MICROBIOLOGICAL QUALITY OF SOME HERBAL FORMULATIONS USED IN THE TREATMENT OF TYPHOID FEVER AND CANDIDIASIS SOLD IN AFIKPO

BY

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ABSTRACT

The present study was aimed at examining the microbiological quality of some herbal formulations used in the treatment of typhoid fever and candidiasis sold in Afikpo. Four samples were purchased from randomly selected sellers, conveyed to the laboratory for microbial analysis. A tenfold serial dilution was performed on each of the samples up to dilution factor of 10^{-3}. An aliquot (0.1ml) of the serially diluted sample (10^{-3}) was inoculated onto solidified media by spread plate method, incubated for 24 hours at 37^{o}C for bacteria isolation and at room temperature for 5 days for fungal isolation. The aerobic count of bacteria isolates showed that Sample B (for typhoid) had the highest count of 7.5 x 10^{3}cfu/ml and the least of 5.0 x 10^{3} from sample D. sample C had no growth. However, the highest coliform count was from sample with B w3.3 x 10^{3} cfu/ml. A total of six (6) bacteria genera were recovered from the samples. Namely Escherichia coli, Pseudomanas sp, Shigella sp, S. aureus, Bacillus sp., Enterobacter aerogenes. Fungal isolates recovered included Aspergillus niger, A. flavus, Penicillium sp. and Rhizopus sp. The percentage-occurrence of fungal isolates indicates that Aspergillus flavus had the highest occurrence of 40.0% while Penicillum sp. (13.3%) had the least occurrence. From the foregoing, we recommend that there is need for government to ensure regular check on the quality control and level of hygiene displayed by the herbal medicine producers to ensure minimal contamination of the products.

Keywords
Microbiological, Herbal formulations, Typhoid fever, candidiasis, coliform,

INTRODUCTION

Background of the Study

Although, oral pharmaceutical preparations are not required to be sterile, they are not supposed to be heavily contaminated by microorganisms of potentially pathogenic organisms including *E. coli, S. aureus* and *Pseudomonas aemginosa* (USP, 2013). This is because apart from the safety of consumers, the presence of high microbial count in any preparation may lead to the proliferation of such organisms within the preparation leading to spoilage (Bloomfield, 2007). This is why pharmaceutical companies are required to adhere to the principles of Current Good Manufacturing Practice (FDA, 2015) and their products must be subjected to total quality, control measures, with the overall drug manufacturing process being made to undergo quality assurance tests at every level. It has to be said however that, only the big pharmaceutical companies have the capacity to adhere to the principles of Current Good Manufacturing Practice.

Okeke and Lamikanra (2001) noted that small pharmaceutical companies involved in the production of orthodox drugs, many of which are found in countries with challenged economies are not able to invest in machinery, controls for production environments and the employment of qualified staff to see that their products are of consistently high quality. The situation is worsened in Nigeria by the existence of a large informal sector which is responsible for small scale production of a large number of unregistered and usually unstandardised medicines using rudimentary equipment and raw materials of plant derivation which are highly susceptible to extensive microbial contamination. In addition, the packaging of these products is often rudimentary with final products being packed in recycled plastic bottles which are frequently unlabelled. These unregistered herbal medicines are outside the control of the relevant regulatory bodies. However, they cannot be ignored as they are available virtually everywhere including and especially in rural areas, which are short of modern pharmaceutical cover.

The widespread use and availability of herbal medicines has been reported to be due to perceived efficacy, safety and absence of side effects from herbal products when compared to orthodox medicines (Kennedy, 2005; Clement *et al*., 2007). The use of herbal medicines is also likely to have increased because of emerging infections such as AIDS and drug resistant malaria (Gyasi *et al*., 2013; Lorenc and Robinson, 2013).

The high cost of hospital consultation and orthodox drugs is an additional reason why herbal therapy may be attracting greater patronage (Gyasi *et al*., 2011). The use of improved packaging materials and increased public awareness through the organization of trade fairs on

traditional medicine and the presence of NAFDAC registration numbers on registered herbal products are also likely factors that have increased the use of herbal products in Nigeria.

The issue of the safety of herbal products is however of great concern to many regulatory bodies who have therefore set out specifications on the quality of herbal products. Some regulatory bodies have given specifications on the microbial load and presence of specific organisms in herbal products (WHO, 2007a; European Pharmacopoeia, 2007).

The high cost of synthetic drugs has created a big health challenge to people especially the poor who are seen to be in the majority in the developing countries like Nigeria. This has led to many looking for alternative means of treatment that rill not only be pocket friendly but also efficacious in meeting their health needs. This search for alternative medicinal solutions has led to the high and ever-increasing demand for traditional medicine. The increase in utilization and awareness of the potency of herbal formulation has led to proliferation of outlets advertising and selling these herbal

MATERIALS AND METHOD

Materials

The material used in this work are herbal medicines purchased from the market, culture media, microscopes and others.

Methods

Sample Collection

Four different samples were purchased randomly from sellers (two for treatment of typhoid fever and two for treatment of candidiasis) with a sterilized sample bottle and then brought to the laboratory for microbial analysis.

Microbiological Analysis

Sample Preparation

A tenfold serial dilution was performed on each of the oral herbal medicines up to dilution factor of 10^4. That is 1ml of the herbal medicine into 9ml of sterilized distilled water.

Medial Preparation

Following manufacturer's instructions, the culture media were prepared. Media used include Nutrient agar for viable count, MacConkey agar for coliform count, Eosin Methylene Blue agar (EMB), Manitol Salt agar, *Salmonela - Shigella* agar and Sabouraud Dextrose Agar (SDA).

Media inoculation

An aliquot (0.lml) of the serially diluted sample was inoculated onto solidified media by spread plate method and incubated for 24 hours at 37°C for bacteria isolation/and at room temperature for 4 to 5 days for fungal isolation. (Cheesbrough, 2000).

Identification of Isolates

i. **Cultural Characteristics:** This was based on their observed colour, size and shape.
ii. **Cellular Characteristics:** This was based on gram reactions of the organisms.
iii. **Biochemical Characteristics:** This was based on the reactions of the isolates on some reagents.

Biochemical Test

The biochemical tests that were used in the identification of the various isolates included as listed below:

Catalase Test, Coagulase Test, Indole Test. Other tests employed in biochemical identification of the isolates were: Voges Proskauer Test, Urease Test, Oxidase test, Citrate Test, Ribose Test, Glucose Test (Cheesbrough, 2000).

RESULTS

Table 1: The Plate Count of Aerobic and Coliform Bacterial of the Samples

Sample	Aerobic Count (Cfu/ml)	Coliform Count (cfu/ml)
A	5.3 x $l0^3$	2.0 x $l0^3$
B	7.5 x 10^3	3.0 x $l0^3$
C	–	–
D	5.0 x $l0^3$	1.0 x $l0^3$

Table 2: Biochemical Features of Bacteria Isolates

Samples	**G. Stain**	**Cat**	**Coag**	**Ind**	**VP**	**MR**	**Ure**	**Oxi**	**Cit**	**Rib**	**Fruc**	**Glu**	**Lac**	**Malt**	**Manno**	**Suc**	**Isolates**
Sample A	–	+	+	+	–	+	–	–	–	+	+	+	+	+	+	–	*Escherichia coli*
	–	+	+	–	+	–	–	–	+	–	–	+	–	–	–	+	*Enterobacter aerogenes*
	–	+	–	–	–	+	–	–	+	–	–	+	–	+	+	–	*Shigella*
	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	*S. aureus*
Sample B	–	+	–	–	+	–	–	–	+	–	–	+	–	–	–	+	*Enterobacter*
	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	*S. aureus*
	–	+	+	+	–	+	–	–	–	+	+	+	+	+	+	–	*Escherichia coli*
	+	+	–	–	+	–	–	+	–	+	+	+	–	+	+	+	*Bacillus* sp
Sample C	–	+	–	+	–	+	–	–	–	+	+	+	+	+	+	–	*Escherichia coli*
Sample D	–	+	–	–	–	+	–	–	–	+	+	+	+	+	+	–	*S.* sp
	–	+	–	–	–	+	–	–	–	–	+	–	–	–	+	–	*Shigella*
	–	+	–	–	–	–	–	+	+	–	–	–	–	–	–	–	*Pseudomonas* sp

Key: **cat - catalase test. Gra = gram stain, M.R. = Methyl Red. Glu. = Glucose utilization test. Coag = coagulase test**

Table 3: Morphological Features of Isolated Fungi

Specimen	Colour of Organism		Suspected Organism
	Top	Reverse	
A	Dark brown to black	Brown	*Aspergillus niger*
	Blue-Green	Reddish-brown	*Penicillium* sp.
B	Yellow	Brown	*A. flavus*
	Gray	White	*A. fumigates*
	White colony		*Rhizopus*

Table 4: Prevalence of Occurrence of Contaminants in Percentage

S/N	Fungi	Number of Occurrence	% of Occurrence
1.	*Aspergillus niger*	4	26.7%
2.	*Aspergillus flavus*	6	40.0%
3.	*PenicIllum* sp.	2	13.3%
4.	*Rhizopus* sp.	3	20.0%
	TOTAL	**15**	**100%**

DISCUSSION, RECOMMENDATION AND SUMMARY

Discussion of Findings

The herbal products collected in this study are in liquid form and they are administered orally. Information from the healer pointed out the herbs are mostly produced for the treatment of typhoid fever and candidiasis. Some hawked herbal products are sold wholly while some are dispensed as requested with or without prescription pattern.

The microbial load of the herbal medicines in this study shows that sample A, B and D had total aerobic count of $5.3x10^3$ cfu/ml, $7.5x10^3$ cfu/ml and $5.0x10^3$ cfu/ml and total coliform count of $2.0x10^3$ cfu/ml, $3.0x10^3$ cfu/ml and $1.0x10^3$ cful/ml respectively but sample C had no growth which may be as a result of preservative used in the formation of the herbal medicine.

Based on WHO Guideline 2007, the total aerobic microbial count of herbal medicine was not more than 10^5 cful/ml, thus the microbial load of these analysed. Herbal products are not within acceptable limit. The contamination of the final products may come from the raw materials, equipment, water, and atmosphere and from personnel Esimone *et al*. (2001). The presence of *Enterobacteriaceae* which is an indicator of feacal contamination and other harmful pathogens like *Escherichia coli, Pseudomanas* sp, *Shigella sp, Staphylococcus aureus, Bacillus* sp., *Enterobacter aerogenes*, shows the degree of contamination of these products. A total of six (6) bacteria isolates were recovered from the oral herbal medicine studied. They include *Escherichia coli, Pseudomanas* sp, *Shigella sp, Staphylococcus aureus, Bacillus* sp., *Enterobacter aerogenes*, and the fungi isolates include *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Aspergillus fumigates,* and *Rhizopus* sp. The findings in this study are in agreement with previous studies in Southeast and southwest Nigeria by (Esimone *et al*., 2002; Idu *et al*., 2010; Braide *et al*., 2013 and Igbeneghu *et al*., 2016). Most organisms isolated like *Escherichia coli,*

Pseudomanas aeruginosa are known to proliferate in portable water; *Staphylococcus aureus*, mucor and Aspergillus are commonly isolated in the air (Underwood, 1999). These pathogenic contaminants could have various health implications on the user of these products. *Escherichia coli* and *Enterobacter aerugenes* are organisms associated with gastrointestinal tract and its presence indicate the likelihood of fecal contamination.

According to Igbenegbu *et al*. (2016), these contaminants could be acquired from the use of water of poor quality for the preparation of the herbal medicine and rinsing of containers. The fungal isolates identified in this study are *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Penicillium* sp. and *Rhizopus* sp, which is in agreement with the work of Esimone *et al*. (2022) and Odedara *et al*. (2014). Herbal medicinal products with fungal contamination with *Aspergillus flavus* and *Aspergillus fumigates* as seen in this study could be as a result of contamination form soil and organic matter, which aremedically important pathogens of human causing invasive *Aspergillosis*. *Aspergillus niger* could be from various samples including sand, air or laboratory contaminants and is the most frequently encountered agent of otomycosis.

The aerobic count of bacteria isolates showed that Sample B had the highest count of 7.5 x 10^3cfu/ml followed by Samples A and D with cfu counts of 5.3 x 10^3 and 5.0 x 10^3 per ml respectively. While sample C had no growth. However, coliform count showed the highest count from sample B 3.3 x 10 cfu/ml followed by sample A and then Sample D with counts of 2.0 x 10 cfu/ml and 1.0 x 10 cfu/ml respectively (table 1).

A total of six (6) bacteria isolates were recovered from the oral herbal medicines studied. They included *Escherichia coli, Pseudomanas* Sp, *Shigella* sp., *S. aureus, Bacillus sp., Enterobacter aerogenes.* The table also shows that sample B has the highest isolates (table 2).

Morphological features of medical isolates indicate that the herbal medicines were contaminated with varying fungus populations which included *Aspergillus* spp. *(Aspergillus niger, A. flavus* and *A. fumigates), Penicillium* sp. and *Rhizopus* sp. Sample A yielded A

niger and *Penicillium* ap. While sample B *A. fumigates, A. flavus and Rhizopus* sp. however, samples C and D yielded no fungal growth (table 3).

The percentage occurrence of fungal isolates indicates *that Aspergillus flavus* had the highest occurrence of 40.0% (6) followed by 26.7% (4), *Rhizopus* Sp. 20,0% and the least is *Penicittum* sp. 13.3% (2) (table 4).

The viable count of 3.3 x 10^3 cfu/ml. followed by sample A and then Sample D with counts of 2:0 x 10 cfu/ml and 1.0 x 10 cfu/ml respectively in this present study as recorded in Table 4.1 is in line with that recorded by Archibong *et al.* (2017), in their study of "Microbiological Assessment of Some Liquid Herbal Medications Sold in Awka Metropolis, Anambra State." Archibong *et al.* (2017), recorded a total bacterial count in the range of 1.0 x 10^3 to 5.9 x 10^3 cfu/ml, theses according to them complied with the WHO limits for bacterial loads. However, Archibong *et al,* (2017) also recorded a total bacterial load range of 1.4 x 10^5 to 2.1 x 10^6cfu/ml which does not comply WHO standard. Five (25%) had no fungal growth, ten (50%) had fungal count between 1.0 x10^2 and 1.0 x l0^3cfu/ml (these complied with the standard) while five (25%) showed counts between 1.4 x 10^3 and 1.4 x 10^5cfu/ml which they remarked did not comply with the standard).

According to the WHO report, there is widespread availability and usage of herbal preparations by a large percentage of persons in many developing countries (Robinson and Zhang, 2011). Some reasons for this have been documented by several authors and these include perceived efficacy, safety and absence of side effects (Kennedy, 2005; Clement *et al.*, 2007; Gyasi *et al.*, 2013; Lorenc and Robinson, 2013). It was observed in this present study that the high patronage of herbal medicines must not be unconnected with the retinue of diseases or infections that its peddlers acclaim it can cure. Oluwatoyin and Adebayo in 2016 made similar observation during their study when their opined that one observation they made suggests that the high patronage of herbal medicine peddlers in Ile-Ife may actually be the appearance of a ready capacity for the treatment or management of all manner of communicable and non-communicable diseases. In this separate studies Gyasi *et al*. (2013); Lorenc and Robinson, (2013) observed that many of the producers of herbal medicine claim to have products for curing AIDS, a condition yet to have a specific orthodox cure.

However, the high incidences of infectious diseases in developing countries also led to the high patronage witnessed by the producers of these herbal medicines, .Our observation is in line with the views of Oluwatoyin and Adebayo in, (2016) when they opined that over 80% of the preparations used in their study were claimed to be for infectious diseases. They

concluded that some of these ailments, for example malaria, typhoid, blood infection (septicaemia) and candidiasis are quite serious and may be life-threatening.

All the herbal preparations used in the study were of assumed to be of acceptable quality as they all had labels and were packed. This is in contrast with the study conducted by Oluwatoyin and Adebayo in 2016 who noted that only 10% of the samples assessed in their study were of acceptable microbial quality; as judged by the absence of any form of labels on this class of herbal samples, it was difficult to determine why these were better than the other 90%. The acceptable samples were similar in appearance and packaging and the vendors were located in the same areas as the unacceptable ones. The absence of a label according to them (Oluwatoyin and Adebayo, 2016) which is one of the characteristics of these unregistered herbal drugs makes it difficult to compare the samples in terms of concentration of herbs or identity of the components of each preparation.

The microbial loads of 90% of the samples assessed in this study were beyond the limits stipulated by the regulatory bodies (WHO, 2007; European Pharmacopoeia, 2007). Apart from the heavy microbial loads, the presence of unacceptable organisms or pathogens was demonstrated in the herbal samples.

The unacceptable organisms recovered included the Gram negative organism's *E. coli*. However, the presence of *E. coli* in this present study cannot be unconnected with unhygienic nature of the handlers arising from faecal matter. Our observation here is in consonance with the views of Edberg *et al.* (2000), who noted in their study that these are organisms associated with the gastrointestinal tract and indicate the likelihood of faecal contamination. To this end, Oluwatoyin and Adebayo (2016) in their study of "Assessment of the microbial quality of some oral liquid herbal medicines marketed in Ile-Ife, South-Western Nigeria" noted that these contaminants could be acquired from the use of water of poor quality for the preparation of the samples and rinsing of containers and concluded that other likely sources are the use of inadequately washed or disinfected plant parts previously exposed to manure.

There is no doubt that the presence of this organisms in this present study is as a result of poor quality preparations. Our observation here agrees with the view of WHO (2007) that the presence of *Escherichia coli* and *Salmonella* spp. has also been stated to be an indication of poor quality of production and harvesting practices.

The plants used in the preparations of these medicinal formulations are assumed to been harvested from soil or in one way or the other had contact with soil as most of the organisms such as *Bacillus* sp. recovered in the present study are soil dwelling organisms.

Our suggestion here is in line with the observation of Adebayo (2016), who during their study of "assessment of the microbial quality of some oral liquid herbal medicines marketed in Ile-Ife, South-Western Nigeria" opined that the recovery of a high number *of Bacillus* species, the frequent predominant aerobic spore-forming bacteria naturally occurring microflora of medicinal plants, supports the fact that vegetative plant parts and roots have been in contact with the soil or dust are among the components of the preparations.

S. aureus recovered from this present study suggest that personnel involved in the preparation chain contributed in the contamination of the products.

Our suggestion is supported with the opinions of Oluwatoyin and Adebayo (2016) when they remarked that the presence of organisms such as *S. aureus* contamination could also have occurred through handling by personnel who carry pathogenic bacteria or normal commensals during harvest/collection, post-harvest processing and the manufacturing process. They went further to conclude that the presence of several contaminants in a single preparation as observed in their study is expected since the preparations usually contain more than one plant or plants parts that have been obtained from multiple harvest sites. The practices of transportation and storage may also cause additional contamination and microbial growth. WHO (2007a) added that the proliferation of microorganisms also results from failure to control the temperatures of liquid form and finished herbal products.

The high load and prevalence of *Aspergillus* spp. isolated from the present study agreed with the study conducted by Ezekwesili-Ofili *et al*. (2014) in their study of the Bioload and Aflatoxin Content of Herbal Medicines from selected States in Nigeria. In this study, they recovered *Aspergillus, Penicillium, Rhizopus, Cladosporium, Geotricum* and *Candida.*

Recommendations

Following the results emanating from the study, we recommend the following:

There is need for government to ensure regular check on the quality control and level of hygiene displayed by the herbal medicine producers to ensure minimal contamination of the products. There is need for evaluation, registration and deregistration of companies that fail to abide by standards as stipulated by the government agencies. There is need for strict use of customized containers by herbal medicine manufacturers and not using already used table water cans in the packaging of herbal medicines. The public should be sensitized on the dangers inherent in the over dependence of the herbal medicines without adequate check on their authenticity.

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