

Available online on 15.09.2022 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2022 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



Open Access Full Text Article



Research Article

The Microbial Quality of Herbal Products

Anie, Oliseloke Clement^{1*}, Egbon, Olukayode Temitope², Enemchukwu, Chizoba Mercy³, Adushoke, Egunor Loveth¹

1. Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Delta State University, Nigeria.

2. Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Federal University, Oye-Ekiti, Nigeria.

3. Department of Pharmaceutical Microbiology and Biotechnology Faculty of Pharmacy Madonna University Elele Rivers State

Article Info:



Article History:

Received 17 July 2022
Reviewed 19 August 2022
Accepted 27 August 2022
Published 15 Sep 2022

Cite this article as:

Anie OC, Egbon OT, Enemchukwu CM, Adushoke, EL, The Microbial Quality of Herbal Products, Journal of Drug Delivery and Therapeutics. 2022; 12(5):64-69

DOI: <http://dx.doi.org/10.22270/jddt.v12i5.5590>

*Address for Correspondence:

Anie, Oliseloke Clement, Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Delta State University, Nigeria.

Abstract

Background: Consumption of herbal products has increased dramatically during the last few decades. Although there have been allegations that herbal products are made in unsanitary conditions resulting in microbial contamination and health hazards consumed by patients.

Aim: The study is aimed at investigating the microbial quality of commonly sold herbal products in Abraka, Delta State, Nigeria.

Methods: A total of seven (7) liquid herbal products (AB, GW, EC, IM, DR, GC and BM) were randomly purchased from local herbal shops and pharmacy shops in Abraka. Samples were aseptically measured out around the zones of a Bunsen flame and the total viable bacteria and fungi counts of samples were determined using serial dilution and spread plate method.

Results: The total bacterial count in the herbal products ranged from 1.8×10^6 to 7.5×10^6 . *Proteus* spp had the highest frequency of occurrence with a percentage of 50% while *Staphylococcus* spp. occurred the least with a percentage of 10% while *Candida* spp. had the highest percentage occurrence for fungi isolates and *Aspergillus niger* and *Aspergillus flavus* occurs less with percentage occurrence of 10 and 20% respectively. The study also revealed that Tarivid, Streptomycin and Gentamycin were susceptible to all bacterial isolated from the herbal products with average zone of inhibition of 18.25 ± 1.39 , 19.75 ± 3.39 and 23.13 ± 2.08 respectively.

Conclusion: The finding of this study revealed that herbal products sold in Abrakawere contaminated with microorganisms, some of which are pathogenic and thus, these herbal products can serve as a means of spreading infections among the consumers.

Keywords: Herbal, microorganism, bacteria, Abraka.

INTRODUCTION

Herbal medicine is an ancient approach that is commonly used in disease treatment. It is also known as complementary and alternative medicine (CAM). It is growing increasingly popular as breakthroughs in analysis and quality control, as well as advances in clinical research, demonstrate the benefits of herbal medicine in the treatment and prevention of diseases. The term "herbal medications" refers to plants and plant components that have been turned into phyto-pharmaceuticals through simple harvesting, drying, and storage methods.¹ Herbal remedies, according to the World Health Organization, contain plant parts or plant material in their raw or processed state as active components and may contain excipients (foreign substances)². Medicinal plant has been defined by World Health Organization (WHO) consultative group as any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.³ It is essential to highlight that herbal medicines do not include any combination of scientifically determined active compounds or separated elements⁴. Globally, the usage of herbal medicine is expanding⁵. In poor nations such as Africa, herbal medicine is used by up to 80% of the population as a

primary source of therapy.⁵ Plants are rich in a wide variety of secondary metabolites such as tannins alkaloids and flavonoids, which have found in vitro to have antimicrobial properties.⁶ Although the World Health Organization (WHO) has urged for the incorporation of herbal medical products (HMPs) into developing nations' primary health care systems, herbalists continue to neglect safety concerns with herbal medication formulations. As a result, the safety of herbal products has become a serious public health problem.⁷

This is due to the fact that microorganisms of various types are normally adherent to the leaves, stems, flowers, seeds, and roots from which herbal medicine can be prepared, and potential pathogens may also be introduced during harvesting, handling, open-air drying, preserving, manufacturing⁷ and the use of contaminated materials for storage.⁸ According to certain findings, consumers may become unwell as a result of ingesting herbs contaminated with pathogenic microbes.⁹ and the presence of antibiotic resistant microbial isolates in HMPs may result in the transfer of antibiotic resistance strains to consumers.¹⁰

Herbal medicine and preparations have been widely used for thousands of years in both developing and developed countries due to their natural origins and lower negative

effects.¹¹ These medications began as crude drugs such as tinctures, teas, poultices, powders, and other herbal concoctions.¹² Plants have been used for medicinal purposes since the beginning of time, and they are the source of much contemporary medicine. Most early medicines, such as aspirin (willow bark), digitoxin (from foxglove), morphine (from the opium poppy), quinine (from cinchona bark), and pilocarpine (jaborandi), were based on clinical, pharmacological, and chemical studies of these traditional medicines, which were derived primarily from plants.¹³

Herbal medicine is still the primary source of basic health care for around 75-80% of the world's population, primarily in underdeveloped nations. This is primarily due to the widespread perception that herbal medications have no negative effects and are inexpensive and widely available. According to the World Health Organization (WHO), the use of herbal treatments outnumbers conventional medications by two to three times.¹³ The World Health Organization recently classified herbal medicines into four different classes based on their origin, evolution, and current forms of use, which include indigenous herbal medicines, herbal medicines in systems, modified herbal medicines, and imported products with an herbal medicine base.

Phytomedicine is now based on traditional medicine (which exists in every continent and in every cultural area of the world). Traditional Ayurvedic medicine in India and Chinese medicine in East Asia are well known and closely related.¹⁴ Herbs are experiencing a resurgence, and a herbal "renaissance" is taking place all over the world. Herbal goods now represent safety in contrast to synthetics, which are viewed as hazardous to both humans and the environment. Although herbs have been valued for millennia for their medicinal, flavorful, and aromatic properties, synthetic products of the modern day have temporarily exceeded their relevance. However, the mindless reliance on synthetics is coming to an end, and people are going back to naturals in the hope of finding safety and security. For health care, plants and plant extracts are used by more than three-quarters of the world's population.

Medicinal plants have been employed as cures for human ailments because they contain medicinal components. Plants produce many organic compounds and substances which are valuable in prevention and in the treatment of various diseases.¹⁵ The problem of germ resistance is worsening, and the future usage of antimicrobial medications is still questionable.¹⁴ As a result, for a long time, plants have been a great source of natural products for sustaining human health, with more thorough studies for natural remedies. The leaf extracts of *A. nilotica* and *S. cordifolia* have the best antibacterial activity against *B. subtilis*. *S. cordifolia* root and leaf extracts were shown to be effective against all of the microorganisms tested. The bark and leaf extract of *A. nilotica* had strong antifungal activity against *A. flavus*, while *Z. mauritiana* and *T. cordifolia* demonstrated significant antifungal activity against *D. turcica*. *S. cordifolia* methanol extract had considerable antifungal efficacy against *F. verticillioides* (Mahesh and Satish).¹⁶

medicine is widespread throughout the world and it can be described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease and which may rely exclusively on past experience and observation handed down from generation, verbally or written.¹⁷

Aim of the Study

The study is aimed at investigating the microbial quality of commonly sold herbal products in Abraka, Delta State, Nigeria.

MATERIALS AND METHOD

Materials

Glass wares used for this study include; petri dish, measuring cylinder, universal bottle, microscope glass slides, glass rod, beakers, Pasteur pipettes and test tubes, equipment use for this study include; autoclave, refrigerator, microscope, incubator, oven, electronic beam balance, Bunsen burner and water bath, distilled water, Lugol' iodine, crystal violet, acetone, alcohol and decolorizer, nutrient agar, peptone water, MacConkey agar, Mannitol Salt agar, Salmonella-Shigella agar and Sabouraud Dextrose agar, foil paper, needle and syringes, (2 ml, 5 ml), white handkerchief, paper tape, detergent, wire loops, cotton wool, Jik bleach

Method

Collection of samples

A total of seven (7) liquid herbal products randomly purchased from local herbal shops and pharmacy shops in Abraka, Delta State, Nigeria were used for this study. The herbal products include; AB, GW, EC, IM, DR, GC and BM. The samples were carefully and aseptically transported to the laboratory in their packages for analysis

Treatment of Samples and Enumerations of associated microorganisms

Samples were aseptically measured out around the zones of a Bunsen flame and the total viable bacteria and fungi counts of samples were determined using serial dilution and spread plate method (Alao, 2017). In the process, each sample was serially diluted to achieve a 1:10,000 dilution and 0.1 ml aliquot of each samples 10^{-4} was spread on to sterilized petri dishes containing different growth media such as nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) (Ciprofloxacin was added to allow the growth of only fungi). The plates were then incubated at 37 °C for 24 hours for bacteria and at room temperature for fungi for 3-5 days respectively. At the end of 24 hours of incubation for bacteria and 3-5 days for fungi, standard bacterial count and fungal count were recorded. The count was expressed as colony forming units (CFU).^{18,19}

Microbial Identification

The emerging discrete colonies upon determination of total viable counts, were separately stored in agar slants and further subcultured into various selective media such as MacConkey agar, Mannitol Salt agar and Centrimade agar. Further identification and biochemical characterization of the bacteria isolated were done using standard microbiological procedures.²⁰ Fungal identification was done by macroscopic (cultural and morphological characterization) microscopic (lactoteroide blue technique and (germ tube test) characterization. These characteristics were compared to features outlines in photographic atlas for microbiology laboratory.²¹ The substantive pure isolates were lastly stored at 4°C until further use.

Biochemical test.

The following biochemical tests were done; Gram-staining, coagulase, oxidase, fermentation, urease, lead acetate/H₂S test, citrate utilization, motility, MR - VP (Methyl Red - Voges Proskauer), catalase, and indole test.^{20,22,23}

Identification of Fungi Isolates

Pure cultures of fungal isolates from Sabouraud Dextrose agar were obtained, characterized and identified based on, colonial morphology, size shape, biochemical characteristics (germ tube and lactophenol blue).²¹

Germ tube test

Germ tube test is a screening test which is used to differentiate *Candida albicans* from other yeast. This test was carried out using an overnight broth of the yeast. 0.5 ml

When *Candida* is grown in human or sheep serum at 37°C for 3 hours, they form a germ tubes, which can be detected with a wet KOH films as filamentous outgrowth extending from yeast cells. It is positive for *Candida albicans*. 0.5 ml of human serum was put into a small test tube. Pasteur pipette was used to touch a colony of yeast and gently emulsify it in the serum. The mixture in the test tube was incubated 37°C for 3 hours. A drop of the serum was transferred into a sterilized slide for examination on a microscope under a low and high-power objective.²¹ A positive organism appeared as germ tube.

Lactophenol cotton blue.

A drop of seventy percent alcohol is placed on a clean microscope slide. Material from cultures of filamentous fungi was removed using a stiff inoculating wire. A coverslip was gently placed onto the slide. The slide was examined using a 10 X and then power 40 X objectives.²¹

Standardization of Inoculum for Sensitivity Test

Nutrient agar plated used in surviving the microorganism, colonies were transferred into peptone water using sterile inoculating wire-loop and incubated each test bacteria 24 hours at 37 °C. The over-night broth cultures were diluted appropriately by gradually adding normal saline to it and the density of the inocula standardized by comparison with 0.5 McFarland standard of Barium Sulphate solution (Cheesbrough, 2010) which is approximately 1.0×10^6 cfu/ml^{24,25, 26}

Antibiotic Susceptibility Test

Antimicrobial sensitivity test of bacteria was one using the disc diffusion method on Mueller Hinton Agar. Bacteria isolated from the herbal preparations were screened for their susceptibilities to the selected antibiotics, clinical laboratory and standard institute (2002a) methods were employed. Antimicrobial sensitivity test of bacteria was one using the disc diffusion method on Mueller Hinton Agar. 0.1ml of the suspension was spread on the agar plate. Commercial antibiotic discs were aseptically placed on the inoculated plates and incubated 37 °C for 24 hours. The zones of inhibition were measured and recorded. The antibiotic discs used were Azithromycin, Gentamycin, Ofloxacin,

Erythromycin, Ciprofloxacin, Imipenam /Cilastatin, Cefuroxime, Augmentin, Cefotaxime, Levofloxacin, Zemdri and Ceftriazone. Metre rule was used to measure the zone of inhibition²²

RESULTS

A total of seven (7) herbal products were microbiologically evaluated. The total bacterial count in the herbal products ranged from 1.8×10^6 to 7.5×10^6 as shown in Table 1. The bacteria and fungi isolated from the various herbal drugs products and their frequency of occurrence is presented in Table 2 and 3 respectively. *Proteus* spp had the highest frequency of occurrence with a percentage of 50% while *Staphylococcus* spp. occurred the least with a percentage of 10%. More so in Table 3 the frequency of occurrence of fungi isolated from the herbal drugs are presented with *Candida* spp. occurring more with percentage of 30% while *Aspergillusniger* and *Aspergillusflavus* had percentage of 10 and 20% respectively.

Tables 5 show the antibiotic sensitivity and resistivity of the standard drugs used in treating the bacteria isolated from the various herbal drugs. The antibiotics used for the susceptibility test include; OFX = Tarivid, PEF = Reflacine, CPX = Ciproflox, AU = Augmetin, CN = Gentamycin, S = Streptomycin, CEP = Ceporex, NA = Nalidixic acid, SXT = Septrin, PN = Amplicin. The standard drugs having the highest zone of inhibition was Tarivid, this was followed by Reflacine and Ciproflox while Septrin had the least zone of inhibition on the organisms isolated from the herbal drugs

Table 1: Total Bacterial Count for the Herbal Product Samples

Herbal Products	Bacterial count (CFU mg/ml)
A	3.4×10^6
B	1.8×10^6
C	2.3×10^6
D	7.5×10^6
E	1.3×10^6
F	2.8×10^6
G	1.9×10^6

Key: A=Adam's bitter, B=Goodwill, C=Elocyin, D=Iba mixture, E=Deep root, F=Goko cleanser, G=Blessed mother

Table 2: Synopsis of bacterial isolates and frequency of occurrence

Suspected Microorganisms	Herbal Drugs							Frequency of Occurrence %
	A	B	C	D	E	F	G	
<i>S. aureus</i>	-	-	-	-	+	-	-	10
<i>Proteus</i> spp	-	+	+	-	+	+	-	50
<i>Pseudomonas</i> spp	-	-	+	-	-	-	+	20
<i>Strep</i> spp	+	-	-	+	-	-	-	20

Key: A=Adam's bitter, B=Goodwill, C=Elocyin, D=Iba mixture, E=Deep root, F=Goko cleanser, G=Blessed mother

Table 3: Synopsis of fungi isolates and frequency of occurrence

Suspected Microorganisms	Herbal Drugs							Frequency of occurrence %
	A	B	C	D	E	F	G	
<i>Candida</i> spp	-	+	+	+	-	-	-	30
<i>Aspergillusniger</i>	+	-	-	-	-	-	+	20
<i>Aspergillusflavus</i>	-	-	-	-	-	+	-	10

Key: A=Adam's bitter, B=Goodwill, C=Elocyin, D=Iba mixture, E=Deep root, F=Goko cleanser, G=Blessed mother

Table 4: Biochemical test results of bacteria identified

Herbal Drugs											Fermentation			Suspected Bacteria
	Citrate	Catalase	Indole	Motility	Urease	H2H	Gram stain	Oxidase	MRVP	Coagulase	G	L	S	
AB														
NA	-	-	-	-	+	-	Rods	-	-	-	A	A	A	<i>Strep. spp</i>
GW														
NA	-	+	-	+	+	+	Rods	-	+	-	AG	A	A	<i>Proteus spp</i>
EC														
NA	+	+	-	+	+	-	Rods	+	+	-	A	-	-	<i>Pseudomonas spp</i>
MSA	+	+	-	+	+	+	Rods	-	+	-	A	A	A	<i>Proteus spp</i>
IM														
NA	-	-	-	-	+	-	Cocci	-	-	-	A	A	A	<i>Strep. spp</i>
DR														
NA	-	+	-	+	+	+	Rods	+	+	-	A	A	A	<i>Proteus spp</i>
MSA	+	+	-	-	-	-	Cocci	-	-	+	A	A	A	<i>S. aureus</i>
GC														
NA	-	+	-	+	+	+	Rods	+	+	-	A	A	A	<i>Proteus spp</i>
BM														
NA	-	+	-	+	+	-	Rods	-	+	-	A	-	-	<i>Pseudomonas spp</i>

Key: NA = Nutrient Agar, MSA= Mannitol Salt Agar

Table 5: Antibiotic susceptibility of bacteria isolated from herbal drugs

Zones of inhibition produced by different antibiotics in the nearest millimeter (mm)										
Samples	Drugs									
	OFX	PEF	CPX	AU	CN	S	SEP	NA	SXT	PN
GW (NA)	20	23	26	14	21	25	-	-	-	18
DR (NA)	17	25	26	-	20	22	-	-	-	15
DR (MSA)										
BM (NA)	18	22	25	-	23	26	-	-	-	19
EC (M/K)	17	-	22	-	12	21	-	-	-	-
EC (NA)	20	24	21	-	16	20	-	-	-	-
IM (NA)	20	25	25	-	22	25	22	-	-	21
AB (NA)	16	22	22	15	23	21	18	-	-	13
GC (NA)	18	20	-	-	21	25	-	-	-	-

OFX = Tarivid, PEF = Reflaxine, CPX = Ciproflox, AU = Augmetin, CN = Gentamycin, S = Streptomycin, CEP = Ceporex, NA = Nalidixic acid, SXT = Septrin, PN = Amplicin

DISCUSSION

Due to the state's vast plant species and sociodiversity, herbal treatments are widely utilized throughout Nigeria, particularly in Delta State. Because of their efficient curative action and low adverse effect, this herbal medicine is used all over the world, not just in Nigeria and Africa.²⁷ Herbal medications are currently utilized in conjunction with synthetic medicines to minimize health-care expenditures for people who have limited access to contemporary health-care facilities due to a lack of health insurance and a lack of education.²⁷ Herbal medications are a cost-effective therapy alternative because they are simple to manufacture or obtain in both locally and in urban area.

Plant-based drugs are increasingly being integrated into poor countries' primary health care systems; yet, safety concerns remain unaddressed.²⁸ Bacterial and fungal contamination is common, particularly in DIY herbal medications, with CFU/g levels above suggested limits.²⁹ Herbal remedies in liquid pharmaceutical form for oral use had the highest microbiological contamination and were also the most popular among the elderly. The failure to manage moisture levels in herbal medications during transportation and storage, as well as the temperatures of liquid forms and finished herbal products, may have resulted in the multiplication of bacteria.²⁹ Furthermore, most medicinal plants are prepared in an open setting under unsanitary conditions, which leads to contamination with diseases that are harmful to public health.

In this study, the microbial quality of seven (7) selected herbal drug sold in Abraka were evaluated. The bacterial count for bacteria in selected herbal drug samples carried out ranges between 1.3×10^6 and 7.5×10^5 CFU/ml; while the total fungal count for the selected herbal drug samples ranges between 1.0×10^6 and 7.0×10^5 CFU/ml. 'The British Pharmacopoeia's standards for microbiological contamination for total aerobic bacteria and fungal counts for herbal products in which boiling water is not added before consumption should not be more than 10^5 and 10^4 CFU/g, respectively,' according to British Pharmacopoeia.³⁰

The pharmacopoeia limit for herbal products was clearly surpassed in all seven (7) herbal products assessed in this study, and as a result, they went below the maximum allowable limit, implying a significant level of microbial contamination. Ideh found high microbiological contamination in 10 of the 12 herbal items tested in a similar investigation.³¹ The permissible reasons for heavy contaminants of the herbal products may as a result of contamination of raw materials, poor hygienic practices during the manufacturing and storage of such products.

The findings of this study revealed the presence of *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp. and *Streptococcal* spp. *Staphylococcus* spp. have been reported to cause Staphylococcal gastroenteritis, scalded-skin syndrome, and folliculitis, among other diseases.³² A study that also evaluated the microbial quality of herbal medicines showed similar results, with 47.6% of samples contaminated with *E. coli*, 33% of samples contaminated with *Salmonella* spp., and 71.4% of samples contaminated with *Staphylococcus* spp.³³ Although the present study showed that the herbal medicinal were contaminated with a higher percentage of *Proteus* spp., while *Staphylococcus* spp. was the least contaminant on the herbal medicine.

Pathogenic bacteria, such as *E. coli*, *Salmonella* spp., *Shigella*, *Staphylococcus* spp. and *P. aeruginosa*, were also found in other studies.³² These contaminations were probably caused by unsafe collection, transportation, drying, preparation, storage or dispensing processes of the herbal medicines. The quality of

the water used in the preparation of herbal medicines may have contributed to the high level of bacterial contamination observed for the homemade herbal medicines. Drinking water should be free from pathogenic microorganisms and bacteria that indicate fecal contamination.³⁴

The British Pharmacopoeia (2013) does not require the identification of fungal colonies, however it is important in determining the danger of examined items. Microscopic investigation revealed fungal contamination, which was similar to another study that found the presence of mycotoxins-producing fungus species such as *Aspergillus niger*, *Aspergillus flavus*, and *Candida* spp. When these species are present in items that are consumed, they can represent a threat. Mycotoxins have also been found in medicinal plant preparations, according to numerous investigations. The severity of mycotoxins is determined by their toxicity, the degree of exposure, the individual's age and nutritional health, as well as any possible synergistic effects of other chemical agents to which they are exposed.³³

In a study³¹ leaving fungal contamination was also seen. This highlights the fact that significantly polluted raw materials from natural sources cannot be used to determine the microbiological purity of medications. If medicinal plants are not properly cleaned before being used for herbal purposes, fungal spores can be dispersed in the air for a short time before being deposited on surfaces. Roots, leaves, and stems of medicinal plants could be a reservoir for these fungal organisms.

In this case, a large microbial burden is unavoidable. Infections of the respiratory tract are a common disease that can emerge as a result of fungal contamination of herbal products, and this has serious public health implications. This means that Tarivid, Streptomycin and Gentamycin were effective against all bacteria identified from herbal items. Although Gentamycin have been classified as vitally important for human medicine by the World Health Organization (World Health Organization, 2018 and 2019)

CONCLUSION

This study revealed that herbal products sold in Abraka were contaminated with microorganisms, some of which are pathogenic and thus, these herbal products can serve as a means of spreading infections among the consumers. Therefore, this study suggest good manufacturing practice should be applied in all aspect right from harvesting of plant materials to finished products, personnel, packaging materials, equipment and environment so as to reduce micro-organism contamination of herbal finished products to the lowest minimum. NAFDAC as a regulatory body should ensure they conduct regular and adequate monitoring of herbal products in order for the herbal products to meet with the minimum microbiological standard before being sold out to the public.

REFERENCES

1. Abel, C. and Busia, K. An Exploration Ethnobotanical Study of the Practice of Herbal Medicine by the Akan People of Ghana. *Alternative Medicine Review*. 2015; 6:70-77
2. WHO Traditional medicine strategy: 2014-2023. Hong Kong, SAR, China: World Health Organization; 2013.
3. Anie CO, Nwabuokeyi IG, Oghenejobo M, Enwa FO, The Antibacterial Effect of the Leaf Extract of *Buchholzia coriariae* (Capparidaceae) on Gram-Negative Nasal Isolates. *Sch. Acad. J. Pharm.*; 2015; 4(4): 226-231
4. Ampofo, J. A., Tetteh, W. and Bello, M. Microbiological Profile of Some Ghanaian Herbal Preparations-Safety Issues and Implications for the Health Professions. *Open Journal Of Medical Microbiology*, 2012; 2:121-130. <https://doi.org/10.4236/ojmm.2012.23018>

5. Olisa, N. S. and Oyelola, F. T. Evaluation of use of herbal medicines among ambulatory hypertensive patients attending a secondary health care facility in Nigeria, *International Journal of Pharmacy Practice*, 2009; 17(2):101-105. <https://doi.org/10.1211/ijpp.17.02.0005>
6. Ibezim EC, Kenechukwu FC, Odimegwu DC, Builders PF, Kabele-Toge B, Anie C, Igwilo CO, Otuu FC, Onyechukwu C. Antimicrobial efficacy of a syrup formulation from methanol extract of *Garcinia kola* seed. *Afr J Pharm Res Dev* 2011; 3(1):22-27
7. Onyambu, M. O., Chepkwony, H. K., Thoithi, G. N., Ouya, G. O. and Osanjo, G. O. Microbial Quality of Unregulated Herbal Medicinal Products in Kenya. *African Journal of Pharmacology and Therapeutics*. 2013; 2(3):70-77.
8. Rajapandian, K., Shanthi, S. and Vidya, S. Assessment of Microbial Quality in Marketed Herbal Drugs Sold in Trichy City. *International Journal of Pharmaceutical, Chemical and Biological Sciences*. 2013; 3(3):894-898.
9. Keter, L., Too, R., Mwikwabe, N., Ndwigah, S., Orwa, J., Mwamburi, E., Korir, R., Mutai, C. Bacteria contaminants and their antibiotic sensitivity from selected herbal medicinal products from Eldoret and Mombasa, Kenya. *Current Research in Microbiology*. 2016; 7(1):18-28. <https://doi.org/10.3844/ajmsp.2016.18.28>
10. Archibong, E. J., Igboeli, C. N., Okoro, N.C. and Obika, I. Microbiological Assessment of some liquid herbal medications sold in Awka metropolis, Anambra state. *Bioengineering and Bioscience*. 2017; 5(3):37-46. <https://doi.org/10.13189/bb.2017.050301>
11. Anjoo, K. Analytical evaluation of herbal drugs. In: Prof. OmboonVallisuta, editor. *Drug discovery research in Pharmacognosy*. 1st edition. Rijeka, Croatia: In Tech Publication. 2012.
12. Balunasa, M.J. and Kinghorn, A.D. Drug discovery from medicinal plants, *Life Sciences*, 2005; 78:431-441. <https://doi.org/10.1016/j.lfs.2005.09.012>
13. Qazi, M.A. and Molvi, K. Herbal Medicine: A Comprehensive Review. *International Journal of Pharmaceutical Research* 2016; 8(2): -5.
14. Siddhartha, S. and Sapna, S. Medicinal Plants and Their Pharmacological Aspects, *FPI* 2017; 1(4):156-170.
15. Clement O. Anie, Matthew I. Arhewoh and Henry A. Okeri. Antimicrobial activity of Crude Extracts of *Diospyros monbuttensis* (Fam: Ebenaceae) Root and Stem Barks. *International Journal of Biomedical Research* 2011; 1:18-24
16. Mahesh, B. and Satish, S. Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. *World Journal of Agricultural Sciences*. 2008; 4(S):839-843.
17. Enwa Felix O, Anie Clement O, Oghenejobo Micheal P & Ilaya Sonia A. Evaluation of the Comparative Activity of Alcohol-Based Hand Sanitizers and Toilet Soaps against some Bacterial Isolates. *Global journal of Science Frontier Research: Biological C*. 2015; 15(3):1-9
18. Okafo S E, Anie CO, Nwanua MC. Formulation and Evaluation of Antimicrobial Topical Creams from Ethanol Extract of *Vernonia ambigua* Leaves. *Nig. J. Pharm. Res*, 2019; 15(2):249-255 <https://doi.org/10.4314/njpr.v15i2.12>
19. Anie, CO. Evaluation of Biofilm and Enterotoxin Producing Capacity of Methicillin-resistant *Staphylococcus aureus* isolated from Healthy Persons. *J. Appl. Sci. Environ. Manage*. 2018; 22(12):1881-1884 <https://doi.org/10.4314/jasem.v22i12.2>
20. Anie, C. O., Jemikalejah, D. J. and Eke CN. Determination Of Microbial Load Of Selected Smoked Fish Sold In Abraka Markets Delta State nig. *J. Pure & Appl. Sci*. 2019; 32
21. Felix O. Enwa, Oghenejobo Michael, Clement O. Anie, Rita A. Ayeh, Antibacterial Screening of the Ethanol and Aqueous Extract of the Fruit Peel of *Persea Americana* Mill against Selected Enteric Bacteria. *Academia Journal of Microbiology Research* 2016; 4(3):040-046.
22. TU Onyekaba, JE Arute, CO Anie. Antimicrobial and phytochemical screening of the methanolic leaf extract of *buchholzia coriacea* (wonderful cola) •ournal of Pharmaceutical and Allied Sciences 2011; 8(1). <https://doi.org/10.4314/jophas.v8i1.68151>
23. C. O. Anie, M. C. Ugwu, E. C. Ibezim and C. O. Esimone. Antibigram of Methicillin- Resistant *Staphylococcus aureus* Isolates among Healthy Human Subjects in Oleh, South-Southern Nigeria *Int.J.Curr.Microbiol.App.Sci* 2017; 6(9):3710-3716 <https://doi.org/10.20546/ijcmas.2017.609.458>
24. Delgado Odongo, N.E (2010). Sustainable improvement of animal production and health. *FAO*.
25. Umair, M., Altaf, M. and Abbasi, A.M. An ethnobotanical survey of indigenous medicinal plants in Hafizabad district, Punjab Pakistan. *PLOS ONE*, 2017; 2. <https://doi.org/10.1371/journal.pone.0177912>
26. World Health Organization, (WHO), (2007). WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Press, Geneva, Switzerland.
27. Esimone, C.O., Oleghe, P.O., Ibezim, E.C., Okeh, C.O. and Iroha, I.R. Susceptibility resistance profile of micro-organisms isolated from herbal medicine products sold in Nigeria. *Afr J Biotechnol*, 2007; 6(24):2766-75. <https://doi.org/10.5897/AJB2007.000-2442>
28. Kaume, L., Foote, J.C. and Gbur, E.E. (2012). Microbiological contamination of herbs marketed to HIV-infected people in Nairobi (Kenya). *S Afr J Sci*, 108(9):80-3. <https://doi.org/10.4102/sajs.v108i9/10.563>
29. Rahimi, S.M., Ebrahimi, M., Barikbin, B. and Zeinali, T. (2019). Evaluation of bacterial and fungal contamination of kitchens of Birjand University of Medical Sciences. *BMC Res Notes* 12(703). <https://doi.org/10.1186/s13104-019-4741-y> <https://doi.org/10.1186/s13104-019-4741-y>
30. Ideh and Ogunkunle, (2019). Botanical Characterization, drug indication and Sustainability status of traditional oral powdered herbal formulation in Ogbomoso, Nigeria. *Journal of Medicinal plants for Economic Development*. 3(1) <https://doi.org/10.4102/jomped.v3i1.67>
31. The British Pharmacopoeia (2013)
32. Maziero, M.T. and Bersot, L.S. (2010). Micotoxinas em alimentos produzidos no Brasil. *Revista Brasileira de Produtos Agroindustriais*, 12(1):89-99. <https://doi.org/10.15871/1517-8595/rbpa.v12n1p89-99>
33. World Health Organization (World Health Organization, 2018 and 2019).