Microbiological Quality Assessment of Herbal Products Produced and Marketed in Gombe Metropolis, North-East Nigeria

Oladosu, O. P.,[1, 2*] Mohammad, F.,[2], Aboh, M. I.,[1] Olatunji, K. T., Izebe, K.,[1] Ya'aba, Y.[1] and Muhammed, S. B.[1]

1. National Institute for Pharmaceutical Research and Development, Department of Microbiology and Biotechnology, PMB 21, Garki, Abuja, Nigeria.
2. Gombe State University, Microbiology Department, Gombe State, Nigeria.

Correspondence author: petyem2001@yahoo.co.uk; +234-8035993159

Abstract: Use of herbal medicines for health management in developing countries like Nigeria no doubt remains an integral part of their life style and because of its wide acceptance; Quality of herbal medicines being produced in these communities requires an urgent attention. Twenty herbal preparations manufactured and marketed in Northeastern Nigeria were randomly collected in Gombe metropolis and analyzed for microbiological quality assessment according to United States Pharmacopeia (USP). Samples were tested for microbial contamination by dilution technique in Tryptic Soy agar and broth (for bacterial count) and Sabouraud dextrose agar and broth (for fungal count) and incubated at 35^{o}C. Post incubation all the microbial contaminants were characterized at least to genera level. The results show that most of the herbal medicines were heavily contaminated with bacteria and fungi at levels far above permissible limit stipulated for oral pharmaceutical preparations and those within acceptable limit have contaminants that are of health concern. A total of 26 bacteria species including *Bacillus subtilis* (23%), *Shigella spp* (4%), *Klebsiella pneumoniae* (11%), *Staphylococcus aureus* (35%), *Proteus* (4%), *Pseudomonas aeruginosa* (8%), *Enterococcus feacalis* (11%) *and Escherichia coli* (4%) and 28 fungal including *Alterneria* spp (3%), *Aspergillus niger* (43%), *Aspergillus flavus* (18%), *Aspergillus fumigatus* (14), *Cladosporium cladosporius* (4%), *Mucor* (11%) spp *and Rhizopus arrhizus* (7%) species were isolated from the preparations. These products are in liquid (L), powder (P), soap (S) and ointment (O) and powdered products (sample P1 – P7) were found to be contaminated with the highest number of bacteria and fungi. In conclusion, 85% of the herbal products studied were found to contain microorganisms that are of health concern; most of the organisms are indicators of poor hygiene and environmental contamination and have compromised the safety of the products. It is recommended that Manufacturers adhere to principle of Good Manufacturing Practice to guarantee safety of herbal medicines marketed in Gombe metropolis.

Keywords: Sterility, Herbal Medicine, Contamination, Bacteria, Fungi

INTRODUCTION

Herbal medicine also called botanical medicine or phytomedicine refers to the use of any plants seed, berries, roots, barks, leaves or flowers for the treatment of illness (Braide *et al.,* 2013). Seventy to eighty percent of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary healthcare (Akerele, 1993). World health organization (WHO, 1998) has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has also encouraged the rational use of traditional plant based medicines by member states and has developed technical guidelines for the assessment of herbal medicine (WHO Guideline, 2000).

The concern over quality of the products is mainly due to their potential contamination, considering their natural origin. Herbal medicines in Nigeria are used in form of various preparations to treat various types of ailments including diarrhea, cough, neonatal fistula, convulsions, skin diseases, urinary tract infection, to decrease kidney stone; to lower cholesterol levels and blood pressure, also used as immune stimulants that help increase resistance to cold, relief from migraine headache and arthritis, healing of wounds; burns; skin ulcers; heart failure; hypertension, typhoid fever, malaria, infertility, fever, waist pain, chest pains, pile insomnia, ulcer, carbuncle, dizziness, blood

prostration etc (Sofowora, 1982, Coon *et al*., Widespread use of herbal medicines, calls for the assurance of sustainable availability of quality and safe herbal medicines to ensure continued access especially for rural communities, without compromising patients safety.

In Nigeria, even though, there is proliferation of herbal products in the market, not so much has been done to guarantee the quality of the products produced for consumption. Some producers of herbal preparations in Nigeria do not have the required expertise to perform quality control on the preparation they produce. This brings about the problem of inconsistency on the quality of the herbal preparation in the country.

Since large number of people in Nigeria rely mostly on the use of traditional medicines for their health management, herbal products then must be of good quality, free of microbial contaminants and meets approved standard. This study aims to determine the microbiological quality of herbal products produced and marketed in Gombe metropolis, North-East Nigeria.

MATERIALS AND METHODS

Sample Collection

Twenty herbal products were collected randomly from retail outlets and herbal shops in Gombe metropolis. Information about therapeutic claims, mode of preparation and preservation additives and uses were retrieved from Traditional Healers using a concise interview form. Gombe is located in the center of North eastern part of Nigeria on Latitude 9”30` and 12”30`N, Longitude 8”5` and 11”45`E, with a land area of 20, 265 km^2. The herbal products collected randomly from markets and herbal shops in Gombe metropolis were 7 liquids (L), 7 powder (P), 3 soaps (S) and 3 ointments (O).

Microbial Load Testing

Sample preparation: The liquid and ointment samples were mixed vigorously, 1 ml was diluted in 9 ml normal saline and further diluted to 10^{-6} by serial dilution in sterile normal saline while 1 g of soap and powder samples was dissolved in 9 ml sterile normal saline respectively and further diluted by serial dilutions to 10^{-6}. Five hundred microliter each of 10^{-2}, 10^{-4} and 10^{-6} dilutions were directly inoculated into a sterile molten Tryptic Soy Agar and Sabouraud Dextrose Agar (45^{o}C), allowed to solidify, dry and incubated at 35^{o}C for bacteria and 25^{o}C for fungi for 14 days. Post incubation, colonies that appeared were counted on colony counter and recorded as total colony forming bacteria. The microbial content was taken as the mean of duplicate determinations. A presumptive bacteria colony was used for bacteria identification by Gram staining and biochemical analysis (catalase, indole, coagulase, oxidase, urease, citrase, triple sugar iron and methyl red voges proskaeur tests). Isolated organisms were inoculated on differential media; Mannitol salt, MacConkey, Eosine methylene blue, Cetrimide and *Salmonella Shigella* agar (Sigma-Aldrich, St. Louis, USA).

Growth suitability test

The growth suitability test of the products was conducted to determine if the products contain any inhibitory substance that can suppress growth of any contaminants. This was carried out by bacteriostasis and fungistasis testing (United States Pharmacopiea, 2018). One hundred gram of powdered and soap samples were dissolved in 9 ml sterile normal saline. One milliliter of each sample was added to 19 ml Tryptic soy and Sabouraud Dextrose agar respectively and inoculated with 100 µl of *Staphylococcus aureus*, *Pseudomonas* and *Aspergillus niger* of 10^2 cfu/ml. The plates were incubated for 3 days for bacteria and 5 days for fungi. Post incubation, the plates were observed for microbial recovery from the products.

RESULTS

The microbial load testing shows that majority of the Herbal products were grossly contaminated by bacterial and fungal contaminations. The analysis of the findings shows that 50% (10) of the products are contaminated with bacteria. Out of the 7 liquid samples, only 1 sample (L7) was contaminated with bacteria (7.22 $x10^4$ cfu/ml), all the powdery samples (P1 – P7) were contaminated with bacteria ranging from 1.35 $x10^4$ cfu/ml – 2.53 x 10^4 cfu/ml, only 2 (O1 and O2) of the 3 ointment samples have bacterial contaminants while soap sample (S1 – S3) were free from bacteria contaminations. Bacteria species found mostly in powdered samples are *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Enterococcus feacalis*. Samples P1 and P2 have the highest number of bacterial contamination, their frequency of appearance are as shown in Table 2. The contaminated ointment samples contained only 2 bacteria species (*B. subtilis* and *S. aureus*) while the only contaminated liquid sample was found to contain *E. coli and S.aureus*. According to WHO Guideline (2007), limit contaminant of aerobic bacteria in herbal medicines is 10^5 cfu/g. The powdered samples being the product with highest number of bacteria contain non permissible bacteria like *S. aureus, P aeruginosa, K. pneumoniae* and *E. feacalis.* Other bacteria identified include E. coli found in ointment samples (O1 and O2).

All the herbal products except the liquid (L1 and L2) and soap samples (S1 – S3), are contaminated with different fungal species; *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus*, *Mucos* spp, *Rhizopus arrhizus*, *Alteneria* spp and *Cladosporium cladosporioides.* The powdered samples (P1 – P7) are found with the highest number of fungi contaminations, while the ointments contained only 1 fungi spp (*A. niger*) as shown in Table 4. Distribution and percentage of bacterial and fungi contaminations are as in Figures 1 and 2.

In the growth promotion test, the herbal preparations contained no inhibitory substances as they supported the growth and recovery of test organisms (*S. aureus, P. aeruginosa* and *A. niger*) except for the ointments in which the test organisms could not reproduce, indicating presence of inhibitory substance and was neutralized by serial dilutions.

DISCUSSION

The herbal products collected in this study are in liquid, powdered, ointment and soap forms and they are administered orally in form of alcoholic solution and water extracted remedies and topical use. Information from the Healers pointed out that the herbs are mostly produced for treatment of malaria, diarrhea, typhoid fever, pile, high blood pressure, ulcer, asthma, body pain and dermatitis. Accordingly, the herbal medicinal products are produced based on traditional folklore which is usually extracted in local gin or water. However, the percentage of the local gin used was not indicated. The herbal products usually contain mono or multi components of plant parts depending on the severity of the disease to be treated. For instance, antimalarial herbal products were found to contain stem, leaves and pulps of fruits from different plants. In addition to plant parts, some preparations also contain animal explant. The herbal products are preserved in different conditions; at room temperatures, refrigerators, by boiling and by addition of preservatives such as edible camphor. Some hawked herbal products are sold wholly while some are dispensed as requested, with or without prescription pattern.

The microbial load of herbal medicines in these study shows that 10 (50%) products were contaminated with different bacterial species and 18 (90%) products were found to be contaminated with different fungal species. Only the soap samples were free of any contamination and this is as reported by Selvamohan, *et al*., (2012) and Oladosu, *et al*., (2018), that soap contains compounds that have the potential to inhibit the growth,

contamination and metabolism of microorganisms or kill them. Based on WHO Guideline 2007, the total aerobic microbial count of herbal medicine was not more than 10^5cfu/g, thus the microbial load of these analyzed Herbal products are not within acceptable limit. The organisms isolated are of growing and culture conditions of medicinal plants but the microflora of the final product may represent contaminants from the raw materials, equipment, water, and atmosphere and from personnel (Esimone *et al.,* 2001). Presence of Enterobacteriaceae which is an indicator of feacal contamination and other harmful pathogens like *Shigella, Pseudomonas, Staphylococcus* spp and *E. coli* shows the degree of contamination of these products. The findings in this study are in agreement with previous studies in Southeast and Southwest Nigeria by Esimone *et al*., 2002, Idu *et al*., 2010, Braide *et al*., 2013, Rajapandiyan *et al.,* 2013, Odedara *et al*., 2014 and Igbeneghu *et al*., 2016. Most organisms isolated like *E. coli, P aeruginosa* are known to proliferate in portable water, *S. aureus, Mucor* and *Aspergillus* are commonly isolated in the air (Underwood, 1999). These pathogenic contaminants could have various health implications on the users of these products. *E. coli* and *Shigella are* organisms associated with gastrointestinal tract and its presence indicate the likely hood of fecal contamination. According to Igbenegbu *et al*., (2016), these contaminants could be acquired from the use of water of poor quality for the preparation of the sample and rinsing of containers. *Staphylococcus spp* which contaminated 34.6% of the samples has been associated with a number of complications especially to immuno-compromised individuals and it is in agreement with report of Archibong *et al*., (2017). Diarrheal episodes of infective aetiology represent around 27% of those reported, leading to a number of serious complications and high mortality rates (Wylie *et al.,* 2005). *Bacillus* spp found in the powdered products is not surprising, it is the most predominantly isolated from herbal preparation and are known to cause gastrointestinal infection which is characterized by diarrhea (Rajapandiyan *et al.,* 2013). *Bacillus* spp produces heat stable spores and causes food borne intoxication when ingested (Cheesbrough, 2000; Pelczar *et al.,* 1993). *Enterococcus feacalis* may cause infections such as urinary tract, biliary tract, ulcer and occasionally endocarditis or meningitis (Cheesbrough, 2000; Prescott *et al*., 1999; Nester *et al*., 1998).

Though a large market exists for herbal preparations as well as finished herbal products, the consumption of these preparations are still associated with risks from contamination. The fungal isolates identified in this study in almost all the samples include *Aspergillus niger, A. flavus, A. fumigatus, Alteneria spp., Cladosporium cladosporioides, Mucor spp. and Rhizopus arrhizus,* which is in agreement with the work of Esimone *et al*., (2002), Idu *et al*., (2010) and Odedara *et al*., (2014). Herbal medicinal products with fungal contamination with *Aspergillus flavus* and *A. fumigatus* as seen in this study could be as a result of contamination from soil and organic matter, which are medically important pathogens of human causing human invasive aspergillosis. *A. niger* could be from various sources including sand, air or laboratory contaminant and is the most frequently encountered agent of otomycosis, *Alterneria spp* could also be as a result of contamination with contaminated soil and are rare cause of onychomycosis. Contamination with *Cladosporium cladosporioides* arises from soil and organic matter and is a well-known thermos-tolerant through human pathogenic species. Most of the findings here agree with reports of other Investigators as stated above.

CONCLUSION AND RECOMMENDATION

The results of this finding shows that 85% of the herbal medicines in this study contain organisms of health concern and it is therefore recommended that Manufacturers of herbal medicines adhere to principles of Good Agricultural and Collection Practices (GACP) elaborated in DARS 952:2015, the principle of Good Manufacturing Practice (GMP) and to regularly subject their products for quality assessment.

Funding: This study is supported by management of Gombe State University, Gombe, Nigeria.

Conflict of interest: The authors declare that there is no conflict of interest.

Table 1: Herbal samples, their therapeutic claims and route of administration

Sample	Therapeutic claims	Route of administration
L1	Typhoid, malaria, pile, high blood pressure.	Oral
L2	Fever, ulcer, cold, cough and back pain.	Oral
	Cough, cold, asthma, and Headache	
L3	Toilet infection.	Oral
	Pile	
L4	Pile	Topical
L5	Ulcer and abdominal disorder	Oral
L6	Dermatitis	Oral
L7	Dermatitis	Oral
P1	Ulcer	Oral
P2	Pile	Oral
P3	Typhoid and malaria	Oral
P4	Cold	Oral
P5	Allergies, ringworm and eczema,	Oral
P6	Prevent cancer, skin disorders, hair lost and obesity.	Oral
P7	NA	Topical
S1	Rashes, back pain, and rheumatism	Topical
S2	NA	Topical
S3	Dandruff	Topical
O1	Eczema, pimples, ring warm and dandruff	Topical
O2	NA	Topical
O3	NA	Topical

Key: L = Liquid, P = powder, S = Soap, O = Ointment, NA = Not available

Table 2: Bacteria load of Herbal products

Sample	Bacteria load Cfu/ml
L1	NG
L2	NG
L3	NG
L4	NG
L5	NG
L6	NG
L7	7.22×10^4
P1	5.38×10^4
P2	3.1×10^8
P3	2.56×10^4
P4	2.58×10^4
P5	3.05×10^6
P6	1.35×10^4
P7	2.53×10^4
O1	5.59×10^8
O2	4.84×10^4
O3	NG
S1	NG
S2	NG
S3	NG

KEY: NG = No bacteria growth, L = liquid, P = powder, O = ointment, S = solid

Table 3: Bacteria species in different herbal products

Samples	**Organisms identified**	**Frequency of bacteria isolated per sample %**
P1	*Bacillus subtilis, Shigella spp., Klebsiella pneumoniae, Staphylococcus aureus.*	4 (40%)
P2	*Bacillus subtilis, Staphylococcus epidermis, Staphylococcus aureus, Proteus spp.*	4 (40%)
P3	*Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae.*	3 (30%)
P4	*Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus feacalis.*	3 (30%)
P5	*Klebsiella pneumoniae, Enterococcus feacalis, Bacillus cereus.*	3 (30%)
P6	*Staphylococcus aureus, Enterococcus feacalis.*	2 (20%)
P7	*Bacillus subtilis, Staphylococcus aureus.*	2 (20%)
O1`	*Bacillus subtilis, Staphylococcus aureus.*	2 (20%)
O2	*Staphylococcus aureus.*	1 (10%)
L7	*Escherichia coli, Staphylococcus aureus.*	2

Key: L = Liquid, **P** = powder, **S** = soap, **O** = ointment

Table 4: Fungal organisms in different herbal products

SAMPLE	Colonial characteristics on SDA	Organism	Frequency of Fungi isolated per sample (%)
L1	NG	NG	0
L2	NG	NG	0
L3	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	1 (14%)
L4	Colonies are dark yellow-green and are granular flat with radial groove.	*Aspergillus flavus*	2 (28%)
	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	
L5	Colonies are floccose pale greyish-brown	*Mucor spp*	2 (28%)
	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	
L6	Colonies are blue green with suede-like surface consisting of dense felt of conidiospore.	*Aspergillus fumigatus*	3 (42%)
	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	
	Colonies are dark yellow-green and are granular flat with radial groove.	*Aspergillus flavus*	
L7	Colonies are blue green with suede-like surface consisting of dense felt of conidiospore.	*Aspergillus fumigatus*	2 (28%0
	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	
P1	Colonies are about 5-8mm high, white cottony at first becoming brownish-grey to blackish-grey.	*Rhizopus arrhizus*	3 (42%)
	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	
	Colonies are dark yellow-green and are granular flat with radial groove.	*Aspergillus flavus*	
P2	Colonies are greyish and are suede-like	*Alteneria* spp	3 (42%)
	Colonies are dark yellow-green and are granular flat with radial groove.	*Aspergillus flavus*	
	Colonies are blue green with suede-like surface consisting of dense felt of conidiospore.	*Aspergillus fumigatus*	

Table 4 continue

P3	Colonies are blue green with suede-like surface consisting of dense felt of conidiospore. - Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus fumigatus* *Aspergillus niger*	2 (28%)
P4	- Colonies are slow growing, blackish-brown in color and suede-like. Colonies are floccose pale greyish-brown	*Cladosporium cladosporioides* *Mucor spp*	2 (28%)
P5	Colonies are floccose pale greyish brown Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Mucor spp* *Aspergillus niger*	2 (28%)
P6	Colonies are dark yellow-green and are granular flat with radial groove.	*Aspergillus flavus*	1 (14%)
P7	Colonies are about 5-8mm high, white cottony at first becoming brownish-grey to blackish-grey - Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Rhizopus arrhizus* *Aspergillus niger*	2 (28%)
S1	NG	NG	0
S2	NG	NG	0
S3	NG	NG	0
O1	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	1 (14%)
O2	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	1 (14%)
O3	Colonies consist of compact basal fetal covered by a dense layer of dark-brown conidial heads.	*Aspergillus niger*	1 (14%)

Key: L = Liquid, P = powder, S = soap, O = ointment, **NG = No fungi growth**

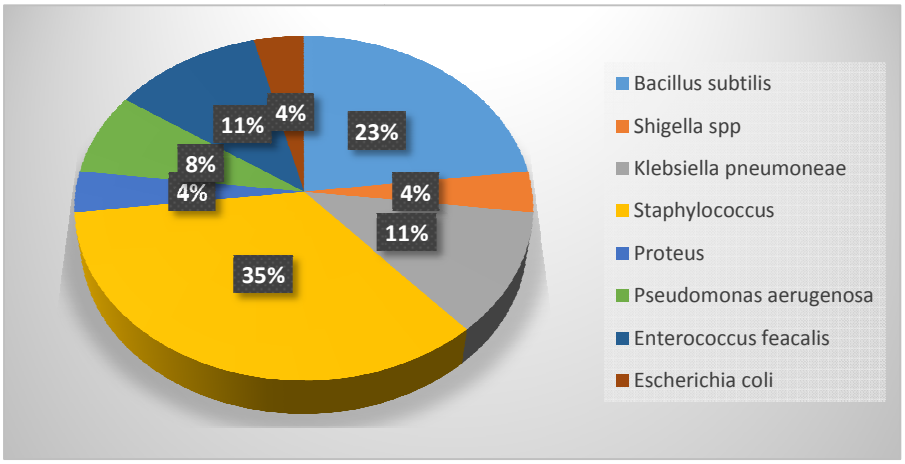

Fig.1: Distribution and percentage of bacterial contaminants in tested samples

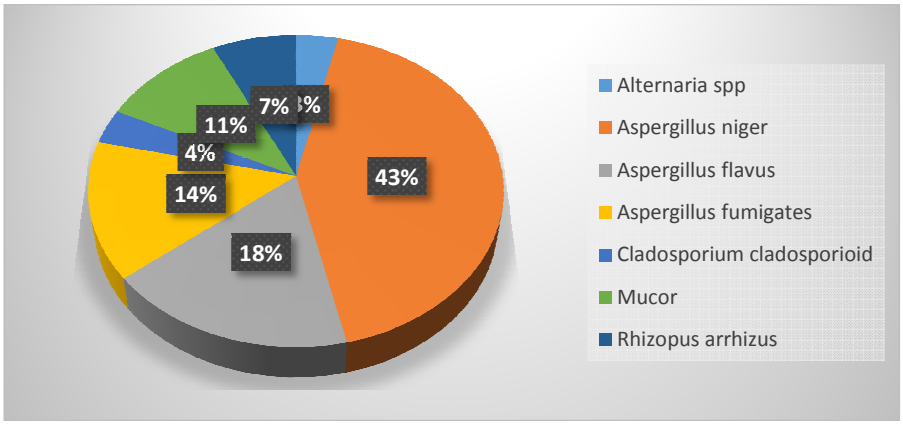

Fig. 2: Distribution and percentage of fungal contaminants in tested samples

REFERENCES

Akerele, O. (1993). Nature's medicinal bounty: don't throw it away. *World Health Forum*. **14**: 390-395.

Archibong Etim, Igboeli J, Chinechendo N., Okoro Nnenna C., and Obika Ifeanyi, (2017). Microbiological Assessment of Some Liquid Herbal Medications Sold in Awka Metropolis, Anambra State. *Bioengineering and Bioscience,* **5(3)**: 37-46.

Braide W., Oranusi S. U., Nwaoguikpe R.N., Offor-Emenike I.U., Nwosu I.L., Akobondu C. I., Chike-Reginald C. and Popgbara L.B. (2013). Evaluation of the microbiological status and antibacterial susceptibility pattern of some herbal remedies administered orally in Nigeria. *Research Journal in Engineering and Applied Sciences* **2(1)**:35-42.

Cheesbrough M. (2000) *District Laboratory Practice in Tropical Countries Part 2*: Cambridge University Press, Cambridge; 1-70.

Coon, J.T., Ernest, E and Parax, G. (2002) "A systemic review of adverse effectand drug interaction." *Drug safety*, **5:** 323- 344.

Esimone, CO, Chah, KF, and Ikejide SC (2001). Microbiological quality of herbal preparations marketed in South East Nigeria**.** *Journal of Natural Remedies,* **2(1):** 42-48.

Idu, M., Erhabor, J. O. and Idele, S. O. (2011). Microbial Load of Some Medicinal Plants Sold in Local Markets of Benin City, Nigeria. *International Journal of Medicinal and Aromatic Plants.* **1(3):**272-277.

Igbeneghu O.A and Adebayo L. (2016). Assessment of the microbial quality

of some oral liquid herbal medicines marketed in Ile-Ife, South-western Nigeria. *African Journal of Microbiology Research*, **10**(**38**): 1618-1624.

Kunle OF, Egharevba HO, and Ahmadu PO (2012). Standardization of Herbal medicines – A review. International Journal of Biodiversity and Conservation 4: 101 – 112.

Mendes, E., Herdeiro, M. T. and Pimentel, F (2010). The Use of Herbal Medicine Therapies by Cancer Patients. *Acta Médica Portuguesa.* **23(5):**901-908.

Nester, E., W., Roberts, C. E., Pearsall, N. N., Anderson, D.G. and Nester, M.T (1998). Microbiology. A Human Perspective, 2nd Edition WBC/McGraws Hills New York, USA, pp. 434 -435.

Odedara O.O. and Memuletiwon E.J., (2014), microbial quality of some locally consumed herbal concoctions in Abeokuta metropolis, Nigeria. *Journal of Natural Science, Engineering and Technology,* **13**:58-66.

Oladosu O. Peters, Umar Y. A, Salawudeen A, Izebe K, Adamu M. T and Aboh M (2018). Antibacterial Activity of Soaps Indigenously Made in Gombe Metropolis, Nigeria. Journal of Natural Remedies, DOI: 10.18311/jnr/2018/22434.

Pelczar, M.J (Jr), Chan, E.C.S and Krieg, N.R 1993). Microbiology: Concepts and Applications, 1st edn. McGraw-Hill Inc., New York, USA. pp. 80-100, 158- 161, 370.

Prescott, L.M., Harley, J.P and Klein, D.A (1999). Isolation of Pure Bacterial Culture from Specimen: Microbiology International, 4th Edition: BostonWCB McGraw's Hill Companies: pp. 714 – 796.

Rajapandiyan, K., Shanthi, S. and Vidya, S. (2013). Assessment of Microbial Quality in Marketed Herbal Drugs Sold in Trichy City. *International Journal of Pharmaceutical, Chemical and Biological Sciences.* **3(3)**:894-898.

Selvamohan V and Sandhya T (2012). Studies on bactericidal activity of different soaps against bacterial strains. Journal of Microbiology and Biotechnology Research 5:646-650

Sofowora, E.A (1994). Medical Plant and Traditional Medicine in Africa. University of Ife Press, Nigeria: pp. 1 – 23.

Underwood E. (1992) Pharmaceutical Microbiology (Hugo WB, Russel AD, ed.) Blackwell Scientific Publication, 5th edn, Oxford 353-368

United States Pharmacopeia (2009). A harmonized standard for sterility test. 10th June, Rev.1.Cor 3.

United States Pharmacopeia USP (2018). Microbiological Tests. In: The National formulary 36, Rockville, MD: US Pharmacopeial Convention, Inc. Vol.4, 2929 – 6017.

World Health Organization (1998). Regulatory situation of herbal medicine: A worldwide review. World Health Organization, Geneva.

World Health Organization Geneva (2000). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine.

World Health Organization. Dept. of Technical Cooperation for Essential Drugs and Traditional Medicine. (2007). Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva: World Health Organization. P 105.

Wylie, J. L. and Norwick, D. L. (2005). Molecular Epidemiology of Community and Health Care-Associated Methicillin Resistant *Staphylococcus aureus* in Manitoba, Canada. *Journal of Clinical Microbiology*. **43(6)**:2830-2836.