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Original Research Article

Bacteriology of Some Liquid Herbal Products Sold in Ilorin-Kwara State Nigeria

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Abstract

Purpose: This study aims to establish the safety and/or potential public health dangers associated with the consumption of liquid herbal preparations (LHP) sold in Ilorin-Kwara State.
Methods: Ten LHPs were randomly collected from three locations, kept under cold chain and transported to the Laboratory. All samples were evaluated for bacterial load using aerobic plate count method and bacterial isolates were presumptively identified using standard microbiological methods. Furthermore, Gram negative bacteria were identified using 12A Microbact ® Identification kits.
Results: Sixty percent (60%) were fresh and faint, 4 (40%) were stale and putrid in smell as well as free of foreign matter. pH and bacterial load of samples ranged from 3.60 to 9.75 and 2.5 x 10^2 to 4.4 x 10^6 CFU/ mL respectively. Five (5) genera of bacteria, namely; *Klebsiella* species 10 (29.41%), *Bacillus subtilis* 8 (23.53%), *Enterobcter* spps. 7 (20.59%), Staphylococcus *aureus* 6 (17.65%) and *Serretia marcescens* 3(8.82%) were isolated from these LHPs. All isolates were resistant (100%) to Sulphamethoxazole trimethoprim combination. Amoxocillin clavulanate was active against 62.50% of *K. pneumonia* and *S. marcescens isolates.* Also 50% of *K. oxytoca* and *E. gergoviae* were susceptible to Amoxocillin clavulanate combination. Approximately, 8 (80%) of LHPs had bacterial load of 2.5 x 10^2 to 4.4 x 10^6 CFU/ mL and 2 (20%) yielded no growth. In addition, 40% of LHPs had bacterial load of 10^6 CFU/mL beyond the 10^4 CFU/mL permissible limit stated by European Pharmacopoeia.
Conclusion: The observed high bacterial load and the presence of *S. aureus* as well as enteric bacteria of public health importance in these LHPs underscore the potential risk inherent in the consumption of these preparations. Therefore, public health awareness campaign on the dangers of unapproved LHPs consumption should be instituted.

Key words: Liquid herbal preparations, bacterial contaminants, Ilorin metropolis

Indexing: Index Copernicus, African Index Medicus

Introduction

The use of plants or plant parts in the treatment, management and prevention of diseases has been well documented by World Health Organization [1]. Reports showed that about 80% of the global population depend directly or indirectly on traditional or herbal medicine for their healthcare needs [2]. This is possibly due to growing misuse of available chemotherapeutic agents with the attendant drug resistance [3] easy accessibility and availability [4], the high cost of orthodox medicines [5, 6] and the emergence of new, re-emerging diseases and unexplained syndrome [1]. This increased interest in the use of herbal medicines necessitated the integration of traditional medicine into the national health system in order to monitor the method of processing and

standardization as contained in Good Manufacturing Practice (GMP) and Good Agricultural Practice (GAP) [7].

In Nigeria, clinicians are apprehensive about prescribing and use of herbal remedies and supplements in the treatment of diseases [8]. The expressed concern over quality of these products has been mainly associated with potential contamination due to their natural origin [5]. Harvesting, handling, storage, production and distribution methods make herbal products easily susceptible to contamination by various microorganisms such as bacteria and fungi; some of which have been documented as potential etiologic agents for infectious diseases [9]. Consumers may be unaware of these microbial contaminants and the health implications [10] as well as drug resistance associated with bacteria isolated from liquid herbal products [11]. Increasing widespread use of herbal preparations among the populace tends to support the need for in-built quality in production of these preparations, standardization and safety in treatment of diseases [12]. Several researchers have reported different types of microbial and non–microbial contaminants associated with herbal preparations [13, 14, 15,]. This study seeks to evaluate the bacteriological quality of selected liquid herbal preparations (LHPs) sold within Ilorin metropolis of Kwara State in Nigeria. This is to establish product safety and/or potential public health dangers associated with the consumption of these products.

Materials and Methods

Collection of liquid herbal preparations

A total of ten (10) liquid herbal preparations (LHPs) with different therapeutic claims were randomly collected from three locations within Ilorin metropolis, namely; Asa – Dam road, Gaa – Odota and Maraba Area. Approximately, one hundred (100) mL each of samples were collected in sterile pre–calibrated transparent bottles. The folkloric use of these products was obtained from the sellers and documented. Other features such as type of package and availability of labels was also examined. Collected samples were transported in cold chain to the laboratory for analysis.

Preparation of culture media

Nutrient agar (NA), Nutrient broth (NB), MacConkey agar (MCA), Mueller Hinton Agar (MHA), Eosin Methylene Blue (EMB), Mannitol Salt agar (MSA) and Simmons Citrate Agar (SCA) (Oxoid Basingstoke, UK) were used. These culture media were prepared according to manufacturer's instructions.

Reagents and Identification Kit

Gram's reagents (Pro-Lab, Canada), 3% hydrogen peroxide (Leyjay, Nigeria), sheep plasma, oxidase stripes and 12A Microbact® rapid test kits (Oxoid Basingstoke, UK) were used.

Antibiotic disc

The Oxoid® (Basingstoke, UK) antibiotic single discs were used for the sensitivity test. The antibiotics discs include ciprofloxacin (5µg), IMP: imipenem (10µg), SXT – sulphamethoxazole trimethoprim (25µg), AMC – amoxicillin clavulanate (30µg), CN – gentamincin (30µg) and CAZ: ceftazidime (30µg)

Physico – Chemical Analysis

Macroscopic Examination

This was performed using the sensory and physical evaluation method [16]. The colour, odour, consistency, method of packaging, and labels were carefully examined.

pH Determination

pH values of all collected samples were also determined in accordance with the protocol described by [17]. Samples with pH values of less than 7 were considered acidic, pH = 7; neutral and greater than 7, alkaline.

Microbiological Analysis

Total Viable Bacteria Count (TVBC)

This was done using the aerobic plate count method in accordance with the standard protocol provided by National Food Safety Standard [18]. Briefly, one milliliter (1 mL) each of the liquid herbal preparations was aseptically introduced into 9 mL of sterile distilled water to prepare a 1 in 10 stock dilution and votexed gently. Other dilutions were further prepared by serial dilution of 10^1 by transferring aseptically 1 ml using a sterile pipette into a second test tube containing 9 ml sterile distilled water to yield 10^2. This procedure was repeated until higher dilutions (10^{10}) were obtained.

One milliliter (1 mL) of each dilution was seeded into 20 mL sterile molten NA sterilized at 121°C for 15 min and cooled to 45°C, plates were homogenized by gentle swirling, then allowed to solidify and incubated at 37°C for 24 hours. After 24 hours, plate count was determined after enumeration of viable colonies using a Colony Counter. TVBC was calculated by multiplying the number of colonies obtained on plates by the dilution factor that yielded growth and expressed in CFU/mL.

Isolation and preservation of Isolates

Colonies were further sub cultured onto MSA, MCA and EMB plates for differentiation. Pure isolates were obtained by repeated sub culturing using streak plate

method [19] and maintained on NA slants stored at 4ºC until required.

Identification of bacteria Isolates

Pure isolates were presumptively identified based on cultural and Gram staining characteristics. Bacterial isolates were further identified using biochemical tests such as catalase, oxidase, coagulase and citrate tests [20]. The oxidase and gram-negative bacteria were further identified using 12A Microbact® rapid identification kit as specified by the manufacturer and confirmed on the Microbact Identification software [21].

Antibiotics Susceptibility Testing (AST) of bacteria isolates

This was done using the modified Kirby-Bauer discs diffusion method [22]. The 0.5 Macfarland standardized bacteria isolates were inoculated onto the surface of MHA plates by flooding method. All inoculated plates were drained and allowed to dry for 30 minutes. Then, selected antibiotics discs were carefully and firmly placed on the inoculated plates using sterile forceps. All prepared plates were allowed to stand for 45 minutes to allow pre – diffusion of antibiotics and incubated at 37º C for 18 hours. After 18 hours, diameters of the zones of inhibition were measured using a ruler and recorded in millimeters (mm). Based on the diameters of zones of inhibition, resistant isolates were selected in accordance with the recommendations of Clinical Laboratory Standard Institute [23].

Data Analysis

Data obtained were analyzed using simple descriptive statistics such as frequency, percentages and tabular presentation.

Results and Discussion

Table 1 shows the distribution of the 10 (ten) liquid herbal preparations evaluated in this study as indicated in their characteristic un-labelled, locally packaged in un-sterilized recycled plastic bottles (Figure 1). This contradicts National Agency for Food and Drug Administration and Control [24] regulations of herbal medicines and related products labelling regulations [14].

The macroscopic properties (color and odour), pH, storage time and microbial load of herbal preparation samples are as shown in Table 2. Their colors varied from greenish, creamy to brownish nature indicating presumptive sources of these herbal preparations from leaf, stem and root parts of plant origin or maybe a consequence of synthetic, chemical adulterants and stimulants. However, this study scope was not designed to elucidate the possibility of such extraneous substances as this would require further studies. Sixty percent (60%) of the samples were fresh and faint, 4 (40%) were stale and putrid in smell as well as free of foreign matter. The color, odour, and taste of these finished herbal products on observation indicate constituent source of one or more herbal plants which affect the quality, stability and safety of finished products [25].

The pH of samples ranged from 3.60 to 9.75. HP 2 was most acidic (pH = 3.60), while HP 6 was the most alkaline (pH = 9.75) and HP 7 and HP 8 were 7.13 and 7.22 were (slightly alkaline) respectively. This is in concordance with the reports of a study by [15] but different from that of [13] who reported that all herbal products used in their study were acidic. The permissible pH range for plants and plant parts products ranges between 4.0 to 7.5 while that of food is given as pH 2 to 9 [26]. Eighty percent (80%) of collected liquid herbal products fell within this range. However, 20% were out of the permissible pH range. This is of concern. The consumption of such products may represent significant health threat to consumers as the excessive consumption of acidic or acid producing foods can alter the optimum pH of the body which in turn affects the digestive, circulatory, respiratory and immune system thereby exposing the body to different diseases including cancer [27].

The bacterial load obtained in all collected LHPs ranged from 2.5 x 10^2 to 4.4 x 10^6 CFU/ mL with 8 (80%) of sample yielding bacterial growth while 2 (20%) yielded no growth. Several other findings have reported varying bacterial load from different herbal remedies [28, 29, 30]. Forty percent, (40%) of our samples were within the permissible limit, 40% were beyond the permissible limit stated by European Pharmacopoeia as reported by Ratajczak *et al.* [31] and more interestingly, 20% of these samples yielded no bacterial count. European pharmacopoeia, [32] specified that total viable aerobic count (TVBC) for aerobic bacteria from herbal products consumed orally, containing natural raw materials for which antimicrobial pre-treatment is not feasible before use should not be greater than 10^4, Gram positive bacteria such as *Staphylococcus aureus*, Enterobacteria and other Gram negative organisms such as *Escherichia coli* and *Salmonella* should be absent. Aside the high bacterial load in 40% of samples, unacceptable bacteria such as *S. aureus* and other enteric bacteria were isolated. Our inability to isolate bacteria from samples (HP 2 and 3) might be due to the observed acidic pH (3.60 and 4.68) since generally bacteria are neutrophiles and grow optimally at neutral pH [33] and suggest possible antibacterial activity attributable to inherent phytochemical constituents such as flavonoids from plant materials used [34]

A total of 34 bacterial species comprising Gram positive (41.18%) and negative (58.82%) were isolated and identified based on colonial characteristics and biochemical reactions (Table 3) bacteria belonging to five (5) genera (Table 4) from the liquid herbal products. *B. subtilis* and *K. pneumoniea* were the most predominant with a percentage frequency of 23.53% each followed by *S. aureus* (17.66%) and the least was *K. oxytoca* (5.89%). Others were *Enterobacter claoca*

(8.82%), *E. gergoviae* (11.76%) and *S. marcescens* (8.82%). The isolation of *B. subtilis* and *K. pneumoniae* in this study was similar to the findings of [14, 35]. These bacteria are of important health significance associated with wide spectra of infections such as opportunistic, bacteremia and septicemia which could be acute, chronic and in severe untreated cases might result to death especially in persons with suppressed or compromised immunity [36].

Table 1: Location, local names and therapeutic claims of liquid herbal products

Sample code	Location	Yoruba	Therapeutic Efficacy claims
HP 1	Maraba	Agbo Iba	Malaria and Fever
HP 2	,,	Agbo Taifodu	Typhoid fever
HP 3	,,	Agbo Jedi – Jedi	Pile treatment
HP 4	,,	Agbo Iba Oju Pipon	Yellow fever
HP 5	Gaa – Odota	Agbo ara riro	Body pain remedy
HP 6	,,	Agbo Iletutu	Convulsion
HP 7	,,	Agbo Inurirun	Stomach upset
HP 8	,,	Agbo opaeyin	Back pain treatment
HP 9	Asa – Dam	Agbo Inutita	Anti – Ulcer
HP 10	,,	Agbo Ida-igbe	Anti – diarrhea

Figure 1: Picture showing Liquid herbal products as packaged by sellers.

Bacillus subtilis commonly are referred to as hay bacilli and are gram positive aerobic spore-forming bacteria occurring naturally in the soil and decomposing plant materials this contributes to its high occurrence in liquid herbal products [28]. *B. subtilis* are of immense industrial and pharmaceutical importance but recently reported to cause diarrhea, severe intestinal inflammatory diseases, urogenital infections, and allergic reactions [37].

S. aureus are the normal body flora found on the skin, armpit and nose suggesting improper handling of herbal products, and thereby putting consumers at risk of Staphylococcal food-borne disease [38]. It is of public health importance as they can cause a wide range of staphylococci infections such as Methicillin and Vancomycin susceptible or resistant infections within the community and hospital settings resulting in serious and fatal infections such as bacteremia, pneumonia, endocarditis, soft tissue infections and osteomyelitis [39].

Enterobacteriaceae such as *Klebsiella*, *Enterobacter* and *Serretia* occur naturally in the environment (water, soil and sewage) and as microflora of gastrointestinal tract of both humans and animals [40]. Their occurrence in this study suggest contamination which could be associated with myriad of factors such as source of plants material (wild or domestic), improper handling of raw plant materials, processing method, dispensing of liquid herbal preparations, poor personal hygiene and use of untreated, contaminated water and inadequate cleaning of both utensils like cutting boards, knives and preparation environment. They are medically associated with the cause of respiratory, gastrointestinal, urinary tract infection [36].

Table 2: Macroscopic properties, storage and pH of samples

Sample code	Colour	Smell	pH	Storage Time (Days)	Mean TVBC CFU/mL^{-1}
HP 1	Greenish	Fresh / Faint	5.92	7	2.80×10^3
HP 2	Creamy	Stale / putrid	5.14	12	3.50×10^3
HP 3	Brownish	Fresh / Faint	3.6	14	No growth
HP 4	Yellowish	Fresh / Faint	4.68	6	No growth
HP 5	Light Brown	Fresh / Faint	5.35	5	2.50×10^2
HP 6	Light Black	Pungent /acrid	9.75	18	3.81×10^4
HP 7	Light Brown	Fresh / Faint	7.13	5	4.40×10^6
HP 8	Brownish	Fresh / Faint	7.22	7	2.70×10^6
HP 9	Light Brown	Stale / putrid	4.92	10	1.80×10^6
HP 10	Creamy	Pungent /acrid	4.75	7	1.20×10^6

Table 3: Colonial characteristics and biochemical reactions for bacteria isolates

Isolates Obtained	Colonial Characteristics	Gram Reaction	Shape	Catalase	Coagulase	Citrate	Oxidase
Staph. aureus	Tiny, circular and golden yellow colonies on MSA	+	Clustered cocci	+	+	NA	-
Bacillus subtilis	Off white, circular, rough and uneven edge on NA	+	Rods	+	NA	+	+
Klebsiella spp	Large, circular, pink and mucoid colonies on MCA	+	Rods	NA	NA	*	-
Enterobacter spp	Small, circular, pink and mucoid colonies on MCA	+	Rods	+	NA	*	-
Serrietia spp	Small pink to dark pink colonies on MCA	+	Rods	NA	NA	*	-

***Keys**: + = Positive; - = Negative; NA = Not applicable; *= Performed using 12A Microbact® Identification Kit*

Table 4: Distribution of bacterial Isolates from liquid herbal products

Genera	Species	Frequency	%
Klebsiella	*K.pneumonia*	8	23.53
	K. oxytoca	2	5.88
Bacillus	*B. subtilis*	8	23.53
Enterobacter	*E. gergoviae*	4	11.76
	E. claoca	3	8.82
Staphylococcus	*S. aureus*	6	17.65
Serretia	*S. marcesens*	3	8.82
Total		34	100

Table 5: Antibiotics resistance profile of bacteria isolated from liquid herbal preparations

	Bacterial Isolates (% Resistance)						
Antibiotics	***K. Pneumonia* n = 8**	***K. oxytoca* n = 2**	***E. claoca* n = 3**	***E. gergoviae* n = 4**	***S. marcescens* n = 3**	***S. aureus* n = 8**	***B. subtilis* n = 6**
CIP	0.00	0.00	0.00	0.00	0.00	16.67	0.00
IMP	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SXT	100.00	100.00	100.00	100.00	100.00	100.00	100.00
AMC	62.50	50.00	37.50	50.00	62.50	0.00	0.00
CAZ	0.00	0.00	0.00	0.00	0.00	16.67	0.00
GEN	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Keys:
CIP: Ciprofloxacin IMP: Imipenem, SXT: Sulphamethoxazole +Trimetoprim, AMC: Amoxocillin Clavulanate CAZ: Ceftazidime, GEN: Gentamicin

The presence of *S. marcescens* in a liquid herbal preparation is alarming because this bacterium which was initially considered a normal flora of the gastrointestinal tract are currently reported as pathogenic capable of invasion, inducing cytotoxicity and inflammation just like other classical enterobacteria [41]. Furthermore, multiple bacteria isolation from 80% of liquid herbal preparations in this study suggests potential contaminations from combination of different plant materials during preparation [25] as these plants basic characteristics

depend on several environmental factors which impact on their final quality [42].

Antibiotic resistance profile of bacteria isolated from the liquid herbal preparations are as shown in Table 5. All isolates were sensitive to Ciprofloxacin, Imipenem, Ceftazidime and Gentamicin. One hundred percent (100%) resistance was observed to Sulphamethoxazole + trimethoprim by all isolates while amoxocillin clavulanate exhibited 62.50% resistance against *K. pneumonia* and *S marcescens*, and 50% resistance against *K. oxytoca* and *E. gergoviae*. This is similar to the findings of [11] which reported antibiotic resistance among bacteria isolated from oral herbal preparations. *S. aureus* was resistant to ciprofloxacin and ceftazidime (16.67%) which suggests the wide use of ciprofloxacin in the treatment of bacterial infections [43] and its co-resistance with a third-generation cephalosporin suggests varying mechanisms of resistance among *S. aureus* [44]. The pattern of antibiotic resistance observed may imply high level of misuse of these antibiotics resulting in selective pressure [45] and consequently pose serious public health problems and high cost of treatment [36].

Conclusion

Eighty percent (80%) of the sampled locally prepared and packaged herbal preparations sold in Ilorin metropolis yielded high degree of bacterial contaminants. With 40% of the LHPs having a bacterial load of 10^6CFU/mL, above acceptable limit of European Pharmacopeia (10^4CFU/mL). The presence of *Staphylococcus aureus* alongside other potentially pathogenic enteric (*S. marcescens*, *Klebsiella and Enterobacter Spp)* and emerging bacteria (*Bacillus subtilis*) of public health importance in these preparations; underscores the potential risk inherent in the consumption of these preparations. Therefore, public health enlightenment campaigns on the dangers of unapproved LHPs consumption should be instituted. In addition, the National Agency for Food, Drug Administration and Control, Nigeria (NAFDAC) should enforce the regulation guiding local herbal practitioners' activities with emphasis on good manufacturing practices (GMPs).

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Conflict of Interest

Authors have declared that no conflict interests exist.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Contributions of authors are as follows; RHB, Research conception and design; RHB, AOO, KOA, OTS – Bench work and data collection; MSD – data analysis and interpretation; RHB – writing the Manuscript; MSD, AA, HYOA, BAL - Revision of the article; JOA – final approval of revised article

References

1. World Health Organization (WHO). *Traditional medicine strategy: 2014-2023. Hong Kong, SAR, China*. 2014.
2. Moody JO, Ajaiyeoba EA, Adeboye JO, Ogundipe O. Standardization and Utilization of Herbal Medicines. In: Challenges of 21st Century: Proceedings of 1st International workshop on Herbal Medicines.1998.
3. Barbour E, Sharif MA, Sagherian VK, Harbe AN, Talkbouk RS and Tasheuk SN. Screening of selected indigenous plants of Lebanon for antimicrobial activity. *J. Ethnopharmacol.* 2004; 93: 1–7.
4. Hack SK. Do not put too much value on conventional medicine. *J. Ethnopharmacol.* 2005; 100: 37 – 39
5. Sofowora A. (1993). Medicinal and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan - Nigeria. 1993 pp 142 – 146.
6. Salie F, Eagles PFK and Beng HML. Preliminary screening of four South African Asteraceae species. *J. Ethnopharmacol.* 1996; *52*: 27-33.
7. World Health Organization (WHO). Regulatory Situation of Herbal Medicines: A Worldwide review Geneva, Switzerland,1998.
8. Pharmanews. The use of Herbal medicines in hospital in Nigeria. *Foremost Health J.* 2010; 10: 2–2.
9. Roy AK and Kumari S. Complementary medicines in Europe. *British Medical Journal* 1999; *309*: 107–111.
10. Adeleye IA, Okogi G and Ojo EO. Microbial contamination of herbal preparations in Lagos, Nigeria. *Journal of Health, Population and Nutrition* 2005; 23(3): 296-297.
11. Adeleke S, and Opiah, TH. In vitro phenotypic antibiotic resistance in bacteria flora of some indigenous orally consumed herbal medicines in Nigeria. *Journal of Rural and Tropical Public Health* 2003; 32:134–140.
12. Falodun A. Herbal Medicined in Africa: Distribution, Standardization and Prospects. *Research Journal of Phytochemistry* 2010; 4(3): 154 –161.
13. Odedara OO and Memuletiwon EJ. Microbial Quality of some locally consumed Herbal concoctions in Abeokuta Metropolis, Nigeria. *J. Nat. Sci. Engr. & Tech.* 2014; 13:58–66.
14. Igbeneghu OA and Lamikanra A. Assessment of the microbial quality of some oral liquid herbal medicines marketed in Ile-Ife *African Journal of Microbiology Research* 2016; 38 (10): 1618–1624. https://doi.org/10.5897/AJMR2016.8237.
15. Raphael O and Emmanuel AO. Quality Assessment of Some Selected Herbal Medicinal Products Consumed in Wukari , Taraba State. *Acta Scientific Microbiology*, 2019; *2*(9): 28–36. https://doi.org/10.31080/ASMI.2019.02.0326.
16. Sharif MK, Butt, SM., Sharif HR. and Nasir, M. *Sensory Evaluation and Consumer Acceptability* 2017. pp 22 - 27.
17. Vijayakumar PP and Adedeji A. Measuring the pH of Food Products. *Agriculture and Natural Resources* 2019; 1: 1–4.

18. National Food Safety Standard (NFSS). Food Microbiological Examination: Aerobic Plate Count. China (No. GB 4789.2), 2010.
19. Sanders ER. Aseptic Laboratory Techniques : Plating Methods Aseptic Laboratory Techniques : Plating Methods. *Journal of Visualized Experiments* 2012: (5): 1–18. https://doi.org/10.3791/3064.
20. Cheesbrough M. District Laboratory Practice in Tropical Countries - Part 2. New York. 2006. 442 p.
21. Olufadi-ahmed HY, Aliyu, A, David MS, Kola-Mustapha, AT and Diyaolu AH. Antibiotic Susceptibility Profile of Uropathogens Isolated from Pregnant Women. *Nig.J. Pharm. Res*, 2019; 15(1): 45–53.
22. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *American Society for Microbiology* 2016; 12: 1–23.
23. Clinical and Laboratory Standards Institute. M100S Performance Standards for Antimicrobial Susceptibility Testing. 2016.
24. National Agency for Food and Drug Administration and Control (NAFDAC): Herbal Medicine and Related Products Labelling Regulations. Abuja - Nigeria, 2019.
25. Pohtam I, Devraj KC, Mahendra PY and Anuradha KN. Recent Updates in Ayurvedic Herbs Standardization. *International Journal of Health Sciences & Research* 2019; *9*(3):252–258.
26. Edebi NV and Gideon OA. Evaluation of pharmacognostical parameters and heavy metals in some locally manufactured herbal drugs. *J. Chem. Pharm. Res*, 2011;3(2) : 88–97.
27. Food Safety and Standard Authority (FSSA). Methods of analysis of foods, fruits and vegetable products, New Delhi, 2015.
28. Oyetayo VO. Microbial Load and Antimicrobial property of two Nigerian Herbal remedies. *Afr. J. Trad. CAM*, 2008; *5*(1): 74–78.
29. Muhammad TH. Studies on Antibiotic Susceptibility of Pathogenic bacteria from liquid herbal preparations sold in Minna metropolis [Dissertation].[Zaria]; Ahmadu Bello University; 2011 Mar. 108 p.
30. Agbo EB and Mboto CI. Phytochemical and Antibacterial Evaluation of Selected Locally Produced Herbal Medicines Sold in Calabar, Nigeria. *Archives of Applied Science Research* 2012; 4(5):1974–1990.
31. Ratajczak M, Kubicka MM, Kaminska D, Sawicka, P and Długaszewska J. Microbiological quality of non-sterile pharmaceutical products. *Saudi Pharmaceutical Journal* 2015; 23: 303–307. https://doi.org/10.1016/j.jsps.2014.11.015.
32. European Pharmacopoeia. European Directorate for the Quality of Medicines EDQM, seventh ed. Strasbourg, 2010. pp 519–520
33. Kim C, Wilkins K, Bowers M, Wynn C, and Ndegwa E. Influence of pH and temperature on growth characteristics of leading foodborne pathogens in a laboratory medium and select food beverages. *Austin Food Sci* 2018; 3(1): 1–8.
34. Minakshi B, Jharna D, Chanbi DE, Nayan T, Partha PK, Kundal N and Manash PS (2016). Phytochemical Analysis of Traditional Medicinal Plants and their Antimicrobial Activity : An Experience from North East India. *Open Access Journal of Pharmaceutical Research* 2016; (1): 1–7.
35. Shah B and Pokhrel N. Microbial Quality and Antibacterial Activity of Herbal Medicines. *Nepal Journal of Science and Technology* 2012;*13*(2): 191–196.
36. Willey JM, Sherwood LM and Woolverton CJ. Prescott, Harley and Klein's Microbiology Seventh Edition. 2008. 1222 p. https://doi.org/10.1016/s0014-5793(94)80077-4
37. Shahcheraghi S, Ayatollahi J and Lotfi M. Applications of *Bacillus subtilis* as an important bacterium in medical sciences and human life . *Tropical Journal of Medical Research*, 2015; 18(1); 1-4. https://doi.org/10.4103/1119-0388.152530
38. Kadariya J, Smith TC and Thapaliya D. *Staphylococcus aureus* and Staphylococcal Food-Borne Disease : An Ongoing Challenge in Public Health. *BioMed Research International*, 2014; (4): 1–10.
39. Schaumburg F, Alabi AS, Peters G and Becker K. New epidemiology of *Staphylococcus aureus* infection in Africa. *Clinical Microbiology and Infection* 2014; 20(7): 589–596. https://doi.org/10.1111/1469-0691.12690.
40. Oliveira RV and Pelli A. Disease Infection by Enterobacteriaceae Family in Fishes : A Review Disease Infection by Enterobacteriaceae Family in Fishes : A Review. *J Microbiol Exp*, 2017; 4(5): 52–54. https://doi.org/10.15406/jmen.2017.04.00128
41. Hejazi A and Falkiner FR. *Serratia marcescens*. *Journal of the Agricultural Chemical Society of Japan* 1997; 46: 903–912.
42. Akerele JO, Ayinde BA and Ngiagah J. Phytochemical and Antibacterial Evaluations of the Stem Bark of Newbouldia laevis against Isolates from Infected Wounds and Eyes. *Tropical Journal of Pharmaceutical Research* 2011; 10: 211–217.
43. Fasugba O, Gardner A, Mitchell BG and Mnatzaganian G. Ciprofloxacin resistance in community- and hospital-acquired *Escherichia coli* urinary tract infections : a systematic review and meta-analysis of observational studies. *BMC Infectious Diseases* 2015; 15: 545–56.
44. Yılmaz EŞ and Aslantaş Ö. Antimicrobial resistance and underlying mechanisms in *Staphylococcus aureus* isolates. *Asian Pacific Journal of Tropical Medicine* 2017; 10(11): 1059–1064. https://doi.org/10.1016/j.apjtm.2017.10.003
45. Sabour PM, Gill JJ, Lepp D, Pacan JC, Ahmed R, Dingwell R and Leslie K. Molecular typing and distribution of *Staphylococcus aureus* isolates in Eastern Canadian dairy herds. *J Clin Microbiol.* 2004; 42: 3449–3455.