

MICROBIAL QUALITY OF SOME HERBAL PRODUCTS IN BENUE STATE

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ABSTRACT

The study was conducted to investigate the microbial qualities of some herbal products in Benue State, Nigeria. A total of twenty-one samples were randomly collected from herbal medicine sales outlets and retail pharmacy outlets and subjected to bacteriological examination using pour plate and modified pour plate method for isolation of bacteria and fungi colonies respectively. Gram reaction, Potassium Hydroxide string techniques and Biochemical analysis were used for identification of bacterial isolates. While fungi colonies were identified using simplified key to classes of fungi. Four microorganisms namely *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and Fungi namely *Aspergillus sp.*, *Mucor sp.*, *Fusarium sp.*, and *Rhizopus sp.* were also found to be implicated in some of the samples examined. The total bacterial counts for *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and Fungi ranged from 1.50×10^3 - 13.0×10^3 , 1.0×10^3 - 4.0×10^3 , 1.0×10^2 - 1.5×10^2 and 1.2×10^4 - 6.0×10^3 cuf/ml respectively. The microbial load of the products varied considerably. Six (28.6%) of the samples were without microbial contamination while fifteen (71.4%) were contaminated by one or more of the microorganisms isolated. 7(33.3 %) of the samples were contaminated by *E. coli*, 1(4.8 %) , 5(23.8%) *Staphylococcus aureus*, 3(27.3%) *Aspergillus sp.*, 4(36.4%) *Mucor sp.*, 2(18.2%) *Fusarium sp.* and 2(18.2%) *Rhizopus sp.* respectively. This research indicated that herbal medicinal products in Benue region of Nigeria required an urgent attention in process improvement to provide better quality products for consumers.

Keywords: Microbial quality, Contamination, Herbal products, Quality control

INTRODUCTION

Herbal medicinal products as form of complementary and alternative medicine are becoming increasingly popular in both developing and developed countries¹. A world survey indicates that about 70 - 80% of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources in their primary healthcare^{2,3}. WHO has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has encouraged the rational use of traditional plants based medicines by member states and has developed technical guidelines for the assessment of herbal medicine^{3,4}.

In Nigeria, there appears to be an overwhelming increase in the public awareness and usage of herbal medicinal products in the treatment and prevention of diseases⁵. This may not be unconnected to the active mass media advertisement embarked upon by the producers and marketers of the herbal medicinal products (HMPs) who have taken the advantage of the relatively high cost of the conventional pharmaceutical dosage forms, inaccessibility of the orthodox medical

services to a vast majority of people particularly in the rural areas and the reservations by the public following the prevalence of fake, substandard or counterfeit drugs in the market. These have placed the HMPs as a ready alternative to conventional dosage forms in the treatment of diseases. With this increased usage, the safety, efficacy and quality of this medicine are of concern for health authorities and professionals. Although herbal remedies are often perceived as being natural and therefore safe, but they are not free from adverse effect which may be due to factors such as adulteration, substitution, contamination, misidentification, lack of standardization, poor preparation, inappropriate regimen and label⁶. In contrast to chemically defined medicinal products, the biopharmaceutical quality of HMPs is often not well documented⁷. The WHO⁴ provides good manufacturing practice guidelines to National Regulatory Authorities, scientific organizations, and manufacturers, to undertake an assessment of the documentation/ submission dossiers in respect of herbal medicinal products. In Nigeria, the National Agency for Food Drug Administration and Control (NAFDAC) is responsible for drug administration and control of the quality of medicinal products including HMPs generally available in the market.

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MATERIALS AND METHODS

Collection of Herbal Products

The total of twenty-one herbal products were randomly collected from various traditional medicine sales outlets in Makurdi, Aliade, Otukpo, Ugbokolo and Orokam areas of Benue State. The samples were transferred to the laboratory in labeled sterile bottles for microbiological analysis.

Total Microbial Count

For total bacterial count, all 21 samples were diluted with phosphate buffer (pH 7.2) to the concentration of 10^{-5} . Subsequently, 1-ml of each dilution was added to two sterile petri dishes. Tryptic soy agar medium was promptly added into each dish, mixed, and the content was allowed to solidify. The plates were then incubated at 37°C for 24 hours. The number of colonies was counted. The average number of microorganisms per gram of each sample was then calculated.

Gram Staining Technique

Gram staining reaction is based on the ability of an organism to resist decolourisation with acetone, alcohol or aniline oil after the initial staining with basic dyes. Using the gram staining technique, smears were made from the colonies isolated, on glass slides and air-dried on a rack for about one hour and fixed by passing it on a flame. Crystal violet was added on the fixed smears and allowed to stand for one minute. The smears were washed with tap water and thereafter, Lugol's iodine was added and allowed to remain on them for one minute. Each prepared smear was washed with tap water and acetone was used to decolourise it until no more colours oozed out of the smear. They were washed with tap water immediately and counter stained with safranin for one minute. They were then washed with tap water and blotted with filter paper and allowed to dry on the bench for about one hour. The prepared slides were examined microscopically using oil immersion objective ($\times 100$)⁸.

KOH String Technique

In the presence of potassium hydroxide, Gram-negative cell walls are broken down, releasing viscid chromosomal material which causes the bacterial suspension to become thick and stringy. Gram-positive organisms remain unaffected; hence, the alternative name for this procedure, the "String-Test".

The KOH string test was performed by mixing a visible amount of growth from a colony on an agar slant in a loopful (3 mm) of 3% aqueous KOH on a glass slide. The KOH – bacterium suspension was mixed continuously with a bacteriological loop in a 2-cm² area on the slide. If such a suspension gels or becomes viscous and strings out when the loop is lifted (Positive KOH reaction), the isolate is Gram negative. Gram positive cells do not form a viscous gel or string out (negative KOH reaction). The cutoff time for negative

reactions was 60s. The result was best observed by raising the loop about 1cm above the slide. Holding the slide at an angle against a dark background also aided the observation^{9, 10}.

Fungal Count

For total fungal count, the diluted samples were prepared following the same procedure as in total bacterial count, replacing Tryptic soy agar medium with Sabouraud dextrose agar medium. The plates were then incubated at 25°C for 5days.

Identification of Fungi

The fungi were identified based on their cultural morphology and microscopic characteristics of their spores and hyphae using the simplified key to the classes of fungi (Division: Mycota) and fungus like organisms devoid of chlorophyll¹¹.

Pathogen Determination

For *Staphylococcus aureus*, 10 mg of the sample was added into TSB and incubated at 37°C for 24 hours. The sample was then streaked on Vogel-Johnson agar and incubated at 37°C for 24 hours. A single colony on each plate was then re-streaked on Mannitol salt agar and incubated at 37°C for 24 hours. After the incubation, the colonial morphology was observed.

For Enterobacteria, the diluted sample was streaked onto MacConkey agar plate. After the incubation at 37°C for 24 hours, the colonial morphology was observed. The colonies were subcultured into Triple sugar iron medium, Eosinmethylene blue agar, and Brilliant green agar for further characterization. Growth of bacteria was determined for Enterobacteria including *E. coli* and *Salmonella typhi*.

RESULTS

The total bacterial counts (TBC) of all the test herbal samples ranged from 1.0×10^3 to 13.0×10^3 cfu/ml. A total of 13 bacteria and 11 fungal strains were isolated from herbal products examined. Fifteen (71.4%) of the sample were contaminated with one or more microorganisms. Seven (33.3%) were contaminated with *E. coli*, and One (4.8%) was contaminated by *Salmonella typhi*, Five (23.8%) were contaminated by *Staphylococcus aureus* and eleven (%) were contaminated with fungi. The results of the present work show that products A to C, were among the products which showed acceptable microbial qualities, though the products were expensive, popular, widely advertised and used in Benue State for treatment of various conditions. The microbial nature and content of the microbial contaminant in the HMPs are presented in Table 1.

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Table 1: Microbial Content of HMPS Viable Count (CFU/ML ORC).

Product code	<i>Staph. aureus</i>	<i>E. coli</i>	Salmonella	Fungi
A	-	-	-	-
B	-	-	-	4.0 x 10 ³
C	-	-	-	-
D	-	13.0 x 10 ³	-	-
E	3.0 x 10 ⁴	7.0 x 10 ³	-	3.0 x 10 ⁴
F	-	-	-	-
C	-	-	-	1.5 x 10 ²
H	1.0 x 10 ³	-	-	-
I	2.0 x 10 ³	1.5 x 10 ²	-	-
J	-	5.0 x 10 ²	-	-
K	-	-	-	-
L	-	-	-	-
M	-	-	-	3.0 x 10 ³
N	-	-	-	1.2 x 10 ⁴
O	4.0 x 10 ³	1.5 x 10 ⁴	-	3.5 x 10 ³
P	-	-	-	3.0 x 10 ³
Q	-	-	-	3.5 x 10 ³
R	-	9.0 x 10 ³	-	1.2 x 10 ⁴
S	-	-	-	-
T	3.0 x 10 ³	2.0 x 10 ³	1.5 x 10 ²	6.0 x 10 ³
U	-	-	-	3.5 x 10 ³

KEY: - no growth; CFU / ML = colony forming unit per ml organisms

DISCUSSION

The present study revealed microbial contaminations in herbal products widely distributed in Benue State. Table 2 shows the product code, the dosage forms, NAFDAC Registration numbers, manufacture and expiry dates of the 21 herbal samples used in this study. Investigation revealed that those samples with NAFDAC Registration Numbers, Manufacture and Expiry dates were more hygienically prepared compared with those without NAFDAC Registration Numbers, Manufacture and Expiry dates. This is evident in samples H, I, O and T without NAFDAC Registration Numbers, Manufacture and Expiry dates. Sample N and F with NAFDAC Registration Numbers, Manufacture and Expiry dates have the least level of microbial contamination. The reason is that, the regulatory body (NAFDAC) helps in the maintenance of hygienic food and drug products in Nigeria. The microbial nature and content of the microbial contaminant in the HMPs are presented in Table 1. The results show that the microbial load of the products varied considerably. Six (28.6%) of the samples were without microbial contamination while fifteen (71.4%) were contaminated with one or more of the micro organisms isolated. Seven (33.3%) of the samples were contaminated by *E. coli*, which is an intestinal bacterium and is an indication of contamination by faeces either from man or animal sources. Only one (4.8%) was contaminated with Five (23.8%) of the products were contaminated by *Staphylococcus aureus* and eleven (52.4%) were contaminated by four different Fungi namely *Aspergillus sp.* 3(27.3%), *Mucor sp.* 4(36.4%), *Fusarium sp.* 2(18.2%) and *Rhizopus sp.* 2(18.2%). The highest microbial impurity was found in sample T followed by sample O and E respectively as shown in Table 1. *Escherichia coli* were the most frequently isolated species resulting from poor hygiene practices and

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Table 2: Product Code, Dosage Forms, Content, NAFDAC Registration, Manufacture and Expiry Dates of 21 Brands of HMPS from the Nigerian Market

Product Code	Dosage form	Date of Manufacture	Expiry Date	NAFDAC REG	Contents	Therapeutic claim
A	Capsul	Aug 2003	Aug. 2007	No	Gingkobilob extract, ganoerma lucidum, schhisand chinensis	Brain fatigue, improvement of thought process
B	Capsule	-	-	No	Lime juice, honey	Catarrh
C	Capsule	June 2004	June 2007	No	Strophantia sarmentosa 75% pyrenanacatha 25%	Chronic fever and cough
D	Solution	-	-	No	Urine	Conjunctivitis (Apdo)
E	Solution	Oct 2002	Oct 2005	No	Hibiscus sabdariffa, sorghum bicolor gongroama, latifolium	Immune booster, energizer, blood normalizer
F	Solution	-	-	Yes	Grinded onions, ginger, garlic, palm oil	Children convulsion
G	Solution	-	-	-	White fluid of snail, honey	Asthma
H	Solution	-	-	No	-	Antimalarial
I	Solution	-	-	No	Honeylime juice	Cantharalbicans
J	Solution	-	-	No	-	Pile, dysentery, other stomach ailments
K	Solution	-	-	No	-	Eyeache, stomach ulcer, waist pain, menstrual pain, Haemorrhoids, pile.
L	Solution	Jan 2004	Dec 2006	No	-	Paralysis
M	Solution	-	-	No	Honey garlic and water	Fever measles skin rashes and small pox
N	Solution	-	-	Yes	Allium sativa, ficus carica, nauclea conbretum micabatum	Antimalarial
O	Solution	-	-	No	Unripe pineapples, unripe pawpaw Lipton tea, sugar cane, lime orange, water	Antimalarial
P	Solution	-	-	No	Alabukun powder	Diarrhoea
Q	Solution	-	-	No	Lime juice and Salt	Stomach ulcer
R	Powder	-	-	No	Unripe plantain water	Heamorrhoids
S	Powder	-	-	No	-	Typhoid fever
T	Powder	-	-	No	Bitter leaves	Diabetes
U	Powder	-	-	No	Irish potato	Pile

improper handling of the products. This is in conformity with earlier study by ^{12, 13}. Similarly, among the group of enteriobacteriaceae though its isolation which usually proves more difficult is an indication of current fecal contamination following improper handling of the products which influence the microbial quality of the herbal products. This present study conforms to previous works by ^{13, 14, 15}. *Mucor sp.*, *Aspergillus sp.* and *Rhizopus sp.* which were principally a storage fungi were found to be implicated in herbal products in this study. The contamination may have resulted from

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exposure to dust and prolonged storage in humid conditions. This report is in agreement with those of ¹⁶. ¹⁷. On the other hand, *Fusarium sp.*, a field fungus was found to contaminate few samples of the herbal products in this study compared to *Mucor sp.* and *Aspergillus sp.* The field fungi (*Fusarium sp.*) as the name implies may have invaded developing or mature plants in the field before harvest which in turn has a serious health implication. This result is in accordance with the earlier study by ^{18,19}. The presence of microbial contamination in herbal products does not only reduce or inactivate the therapeutic activity of the products but some infectious outbreaks are found to be associated with the use of heavily contaminated herbal products therefore, the need for constant monitoring and control of quality of the herbal medicinal products manufactured, sold, advertised, and used in Benue, Nigeria by appropriate agencies like National Food, Drug, Administration and Control (NAFDAC) is of utmost significant.

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