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Microbial Assessment of Regulated and Unregulated Herbal Medicines in Delta State, Nigeria

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ABSTRACT

This study investigated the microbiological status of herbal drugs sold in Delta State. Fifty-eight herbal products of which 31.03% were regulated by the National Agency for Food and Drug Administration and Control and 68.97% unregulated. Of the unregulated herbal products, seven products were unpackaged prepared by street vendors and 33 were packaged. Total aerobic viable counts for bacteria and fungi were analyzed according to standards stipulated by World Health Organization for microbial quality of herbal products. Selective media were used for detecting specific organisms. Identification of bacteria and fungi was carried out using prescribed standard methods. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were not detected in any of the regulated herbal products. *Salmonella* spp. and *Shigella* spp. were not detected in any of the herbal products. *Bacillus* spp. and *Aspergillus* spp. were the most predominant organisms. Some herbal products did not yield bacteria and fungi growth. However, the microbial load of those that yielded growth were more than the permissible range. This study revealed the poor microbial quality of these herbal drugs. Although effective for treating diseases, yet the method of production needs to be improved following good manufacturing process and standardization for the safety and well-being of unsuspecting populace.

Key words : Herbal medicine, regulated, microbial quality, contaminants

INTRODUCTION

Herbs are sources of health care available to man since antiquity. Most herbal drugs are efficacious and constitute therapeutic source to human ailments. Many reports exist to show that herbal drugs have antimicrobial activity. This notion gives the impression and perception that herbal medicines are safe. Herbal medicine formulation depends on various factors including geography, soil, water, transport and storage conditions surrounding their formulations (Dghaim *et al.*, 2017). The therapeutic effect and efficacy of herbal medicines have been reported. However, safety of phytomedicine is a problem. According to World Health Organization (WHO), drugs used for treatment of ailment must be free from contaminants. Herbal medicines are prone to different forms of contaminations including microbial, heavy metals, toxins and agrochemical residues (Shu *et al.*, 2019). Herbal medicine constitutes the drug which many people use to manage their health conditions. Bioactive features of herbal drugs have been studied widely, however, there are few literatures on the determination of microbial quality of herbal drugs consumed by the general population especially in Delta State, Nigeria. Earlier studies showed that there was paucity of data showing microbial quality of herbal drugs (Adomi, 2014) making herbal drugs not to be taken by young people or youths. The elderly population uses more of these products (de Sousa Lima *et al.*, 2020). The more reason drug should be sterile because this group of people have weak immune system.

Although, there are regulating bodies which regulate safety and efficacy of herbal drugs. It is worthy to note however that not all herbal medicines available to the general public are regulated by these bodies. Those which are regulated could pass the hygienic test but many herbal drugs available to the populace are not validated. Vended herbal remedies are common in our cities and towns. Therefore, this study was designed to investigate microbial quality of regulated and unregulated herbal medicines in Delta State.

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MATERIALS AND METHODS

A total of 58 herbal drugs; solid (17), semi-solid (6) and liquid (35) products administered orally or topically with or without further processing were purchased from Abraka and Effurun. Eighteen of them were registered and 40 were unregistered while seven were homemade. The homemade were bought into sterile cellophane and packaged in ice pack to the laboratory for analysis.

Various media were prepared according to the manufacturer's instructions. The media included nutrient agar, Saboraud dextrose agar eosin-methylene blue (EMB) agar, Salmonella Shigella agar (SSA), centrimide agar and mannitol salt agar. The microbiological quality of the herbal medicines was tested according to the methods prescribed by WHO (2007) and British Pharmacopoeia (2007). The number of bacteria and fungi which can grow aerobically in 1 g or 1 ml of herbal product was counted. Specific organisms *Salmonella* spp.*, Shigella* spp.*, Pseudomonas aeruginosa, Escherichia coli* were determined with selective media.

The microorganisms were characterized according to methods described by Nwankwo and Olume (2019).

RESULTS AND DISCUSSION

The microbiological analysis of unregulated and regulated drugs sold in Delta State, Nigeria is presented in Tables 1 and 2. Table 1 shows the microbial quality of unregulated herbal drugs of which the microbial quality HDUR 8 and HDUR 15 were too numerous to count (TNTC). Similarly, the total mold/yeast count was 1.5×10^5 and nil, respectively, for the two herbal products. It is important to note that the two herbal products were solids (powdery) of the 40/58 (68.97%) unregulated drugs. Seven 7/40 (17.5%) out of the unregulated were homemade and not packaged into bottles or any pack. Usually, specific quantities were dispensed in disposable cups to people as they come for patronage. There was no microbial growth for eight of the regulated drugs. The herbal drug bearing the label HDUR 9 had the highest mold/yeast count while no mold/yeast was counted for 18 (45.00%) of the unregulated drugs. This finding agreed with that of Yaaba *et al.* (2020) who also found that some of the herbal drugs investigated did not yield any bacterial and fungal growth, thereby indicating the good microbial quality of such drugs. However, other herbal products had bacteria and fungi counts between 1.0×10^2- 4.5×10^5. Liquid herbal preparations (homemade) had more bacterial population than the other packaged unregulated drugs (Fig. 1). Also, the microbial population of liquid unregulated drugs was more compared to solid herbal drugs. Similar reports were obtained from previous studies which observed that liquid distillates contained bacteria and fungi but dried samples of herbal plant did not yield microbial growth (Alavi *et al.*, 2017). de Sousa Lima *et al.* (2020) also stated that microbial contamination was higher above standards recommended by national and international regulating bodies (WHO, 2007, European pharmacopoeia 6-0) in their study. According to them, microbial population may have increased due to water availability to the organism which got through products during preparation. The hygienic conditions for which these herbal drugs were prepared were questionable especially those not regulated. Microorganisms are present everywhere except areas where deliberate methods are used to eliminate, or hinder their growth. The vended herbal drugs were always exposed to the environment (air) especially when there was a ready buyer. The unpackaged state of these drugs served as avenue for microbial inoculation and growth especially in the liquid herbal products. In Table 2, three (16.67%) of the 18 regulated drugs studied were sterile, devoid of both fungi and bacteria. The following herbal drugs HDR 6, HDR 10 and HDR 16 had no bacteria present while in others, bacteria count ranged from 2.0 to 8.0×10^4. Similar result was obtained for mold and yeast count. HDR 4, HDR 10, HDR 12, HDR 13, HDR 14, HD 15 and HDR 17 were sterile. The highest mold/yeast count was observed for HDR 6 and HDR 8.

Out of the herbal products, 29.31% were solids (powdery) in which boiled water was added before consumption. For these herbal products, microbial quality ranged between 1.0×10^2- 9.0×10^4 for bacteria and 1.0×10^2- 3.0×10^4 for fungi which were within standards recommended by British Pharmacopoeia category 4(A) for herbal drugs in which boiling water was added before use. According to the standard, microbial population should not be more than 10^5 and 10^7 CFU/g, respectively, for

Table 1. Microbial quality of unregulated drugs

Sample code	Use	Nature	Aerobic microbial counts (CFU/ml OR CFU/g) 10^4	Total fungal counts	Microorganisms identified
HDUR 1	Oral	Liquid	2.0	1×10^4	*Rhizopus* spp., *S. aureus*
HDUR 2	Oral	Liquid	7.0	1×10^5	*Neurosora* spp., *Fusarium* spp., *Botrytum* spp., *Penillium* spp., *Bacillus* spp.
HDUR 3	Oral	Liquid	5.8	1×10^5	*Fusarium* spp., *Pseudomonas* spp.
HDUR 4	Oral	Liquid		1×10^2	*Aspergillus* spp., *S. epidermis*
HDUR 5	Oral	Liquid		1×10^3	*Neurospora* spp., *Klebsiella* spp.
HDUR 6	Oral	Liquid	4.0	1×10^5	*Fusarium, S. aureus, Proteus* spp., *E coli, Pseudomonas, Bacillus*
HDUR 7	Oral	Solid	4.0	1×10^2	*Aspergillus* spp., *Pseudomonas, Bacillus spp., E. coli*
HDUR 8	Oral	Solid	TNTC	1.5×10^5	*Aspergillus, Rhizopus, Bacillus* spp., *E. coli*
HDUR 9	Oral	Liquid	2.0	1×10^7	*Rhizopus* spp., *S. aureus*
HDUR 10	Oral	Liquid	7 .0	1×10^4	*Neurosora* spp., *Fusarium* spp., *Rhizopus* spp., *Penillium* spp., *Bacillus ssp.*
HDUR 11	Oral	Liquid	5.8	5×10^4	*Fusarium* spp., *Pseudomonas* spp.
HDUR 12	Oral	Liquid		5×10^2	*Aspergillus* spp., *S. epidermis*
HDUR 13	Skin	Molten		1×10^3	*Mucor* spp., *Bacillus* spp.
HDUR 14	Oral	Solid	4.0	1×10^3	*Alternaria* spp., *Fusarium* spp., *Mucor* spp., *Aspergillus* spp., *Penicilluim* spp., *Bacillus* spp.
HDUR 15	Oral	Solid	TNTC	-	*Bacillus* spp., *Proteus* spp., *E. coli*
HDUR 16	Oral	Liquid	9.0	-	*E. coli, Bacillus* spp., *Bacillus* spp.,
HDUR 17	Oral	Molten	2.6	-	*Bacillus* spp.
HDUR 18	Oral	Liquid	3.6	-	*Bacillus* spp., *P. aeruginosa, Bacillus* spp.
HDUR 19	Oral	Liquid	1.5	-	*P. aeruginosa, Bacillus* spp.
HDUR 20	Oral	Liquid		-	*Bacillus* spp.
HDUR 21	Oral	Molten		1×10^3	*Mucor* spp.
HDUR 22	Oral	Liquid	-	-	-
HDUR 23	Skin	Molten	-	-	-
HDUR 24	Oral	Solid	-	-	-
HDUR 25	Oral	Solid	-	-	-
HDUR 26	Oral	Solid		1.5×10^3	*Mucor* spp.
HDUR 27	Oral	Solid		-	-
HDUR 28	Oral	Solid	9.0	3.0×10^4	*Aspergillus* spp., *Penicillium* spp.
HDUR 29	Skin	Solid		-	-
HDUR 30	Oral	Molten	3.2	1.2×10^4	*Fusarium* spp.
HDUR 31	Skin	Solid	9.1	3.0×10^4	*Rhizopus* spp.
HDUR 32	Oral	Solid	-	-	-
HDUR 33	Skin	Molten	- 105.0	-	-
HDUR 34	Oral	Liquid	14.0	1×10^4	*Bacillus* spp., *E. coli, A. niger*
HDUR 35	Oral	Liquid	22.0	-	*E. coli, Pseudomonas* spp.
HDUR 36	Oral	Liquid	31.0	-	*Bacillus* spp., *S. aureus*
HDUR 37	Oral	Liquid	17.0	1×10^4	*Aspergillus* spp., *Rhizopus* spp., *Bacillus* spp., *E. coli*
HDUR 38	Oral	Liquid	10.0	1×10^4	*Rhizopus* spp., *Proteus* spp., *Bacillus* spp.
HDUR 39	Oral	Liquid	45.0	-	*Bacillus* spp., *P. aeruginosa, S. aureus*
HDUR 40	Oral	Liquid	45.0	-	*P. aeruginosa, E. coli*

bacteria and fungi, while *E. coli* should not be more than 10^2. This study was consistent with earlier study which revealed that microbial count and microorganisms were within WHO standard (Nwankwo and Olume, 2019). This report indicated that these herbal medicines were safe for the treatment of ailments to which they were prescribed for.

Tables 3 and 4 show the results of selective media used to access coliform count and presence of *Staphylococcus aureus, Pseudomonas aeruginosa*, *Salmonella* spp. and *Shigella* spp. The homemade (unregulated drugs) not packaged either in bottle or paper were having large population of *S. aureus* count compared to the one packaged (HDUR 5, HDUR 6, HDUR 13 and HDUR 14) though unregulated. In the regulated drugs, there was no bacterial count for *S. aureus, P. aeruginosa*, *Salmonella* spp. and *Shigella* spp. but *E. coli* count with detected for HDR 8, HDR 12, HDUR 14 and HDUR 8, HDUR 16; HDUR 18 in both regulated and unregulated, respectively. Other researchers detected *E. coli* in their research.

Table 2. Microbial quality of regulated drugs

Sample code	Use	Nature	Aerobic microbial counts (CFU/ml OR CFU/g) 10^4	Total fungal counts	Microorganisms identified
HDR I	Oral	Liquid	5.0	2×10^4	*Bacillus* spp.
HDR 2	Oral	Liquid	4.0	1×10^4	*Rhizpus* spp. *Aspergillus* spp., *S. aureus*
HDR 3	Oral	Liquid	4.0 9.0	1×10^4	*Neurosora* spp., *Aspergillus* spp., *E. coli*
HDR 4	Oral	Liquid	2.0 15.0	-	*P. vulgaris, Providentia* spp.
HDR 5	Oral	Liquid	2.0	1×10^4	*Penicillium* spp., *Aspergillus* spp., *S. epidermis*
HDR 6	Oral	Solid	-	1×10^4	*Alternaria* spp., *A. niger, E. coli, P. aeruginosa*
HDR 7	Oral	Liquid	7.0	1×10^4	*Aspergillus* spp. *Bacillus* spp., *Proteus* spp., *E. coli*
HDR 8	Oral	Solid	5.0	1×10^4	*A. niger, P. vulgaris, E. coli, Proteus* spp.
HDR 9	Oral	Liquid	5.0	1×10^4	*Aspergillus* spp., *Candida* spp., *Bacillus* spp.
HDR 10	Oral	Liquid		-	-
HDR 11	Oral	Liquid	3.0	1×10^4	*Botrytium* spp.
HDR 12	Oral	Liquid	16.0	-	*Bacillus* spp. *E. coli*
HDR 13	Oral	Liquid	1.5	-	*Proteus* spp.
HDR 14	Skin	Solid	3.7	7	*Bacillus* spp. *Staphylococcus* spp.
HDR 15	Oral	Solid	-	-	-
HDR 16	Oral	Solid		1×10^4	*Mucor* spp.
HDR 17	Skin	Solid	3.1	-	-
HDR 18	Oral	Liquid	8.0	1×10^4	*Fusarium* spp.

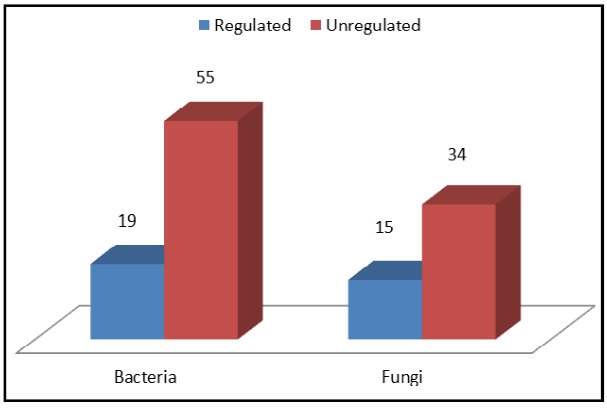

Fig. 1. Microbial population in regulated and unregulated drugs.

Hassan *et al.* (2021) detected 24.4%, Shiaka *et al.* (2018) detected *E. coli* in all herbal samples in their study but 22.4% in this study. *Pseudomonas aeruginosa* was detected in 9/74 (12.16%) herbal products. No *P. aeruginosa* was detected in regulated drugs in this study (Figs. 2 and 3). The percentage of *P. aeruginosa* in this study was low compared to other study of 20% (Onyemelukwe *et al.*, 2019). The most prevalent bacteria was *Bacillus* spp. 25/74 (33.78%) which agreed with study of Nwankwo and Olime (2019) whose studies detected mostly *Bacillus* species. *S. aureus* was 12/74 (16.21%), *Proteus* spp. 8/74 (10.81%) while *Klebsiella* spp. and *Providencia* spp. was 1/74 (1.35%). *Salmonella* and *Shigella* spp. were not detected in any of the herbal drugs which contrasted studies of Adounkpe *et al.* (2017) and de Sousa Lima *et al.* (2020) but agreed with Shu *et al.* (2019) who did not detect *Salmonella* spp. and *Shigella* spp. in herbal products studied. The fungi population from highest to lowest included *Aspergillus* spp. 15/49 (30.61%), *Rhizopus* spp. 10/49 (20.41%), *Fusarium* 8/49 (16.32%) and *Mucor* 4/49 (8.16%). Detecting these fungi in herbal products is not only dangerous but may pose potential health risk to the consumers since these products are taken orally and administered topically on the skin. These fungi produce mycotoxins which are carcinogenic. *Aspergillus* spp cause diseases in immunocompromised population having noted that the elderly population take more of these drugs especially in the developing world.

The presence of the mentioned organisms showed that these herbal products need to be standardized during processing, production and packaging. When the manufacturers follow good manufacturing practices, standardization of the materials for product and packing, these herbal products may meet standards set by regulating agents. The higher population of microbes in the unregulated vended herbal medicine showed that there was need for education of these producers on good manufacturing practices for the safety of unsuspecting population who patronize these vendors which are very common in most cities

Table 3. Specific organisms from unregulated drugs

Sample code	*E. coli* count	*S. aureus* count	*P. aeruginosa* count	*S. shigella* count
HDUR 1			-	-
HDUR 2			-	-
HDUR 3			-	-
HDUR 4			-	-
HDUR 5	-	8.0	-	-
HDUR 6		5.0	-	-
HDUR 7			-	-
HDUR 8	2.0		-	-
HDUR 9			-	-
HDUR 10			-	-
HDUR 11			-	-
HDUR 12			-	-
HDUR 13		7.0	-	-
HDUR 14		3.0	-	-
HDUR 15			-	-
HDUR 16	10.0		-	-
HDUR 17			-	-
HDUR 18	6.0		-	-
HDUR 19			-	-
HDUR 20			-	-
HDUR 21			-	-
HDUR 22			-	-
HDUR 23			-	-
HDUR 24			-	-
HDUR 25			-	-
HDUR 26			-	-
HDUR 27			-	-
HDUR 28			-	-
HDUR 29			-	-
HDUR 30			-	-
HDUR 31			-	-
HDUR 32				-
HDUR 33			-	-
		10^5	-	-
HDUR 34		14.0		-
HDUR 35		22.0	-	-
HDUR 36		31.0	-	-
HDUR 37		17.0	-	-
HDUR 38		10.0	-	-
HDUR 39		45.0	-	-
HDUR 40		45.0	-	-

in Delta State and even Nigeria. Methods to ensure microbiological safety of the drug need to be published widely to get vendors intimated and health of the entire population.

CONCLUSION

Herbal products sold in Delta State are promising and show minimal microbial contamination. *Salmonella* spp. and *Shigella* spp. were absent in all drugs. Contrarily, *P. aeruginosa* was detected in regulated drugs. Unpackaged unregulated drugs were

Table 4. Specific organisms from regulated drugs

Sample code	*E. coli* count	*S. aureus* count	*P. aeruginosa* count	*S. shigella* count
HDR I	-		-	-
HDR 2			-	-
HDR 3			-	-
HDR 4			-	-
HDR 5			-	-
HDR 6			-	-
HDR 7			-	-
HDR 8	5.0		-	-
HDR 9			-	-
HDR 10			-	-
HDR 11			-	-
HDR 12	2.0		-	-
HDR 13			-	-
HDR 14	1.0		-	-
HDR 15			-	-
HDR 16			-	-
HDR 17			-	-
HDR 18			-	-

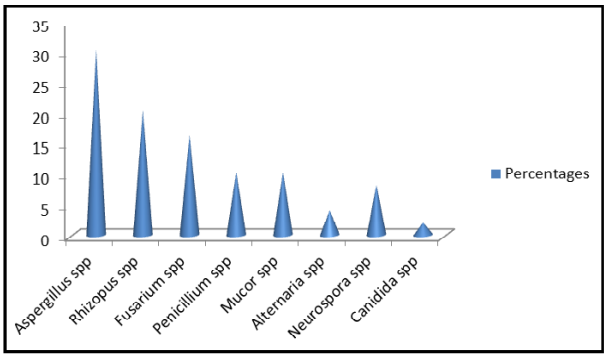

Fig. 2. Percentage population of fungi in the herbal drugs.

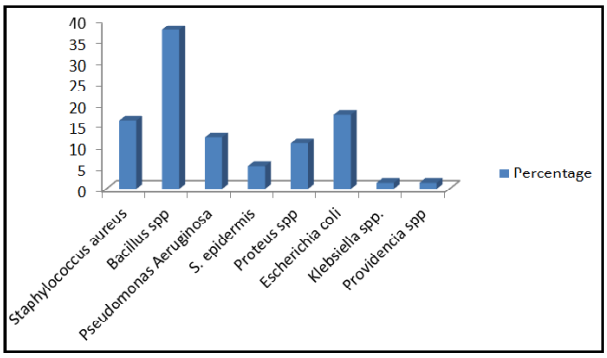

Fig. 3. Percentage population of bacteria in the herbal drugs.

contaminated with *S. aureus*. Herbal products need to be standardized using scientific based methods in processing, packaging and production. Packaging of herbal products in sterile pack could reduce the population of organisms in homemade drugs. The vendors of herbal products need to be enlightened on

good manufacturing practices for safe herbal products for the benefit of the public.

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