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Research Article

MICROBIAL LOAD OF SOME POLYHERBAL PRODUCTS FROM LAGOS STATE, NIGERIA.

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ABSTRACT

This study investigated the microbial contamination of commonly used polyherbal products in Lagos State. Thirty polyherbal products were purchased from different vendors. Using standard microbiological techniques for microbial analysis, the bacteria isolates used included *Bacillus* sp (100%), *Flavobacterium* sp (30%), *Pseudomonas* sp (50%) and *Staphylococcus* sp (33.3%) while the fungi isolates used were *Fusarium* sp (10%), *Aspergillus flavus* (43.3%), *Penicilium* sp (13.3%), *Geotrichum candidum* (3.3%), *Mucor* sp (3.3%), *Aspergillus oryzae* (23.3%) and *Aspergillus niger* (3.3%). The total bacteria counts ranged from $2.5x10^3$ to $6.4x10^9$ cfu/ml while fungal counts ranged from $9.5x10^3$ to $3.5x10^9$ cfu/ml. This study has shown that most polyherbal preparations sold in Lagos State are of poor microbiological standards which may be due to poor sanitary and inappropriate hygienic measures. Improved hygiene standards of polyherbal products is recommended.

KEYWORDS: Microbial load; Polyherbals; major markets; Lagos; Nigeria.

INTRODUCTION

An herb is a plant or plant part used for its scent, flavor, or therapeutic properties. Herb includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and barks. The use of herbs for treating various diseases predates human history and forms the origin of most of the modern medicine. Long before the advent of modem medicine, herbs were the mainstream remedies for nearly all ailments [1]. People commonly diagnosed their own illnesses, prepared and prescribed their own herbal medicines, or bought them from the local apothecaries. Herbal medicine is defined as a branch of science in which plant based formulations are used to alleviate diseases [2].

Herbal medicine is most often polyherbal, being prepared from mixtures of different plant parts obtained from various plant species and families and may contain multiple bioactive constituents that could be difficult to characterize [3]. Phytomedicines are preparation consisting of complex mixture of one or more plant materials [4]. This is synonymous to traditional medicine (herbalism) which is the most ancient method of curing diseases and it has been said that plants are the very first and only true medicines ever used [5]. The bioactive principles in most herbal preparations are not always known and there could be possibilities of interaction with each other in solution. The quality as well as the safety criteria for herbal drugs may be based, therefore, on a clear scientific definition of the raw materials used for such preparations [3]. Plants and herbs derived medicines are popularly known as "Herbal medicine" and are generally regarded as safe; based on their long-standing use in various cultures [6]. According to the World Health Organization (WHO), herbal medicines are medications prepared from one or more herbs or plant parts (roots, stem bark, seeds and/or fruits). Herbal medicines have been employed since prehistoric era by the traditional medical practitioners for the treatment of various diseases. They remain the main stay of health care system in the developing countries and are gaining increasing popularity in the developed countries

where orthodox medicines are predominantly used. The use of medicinal plants as therapeutic or dietary supplements goes back beyond recorded history, but has increased substantially in the last decades [7][8]. Medicinal plants play a major role in the health care sector of developing nations in the management of diseases. In Nigeria, there appears to be an overwhelming increase in the public awareness and usage of herbal medicinal products in the treatment and/or prevention of diseases as a result of active mass media advertisement embarked upon by the producers and marketers of herbal products. The growing popularity has been linked with its typically low side effect profiles, low cost and high level of acceptance by patients and the majority of the population [9][10]. Some managed care organization now offer herbal preparations as an expanded benefit [11]. The use of herbal and traditional medicines raises concerns in relation to their safety, however, there is a wide misconception that "natural" means "safe"[8]

The contamination of herbal remedies with heavy metals may be due to soil and atmospheric contamination which poses a threat to its quality and safety. Medicinal plants are normally contaminated with toxic metals during growth, development and processing [12]. Indeed the issues of safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals. This could be due to lack of standards for herbal products. To maximize the potential of African traditional medicines as a source of healthcare, the safety, efficacy and quality need to be assessed [13]. Some of the shortcomings of herbal medicines are scientific proof, imprecise dosage, imprecise diagnosis and unhygienic condition under which the herbal products are produced as well as the contaminated environment (soil and air) where the plants are grown [14].

Herbal preparations are used in different forms and may carry a large number of microbes originating from soil usually adhering to various parts of herb. The commonly used herbal materials include chewing sticks, herbal pastes, powders, herbal mixtures and suspensions. Most of these herbal materials are prepared and sold under unhygienic conditions [15]. A number of oral health care materials are hawked when not packaged and this raises the possibility of contamination. Most of these materials are used directly without further processing (for example chewing sticks) thereby increasing the risk of disease transmission. Herbal preparations are used in different forms and may carry a large number of microbes originating from soil usually adhering to various parts of herb. The contaminants that present serious health hazard are pathogenic bacteria such as *Salmonella, Escherichia coli, Staphylococcus aureus, Shigella* species and other Gram positive and Gram negative strains of bacteria [13].

Biological contamination refers to contamination of herbaceous plants by microorganisms such as bacteria, fungi (moulds), viruses, protozoa, insects (eggs and larvae) and other organisms. The presence of pathogenic microorganisms in herbs might pose a risk to public health and affects the quality of the products [16]. Pathogenic microorganisms include; Salmonella spp, Escherichia coli, Listeria Monocytogenes and spore-forming microorganisms such as Bacillus cereus and Clostridium perfringens [17]. Enterobacteriaceae spp and Pseudomonasspp are the two groups of bacteria found commonly on the harvested plant surface and these can rise to the problem of damage and deterioration in the quality of food. Thus, it's important to monitor the bacteria contamination in any herbal product [18].

STUDY AREA

The study was carried out in Lagos state, Nigeria. The markets surveyed were Agege market, Ikeja market, Temidire market, Abibatu mogaji market, Alimosho market. Lagos State is a commercial city in Nigeria, located in the south western part of the country. Lagos State is arguably the most economically important state of the country. The estimated population is disputed between the official Nigerian Census of 2006, and a much higher figure claimed by the Lagos State Government, which is 9,019,534. Lagos state was created on May 27, 1967. The capital of Lagos state is Ikeja. Lagos state is made up of 20 local government and 37 local council development areas (LCDA). The local government areas include; Alimosho (1,277,714), Ajeromi-Ifelodun (684,105), Kosofe (665,393), Mushin (633,009), Oshodi-Isolo (621,509), Ojo (598,071), Ikorodu (535,619), Surulere (503,975), Agege (459,939), Ifako-Ijaye (427,878), Shomolu (402,673), Lagos mainland (317,720), Ikeja (313,196), Amuwo-Odofin (318,166), Eti-Osa (287,785), Badagry (241,093), Apapa (217,362), Lagos Island (209,437), Epe (181,409) and Ibeju-Lekki (117,481). Lagos State is located in the south-western part of the Nigerian Federation. On the North and East it is bounded by Ogun State. In the West it shares boundaries with the Republic of Benin. Behind its southern borders lies the Atlantic Ocean. 22% of its 3,577 km^2 are lagoons and creeks [19]

Figure 1:*A Map Of Lagos State Showing The Local Government Areas And The Study Area*

Represents study area.

MATERIALS AND METHOD

Samples Collection

Thirty polyherbal preparation samples were randomly purchased from different locations in Lagos state. The samples were taken to the laboratory for microbiological analysis.

Preparation of samples and culturing

One ml of each sample was aseptically introduced into 9 ml of sterile distilled water, properly shaken before a 10 fold serial dilution, up to 10^{-8}, was performed.

Preparation of culture medium

The media used for the analysis were nutrient agar and potato dextrose agar

Nutrient Agar

This medium was prepared from commercially available dehydrated powder, available from most suppliers of culture media. In the preparation, 28g of nutrient powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. This was stirred and autoclaved at 121^{o}C for 15 minutes and then cooled to 50^{o}C, before pouring into Petri dishes.

Potato dextrose agar

This medium was used for isolation of fungi from the polyherbal samples and for the preparation of pure cultures. The medium was prepared from commercially produced dehydrated medium following the manufacturer's instruction. 39g of Potato Dextrose agar powder was dissolved in 1 litre of distilled water in a sterile conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and autoclaved at 121^{0}C for 15 minutes. The medium was cooled after autoclaving to 50^{0}C and then dispensed aseptically into sterile Petri dishes containing 0.1 ml of the prepared sample. Streptomycin (0.1w/v) was added to the medium to prevent the growth of bacteria.

Determination of total viable count

Pour plate method was used for microbial enumeration. 0.1 ml from each dilution was pipetted into sterile Petri dish and labelled as such. 20 ml of prepared agar was dispensed into the various Petri plates. The nutrient agar plates were incubated at 37^{0}C for 24 hours while the potato dextrose agar was kept at room temperature for 48 to 72 hours.

Developed colonies were counted to obtain total viable count. Discrete distinct colonies were purified by sub-culturing into nutrient agar plates using the streak plate method.

Procedure For Identification Of The Organisms

The bacterial isolates were characterized and identified based on their cultural characteristics and biochemical reaction as follows:

Gram reaction

The Gram staining techniques was done on the basis of the component of the cell wall Organisms which retained the colour of the initial stain are called gram positive while those that do not retain the primary stain when decolorized are gram negative. The non-retention of the stain is due to the cell composition. Gram staining reagents include; crystal violet (primary dye stain), iodine (as mordant), 70% alcohol (decolorizer) and safranin (counter stain)

Procedures; a drop of sterile distilled water was placed on a clean grease free slide. The inoculating wire loop was flamed until red hot. The loop was allowed to cool and a small portion of the organism to be gram stained was picked and smeared in the drop of water on the slide. The slide was then air dried. It was heat fixed by passing it gently over flame.

The smear was stained with 1% crystal violet for 1 minute and washed with distilled water. Gram iodine was added as a mordant for one minute. This was drained off and 70% alcohol was added for 30 seconds. This acted as a decolorizer. The above is termed primary staining. The slide was then rinsed with distilled water. The slide was finally flooded with counter stain, safranin for 1 minutes and washed off with distilled water and air dried. The slide was observed under the microscope oil immersion x 100 objective lens. The Gram positive organisms appeared purple while the Gram negative organisms appeared red.

Motility test

The test organism was inoculated into peptone water and then incubated aerobically at 35^0C for 24 hours. After overnight incubation, a drop of the bacteria culture broth was placed in the center of a clean, grease free slide and covered with a clean Vaseline bound cover slip. The prepared slide was observed under microscope using x40 objective with the condenser iris closed sufficiently to give good contrast. Motile organism was seen moving randomly in the deep of the liquid.

BIOCHEMICAL TEST

Oxidase test

This test was carried out to detect the production of the enzyme oxidase by the bacteria isolates.

Procedures

piece of filter paper was soaked in a few drop of oxidase reagent (Tetramethyl- p- phenyl-diamine-dihydrochloride). A colony of the test organism was then smeared on the soaked filter paper. An oxidase producing organisms on the filter paper oxidized the phenyl-diamine in the reagent to deep purple colour. This change in colour to deep purple within l0 seconds indicated positive result.

Catalase test

This test is used to demonstrate the presence of enzyme catalase, which catalyzes the release of oxygen from hydrogen peroxide.

Procedure

The pure culture of the test organism was placed and added to a drop of 3% hydrogen peroxide solution on a clean slide. The production of gas bubble from the surface indicates positive result.

Coagulase test

This test is carried out to determine the enzyme coagulase. The test distinguishes pathogenic Staphylococcus aureus from other non-pathogenic strains of Staphylococcus.

Procedure

a colony of the test organisms was emulsified with sterile normal saline solution on a clean slide using a sterile wire loop. A drop of human plasma was added and mixed with emulsion. The positive coagulase organisms showed clumping while negative coagulase organisms showed no clumping.

Indole test

This test helps in the identification of Enterobacteria.

Procedure

The test organism was inoculated into bijou bottle containing 3ml of tryptone water and incubated at 37°C for up to 48hours. 0.5ml of kovac's reagent which contains 4-p-dimethylaminebenzaldehyde was added. The test tube was shaken gently. The development of red coloration on the surface of the medium within l0minutes indicated a positive reaction of indole production; while no colour change indicated negative reaction.

This was carried out to differentiate gram position from gram-negative organisms.

A wire loop was sterilized in Bunsen burner and allowed to cool, then a loopful of growth was collected from the agar plate, smeared on a glass slide and then heat fixed by passing over a flame three times. The smear was flooded with crystal violet for 60 seconds, after which it was rinsed with distilled water. Iodine was added for 60 seconds and then washed off; it was decolorized with alcohol until no colour runs off the slide and rinsed immediately. Safranin was finally added for 1minute and then washed off with distilled water. The slide was kept in a rack to air dry after wiping the back with cotton wool.

The stained smear was then examined microscopically under oil immersion at 100x objective lens. Gram – positive bacteria appeared dark purple while gram-negative bacteria appeared red.

Urease test

This test was aimed at identifying Enterobacteria that produce urease enzyme, which hydrolyze urea to give ammonia and carbondioxide. The test organism was inoculated onto Christensens urea agar in a bijou bottle using a sterile wire loop and incubated at 35^0C - 37^0C for 18 - 24hours and examined, thereafter a pink color in the medium showed positive test.

Citrate test

This test is based on the ability of an organism to use citrate as its source of carbon. It was used to identify the Enterobacteria.

Simon's citrate agar medium was prepared in a slant bijou bottle, then using a sterile wire loop, organism was inoculated onto the slant medium and incubated at 37^0C for 48hours after which it was examined for color formation. A bright blue color in the medium gave a positive citrate test.

Carbohydrate fermentation test

This test is used to determine the ability of bacteria to utilize different sugars. Examples are mannitol, glucose, lactose and sucrose.

The four sugar solutions were prepared and poured into test tubes well stopped with Durham tube for gas collection. The sugar was autoclaved after which a loopful of test organisms was introduced into the sugar solution. A change in color from pink to yellow shows fermentation and collection of gas bubbles in the Durham tube which pushes the tube upward, shows gas production which is a positive test. A control was set up without the organism inoculated.

Identification of fungi isolates

The fungi isolates were examined macroscopically using their cultural characteristics and microscopically using lactophenol blue.

Lactophenol stain

A drop of lactophenol blue stain was placed on a clean grease free sterilized glass slide and after this a sterile inoculating wire loop was used to pick the mycelium unto the glass slide from the mould culture .The mycelium was spread evenly on the slide and then covered with cover slips gently and then allowed to stay for some seconds before observing under x40 under the microscope. The colonial and morphological characteristics of each isolate were determined, appearance of special structures including the nature of spore / ascospores if produced. The growth, the appearance of the colony from initial to the time of maturity was also taken into consideration as well as the presence or absence of septate hyphae.

RESULTS

The compositions, indications and dosage of 30 common polyherbal samples in major markets in Lagos state are shown on Table 1. Table 2 shows the total bacterial and fungal counts in the polyherbal samples. Table 3 highlights the cultural, morphological and biochemical characteristics of the bacterial isolates. Table 4 outlines the identification of the fungal isolates. Table 5 outlines the probable bacteria contaminants associated with different polyherbals. Table 6 depicts the fungi contaminant present in polyherbal products. Table 7 shows the percentage distribution of microbial isolate among the different samples. Table 8 shows the percentage occurrence of microbial isolates in the polyherbal samples. Table 9 evaluates the concentrations of heavy metals associated with polyherbal products.

Table 1: Composition, indication and dosage of commonly consumed polyherbal in Lagos state

The table above show the composition of some of the polyherbals. The compositions of some other polyherbals were not disclosed by the sellers and manufacturers.

Polyherbal samples	Compositions	Indications	Dosage
H1	Aloe vera, Allium sativum, Zingiber officinale, Eremomastax speciosa, Garcinia kola.	Malaria, typhoid.	One cup twice a day.
H2	Gossypium hirsutum, Eremomastax speciosa	Irregular and painful menstruation.	One cup daily.
H3	Not disclosed.	Diabetes, chronic ulcer, pile, malaria, typhoid, worm expellant, itching, nettle rash, Eczema. Rheumatic pain, hypertension, eye infection.	Adult; two tablespoons daily.
H4	Callichilia barteri, Pachylobus edulos, Lecaniodiscus, Allium sativum, Zingiber officinale, Monodora, Khaya ivorensis, Piper nigrum, Eugenia	Energy booster, general over all well-being.	Adult; Four tablespoons daily.

	catyophyli.		
H5	Not disclosed.	Acute stomach ache, painful and irregular menstruation	Two tablespoons daily.
H6	Not disclosed.	Typhoid	Two cups twice daily.
H7	Not disclosed.	Typhoid, malaria,	One cup three times daily
H8	Seccharum officinarum, Ocimum basilicum.	Diabetes, stomach disorder, pile.	Four tablespoons daily.
H9	Not disclosed.	Dysentery, cough, pile, convulsion, chest pain, snake or scorpion bite, stomach ache, rheumatism	One tablespoon daily.
H10	Not disclosed	Whooping cough, tuberculosis.	Two tablespoon twice daily.
H11	Not disclosed	Malaria, typhoid, general body well-being.	One cup daily.
H12	Not disclosed	Diabetes	One cup twice daily.
H13	Azadirachta indica, Citrus aurantiifolia, Eremomastax speciosa, Garcinia kola, Newbouldia leavis.	Malaria and typhoid.	One cup three times daily.
H14	Not disclosed.	Malaria	One cup twice daily
H15	Newbouldia leavis, Eremomastax speciosa, Callichilia barteri.	All sexually transmitted diseases. Immune booster.	Two tablespoons twice daily.
H16	Gossypium hirsutum, Eremomastax speciosa and Aloe vera	Irregular menstruation.	Four tablespoons daily.
H17	Not disclosed	Pile, heamorhoid, waist pain, constipation, irregular menstruation, obesity.	Four tablespoons daily, after meal.
H18	Ocimum gratissimum, Vernonia amygdalina Eremomastax speciosa	Vagina itching, burning and irritation, chronic pile and aids digestion.	Two cups daily.
H19	Psidium guajava, Carica papaya, Mangifera indica, Citrus aurantiifolia.	Malaria	Two cups twice daily.
H20	Citrus aurantiifolia, Garcinia kola, Cymbopogon citrates.	Malaria.	Two cups twice daily.
H21	Moringa oleifera, Zingiber officinale, Allium sativum, Garcinia kola.	Malaria, typhoid.	Two cups twice daily.
H22	Not disclosed.	Pile, dysentery.	Four tablespoons daily.
H23	Not disclosed	Vagina discharge, itching, burning sensation.	Five tablespoons daily
H24	Sorghum bicolor, khaya grandifoliola, Cassia sieberiana, Staudtia stipitata, Alstonia cognensis, Ocimum basilicum, Mangifera indica, Cyathula prostrate, Securidaca longepedunculata, Seccharum officinarum.	Pile, dysentery, constipation, diarrhea, irregular menstruation, men turgidity, withdraws protruding rectum, waist and stomach ache.	Adult; four tablespoons twice daily, children; two tablespoons twice daily.
H25	Cassia siamea, Cassia alata, Cassia augustifolia, Aloe vera, Gongronema latifolium, Anthocelesta djalonensis, Moringa lucida, Citrulus lanatus, Xylopha aethloica, Kyaha sengaiensis, Garcinia kola.	Infertility, Menstrual dysfunction, STD, Mouth odor, Acute stomach ache, Obesity,	Adult; Four tablespoons daily. Children; One tablespoon daily
H26	Eremomastax speciosa, Garcinia kola, Allium sativum, Irvingia	Stomach disorder, indigestion, peptic/duodenal	Adult; two tablespoons daily after meal. Children;

	gabonensis,	ulcer, improves blood circulation, eliminates pain and discomfort.	one tablespoon daily after meal.
H27	Not disclosed.	Chronic hypertension, palcipitation of the heart.	One cup daily.
H28	Garnia nigrum, Zysigium aromaticum, Piper nigrum, Microdesmis puberula, Ephobia lateriflora, Uvaria chame, Securidaca longependunculata, Treculia Africana, Allium sativum, Zingiber officinale,	Pile, Haemorhoid, Waist pain, Enhances effective digestion, Reduces fatique, Improve libido, Strengthens the heart and cardiac muscules, Reduces blood sugar.	Adult; Four tablespoons daily. Children; two tablespoons daily.
H29	Khaya grandifolia, Crotan lobatus, Anthocleisa vogelii, Eremomastax speciosa	Glaucoma, Eye ulcer, cataract.	Adult; Four tablespoons daily every two days. Children; One tablespoon daily every two days.
H30	Olax subscorpioidea, Kigelia africana, Securidaca longepedunculata, Allium ascalonicum Eremomastax speciosa.	Purifies the blood, Eliminates toxic material from the blood vessels, Reduces accumulated fat and cholesterol level, prevents oedema, numbness, and reduces blood sugar.	Adult; five tablespoons daily. Children; one tablespoon every two days.

Table 2 below shows the total bacterial and fungal counts of all the polyherbal samples.

Table 2: *Total bacteria and fungi counts.*

Polyherbal samples	Total Bacterial Counts (cfu/ml)	Total fungi counts (cfu/ml)
H1	$5.5X10^6$	$5.8X10^5$
H2	$3.3X10^6$	$4.3X10^6$
H3	$8.0X10^4$	$7.0X10^4$
H4	$4.6X10^5$	$3.6X10^5$
H5	$7.2X10^8$	$8.2X10^7$
H6	$2.2X10^7$	$1.2X10^6$
H7	$9.7X10^6$	$7.7X10^7$
H8	$9.0X10^8$	$3.0X10^8$
H9	$2.5X10^3$	$9.5X10^3$
H10	$8.3X10^6$	$2.3X10^6$
H11	$4.2X10^9$	$4.2X10^9$
H12	$7.0X10^7$	$5.0X10^7$
H13	$5.0X10^7$	$1.0X10^7$
H14	$3.2X10^4$	$4.2X10^4$

H15	$1.0X10^{6}$	$2.0X10^{6}$
H16	$4.9X10^{6}$	$6.9X10^{6}$
H17	$3.7X10^{5}$	$3.7X10^{5}$
H17	$8.7X10^{8}$	$6.7X10^{8}$
H19	$6.4X10^{9}$	$1.4X10^{9}$
H20	$5.5X10^{6}$	$7.5X10^{6}$
H21	$7.8X10^{7}$	$4.8X10^{7}$
H22	$6.5X10^{6}$	$7.5X10^{6}$
H23	$6.1X10^{7}$	$5.1X10^{7}$
H24	$5.6X10^{5}$	$4.6X10^{5}$
H25	$2.6X10^{5}$	$3.6X10^{5}$
H26	$6.7X10^{5}$	$1.7X10^{5}$
H27	$3.9X10^{5}$	$7.9X10^{5}$
H28	$1.0X10^{4}$	$3.0X10^{4}$
H29	$4.9X10^{5}$	$2.9X10^{5}$
H30	$7.3X10^{5}$	$5.3X10^{5}$

Table 3: : Cultural, morphological and biochemical characteristics of the bacterial isolates

Parameters	B1	B2	B3	B4
Cultural				
Elevation	Convex	Flat	Convex	Low convex
Margin	Entire		Entire	Entire
Colour	Yellow	Cream	Green	Yellow
Shape	Circular	Irregular	Circular	Circular
Morphological				
Gram reaction	+	+	-	_
Cell arrangement	Cluster	Chain	Singly	Singly
Motility +	-	+	-	
Cell type	Cocci	Rod		Bacilli
Biochemical				
Catalase	+	-	+	+
Indole	-	-	-	-
Urease	-	-	-	-
Oxidase	-	+	+	+
Coagulase	+	-	-	-
Citrate	+	-	+	-
Carbonhydrate fermentation (Sucrose)	+	+	-	-

Key B1 = *Staphylococcus* sp., B2 = *Bacillus* sp., B3 = *Pseudomonas* sp., B4 = *Flavobacterium* sp.

Table 4:*Identification of fungal isolates*

Cultural	Microscopic	Fungal isolates
Cottony white colony with reverse side creamish	Macro conidia formed with chlamydospores arising in the mycelium and conidia	Fusarium sp.
Yellowish- green colony	Septate hyhae, Conidiosphores, Columnar cooidial bead	Aspergillus flavus
Light greenish colony	Non septate hyphae presence of conidiophores	Penicillium sp.
Whitish colony	Septate hyphae and Presence of arthroconidia	Geotrichum candidum.
White colony	Non septate, single columellate sporagiophores	Mucor sp
Dark green colonies	Conidia in balls	Aspergillus oryzae
Black compact colonies	Septate hyphae, conidiophores and branched foot cells	Aspergillus niger

Table 5:*Heterotrophic bacterial counts of polyherbal samples obtained from major markets in Lagos state.*

Samples	Heterotrophic bacterial count (cfu/ml)	Predominant bacterial species isolated
H1	$5.5X10^6$	Bacillus spp. Flavobacterium spp.
H2	$3.3X10^6$	Bacillus spp. Pseudomonas spp.
H3	$8.0X10^4$	Bacillus spp.
H4	$4.6X10^5$	Bacillus spp., Staphylococcus spp.
H5	$7.2X10^8$	Bacillus spp., Staphylococcus aureus. Flavobacterium spp.
H6	$2.2X10^7$	Bacillus spp., Staphylococcus spp. Pseudomonas spp.
H7	$9.7X10^6$	Bacillus spp. Pseudomonas spp.
H8	$9.0X10^8$	Bacillus spp., Staphylococcus spp. Pseudomonas spp.
H9	$2.5X10^3$	Bacillus spp.
H10	$8.3X10^6$	Bacillus spp. Flavobacterium spp. Pseudomonas spp.
H11	$4.2X10^9$	Bacillus spp., Staphylococcus spp. Pseudomonas spp. Flavobacterium spp.
H12	$7.0X10^7$	Bacillus spp. Flavobacterium spp.
H13	$5.0X10^7$	Bacillus spp., Staphylococcus spp.
H14	$3.2X10^4$	Bacillus spp. Pseudomonas spp.
H15	$1.0X10^6$	Bacillus spp.
H16	$4.9X10^6$	Bacillus spp. Flavobacterium spp., Pseudomonas spp.
H17	$3.7X10^5$	Bacillus spp., Pseudomonas spp.
H18	$8.7X10^8$	Bacillus spp., Staphylococcus spp. Pseudomonas spp. Flavobacterium spp.
H19	$6.4X10^9$	Bacillus spp., Staphylococcus spp. Pseudomonas spp., Flavobacterium spp.
H20	$5.5X10^6$	Bacillus spp., Pseudomonas spp.

H21	$7.8X10^{7}$	Bacillus spp., Staphylococcus spp., Pseudomonas spp., Flavobacterium spp.
H22	$6.5X10^{6}$	Bacillus spp., Pseudomonas spp.
H23	$6.1X10^{7}$	Bacillus spp. Pseudomonas spp.
H24	$5.6X10^{5}$	Bacillus spp.
H25	$2.6X10^{5}$	Bacillus spp.
H26	$6.7X10^{5}$	Bacillus spp.
H27	$3.9X10^{5}$	Bacillus spp., Staphylococcus spp.
H28	$1.0X10^{4}$	Bacillus spp.
H29	$4.9X10^{5}$	Bacillus spp.
H30	$7.3X10^{5}$	Bacillus spp.

The table above shows the heterotrophic bacterial counts in the polyherbal samples. A total of four bacteria (*Bacillus* spp., *Flavobacterium* spp., *Staphylococcus* spp.,*Pseudomonas* spp. *Bacillus* spp. is present in all the samples.

Table 6:*Total fungal propagule counts of polyherbal samples obtained from major markets in Lagos state*

Samples	Fungal count (cfu/ml)	Predominant fungal species isolated
H1	$5.8X10^{5}$	Aspergillus flavus
H2	$4.3X10^{6}$	Penicillium spp
H3	$7.0X10^{4}$	Aspergillus niger
H4	$3.6X10^{5}$	Fusarium spp
H5	$8.2X10^{7}$	Aspergillus oryzae
H6	$1.2X10^{6}$	Aspergillus flavus
H7	$7.7x10^{7}$	Aspergillus flavus
H8	$3.0X10^{8}$	Aspergillus flavus
H9	$9.5X10^{3}$	Fusarium spp
H10	$2.3X10^{6}$	Aspergillus flavus
H11	$4.2X10^{9}$	Aspergillus oryzae
H12	$5.0X10^{7}$	Aspergillus oryzae
H13	$1.0X10^{7}$	Aspergillus flavus
H14	$4.2X10^{4}$	Aspergillus flavus
H15	$2.0X10^{6}$	Aspergillus oryzae
H16	$6.9X10^{6}$	Aspergillus flavus
H17	$3.7X10^{5}$	Penicillium spp
H18	$6.7X10^{8}$	Apergillus oryzae
H19	$1.4X10^{9}$	Penicillium spp
H20	$7.5X10^{6}$	Aspergillus flavus
H21	$4.8X10^{7}$	Geotricum candidum
H22	$7.5X10^{6}$	Penillicum spp
H23	$5.1X10^{7}$	Fusarium spp
H24	$4.6X10^{5}$	Apergillus oryzae
H25	$3.6X10^{5}$	Mucor spp
H26	$1.7X10^{5}$	Aspergillus flavus

H27	$7.9X10^5$	Aspergillus flavus
H28	$3.0X10^4$	Aspergillus oryzae
H29	$2.9X10^5$	Aspergillus flavus
H30	$5.3X10^5$	Aspergillus flavus

The table above shows the heterotrophic bacterial counts in the polyherbal samples. A total of seven fungi were isolated. Which include; *Aspergillus flavus., Aspergillus oryzae., Mucor spp Fusarium spp., Penicillium spp., Geotricum candidum, Aspergillus niger.*

Table 7: Presence of microbial isolate in the different samples

	B1	B2	B3	B4	F1	F2	F3	F4	F5	F6	F7
H1	+	+	-	-	-	+	-	-	-	-	-
H2	+	-	-	+	-	-	+	-	-	-	-
H3	+	-	-	-	-	-	-	-	-	-	+
H4	+	-	+	-	+	-	-	-	-	-	-
H5	+	+	+	-	-	-	-	-	-	+	-
H6	+	-	+	+	-	+	-	-	-	-	-
H7	+	-	-	+	-	+	-	-	-	-	-
H8	+	-	+	+	-	+	-	-	-	-	-
H9	+	-	-	-	+	-	-	-	-	-	-
H10	+	+	-	+	-	+	-	-	-	-	-
H11	+	+	+	+	-	-	-	-	-	+	-
H12	+	+	-	-	-	-	-	-	-	-	-
H13	+	-	+	-	-	+	-	-	-	-	-
H14	+	-	-	+	-	+	-	-	-	-	-
H15	+	-	-	-	-	-	-	-	-	+	-
H16	+	+	-	+	-	+	-	-	-	-	-
H17	+	-	-	+	-	-	+	-	-	-	-
H18	+	+	+	+	-	-	-	-	-	+	-
H19	+	+	+	+	-	-	+	-	-	-	-
H20	+	-	-	+	-	+	-	-	-	-	-
H21	+	+	+	+	-	-	-	+	-	-	-
H22	+	-	-	+	-	-	+	-	-	-	-
H23	+	-	-	+	+	-	-	-	-	-	-
H24	+	-	-	-	-	-	-	-	-	+	-
H25	+	-	-	-	-	-	-	-	+	-	-
H26	+	-	-	-	-	+	-	-	-	-	-
H27	+	-	+	-	-	+	-	-	-	-	-
H28	+	-	-	-	-	-	-	-	-	+	-
H29	+	-	-	-	-	+	-	-	-	-	-
H30	+	-	-	-	-	+	-	-	-	-	-

+: Present. - : Absent.

Key:

B1= Bacillus spp

B2=Flavobacterium spp

B3=Staphylococcus spp

B4=Pseudomonas spp

F1= Fusarium spp

F2= Aspergillus flavus

F3= Penicilium spp

F4= Geotrichum candidum

F5= Mucor sp

F6= Aspergillus oryzae

F7 = Aspergillus niger

Table 8:Percentage occurrence of Microbial isolates in polyherbal samples obtained from different markets in Lagos state, Nigeria

Microbial isolates	Occurrence
Bacillus sp	100.0%
Flavobacterium sp	30.0%
Staphylococcus sp	33.3%
Pseudomonas sp	50.0%
Fusarium sp	10.0%
Aspergillus flavus	43.3%
Penicilium sp	13.3%
Geotrichum candidum	3.3%
Mucor sp	3.3%
Aspergillus oryzae	23.3%
Aspergillus niger	3.3%

The table above shows the presence of microbial isolates in the polyherbal samples and the percentage of occurrence. Bacillus occur in all the polyherbal samples. *Aspergillus niger, Geotrichum candidum and Mucor sp.* occur the least.

DISCUSSION

Herbal medicines especially those containing combinations, are readily used by humans in different parts of the world in general and in particular Lagos State, Nigeria. This high demand for herbal products is due to the fact that it is natural and no likely to cause serious side effects compared to conventional drugs. Despite these, citizens should not be unaware of the possible contamination of these products by microorganisms especially bacteria and fungi and also heavy metals contamination [20]. The composition, indication and dosage of the 30 polyherbal samples are enumerated above. The recommended dosage cup was not specific. This research work has focussed on the microbiological contamination of different polyherbal preparations sold in Lagos State, Nigeria.

The result showed high microbial contamination in all the polyherbal samples, with total bacteria counts ranging from $2.5x10^3$ in H9 to $6.4x10^9$ in H19 and total fungi counts ranging from $9.5x10^3$ in H9 to $3.5x10^9$ in H11 as compared to the specific standard limit of $1x10^5$ cfu/ml for bacteria and fungi recommended by [21][22]. All the polyherbal samples analyzed were grossly contaminated and the microorganisms identified include; *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp. and Flavobacterium sp. While fungal isolates included Aspergillus flavus, Penicillium sp, Aspergillus niger, Fusarium sp, Aspergillus oryzae, Geotricum candidum and Mucor sp.

The percentage microbial contamination of the polyherbal samples is as follows; Bacillus sp (100%), Flavobacterium sp (30%), Pseudomonas sp (50%), Staphylococcus sp (33.3%), Fusarium sp (10%), Aspergillus flavus (43.3%), Penicilium sp (13.3%), Geotrichum candidum (3.3%), Mucor sp (3.3%), Aspergillusoryzae (23.3%), Aspergillus niger (3.3%). Bacteria that are of health importance such as Bacillus species were isolated from the polyherbal samples. The presence of Bacillus species may be as a result of inadequate heat processing, improper handling of products and contaminated processing equipment [23].

This result is in accordance to [14]when the microbial load of two Nigerian herbal remedies from Ondo state, Akure was analyzed, a total of 7 microorganisms were isolated from the two herbal products; 4 fungi (Basidiobotrytis sp, Oedocephalum sp, varicosporium sp and Articulospora inflata) and 3 bacteria (Bacillus subtilis, Bacillus coagulans and Bacillus cereus). This is similar to the research carried out by [24], in Benin City Edo state, where the bacterial population isolated includes; Staphylococcus aureus (50.0%), Staphylococcus epidermidis (25.0%), Escherichia coli (10.0%) and Bacillus subtilis (40.0%) while fungal

isolates consist of Aspergillus niger (85.0%) and Penicillium species (50.0%). Similar finding was also observed by [25] when a research was carried out on 15 randomly selected medicinal plant samples collected from the local markets in Benin City. The mean heterothrophic bacterial count ranged from 1.73 x 10^4 (Schrebera arborea) to 2.65 x 10^5cfu/g (Tetrapleura tetraptera).

In contrast to [15], where the microbial contamination of some hawked herbal products in Ado-Ekiti were analyzed, the total bacterial counts of all the test herbal samples ranged from 4.0 x 10^4 to 1.7 x 10^6 cfu/ml. A total of 23 bacteria and fungi (20 bacteria and 3 fungal strains) were isolated from the herbal products examined. Six (60%) of the sample were contaminated with *Escherichia coli* (3.7 x10^4– 2.8 x 10^5 cfu/ml) which is an intestinal bacterium and an indication of feacal contamination. Four (40%) were contaminated with Salmonella spp and Pseudomonas aeruginosa in the range of 2.8 x 10^4 to 8.0 x 10^4 cfu/ml. Three (30%) were contaminated with Staphylococcus aureus (1.0 x 10^4- 2.8 x 10^4cfu/ml), two (20%) were contaminated with Serratia marcescens, three (30%) were contaminated with Klebsiella pnemoniae, one (10%) contained proteus mirabilis.

In accordance with the work of [24]when medicinal herbs sold in Bini markets were analyzed, the presence of microorganisms and bacteria count of the different plant materials shows that the total heterotrophic bacterial counts ranged from 6.25Ч10^2 cfu/g in Butryosermum paradoxum, to 9.80Ч10^4 cfu/g in Aframonum meleguata and fungal counts ranged from 2.33Ч10^2cfu/g in Khaya ivorensis to 5.00Ч10^5cfu/g in Lannea welwitschii.The staphylococcal and coliform counts ranged from 1.41Ч10^2cfu/g in *Oyekca goreto* 4.78Ч10^4cfu/g in *Hunteria umbellate* while only two samples, *Khaya ivorensis* and *Irvingia gabonesis*, showed presence of coliform counts. Contamination of polyherbals by these microorganisms may have been due to poor sanitary measures and inadequate hygienic practices during preparation [18]

CONCLUSION

This work has shown the presence of microbial contaminants in some polyherbal products sold in Lagos State, Nigeria. The thirty polyherbal samples analysed were shown to be heavily contaminated by microorganisms including bacteria and fungi thereby making these products unsafe microbiologically

RECOMMENDATION

Adequate sanitary measures and hygienic practices should be observed during harvesting, handling and processing of herbal product in order to curb microbial contamination of the products.

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