

## EVALUATION OF THE MICROBIOLOGICAL STATUS AND ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF SOME HERBAL REMEDIES ADMINISTERED ORALLY IN NIGERIA

<sup>1</sup>W.Braide., <sup>1</sup>S. U. Oranusi., <sup>2</sup>R.N. Nwaoguikpe., <sup>3</sup>I.U. Offor-Emenike., <sup>4</sup>I.L. Nwosu., <sup>1</sup>C. I. Akobundu <sup>1</sup>C. Chike-Reginald and <sup>5</sup>L.B. Ppogbara

<sup>1</sup>Department of Microbiology,

, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria

<sup>2</sup>Department of Biochemistry,

Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria.

<sup>3</sup>Department of Biology,

Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria.

<sup>4</sup>Department of Medical Laboratory Sciences,

Abia State College of Health Technology.

<sup>5</sup>Department of Science Laboratory Technology,

Rivers State Polytechnic, Bori.

**Corresponding Author:** *W.B. Braide*

### ABSTRACT

The use of herbal remedies in preventive and curative medicine dates back to the primitive era and progressively gave birth to the modern day chemotherapy and medicine. Investigation into the microbiological quality of ten well packaged herbal drugs produced and commonly administered in Nigeria was carried out using standard methods. Antibiotic susceptibility test was demonstrated by Kirby- Bauer method and McFarland standard. Aliquot portions of decimally diluted drug suspensions were inoculated onto bacteriological and mycological media. Total counts were determined and expressed as colony forming units per grams /milliliters. Total heterotrophic and coliform bacteria count was  $4.3 \times 10^7$ - $2.61 \times 10^{11}$  and  $1.0 \times 10^7$ -  $1.87 \times 10^{10}$  on respectively. Total heterotrophic fungi count was  $3.0 \times 10^7$ -  $1.55 \times 10^{10}$ . Five species of bacteria, namely, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Corynebacterium diphtheriae* and *Micrococcus luteus* and five species of fungi, namely, *Aspergillus flavus*, *Penicillium notatum*, *Rhizopus stolonifer*, *Mucor* and *Saccharomyces* species were isolated from the herbal remedies. Most of the isolates are resident in the soil, water, air and vegetations, and their public health implications had been reported. *Staphylococcus aureus* produce potent enterotoxins associated with food borne intoxication, toxic shock syndrome and staphylococcal scalded skin syndrome. *Bacillus* species, an endospore former also produce an exotoxin implicated in food borne infection. The presence of *Enterococcus faecalis* indicates fecal contamination. Some species of *Aspergillus*, *Penicillium* and *Rhizopus* are known to produce mycotoxins that cause cancer and other mycotoxicoses as well as mycotic infections of the liver, kidney and skin. *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus* sp were susceptible to seven of the ten oxid commercial antibiotics. The high incidence of bacteria and fungi fall short of international standard and portends danger to consumers. Contamination may result from inadequate sanitary measures employed during production, packaging and storage. Good manufacturing practices (GMP) are recommended to ensure products with wholesome quality that meets international safety standards.

©Emerging Academy Resources

**KEYWORDS:** Herbal Medicinal Products, Microbiological Status, Antibacterial Susceptibility Pattern.

### 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. Many well established medicine comes from plants. For example morphine comes from poppies, aspirin from willow bark, ephedrine

from ephedra and digoxin from foxgloves. Long practiced outside conventional medicine, herbalism is becoming more mainstream as up-to-date analysis and research show their value in the treatment and prevention of diseases. More than 80% of the world's population uses herbal medicines in one form or another, from China to Australia and Europe to

Africa. There is evidence that the Chinese, Persians, Indians and Americans have used medical herbs for centuries. Scientist observed that people in different parts of the globe tended to use the same or similar plants for the same purpose (Castleman, 2001; Sofowara, 1993).

Substances derived from plants remain the basis for a large proportion of the commercial medications used today for the treatment of asthma, premenstrual syndrome, eczema, rheumatoid arthritis, migraine, menopause symptoms, chronic fatigue and irritable bowel syndrome. Herbs had been and still used in the treatment of typhoid fever, malaria, infertility, fever, waist pain, chest pains, pile insomnia, ulcer, carbuncle, dizziness, blood prostration etc (Coon *et al.*, 2002). Tapsel *et al.* (2006) and Castleman (2001) have independently reported on the *in vitro* applications of plants extracts in the treatment of diseases associated with *Mycobacterium tuberculosis*, *Staphylococcus aureus* and several other gram positive bacteria and fungi. It has been claimed that the active components in some plants act by inhibiting bacterial DNA dependent RNA polymerase inhibition of cell wall synthesis, damage to the cytoplasm membrane, inhibition of nucleic acid and protein synthesis and inhibition of specific enzyme system of microorganisms (Barret *et al.*, 1999).

During preparation, handling and storage of herbs by local herbalist, the chances of the final products being contaminated is very high. The roots, stems, barks and leaves of plants harbour a lot of microorganisms (Adeleye *et al.*, 2005; Braide *et al.*, 2008). In most cases the water used for washing and preparation of the herbs may not be sterile. During drying soil and air microorganisms may recontaminate the final products.

In Nigeria herbal practitioners have capitalized on the poor health conditions of the masses and high cost of synthetic orthodox medicine by organizing herbal trade fare indiscriminately. The probability of a patient on herbal remedies contracting more deadly diseases cannot be totally ruled out considering the unhygienic and crude method of production and storage. This report evaluates the microbiological status of some herbal remedies consumed by vast population of Nigerians. The antibiogram of the bacterial isolates was also determined.

## MATERIALS AND METHODS

### Description and collection of samples

Ten herbal samples neatly packaged in sachets and bottles were randomly purchased from trade fare centers, herbal stores and motor parks in Owerri, Imo State, Eastern Nigeria. The herbs are used in the treatment of typhoid fever, sexually transmitted diseases, pile, stomach aches, diabetes, headache, skin infection, toothache among others.

### Preparation and Inoculation of Samples

Ten grams of finely grind powder and ten milliliters of liquid samples were dispersed in 90mls of peptone water to obtain  $10^{-1}$  dilution. Further dilutions were made decimally until  $10^{-8}$  dilution was obtained (Pelczar and Chan, 1977; Beishir, 1987; Pelczar *et al.*, 1993). Aliquot portion (0.1ml) of the 7<sup>th</sup> and 8<sup>th</sup> dilution was inoculated onto MacConkey and Nutrient agar respectively. The same quantity of the 5<sup>th</sup> dilution was inoculated onto Potato Dextrose Agar (PDA). Inocula were spread evenly and plates incubated at appropriate temperature and time (Beishir, 1987; Cheesbrough, 2000).

### Enumeration and Characterization of Isolates

Bacteria count was done using a Gallenkamp colony counter while fungi count was done with the aid of hand lens. Total colony count was expressed as colony forming units per gram/milliliters for powder and liquid sample respectively. Isolates were characterized on the basis of colonial, microscopic and biochemical methods (Harrigan and McCance, 1990; Prescott *et al.*, 1999; Abbey, 2007). The identities of the isolates were determined with reference to standard manuals (Barnett and Hunter, 1987; Buchannan and Gibbon, 1974, Harrigan and McCance, 1990).

### Antibiotic Susceptibility Test

This was done by adopting Kirby-Bauer disc diffusion method in accordance with McFarland standard. Oxoid disc impregnated with different concentrations of the antibiotics was placed on a 24h old culture plate of three bacteria isolated from HMP. Zone of inhibition (mm) was recorded after 48h (Cheesbrough, 2000).

Table 1 show the total microbial population obtained from three culture media. The microbial load is high above recommended limit (WHO, 1998, 2000). Colonial and microscopic characteristics of bacterial isolated on nutrient are shown in Table 2. Biochemical characteristics of the bacterial isolates from the herbal products are shown in Table 3.

The cell morphologies and microscopic characteristics as well as biochemical characteristics of bacteria isolated on MacConkey agar is shown on Table 4 and Table 5 respectively. Table 6 show the colonial and microscopic characteristics of fungi isolated on the herbal remedies.

Table 7 show the antibacterial susceptibility test of three bacteria isolated from the products. The percentage occurrence of bacteria and fungi species isolated on the herbal products is shown in Fig. 1 and Fig 2.

**RESULTS**

**Table 1: Total microbial population from Herbal materials**

Sample codes	Total bacterial counts on nutrient agar	Total Bacteria counts on MacConkey agar	Total Fungal counts on PDA
HEBA	$2.56 \times 10^{11}$	$1.7 \times 10^9$	$6.5 \times 10^9$
HEBB	$1.98 \times 10^{11}$	$2.5 \times 10^9$	$2.9 \times 10^9$
HEBC	$2.01 \times 10^{11}$	$7.3 \times 10^9$	$3.6 \times 10^9$
HEBD	$4.1 \times 10^{10}$	$1.87 \times 10^{10}$	$1.55 \times 10^{10}$
HEBE	$2.61 \times 10^{11}$	$4.0 \times 10^9$	$1.27 \times 10^{10}$
HEBF	$7.6 \times 10^8$	$1.0 \times 10^7$	$6.8 \times 10^7$
HEBG	$4.3 \times 10^7$	$1.0 \times 10^7$	$4.6 \times 10^7$
HEBH	$3.8 \times 10^8$	$7.0 \times 10^7$	$7.2 \times 10^7$
HEBI	$8.0 \times 10^7$	$8.0 \times 10^7$	$9.5 \times 10^7$
HEBJ	$6.0 \times 10^7$	$2.0 \times 10^7$	$3.0 \times 10^7$

**Table 2: Colonial and microscopic characteristics of bacteria isolated on nutrient agar**

Colony Code	Colonial Features	Mot	Gram Stain	Spore	Flagellum	Capsule	Probable Identity
HEBA1	Shiny and smooth goldenyellowcolonies	-	+S	-	-	-	<i>Staphylococcus</i> sp
HEBA2	Shiny and smooth cream colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBB1	Shiny and smooth golden yellow colonies	-	+S	-	-	-	<i>Staphylococcus</i> sp
HEBB2	Shiny and smooth cream colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBB3	Small, smooth and shiny yellow colonies	-	+S	-	-	-	<i>Micrococcus</i> sp
HEBB4	Large flat irregular cream colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBC1	Shiny and smooth golden yellow colonies	-	+S	-	-	-	<i>Staphylococcus</i> sp
HEBC2	Large flat irregular cream colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBD1	Shiny and smooth golden yellow colonies	-	+S	-	-	-	<i>Staphylococcus</i> sp
HEBD2	large flat irregular Cream colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBE1	Small umbonate Cream colonies	-	+R	-	-	-	<i>Corynebacterium</i> sp
HEBF1	large flat irregular Cream colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBF2	Small smooth and Shiny yellow Colonies	-	+S	-	-	-	<i>Micrococcus</i> sp
HEBF3	Shiny and smooth Cream colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBF4	shiny and smooth Golden yellow colonies	-	+S	-	-	-	<i>Staphylococcus</i> sp
HEBG1	large flat irregular Cream colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBG2	Shiny and smooth Cream colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBG3	Shiny and smooth Golden yellow Colonies	-	+S	-	-	-	<i>Staphylococcus</i> sp
HEBH1	large flat irregular Cream colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBH2	Shiny and smooth Cream colonies	-	+S	-	-	-	<i>Enterococcus</i> sp

HEBH3	Small smooth and Shiny yellow Colonies	-	+S	-	-	-	-	-	-	-	-	-	-	<i>Micrococcus</i> sp
HEB11	large flat irregular Cream colonies	+	+R	+	+	+	+	+	+	+	+	+	+	<i>Bacillus</i> sp
HEB12	Small smooth and Shiny yellow Colonies	-	+S	-	-	-	-	-	-	-	-	-	-	<i>Micrococcus</i> sp
HEB13	Shiny and smooth Cream colonies	-	+S	-	-	-	-	-	-	-	-	-	-	<i>Enterococcus</i> sp
HEBJ1	large flat irregular Cream colonies	+	+R	+	+	+	+	+	+	+	+	+	+	<i>Bacillus</i> sp
HEBJ2	Shiny and smooth Cream colonies	-	+S	-	-	-	-	-	-	-	-	-	-	<i>Enterococcus</i> sp

Mot, motility; R, rod shaped; S, spherical shsped.

Table 3: Biochemical characteristics of bacteria isolated in nutrient agar

Colony Code	CAT	OXI	COAG	IN	MR	VP	CIT	GLU	SUC	MAL	LAC	MANN	IDENTITY OF ISOLATES
HEBA1	+	-	+	-	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
HEBA2	-	-	-	-	+	-	-	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBB1	+	-	+	-	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
HEBB2	-	-	-	-	+	-	-	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBB3	+	-	-	-	+	-	+	-	-	-	-	-	<i>Micrococcus luteus</i>
HEBB4	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBC1	+	-	+	-	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
HEBC2	+	-	-	-	-	+	-	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBD1	+	-	+	-	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
HEBD2	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBE1	+	-	-	-	-	+	+	+	+	-	+	+	<i>Corynebacterium</i> sp
HEBF1	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBF2	+	-	-	-	+	-	+	-	-	-	-	-	<i>Micrococcus luteus</i>
HEBF3	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBF4	+	-	+	-	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
HEBG1	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBG2	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBG3	+	-	+	-	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
HEBH1	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBH2	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
NAH3	+	-	-	-	+	-	+	-	-	-	-	-	<i>Micrococcus luteus</i>
HEB11	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEB12	+	-	-	-	+	-	+	-	-	-	-	-	<i>Micrococcus luteus</i>
HEB13	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBJ1	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBJ2	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>

Cat, catalase; Oxi, oxidase; Coag, coagulase; In, indole; MR, methyl red; VP, Voges Proskauer; Cit, citrate; Glu, glucose; Suc, sucrose; Mal, maltose; Lac, lactose; Mann, mannitol.

Table 4: Cell Morphology and Microscopic Characteristics of Bacteria Isolated in MacConkey Agar

Colony Code	Colonial features	Motility	Gram Stain	Spore	Flagellum	Capsule	Probable Identity
HEBAX	Round pink colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBAY	Pink umbonate colonies	-	+R	-	-	-	<i>Corynebacterium</i> sp
HEBBX	Round pink colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBBY	Irregular grey colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBCX	Round pink colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBCY	Irregular grey colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBDX	Rose pink colonies	-	+R	-	-	-	<i>Staphylococcus</i> sp
HEBDY	Pink umbonate colonies	-	+R	-	-	-	<i>Corynebacterium</i> sp
HEBEX	Round pink colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBEY	Pink umbonate colonies	-	+R	-	-	-	<i>Corynebacterium</i> sp
HEBFX	Pink umbonate colonies	-	+R	-	-	-	<i>Corynebacterium</i> sp
HEBGX	Round pink colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBHx	Irregular grey colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBHY	Round pink colonies	-	+S	-	-	-	<i>Enterococcus</i> sp
HEBHZ	Pink umbonate colonies	-	+R	-	-	-	<i>Corynebacterium</i> sp
HEBIX	Irregular grey colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBIY	Pink umbonate colonies	-	+R	-	-	-	<i>Corynebacterium</i> sp
HEBJX	Irregular grey colonies	+	+R	+	+	-	<i>Bacillus</i> sp
HEBJY	Rose pink colonies	-	+S	-	-	-	<i>Staphylococcus</i> sp

Table 5: Biochemical characteristics of bacteria isolated in MacConkey Agar

Colony Code	Cat	Oxi	Coag	In	MR	VP	Cit	Glu	Suc	Mal	Lac	Mann	Identity of Isolate
HEBAX	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBAY	+	-	-	-	-	+	+	+	+	-	+	+	<i>Corynebacterium</i> sp
HEBBX	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBBY	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBCX	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBCY	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBDX	+	-	+	-	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
HEBDY	+	-	-	-	-	+	+	+	+	-	+	+	<i>Corynebacterium</i> sp
HEBEX	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBEY	+	-	-	-	-	+	+	+	+	-	+	+	<i>Corynebacterium</i> sp
HEBFX	+	-	-	-	-	+	+	+	+	-	+	+	<i>Corynebacterium</i> sp
HEBGX	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBHx	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBHY	-	-	-	-	+	-	+	+	+	+	+	+	<i>Enterococcus faecalis</i>
HEBHZ	+	-	-	-	-	+	+	+	+	-	+	+	<i>Corynebacterium</i> sp
HEBIX	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBIY	+	-	-	-	-	+	+	+	+	+	+	-	<i>Corynebacterium</i> sp
HEBJX	+	-	-	-	-	+	+	+	+	+	+	-	<i>Bacillus subtilis</i>
HEBJY	+	-	+	-	+	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>

Table 6: Colonial and microscopic characteristics of Fungi isolated on Potato Dextrose Agar Medium

Colony Code	Colonial Characteristics	Microscopic Appearance	Identity of isolates
HEBAA	Round flat cream colonies	Gram positive large oval budding cells	<i>Saccharomyces cerevisiae</i>
HEBAB	Irregular cream colonies	Gram positive ellipsoidal budding cells	<i>Saccharomyces ellipsoideus</i>
HEBBA	Round flat cream colonies	Gram positive large oval budding cells	<i>Saccharomyces cerevisiae</i>
HEBBB	Irregular cream colonies	Gram positive ellipsoidal budding cells	<i>Saccharomyces ellipsoideus</i>
HEBBC	Short white filamentous hyphae	Non-septate hyphae	<i>Mucor</i> sp
HEBCA	Irregular cream colonies	Gram positive ellipsoidal budding cells	<i>Saccharomyces ellipsoideus</i>
HEBCB	Round flat cream colonies	Gram positive large oval budding cells	<i>Saccharomyces cerevisiae</i>
HEBDA	Short white filamentous hyphae	Non-septate hyphae	<i>Mucor</i> sp
HEBDB	Tall white filamentous hyphae	Non-septate hyphae	<i>Rhizopus stolonifer</i>
HEBDC	Black spores on short white hyphae	Hyphae septate conidia on sterigma	<i>A.flavus</i>

Table 6: Colonial and microscopic characteristics of Fungi isolated on Potato Dextrose Agar Medium (continuation)

HEBEA	Round flat cream colonies	Gram positive large oval budding cells	<i>Saccharomyces cerevisiae</i>
HEBEB	Irregular cream colonies	Gram positive ellipsoidal budding cells	<i>Saccharomyces ellipsoideus</i>
HEBEC	Short white filamentous hyphae	Non-septate hyphae	<i>Mucor</i> sp
HEBED	Green Spores with white hyphae	Hyphae Septate conidia arranged like mop-head	<i>P. notatum</i>
HEBFA	Short white filamentous hyphae	Non-separate hyphae	<i>Mucor</i> sp
HEBFB	Tall white filamentous hyphae	Non-separate hyphae	<i>Rh. stolonifer</i>
HEBFC	Round flat cream colonies	Gram positive large oval budding cells	<i>Saccharomyces cerevisiae</i>
HEBGA	Irregular cream colonies	Gram positive ellipsoidal budding cells	<i>Saccharomyces ellipsoideus</i>
HEBGB	Round flat cream colonies	Gram positive large oval budding cells	<i>Saccharomyces cerevisiae</i>
HEBGC	Green Spores with white hyphae	Hyphae Septate conidia arranged like mop-head	<i>P. notatum</i>

Table 7: Antibiotic susceptibility test of three bacteria isolated on herbal medicinal products

Antibiotics (µg)	zone of inhibition (mm)		
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Enterococcus faecalis</i>
AMX	-	30	20
OFL	15	18	-
STR	-	10	12
CHF	30	-	10
CEF	20	-	-
GEN	12	14	16
PFX	12	-	10
COT	-	20	-
CPX	16	22	14
ERY	18	10	10

AMX, amoxicilin (25µg); OFL, ofloxacin (5µg); STR, streptomycin (30µg); CHF, chloramphenicol (µg); CEF, ceftriazone (30µg); GEN, gentamycin (10µg); PFX, pefloxacin (5µg); COT, cotrimaxozole (25µg); CPX, ciprofloxacin (10µg); ERY, erythromycin (5µg)

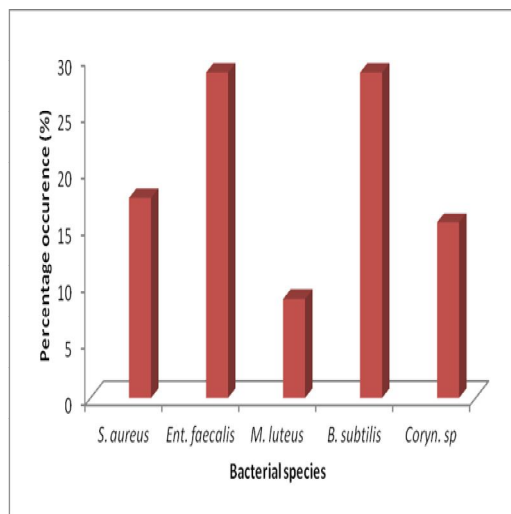


Fig 1: Percentage occurrence of bacteria isolated from herbal samples

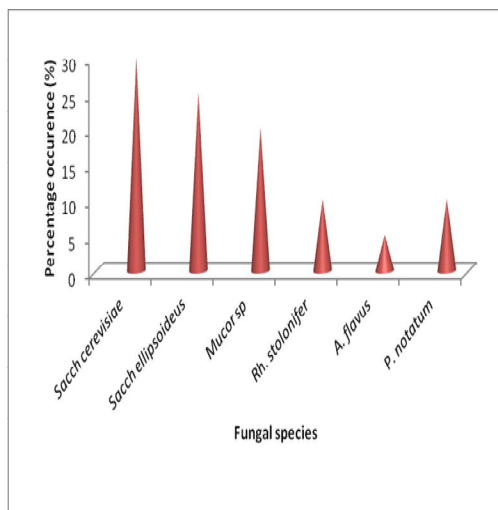


Fig 2: Percentage occurrence of fungi isolated from herbal samples

**DISCUSSION**

The herbal medicinal products analyzed showed gross contamination of bacteria and fungi (Table 1). Five genera of bacteria and six genera of fungi were isolated (Tables 2, 3, 4, 5, 6). A total of forty five bacterial species were isolated. Eight (17.7%) were *Staphylococcus aureus*, thirteen (28.8%) were *Enterococcus faecalis*, four (8.8%) were *Micrococcus luteus*, thirteen (28.8%) were *Bacillus subtilis* and

seven (15.5%) were *Corynebacterium* sp (Fig 1). Out of twenty fungal species isolated from the herbal medicinal product, six (30%) were *Saccharomyces cerevisiae*, five (25%) were *Saccharomyces ellipsoideus*, four (20%) were *Mucor* sp and two (10%) were *Penicillium notatum* (Fig 2). Some of the bacteria and fungi isolated on the herbal medicinal products are normal flora of the soil, water and vegetation (Pelczer *et al.*, 1986; Nester *et al.*, 1998;

Braide *et al.*, 2008); atmosphere, harvesting, poor drying, processing, storage and improper handling influence the microbiological quality of herbal drugs (Okunlola *et al.*, 2007).

Okunlola *et al.* (2007) and Abba *et al.* (2009) had independently reported large scale contamination of herbal remedies sold in Benin and Kaduna metropolis respectively. The high incidence of *Staphylococcus aureus* was also reported by Okunlola *et al.* (2007) and Abba *et al.* (2009).

These bacteria constitute the intestinal flora of human and other animals and are therefore used as indicator organism and as an index of possible contamination by human pathogen (Prescott *et al.*, 1999; Nester *et al.*, 1998). *Enterococcus faecalis* 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). *Bacillus* sp produces heat stable spores and causes food borne intoxication when ingested (Cheesbrough, 2000; Pelczar *et al.*, 1993). *Corynebacterium* sp produces toxins which can cause toxemia with fetal cardiac and neural complication (Cheesbrough, 2000; Pelczar *et al.*, 1986).

The significance of the faecal bacteria is that if these specific bacteria are present, then other harmful microorganism may also be present such as *Salmonella* (Forest, 2004).

Fungal species such as *Aspergillus Penicillium*, *Mucor* and *Rhizopus* may endanger the health of consumers as they have been implicated in human pathogenicity. They produce potent mycotoxins that have been implicated in carcinogenicity, dermatitis, hepatotoxicity and nephrotoxicity (Pelczar *et al.*, 1997; Frazier and Westhoff, 1978).

*Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis* responded positively (susceptible) to seven out of the ten antibiotics and therefore strongly recommended for the treatment of suspected cases of infections arising from the intake of contaminated herbal remedies.

Good manufacturing practice (GMP) is strongly advocated to produce herbal remedies with wholesome quality.

## CONCLUSION

Large microbial population and types were isolated from some herbal medicines consumed in Nigeria. The pathogenic effects of some isolates have been discussed. The need for constant monitoring and control of the standard of herbal remedies available in the Nigerian market is strongly advocated to curb the menace and maintain correct quality, safety and efficacy of the final herbal preparation

## REFERENCES

Abba, D., Inabo, H.I., Yakubu, S.E, and Olontola, S.O. (2009). "Contamination of Herbal Medicinal Product Marketed in Kaduna Metropolis with Selected Pathogenic Bacteria". "African Journal of Traditional, Complementary and Alternative Medicine", **6**(1)70 – 77.

Abbey, S.D. (2007). Foundation in Medical Mycology, 4<sup>th</sup> edn Kenalf Publication, Port Harcourt, Nigeria, p 22-30.

Adeleye, I. A., Okogi, G and Ojo, E.O (2005). Microbial contamination of herbal preparation in Lagos, Nigeria. Journal of Health Population and Nutrition, **23**(3): 296-297.

Barnet, H.I. and Hunters, B.B., (1987). Illustrated Genera of Imperfecti Fungi, 4<sup>th</sup> Edition Macmillan Publishing Company New York, USA, pp. 106, 130.

Barret, B.J., Kafer, D. and Rabago, D. (1999). "Assessing the Risk and Benefits of Herbal Medicine and Overview of Scientific Evidence" C. Attern Health Med., **4**:40 – 49.

Beishir, I. (1987). Microbiology in Practice. A Self-Instructions Laboratory Course, 4<sup>th</sup> edn. Harper and Row Publishers, New York, pp 96-111, 120-130, 238-272.

Braide, W., Sokari T. G., Nwaoguikie, R.N. and Okorundu, S. I. (2008) "Microbes from soil Associated with Metamorphosing Moth Larvae, Current Trends in Microbiology, **4**:11 – 14.

Buchanan, R. E. and Gibbon, N. E. (1974). Bergeys Manual of Determination Bacteriology, Williams and Wilkin Baltimore, USA.

Castleman, M. (2001). "The New Healing Herbs". The Classic Guide to Nature's Best Medicine Featuring the Top 100 Time-Tested Herbs' Rodale Chess Brought.

Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press, UK. pp 157-234.

Coon, J.T., Ernest, E. and Parax, G. (2002) "A systematic Review of Adverse Effects and Drug interaction. Drug Safety, **5**:323 – 344.

Forest, J (2004). Faecal coliforms Hygienic Laboratory Manual Vol 36. University of Iowa, pp. 2 – 4.

Frazier, W.F. and Westhoff, P.C. (1978). Food Microbiology, 3<sup>rd</sup> Edition, Tata McGraw Hill Publishing Co-Limited, New Delhi, India pp. 17 – 64, 456.

- Harrigan, F.W and MacCance, M.E. (1990). *Laboratory Methods in Food and Dairy Microbiology*, 8<sup>th</sup> Edition Academic Press Inc, London Pp. 7 – 23, 286 – 303.
- Nester, E., W., Roberts, C. E., Pearsall, N. N., Anderson, D.G. and Nester, M.T. (1998). *Microbiology. A Human Perspective*, 2<sup>nd</sup> Edition WBC/McGraws Hills New York, USA, pp. 434 -435.
- Okunlola, A., Babatunde, A.A, and Oluwatoyin, A.O. (2007). "Evaluation of Pharmaceuticals and Microbial Quality of some Herbal Medicinal Product in South Western Nigeria" *Tropical Journal of Pharmaceutical Research*, **61** (1):661-670.
- Prescott, L.M., Harley, J.P and Klein, D.A (1999). *Isolation of Pure Bacterial Culture from Specimen: Microbiology International*, 4<sup>th</sup> Edition: Boston WCB McGraw's Hill Companies: pp. 714 – 796.
- Pelczar, M.J. and Chan, E.C.S. (1977). *Laboratory Exercise in Microbiology*. Bank Pot. Inc, New York, USA.
- Pelczar, M.J (Jr)., Chan, E.C.S. and Krieg, N.R. (1986). *Microbiology*, 5th ed. McGraw-Hill Book Co., New-York, USA. pp.37-50,133-146.
- 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.
- Sofowara, A. (1993). "Medicinal Plant and Traditional Medicine in Africa" Ibadan Spectrum Books Ltd Pub, Ibadan, Nigeria. pp. 50 – 195.
- Tapsel, L.C., Hemphill, I. and Cobiac, L. (2006). "Health Benefits of Herbs and Spices. The Past, the Present, the Future" *Med. J. Aust.* **185** (4): 24.
- World Health Organization, WHO (1998). "Regulatory Situation of Herbal Medicine: A World Wide Review" Retrieved July 1, 2012 from <http://en/Wikipedia/wiki>.
- World Health Organization (2000). 'General Guidelines' for Methodologies on Research and Evaluation of Traditional Medicine Retrieved, June 2012 from <http://en/Wikipedia>.
- <http://www.who.int/mediacentre/factsheets/fs/34/eni>.