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Microbiological quality of herbal preparations marketed in South East Nigeria

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Abstract

Objective: The present work is a microbiological quality control study on some herbal preparations marketed in south eastern Nigeria. Materials and methods: Ten solid and ten liquid herbal preparations collected from different herbalists in south eastern Nigeria were assayed for their bacterial and fungal load using the spread plate technique. All the microbial contaminant were characterised at least to genera level. Results: The herbal preparations were heavily contaminated with bacteria and fungi at levels far above the officially stipulated limit for oral pharmaceutical preparations. A total of 45 bacterial (including *E. coli*, *Klebsiella, Pseudomonas, Proteus, Streptococcus,* and *Staphylococcus*) and 20 fungal (including *Candida, Microsporium* and *Curvularia*) strains were isolated from the preparations. Conclusions: Due to the high degree of microbial contamination of these herbal medicines, and the pathogenic potentials of many of the isolates, it is very necessary that herbalists should be enlightened about Good Manufacturing Practice (GMP). Also regular microbiological monitoring of herbal preparations marketed in Nigeria is advocated.

Keywords : Microbiological quality control, Herbal medicines, South eastern Nigeria.

1. Introduction

The plant kingdom has served as the best source of natural remedies since time immemorial [1, 2]. In many countries particularly Asian countries, herbal medicine (HM) has become an integral part of the health care delivery system on the same basis as orthodox medicine [3,4]. In most African countries including Nigeria, HM is recognized as an important component of health care system especially among rural dwellers who constitute about 70% of the population [5, 6].

In recognition of the inherent value of HM to primary health care, the World Health Organisation (WHO) has advocated for proper

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identification, sensible exploitation, scientific development and appropriate utilization of herbal medicines that provide safe and effective remedies in medicare [7].

In order to promote the effective production and utilization of HM therefore, the WHO has published series of guidelines for the assessment of HM [7-9]. An essential part of these WHO requirements is the assessment of the microbiological purity of HM.

Presence of microorganisms in pharmaceuticals is undesirable because they cause spoilage of the product and also because they constitute serious health hazards to the consumer or patient [10-12]. Several cases of infections caused by the administration of non-sterile preparation contaminated with microorganisms have been documented [11-13].

Most developed countries have adopted microbiological standards as well as the application of Good Manufacturing Practice (GMP) in order to guarantee the hygienic quality of non-sterile pharmaceutical preparations [14,15].

In Nigeria the increasing cost of orthodox medication coupled with the object poverty among the rural dwellers has forced a large population of these rural farm families to resort to traditional or herbal medicines to relief them of their ailments. This increase in demand for the HM has resulted in an appreciable change in the quality of Traditional Herbal Medicine Practice [5,16].

It is therefore necessary to monitor, on a regular basis, herbal preparations in our markets to ensure their quality, efficacy and safety. This study is designed to evaluate the degree of microbial contamination of some herbal preparations prepared by herbalist in Southeast Nigeria.

2. Materials and Methods

2.1 Herbal Samples

Ten (10) solid and ten (10) liquid herbal preparations collected from different herbalists in southeast Nigeria were used in the study. The samples, their therapeutic claims and routes of administration are presented in Table 1. Water was the vehicle used in the preparation of the liquid samples.

2.2 Determination of microbial load of herbal samples

2.2.1 Liquid samples

Each sample was shaken vigorously; 1ml pipetted and subjected to a 10-fold serial dilution in sterile normal saline. A 0.02 ml volume of 10^{-6} dilution was spread plated on well-dried surface of nutrient agar (for bacterial count) and Sabouraud dextrose agar (for fungal count). Each sample was inoculated in triplicate. The nutrient agar plates were incubated at 37°C for 24 to 48 h while the SDA plates were incubated at 28°C for up to 5 days. The number of colonies on each plate was determined and the mean for each sample calculated and expressed as mean colony forming units (cfus) per ml.

2.2.2 Solid samples

For each sample 1 g portion was weighed out, dissolved in 9 ml sterile normal saline and 10-fold serial dilution also carried out. A 0.02 ml volume of 10^{-3} dilution was inoculated and incubated as described above. Mean counts were expressed as cfus/ml.

2.3 Isolation and identification of microbial contaminants in the herbal preparations

A loopful of each of the liquid sample was streaked on cetrimide agar, mannitol salt agar, McConkay agar, sodium azide crystal violet agar and Sabouraud dextrose agar. Isolated organisms

Table 1.
List of the studied herbal preparations marketed in southeast Nigeria showing their therapeutic claims and route of administration

Sample	Therapeutic Claim(s)	Route of administration
L1	Eye and ear infections	Intra ocular or intra auricular
L2	Blood pressure	Oral
L3	Antihemorrhoidal	Oral
L4	Cough	Oral
L5	Dysmenorrhoea & GIT disturbance	Oral
L6	High blood pressure, insomnia, anxiety/ worries and disease prevention	Oral
L7	Malaria	Oral
L8	Tonic for freeing bowel	Oral
L9	Tonic for pregnant women	Oral
L10	Antihemorrhoidal	Oral
S1	Gonorrhea	Oral
S2	Antidiabetic	Oral
S3	Charm & antidote for Poison	Topical/Oral
S4	High blood pressure	Oral
S5	Wound & cough	Topical
S6	Cough	Oral
S7	Dysentery	Oral
S8	Indication undisclosed	Intranasal
S9	Headache	Intra ocular, intranasal and intra auricular (mix with water)
S10	Undisclosed	Topical

L = Liquid; S = Solid

were identified by their morphological, physiological and biochemical characteristics [17,18].

3. Results

Microbial contaminants isolated from the herbal preparations are shown in Table 2. The mean bacteria counts for the liquid samples varied from 1.0 x 10^7cfu/ml to 2.0 x 10^9 cfus/ml while the mean counts for the solid samples varied from 5.0 x 10^7 cfus/ml to 4.0 x 10^9 cfus/ml. Three of the ten liquid herbal preparations had no fungal growth while each of the remaining seven samples had mean count of 5.0 x 10^7 cfus/ml. Two of the solid preparations had no fungal growth while the mean count of the remaining eight samples ranges from 5.0 x 10^7 to 4.0 x 10^9 cfus/ml. More than one microorganism contaminated most of the samples. Of the 20 preparations examined 16 (80%) were contaminated with Gram negative rods. Gram positive rods were isolated from 12 (60%) of the samples [6 liquids and 6 solids] while 9 (45%) of the 20 samples [2 liquid and 7 solid] were contaminated with Gram positive cocci.

A total of 45 bacterial and 20 fungal strains were isolated from the herbal preparations (Table 3). The 45 bacterial strains were

Table 2
The microbial load and the various contaminants of some herbal preparations marketed in southeast Nigeria

Sample	Bacteria	Mean bacterial count (cfus/ml)	Fungi	Mean fungal count (cfus/ml)
L1	*E. coli, Bacillus* spp	$8.5x10^8$	*Candida* spp, *Mucor* spp	$5.0x10^7$
L2	*Proteus* spp, *Micrococus* spp, *E. coli*	*Trichophyton* spp $2.5 x10^8$	*Penicillium* spp, *Mucor* spp	$5.0x10^7$
L3	*Proteus* spp, *Streptococcus* spp	$9.5 x10^8$	*Curvularia* spp	$5.0x10^7$
L4	*Citrobacter* spp	$5.5 x10^8$	*Aspergillus* spp	$5.0x10^7$
L5	*Pseudomonas* spp, *Bacillus* spp, *E. coli*	$7.5 x10^8$	*Aspergillus* spp	$5.0x10^7$
L6	-	-	-	-
L7	*Pseudomonas* spp, *Klebsiella* spp, *Providentia* spp	$1.0 x10^8$	*Penicillium* spp	$5.0x10^7$
L8	*Bacillus* spp	$2.2 x10^9$	-	-
L9	*Citrobacter* spp, *Corynebacterium*, *Bacillus* spp	$3.5 x10^9$	*Trichophyton* spp	$5.0x10^7$
L10	*Bacillus* spp, *E. coli*, *Alcaligenes* spp	$1.1x10^9$	-	-
S1	*Proteus* spp, *Staphylococcus* spp, *Micrococus* spp	$5.5 x10^8$	*Trichophyton* spp	$5.0x10^7$
S2	*Proteus* spp, *E. coli*, *Bacillus* spp	$2.5 x10^8$	-	-
S3	*Bacillus* spp, *Staphylococcus* spp	$1.1 x10^9$	*Mucor* spp	$5.0x10^7$
S4	*Proteus* spp, *Staphylococcus* spp, *Bacillus* spp	$3.5 x10^8$	*Petriellidium* spp, *Trichophyton* spp	$5.0x10^7$
S5	*Staphylococcus* spp, *Enterococcus* spp, *Klebsiella* spp	$3.5 x10^8$	*Trichophyton* spp	$5.0x10^7$
S6	*Proteus* spp, *Bacillus* spp	$5.0 x10^7$	Not identified	$5.0x10^7$
S7	*Staphylococcus* spp, *Pseudomonas* spp	$3.5 x10^8$	*Trichophyton* spp, *Microsporium* spp	$9.5x10^8$
S8	*Bacillus* spp, *Proteus* spp	$1.7 x10^9$	*Trichophyton* spp	$5.0x10^7$
S9	*E. coli*, *Micrococus* spp	$2.7 x10^8$	*Candida* spp	$3.5x10^9$
S10	*Bacillus* spp, *Staphylococcus* spp, *Klebsiella* spp	$3.5 x10^9$	-	-

distributed into the following genera: *Bacillus* (11), *Proteus* (7), *Staphylococcus* (6), *Escherichia coli* (6), *Pseudomonas* (3), *Klebsiella* (3), *Micrococcus* (3), *Corynebacterium* (1), *Streptococcus* (1), *Enterococcus* (1), *Alcaligenes* (1), *Citrobacter*

(1) and *Providencia* (1). The fungal strains were distributed into the following genera: *Trichophyton* (7), *Mucor* (3), *Penicilliun* (2), *Aspergillus* (2) *Petricellium* (1), *Candida* (2), *Microscoprium* (1), *Curvularia* (1) and unidentified (1)

4. Discussion

The results of this study have shown that majority of the herbal preparations examined were grossly contaminated by bacterial and fungal agents. The mean microbial counts of the preparations were generally higher than the accepted values for non-sterile pharmaceuticals [15,19].

A number of sources of contamination of herbal preparation especially during preparation have been identified [20-22]. The microflora of the final product may represent contaminants from the raw materials, equipment, water, and atmosphere and from personnel [20-23]. Microorganisms such as *Escherichia coli,* and *Pseudomonas spp* reported in this study are generally known to proliferate in potable, distilled and de-ionized water while *Bacillus spp, Staphylococcus, Streptococcus, Corynebacterium, Aspergillus* and *Mucor* are commonly isolated from air [23]. The most common source of post preparation herbal contamination is the packaging vessels.

In order to enhance consumer acceptability, most herbalists in Nigeria have adopted the use of bottles and plastic containers as packaging vessels for their preparations. Unfortunately these vessels are not subjected to any form of sterilization after washing them. Contamination of the preparation coupled with the humid tropical environments may result in the proliferation of microbial contaminants in the herbal remedies; this probably explains the high microbial counts recorded in this study.

Such high levels of microbial contamination have been shown to result in spoilage and degradation of the products or may constitute a health hazard to the user [11-13, 24]. Most herbal preparations are made up of different components of various plant species and these preparations are not standardized with respect to colour, taste, consistency etc. Unlike the orthodox drugs, changes in the appearance, odour, taste etc of HM due to spoilage are hardly readily detected by the patients.

Table 3.
Genera of Microorganisms isolated from some herbal Preparations marketed in Nigeria

Microorganism	**Liquid**	**Solid**	**Total**
Bacteria			
Bacillus. spp.	5	6	11
Corynebacterium spp.	1	0	1
Staphylococcus spp.	0	6	6
Streptococcus spp.	1	0	1
Enterococcus spp.	0	1	1
Micrococcus spp.	1	2	3
Escherichia coli spp.	0	2	6
Proteus spp.	2	5	7
Pseudomonas spp.	2	1	3
Klebsiella spp.	1	2	3
Alcaligenes spp.	1	0	1
Citrobacter spp.	1	0	1
Providentia spp.	1	0	1
Total	**16**	**25**	**45**
Fungi			
Candida spp.	1	1	2
Mucor spp.	2	1	3
Trichophyton spp.	2	5	7
Penicillium spp.	2	-	2
Curvularia spp.	1	-	1
Aspergillus spp.	2	-	2
Microsporium spp.	-	-	1
Petriellidium spp.	-	1	1
Unidentified	-	1	1
Total	**10**	**9**	**20**

Among the microorganisms isolated from the HM, *Bacillus, Staphylococcus, Proteus* species, *E. coli* and *Trichophyton* were the major contaminants. *Bacillus* and *Staphylococcus* species have been found to be the bacteria most frequently isolated from non-sterile orthodox pharmaceuticals [10].

Although the pathogenicity of these organisms was not assessed, species of these agents have been incriminated in serious human infections [24-28]. Bacillus spp are widely distributed in the soil, dust, air and water and they are resistant to environment destructive factors [24,29,30].

Sample S_5, a solid preparation used in the treatment of wounds, was found to be contaminated with *Staphylococcus* species. This micro organism is known to be one of the principal organisms associated with wound infections. Its isolation from a preparation used in the treatment of wounds has grave clinical significance.

Also sample L_1 whose mode of administration is by instillation into either the eye or ear was found to be contaminated by *E. coli, Bacillus spp, Candida spp* and *Mucor spp.* Ideally, drugs instilled into the eyes are officially required to be sterile while those instilled into the ears should be totally devoid of enterobacteriaceae such as *E. coli* [19]. This sample clearly falls short of this requirement. Sample L_6 did not contain any bacteria or fungi.

Actually, this sample was aesthetically packaged and the product label indicated that it contained a preservative. It is possible that the manufacturers of this preparation observed Good Manufacturing Practice (GMP), which eventually reflected in the high microbial purity of their product. It is widely advocated that GMP exercised throughout production of non-sterile pharmaceuticals will eventually curb risk of microbial contamination.

Generally, although the microorganisms reported in this study may be considered as non-pathogenic agents, they can constitute a serious health hazards especially in severely debilated or immuno-compromised patients.

The results of this study indicate a high contamination rate of the herbal preparations examined. The organisms isolated from the preparations are potential pathogens. The results call for the creation of awareness among herbalists of the principles of Good Manufacturing Practices (GMP). Also regular microbiological monitoring of herbal preparations in the country is advocated. Results of such monitoring should be channeled to the traditional medicine practitioners or herbalists through their national body.

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