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Bacteriological Investigations on the Quality of Herbal Medicinal Concoctions Vended in Gombe Markets.

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ABSTRACT

Background: The use of medicinal plant products for the treatment of diseases is still highly valued globally due to several rationales, especially in low-income countries. The recognized potency of herbal medicines based on their vast pharmacological functions has enabled their uses to produce modern pharmaceutical drugs. However, the unregulated production and widespread use of non-sterile pharmaceutical medicinal products like herbal medicinal concoctions have instigated public health experts to question their quality and wholesomeness for consumption. This prompted the study aimed at investigating the quality of herbal medicinal concoctions vended in Gombe markets.
Methods: A total of sixty (60) herbal medicinal concoctions were randomly sampled from four different vending outlets or markets for this study. Microbiological culturing techniques which include ten-fold serial dilution and spread-plating method were adopted for the isolation of bacteria in herbal medicinal concoction samples, and viable plate counts were established in colony-forming units per mL according to standard practice. Identification of bacterial isolates was achieved by colony morphology determination/macroscopy, gram staining and microscopy, while the identified bacteria were further confirmed using several biochemical analyses.
Results: The mean viable plate count for all the different concoctions sampled was high and above the safe level of the established standards for non-sterile herbal preparations. Consequently, this initially signifies the unsafe status of the herbal concoctions for public consumption. Also, eight (8) bacteria genera/species among which are pathogenic strains were isolated and identified from the herbal concoctions. These bacterial contaminants with different percentages of occurrences include *Enterobacter* spp., *Escherichia coli, Staphylococcus aureus, Shigella* spp., *Staphylococcus epidermidis, Klebsiella pneumoniae, Citrobacter freundii, Salmonella typhi.*
Conclusion: Herbal or medicinal concoctions vended in different markets within the Gombe metropolis where samples were collected were grossly contaminated beyond the established allowable limits for non-sterile pharmaceuticals, thus depicting the unsafe quality status of the medicinal products for consumption.

1. INTRODUCTION

The traditional use of herbs and medicinal plants for the treatment of illnesses is still in practice despite the advancement in modern medicine; though several factors among which are poverty, cultural beliefs and illiteracy have enabled the persistent use and increase the patronage of herbal medicinal products. In Nigeria, the increasing price of medication and poverty has forced a large population to resort to herbal medicines for treatment. The medicinal value of herbs and other plants has been identified, recognized, and used since the beginning of human history[1]. In ancient days, the use of herbs for medicinal purposes was based on instinct and experiences where people strongly believed in the curative properties of plants and thus were widely used for numerous health disorders[2]. This ancient use of herbs and medicinal plants was an integral part of the development of modern civilization of which several potent herbs and medicinal plants have been exploited for modern medicine based on their verified active constituents. To support this, Rate *et al.*[3]

stated that about 25 % of prescribed drugs in the world are sourced from plants; also, the phytochemical ingredients of medicinal plants are distinguished as the basis for the long aged traditional herbal medicine currently sustained in many parts of the world[4]. For mention, in the US, ~25 % of prescribed medicinal products contain a minimum of one active constituent extracted from crop products[5].

The numerous herbs and medicinal plant parts (e.g., leaves, stems, barks, roots, fruits, and flowers) extracts are often utilized as potent raw medicines which possess varieties of pharmacological functions from which plant-derived drugs are produced based on therapeutic and traditional healings antecedents. This shows the use of medicinal plants has attained a long-standing sustainable role in health systems all over the world. Consequently, the current implication now arises where herbal or medicinal plant products such as herbal or medicinal concoctions are often produced in local settings or at a factory scale and portrayed as drugs for the treatment of several diseases. Besides, since medicinal plants possess therapeutic properties or exert beneficial pharmacological effects on the human and animal bodies[6], the rational understanding is that herbal or medicinal concoctions are supposed to exert the same therapeutic and antimicrobial properties when consumed.

Herbal or medicinal concoctions are preparations or mixtures mostly derived from either a single or multiple herbs or/and medicinal plants via different phases of processing. Oluyemi *et al*.[7] referred to herbal medicine as preparations and finished products which contain plant parts and materials as their active ingredients and are used for the treatment of diseases. Often, herbal products are prepared by subjecting herbs to different processes such as steaming, decocting, infusing in water, roasting, squeezing, baking, or sweetening with honey, and alcohol extraction[8]. These medicinal concoctions are mostly street-vended in cities of most developing countries for the supposed treatment of certain infections like enteric fever, *Staphylococcus* infections and other infectious and non-infectious diseases.

Presently, the never-ending production and sales of herbal or medicinal concoctions in our society today is startling, because its high patronage cut across gender, age, social and political status. Meanwhile, producers and vendors have relied on sales opportunities in villages and cities for economic reasons while the end users clinched on the believed or tested efficacy of herbal concoctions. However, the unhygienic processing, handling, and packaging of herbal or medicinal concoctions which could be sources for microbial contamination, coupled with the unregulated production and sales of different herbal concoctions has called for public health concerns. The public health repercussion of consuming unsafe and unwholesome herbal or medicinal concoctions could result in catastrophic public health issues of great concern such as an outbreak of foodborne infection, microbial food poisoning, body organ damage, adverse allergy and many more. In this regard, with the aforementioned valid insinuations and owing to the large public patronage of herbal concoctions, it is of great public health relevance to examine the bacteriological quality status of different herbal or medicinal concoctions sold within the Gombe metropolis to ascertain the safety of consumption. Additionally, this study will unravel bacterial infectious agents contained in the various types of herbal or medicinal concoctions to further prospect on public health implications.

2. MATERIALS AND METHODS

2.1 Sample Collection

Sixty (60) herbal or medicinal concoction samples were randomly collected from four different markets for the bacteriological analysis. For the sampling criteria, five (5) different types of herbal or medicinal concoctions were purchased from four (4) dissimilar vending locations which include Gombe old market, Gombe new market, Pantami market and Nassarawo market. Triplicates of the five (5) different types of herbal medicinal concoctions were collected from each market which gave a total of 60 samples analysed in this study.

The five different purposed common medicinal preparations sampled for this study were sold for the treatment of typhoid fever, malaria, ulcer and other health issues like haemorrhoids and severe cough. These samples were aseptically transferred into a new and sterile pre-labelled sampling bag and immediately taken to the laboratory in an ice cooler at 4 °C for microbiological analysis. Samples not analysed immediately were also preserved at 4 °C in the refrigerator for not longer than 12 hours before analysis.

2.2 Isolation of bacterial infectious agents from herbal/medicinal concoctions

The conventional culturing approach was used for the isolation of bacteria from the sampled medicinal concoctions by adopting the following standard microbiological techniques: Per the standard serial dilution practice, ten-fold serial dilution was completed by initially taking 1 mL of herbal or medicinal concoction

sample (as stock) and dispensed into 9 mL distilled water to obtain 10^{-1} dilution. Further dilution from 10^{-1} to 10^{-10} dilutions was attained serially as described by Ibrahim *et al*.[9] to obtain discrete colonies when plated. After serial dilution, 0.1 mL from the 10^{-7} and 10^{-8} dilutions were spread-plated separately onto plate count agar for the viable plate count, and further plated on Nutrient agar and other selective and differential media (Mannitol salt agar, MacConkey agar, S*almonella Shigella* agar and Esoine methylene blue agar) for selective cultivation and easy identification of specific bacteria based on their distinguishing biochemical properties. All inoculated plates were incubated for 24 hours at 37 °C and subsequently examined for colony morphology. Colonies obtained on PCA were counted using an illuminated colony counter and viable count in CFU/mL was determined as per standard calculation by Miles and Misra[10].

2.3 Identification and confirmation of bacteria isolated from herbal medical concoctions.

Various microbiological techniques described below were applied for the microbial identification and confirmation of bacteria isolated from the sampled herbal medicinal concoctions.

2.3.1 Colony morphology of bacteria isolates

The macroscopic examination was carried out on the colonies from the culture plates to observe the colony characteristics and morphology of the bacteria isolated. Colony features observed include colony size, shape, colour, elevation, margin consistency haemolysis, pigmentation and odour of the colonies produced especially on differential and selective media.

2.3.2 Gram reaction and microscopic morphology of bacteria isolates

Bacterial isolated were subjected to gram staining following gram staining techniques described by Cheesbrough[11] to determine the bacterial cell wall's gram reaction. Subsequently, both gram reaction and microscopic cell morphology were deduced by observing the gram-stained cells under the microscope at x100 objectives under oil immersion.

Biochemical characterization of bacterial isolates

Aside from the above-mentioned identification of bacteria isolated from the herbal or medicinal concoction, confirmation of the bacterial isolates is paramount, and this was achieved by adopting the confirmatory biochemical tests. These biochemical tests which include the catalase test, coagulase test, citrate utilization test, urease test, indole test, oxidase, Kligler iron agar (KIA), motility and methyl red were carried out as described by Cheesbrough[11]. Bacterial identification was based on a scheme in Bergey's manual of determinative bacteriology[12,13], based on results from colony morphology, gram staining and microscopy, and confirmatory biochemical tests.

3. RESULTS

The bacteriological assessment of sixty (60) samples of herbal medicinal concoctions collected from four (4) different markets produced results shown in the tables below:

Table 1: Therapeutic types and details of Herbal/Medicinal Concoctions Sampled within Gombe Metropolis all devoid of an ingredient list and regulatory approval by NAFDAC.

Sample	Therapeutic claim	Formulation type	Administration mode
1	Pile/Hemorrhage	Suspension	Oral
2	Typhoid fever	Solution	Oral
3	Ulcer	Solution	Oral
4	Cough	Solution	Oral
5	Malaria	Solution	Oral

Table 1 provides information about the five (5) different types of herbal or medicinal concoctions sampled from four (4) different markets (Gombe old market, Gombe new market, Pantami and Nassarawo markets) within the Gombe metropolis. Table 1 denotes that all the five different purposed herbal concoctions sampled for this study were prepared liquid mixture (in solution) herbal concoctions with varied claims of therapeutic functions such as treatment of typhoid fever and others. It is noteworthy to state that all the herbal concoction samples were not registered with National Agency for Food and Drug Administration and Control (NAFDAC). It must be acknowledged that the compositions of these herbal concoctions in terms of herbs and medicinal plant sources were not probed in this study.

Table 2: Mean Viable Plate Count of herbal concoction samples from four (4) different markets within the Gombe metropolis.

Sample	Treatment claim	Mean viable plate counts of herbal concoction (CFU/mL)			
		GOM	GNM	PM	NM
1	Pile	1.0×10^9	1.6×10^9	1.4×10^9	1.9×10^9
2	Typhoid fever	2.1×10^9	1.4×10^9	1.5×10^9	1.2×10^9
3	Ulcer	1.9×10^9	1.7×10^9	1.9×10^9	1.5×10^9
4	Cough	1.1×10^9	1.9×10^9	1.7×10^9	1.5×10^9
5	Malaria	2.0×10^9	2.4×10^9	1.0×10^9	1.6×10^9

Viable plate count established in colony forming units per millilitre (CFU/mL). GOM = Gombe old market, GNM = Gombe new market, PM = Pantami market, NM = Newmarket.

Table 2 shows the mean viable plate count (for aerobic mesophilic bacteria) in CFU/mL for all five (5) different types of herbal concoctions sampled from the four (4) different markets within the Gombe metropolis. Irrespective of the sample location, the lowest and highest mean viable plate count for the herbal medicinal concoctions bacteriologically analysed include 1.0×10^9 and 1.9×10^9, 1.2×10^9 and 2.1×10^9, 1.5×10^9 and 1.9×10^9, 1.1×10^9 and 1.9×10^9, 1.0×10^9 and 2.4×10^9 for pile/haemorrhage, typhoid fever, ulcer, cough and malaria respectively.

Table 3: Average of Total Viable Counts for the Five Different Herbal Concoctions

Samples	Treatment claim	Average viable plate counts across all vending locations
1	Pile	1.5×10^9
2	Typhoid fever	1.5×10^9
3	Ulcer	1.7×10^9
4	Cough	1.5×10^9
5	Malaria	1.7×10^9

Viable plate count in colony forming units per millilitre (CFU/mL).

Table 3 realized from table 2 shows the average viable plate counts (of herbal concoctions) for all the entire four (4) vending locations within the Gombe metropolis. This table recognised that the herbal concoction for the treatment of ulcer and malaria has the highest aerobic mesophilic bacteria load with a plate count of 1.7×10^{11} followed by others. Though, a higher separate count from a single vending point could interfere with the average values obtained.

Table 4: Identity of Bacteria Isolated from Herbal Medicinal Concoctions and Percentage of Occurrence.

Identity of bacteria isolate	Percentage of occurrence (%)
Enterobacter spp	17
Klebsiella pneumonia	16
Shigella spp	14
Staphylococcus aureus	12
Staphylococcus epidermidis	12
Citrobacter freu ndii	12
Escherichia coli	9
Salmonella typhi	8

Table 4 shows the identity of various bacteria isolated from herbal samples bacteriologically analysed in this study. The table also presented the percentage of occurrence of bacterial isolates in the entire Sixty (60) herbal medicinal concoctions sampled across four markets within the Gombe metropolis.

This study (Table 4) exposed eight (8) different bacteria (genera/specie) which includes *Enterobacter* spp., *E. coli, S. aureus, Shigella* spp., *S. epidermidis, K. pneumoniae, C. freundii,* and *S. typhi* isolated and identified from the different herbal or medicinal concoctions vended in four (4) markets within Gombe metropolis. Though *Enterobacter* spp (17 %) produced the highest percentage of occurrence, trailed closely by *K. pneumonia* (16 %), then *Shigella* spp (14 %), and others with the same percentage of occurrence (12 %) are *S. aureus, S. epidermidis* and *C. freundii. E. coli* and *S. typhi* produced the lowest percentage of occurrence of 9 % and 8 % respectively.

4. DISCUSSION

This study portrays varied bacterial counts (Table 2), mostly the viable mesophilic bacteria in the different herbal concoctions vended in various outlets within the Gombe metropolis. The viable plate counts (Table 2) of these herbal concoctions are very high and this simply depicts a very high bacterial load or microbial contamination. The established safe level for non-sterile herbal preparation is 10^7 bacteria per mL of herbal preparation while the safe level for fungal count is 10^5 fungi per mL. Also, the limit of microbial contaminants for aerobic bacteria in herbal medicines is 10^5 CFU/g[14]. In relation to the European pharmacopoeia and WHO standards, it can be rightly declared that all the assorted herbal concoctions for the treatment of various ailments analysed in this study are very unsafe for consumption as their viable counts (Table 2) exceed these allowable limits.

From another standpoint, these herbal or medicinal concoctions can as well be viewed as a ready-to-drink item since the majority of herbal mixtures are vended as such and consumed without further processing of the medicinal products. Besides, many of these herbal concoctions have been subjected to various stages of processing which include heating, boiling or cooking, roasting and others basically to extract the medicinal constituents, consequently reducing the microbial load of the herbal mixture. In consideration of the allowable limits for ready-to-eat food, the International Commission on Microbiological Specifications for Food (ICMSF) reported the limit in order of $\leq 10^3$ for total aerobic bacterial and fungal counts for any ready-to-food to be acceptable, and $\leq 10^4$ to $\leq 10^5$ as tolerable limit[15]. Still, the viable plate counts for the herbal products analysed in this study are far in excess of the allowable limit specified by the International Commission on Microbiological Specifications for Food (ICMSF). Based on these established standards, these herbal concoctions are considered unacceptably contaminated beyond acceptable limits and hence unsafe for consumption. Related studies (e.g.,[16-17]) have also reported high microbial load in various types of herbal preparation vended to consumers. In comparison with previous studies, Ebo *et al*.[16] related that 50 % of the total herbal samples analysed in their study were not within the safe level of 10^7 bacteria per mL, while Stevic *et al*.[18] reported the highest contaminants of herbal drugs where the microbial load exceeded the acceptable limit. Admissibly, the high mean viable plate counts of herbal concoctions above acceptable limits of established standards presented in this study is in accordance with several previous studies[17-20]. Consequently, this generally relates to the unsafe status of most herbal preparations vended for public consumption. And one of the chief implications of the consumption of unsafe food and medicinal products is that it causes various foodborne diseases[9].

Also, table 2 shows variations in the mean viable counts of the same type of herbal concoction across all the different vending outlets, indicating varied bacterial loads in samples collected. This is only reasonable as the concoctions are unique in their therapeutic claim but may vary in composition and mode of preparation as mostly stated by the producers.

The presence of bacteria variety including pathogenic or infectious ones (e.g., *S. typhi, Shigella* spp, *K. pneumonia, S. aureus, S. epidermidis*) especially emphasized the unsafe status of these herbal or medicinal concoctions for human consumption. Collaboratively, this further attested to the earlier assertion regarding the unwholesome and unsafe quality of these herbal products regarding their mean counts exceeding the allowable limits. Also, in agreement with the findings of this study, related research by Okunlola *et al*.[20] also isolated similar genera or species of bacteria from herbal preparations or products.

It is noteworthy to mention that the high microbial count above the acceptable limits observed in this study, and the presence of several bacterial contaminants in the herbal concoctions could be mostly due to the varied sources of contamination of the medicinal product. These contamination sources could be the unhygienic preparation of herbal concoctions, poor handling, packaging and importantly, the water used for the preparation of herbal concoctions. Also, microbial contamination may originate from the plants and other materials for compounding the herbal concoctions - this is reasonable since herbs or medicinal plants are originally exposed to pathogenic microorganisms from the air, soil and water[21]. These mentioned sources of microbial contamination of herbal concoctions have been backed by several studies, as Ola *et al*.[22] stated that microbial contaminants are introduced into herbal preparations during the handling, preparation, storage and harvesting of herbal medicine.

Stevic *et al*.[18] and Okunlola *et al*.[20], also reiterated that harvesting, soil, drying, storage and poor handling of herbal plants can influence the microbial quality of herbal medicines. Additionally, bacterial contaminants are

introduced by personnel during processing[23]while Abba *et al*. [24] reported the presence of various bacteria in herbal products may be attributed to the preparation methods, equipment/utensils and materials used during the production process. Safety issues concerning herbal drugs or medicines are being ignored by herbalists (producers) whose method of concocting herbal mixtures for public use is frequently unhygienic which leads to microbial hazards[25]. Therefore, these diverse microbial contamination sources of herbal concoctions have explained the high microbial loads of the medicinal products as mostly reported.

Relating the contamination sources to the bacteria contaminants identified in this study, *S. aureus* is commonly found in the nose, throat, hair, and skin of more than 50 % of healthy individuals[20]. Therefore, its occurrence in herbal products could be linked to poor hygiene and handling of herbal products by the producers. Also, the *Enterobacter* spp. and *K. pneumoniae* with the highest occurrences of 17 % and 16 % respectively, and other members of the coliform group identified such as the *Citrobacter freundii* (12 %) and *E. coli* (9 %) commonly suggest contamination from faecal and non-faecal contamination sources. These microbial contaminants mostly originate from faecal-contaminated water and soils, poor handling, unhygienic processing and packaging, raw materials, and others. This assertion is in agreement with Okunlola *et al*.[20] and Ujam *et al*.[26] who reported that *E. coli* is an intestinal bacterium indicating faecal contamination, thus its presence in the herbal concoctions implies poor hygiene and improper handling of herbal products.

This study proved the presence of infectious bacterial agents in various herbal concoctions vended for public consumption within the study area, - this is mostly expected; as microbial contaminations are commonly associated with herbal products in large amounts in some cases and may include pathogenic strains[27].

The occurrence of pathogenic microbes and opportunistic pathogens in herbal medicinal concoctions signifies a serious public health concern, as the consumption of these objectionable medicinal products could lead to foodborne diseases, microbial food poisoning, and other health-related issues. The daring use of highly pathogen-contaminated natural medicinal materials has been associated with numerous fatal infectious outbreaks[23]. Again, the microbial contaminants that cause serious health hazards are pathogenic bacteria such as *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Escherichia coli*, and other pathogenic Gram-positive and Gram-negative bacteria strains[20,28]. For instance, *S. aureus* has been an agent of food poisoning, especially the toxin-producing strains and its presence in the food might pose a health hazard to the consumers of contaminated food[9, 29]. Also, *S. aureus* is reported to be associated with several health complications, especially in immune-compromised individuals[30-31]. Importantly, the presence of *S. typhi* in the herbal concoctions as reported in this study and many others[26] is of great public health implication, as this bacterial infectious agent causes enteric fever, and would cause gastroenteritis when ingested at very low doses[17]. Moshood *et al*.[29] have also cited these infectious bacterial agents as the causes of dysentery, travellers' diarrhoea, abdominal disorder and pains, sore throats, staphyloenterotoxemia, salmonellosis, fever and many others.

Many studies have established the antimicrobial potentials and significance of medicinal or herbal plants based on the susceptibility to a wide range of disease-causative microorganisms to the herbal plants and extract. Consequentially, the herbal concoction for the treatment of typhoid fever is supposed to destruct *S. typhi* which is the infectious agent causing typhoid, however, the presence of *S. typhi* in this particular concoction could be questionable and may prompt the issue of the efficacy of the herbal concoction for the treatment of typhoid fever.

Again, the safety, quality and efficacy of herbal medicines have been an imperative concern to health professionals[20], thus further implications of microbial contaminant's presence in herbal concoctions have been emphasized and reported. Noor *et al*.[23] related that microbial pathogens in non-sterile herbal drugs can inactivate or reduce their therapeutic activity and may have a potentially adverse effect on the consumers. Also, some serious adverse effects from the consumption of unwholesome medicinal concoctions may arise from several factors such as incorrect preparations, wrong dosage, adulterations, and lack of standardization, substitutions, contaminations, misidentification, inappropriate labelling, and advertisement[32-33].

Another implication is the increase in antibiotic resistance, the consumption of herbal concoctions containing antibiotic-resistant strains could lead to infections by antibiotic-resistant microorganisms thereby causing a challenging therapy of the disease caused. Though antibiotic resistance is a natural biological phenomenon frequently enhanced as a consequence of the adaptation of infectious agents to antimicrobial drug exposure in humans

or agriculture, also the common use of disinfectants[34]. Thus, now widely accepted that the use of antimicrobials is the single most important factor responsible for increased antimicrobial resistance[35].

Additionally, the proliferation of toxigenic mycoflora and the occurrence of mycotoxins in herbal or medicinal products has widely been reported in many studies[36], and the genus *Aspergillus* and *Penicillium* are the two major groups reported to produce mycotoxins[37]. Stevic *et al*.[18], also reported mould as the highest contaminant of herbal products or drugs in terms of microbiological quality where the fungal load exceeded the acceptable limit. The presence of fungi in herbal drugs will not only diminishes their quality but could lead to the production of toxic metabolites such as mycotoxins[23]. Therefore, mycotoxins in herbal concoctions could cause adverse health issues when ingested. However, this study does not entirely cover this aspect of research.

5. CONCLUSION

Herbal or medicinal concoctions vended in different markets within the Gombe metropolis where samples were collected were grossly contaminated beyond the established allowable limits for non-sterile pharmaceuticals, thus depicting the unsafe quality status of the medicinal products for consumption. The adoption of standard manufacturing practices and good hygiene are therefore recommended to improve the safety and acceptability of herbal preparations. The production of herbal or medicinal concoctions should be standardized and regulated by national regulatory bodies, and manufacturers coerced to adhere to quality control measures to guarantee the quality and safety of the medicinal products.

References

1. Falodun, A. (2010). Herbal medicine in Africa-distribution, standardization, and prospects. *Research Journal of Phytochemistry*, *4*(3), 154-161. DOI:10.3923/RJPHYTO.2010.154.161
2. Shinwari, Z. K. (2010). Medicinal plants research in Pakistan. *Journal of Medicinal Plants Research*, *4*(3), 161-76.
3. Rates, S.M. (2001). Plants as source of drugs. *Toxicon*, 39:03–13. https://doi.org/10.1016/S0041-0101(00)00134-9
4. Ugochukwu, S. C., Uche, A., & Ifeanyi, O. (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. *Asian journal of plant science and research*, *3*(3), 10-13. www.pelagiaresearchlibrary.com
5. Tapsell, L. C., Hemphill, I., Cobiac, L., Sullivan, D. R., Fenech, M., Patch, C. S., ... & Inge, K. E. (2006). Health benefits of herbs and spices: the past, the present, the future. *Medical Journal of Australia*, 185(4): 24-29. Available online at https://ro.uow.edu.au/hbspapers/1397
6. Ghani, A. (2003). *Medicinal Plants of Bangladesh Chemical constituents & uses*. (2nd edition) Asiatic Society of Bangladesh.
7. Oluyem, J., Yinusa, M.A., Raji, A., & Adejoke, J. (2016). Utilization of Herbal Medicine Among Inhabitants of an Urban Centre in North-Central Nigeria. *Algerian Journal of Natural Products, 4*, 367-378.
8. Ayantade, D., Victor, A., Aderonke, O.A., (2022). Microbial quality and antimicrobial evaluation of some herbal concoctions in a Rural Town in Nigeria. *World Journal of Biology, Pharmacy and Health Sciences*, 04(03), 048–058. https://doi.org/10.30574/wjbphs.2020.4.3.0098
9. Ibrahim, H.I., Abdulrasheed, M., Hamza, J.A., Mohammed, S.A., Ibrahim, S., Babayo, C., Nicholas, M.P. (2020). Isolation and Identification of Microorganism from Street Vended Ready-to-Eat Food in Gombe Metropolis, Nigeria. *UMYU Journal of Microbiology Research*, *5*(2)*26-32.* https://doi.org/10.47430/ujmr.2052.004
10. Miles, A. A., Misra, S. S., & Irwin, J. O. (1938). The Estimation of the Bactericidal Power of the Blood. *The Journal of Hygiene,* 38(6), 732–749. http://www.jstor.org/stable/3859638
11. Cheesbrough, M. (2006). *District Laboratory practice in Tropical countries, part 2*, Cambridge University press, UK.
12. Buchanna, R.E., Gibbon, N.E. (1973). *Bergy's Manual of Determination Bacteriology,* Williams and Wilkin Baltimore, USA.
13. Holt, J.G., Kneg, N.R., Sneath, P.H., Stanly, J.J., Williams, S.T. (1994). *Bergeys Manual of Determinative Bacteriology*, Wilkins Publishers, Baltimore.
14. World Health Organization. (2007). Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva.
15. Cary, N.C. (1996). Microbiological Specifications of Pathogens. *International Commission on Microbiological Specifications for Food Microorganisms in Foods*, 1996;89.
16. Ebo, P.U., Mukhtar, M.D., Taura, D.W. (2012). Evaluation of Microbiological Safety of Herbal Concoctions Sold in Some Markets in Gabasawa

and Nassarawa Local Government Areas in Kano State. *Bayero Journal of Pure and Applied Science*, 5(1):127 – 131 http://dx.doi.org/10.4314/bajopas.v5i1.23

17. Nwankwo, E.O, John, C. (2017). Evaluation of Pathogenic Bacterial Contamination and Antimicrobial Activity of Some Liquid Herbal Medicinal Products Sold in Umuahia, Abia State, South-Eastern Nigeria. *Journal of Current Biomedical Research*, 1(1):55-64.https://journals.unizik.edu.ng/index.php/jcbr/article/view/715
18. Stević, T., Pavlović, S., Stanković, S., Šavikin, K. (2012). Pathogenic microorganisms of Medicinal Herbal Drugs. *Archive of Biological Science Belgrade*, 64(1):49-58. https://doi.org/10.2298/ABS1201049S
19. Esimone, C. O., Chah, K. F., & Ikejide, S. C. (2002). Microbiological quality of herbal preparations marketed in South- East Nigeria. *Journal of Natural Remedies*, 42-48.
20. Okunlola, A., Adewoyin, B. A., & Odeku, O. A. (2007). Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in south- western Nigeria. *Tropical Journal of Pharmaceutical Research*, *6*(1), 661-670. **DOI:** 10.4314/tjpr.v6i1.14644
21. Ejukonemu, F. E., & Isaac, I. O. (2019). Physicochemical and microbiological evaluation of Agbo (herbal decoction) sold in some parts of Warri metropolis, Delta-State, Nigeria. *Infection*, *4*, 0-7.
22. Ola, I. O., Omomowo, I. O., Aina, D. A., Majolagbe, N. O., & Oladipo, E. K. (2013). Bacteriological evaluation and antimicrobial sensitivity test of common herbal concoctions in Ogbomoso metropolis. *African Journal of Microbiology Research*, *7*(48), 5479-5483.
23. Noor, R., Huda, N., Rahman, F., Bashar, T., & Munshi, S. K. (2013). Microbial contamination in herbal medicines available in Bangladesh. *Bangladesh Medical Research Council Bulletin*, *39*(3), 124-129. https://doi.org/10.3329/bmrcb.v39i3.20313
24. Abba, D., Inabo, H., Yakubu, S., & Olonitola, O. (2009). Contamination of herbal medicinal products marketed in Kaduna metropolis with selected pathogenic bacteria. *African Journal of Traditional, Complementary and Alternative Medicines*, *6*(1). **DOI:** 10.4314/ajtcam.v6i1.57076
25. Aarland, R. C., Rivera-Cabrera, F., Perez-Flores, L. J., de Leon Sanchez, F. D., & Mendoza-Espinoza, J. A. (2014). Relevance of chemical standardization and innocuousness in the process of development of herbal medicines: a review. *Asian Journal of Plant Sciences*, *13*(1), 1-7. https://doi.org/3923/ajps.2014.1.7
26. Ujam, N. T., Oli, A. N., Ikegbunam, M. N., Adikwu, M. U., & Esimone, C. O. (2013). Antimicrobial resistance evaluation of organisms isolated from liquid herbal products manufactured and marketed in South-Eastern Nigeria. *British Journal of Pharmaceutical Research*, *3*(4), 548.
27. Walther, C., Marwa, K. J., Seni, J., Hamis, P., Silago, V., Mshana, S. E., & Jande, M. (2016). Microbial contamination of traditional liquid herbal medicinal products marketed in Mwanza city: magnitude and risk factors. *Pan African Medical Journal*, *23*(1).
28. Arias, M. L., Chaves, C., & Alfaro, L. D. (1999). Microbiological analysis of some herbal infusions used as medicines. *Revista Biomédica*, *10*(1), 1-6.
29. Yusuf, M. A., Hamid, T. A., & Hussain, I. (2012). Isolation and identification of bacteria associated with balangu (roasted meat product) sold in Bauchi. Nigeria. *Journal of Pharmacy*, *2*(6), 38-48.
30. Archibong, E. J., Igboeli, C. N., Okoro, N. C., & Obika, I. (2017). Microbiological Assessment of some liquid herbal medications sold in Awka metropolis, Anambra state. *Journal of Bioengineering and Bioscience, 5*(3), 37-46. https://doi.org/10.13189/bb.2017.050301
31. Glória, M. L. S. (2020). Microbial contamination of herbal medicines: a review. http://hdl.handle.net/10183/220586
32. Lau, A. J., Holmes, M. J., Woo, S. O., & Koh, H. L. (2003). Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography–mass spectrometry–mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *31*(2), 401-406. https://doi.org/10.1016/S0731-7085(02)00637
33. Oluyege, J. O., & Adelabu, D. M. (2010). Microbial contamination of some hawked herbal products in Ado-Ekiti, Nigeria. *Continental Journal of Microbiology*, *4*(8), 8-14.
34. Thillaivanan, S., & Samraj, K. (2014). Challenges, constraints and opportunities in herbal medicines-a review. *International journal of Herbal medicine*, *2*(1), 21-24.

35. Sosa, A. D. J., Byarugaba, D. K., Amábile-Cuevas, C. F., Hsueh, P. R., Kariuki, S., & Okeke, I. N. (Eds.). (2010). *Antimicrobial resistance in developing countries* (pp. 3-7). New York: Springer. https://doi.org/10.1007/978-0-387-89370-9_2

36. Alwakeel, S. S. (2009). The effect of mycotoxins found in some herbal plants on biochemical parameters. *Pakistan Journal of Biological Sciences*, *12*(8), 637-642.

37. Rodríguez-Amaya, D. B., & Sabino, M. (2002). Mycotoxin research in Brazil: the last decade in review. *Brazilian Journal of Microbiology*, *33*, 1-11.