *P. aeruginosa*. Microbial contamination of liquid herbal preparations may potentially endanger health or cause severe infection if administered orally or through any means by which the organism may have exposure to the body[3,4,16,22]. A total of 15 samples consisting of herbal blood tonics and herbal preparations for piles, typhoid, and sexually transmitted infections were randomly chosen for this study (Table 1) and assessed for the presence and levels of microbial contamination.

Primary packages are expected to offer finished medicinal products a barrier to the contamination with microbes, which can be found in the natural environment and atmosphere. All the sampled herbal products (100%) passed the seal integrity test (Table 2). This indicates that their primary packages serve as a good barrier for preventing the ingress of microbes from the immediate environment and atmosphere into the finished herbal products; therefore, any microorganisms that could be found in the herbal product could have been introduced during the production processes or contaminated from its source, the starting raw materials[11,12].

Microbial contamination limits in herbal medicinal products set by the European Pharmacopoeia (EP), British Pharmacopoeia (BP), United States Pharmacopoeia (USP), and WHO help in the maintenance of product safety and efficacy, ultimately safeguarding the health of the consumer. A majority (67%) of the sampled herbal products were contaminated with microorganisms, whereas the remaining 33% showed no growth of any microorganism (Table 3). All the sampled herbal products (100%) were not contaminated with *P. aeruginosa* (Table 4) and hence passed the microbial limit of *P. aeruginosa* contamination in herbal medicinal products as specified by the British Pharmacopoeia[15,19-21]. *P. aeruginosa* is an opportunistic pathogen and a major cause of nosocomial infections; therefore, their absence in all the sampled herbal products is a desirable attribute. According to the British Pharmacopoeia, *S.* typhi should not be present in oral herbal preparations[23]. According to our findings, only one product (HM14) was contaminated with *S.* typhi (Table 4). This has grave consequences on the consumers of this product (HM14) since *S.* typhi is known to cause typhoid fever with debilitating effects. However, 93% of the sampled herbal products did not contain *S.* typhi and complied with the specifications set by the British Pharmacopoeia[15,19-21]. While the British Pharmacopoeia stipulates that oral herbal preparations should not contain *E. coli,* the WHO recommends a limit of not more than 1×10^1 Cfu/mL. Based on the limits set by the British Pharmacopoeia[15,19,21], 40% of the sampled products were contaminated with *E. coli*. Conversely, 20% of the herbal products exceeded the recommended WHO limits for *E. coli* (Table 4)[20]. *E. coli* has been implicated in common bacterial infections, including cholecystitis, cholangitis and urinary tract infections. This may result in a blind spot in the clinics, where clinicians may attribute complication of traditional medicine consumption to be the cause of such disease conditions while the reason could be microbial contamination. Their presence in herbal medicines may exacerbate the disease conditions of consumers with dire consequences. Only one sampled product (HM11) was contaminated with *S. aureus* (Table 4) and failed the specifications set by the British Pharmacopoeia and the WHO[15,19-21]. *S. aureus* has the potential of causing a wide range of conditions and life-threatening diseases, including skin infections, abscesses, pneumonia, and meningitis. Thus, the presence of this microorganism in HM11 can potentially aggravate the existing pathological condition(s) of the consumer or result in the development of life-threatening complications. Eight (53%) out of the 15 samples showed fungal growth, with 5 (33%) of the infected samples (HM4, 5, 6, 9, and 13) showing colony forming units which complied with the British Pharmacopoeia and the WHO specifications while the remaining infected samples (HM7, 11, and 14) exceeded the set limits[15,19-21]. High levels of fungi in oral preparations can cause candidiasis of the mouth, throat, and esophagus. They are also known to cause skin infections and, in extreme cases, pneumocystis pneumonia[24]. Thus, high levels of fungi in these herbal medicinal products (HM7, 11, and 14) will have negative health effects on the consumers, which may manifest as new disease condition(s), such as pyelonephritis and gastrointestinal hemorrhage. Ultimately, patients taking two or more of the sampled herbal products (contaminated with microorganisms) for the treatment of different ailments may be at risk of contracting serious infections and complicating their disease conditions due to the presence of these pathogenic microorganisms in their gastrointestinal tract and eventually in their blood.

5. Conclusion

The microbial quality of the sampled herbal formulations was evaluated and the microbes found to be present in the various herbal samples included *E. coli*, fungi, *S.* typhi, *P. aeruginosa,* and *S. aureus*. Only 33% of the sampled herbal products were not contaminated with microorganisms. Producers of herbal liquid preparations in Ghana should be educated on the need to enforce good manufacturing practices, effective harvesting practices, and safe handling and storage of liquid herbal medicinal products. Periodic sanitization and education courses should be carried out by various regulatory and research agencies, such as the FDA of Ghana and the Ghana standards authority, using easy-to-interpret flyers as well as online and in-person refresher courses.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data used to support the findings of this study are included in the article and also available from the corresponding author on reasonable request.

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