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Assessment of microbiological status of some herbal medicines sold in Calabar, Nigeria

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ABSTRACT

Aim: The upsurge of cases of adverse reactions, complications and herbal drug- induced infections in developing countries, due to the use of herbal remedies , have generated deep public health concern about the microbiological status and safety of the products. The aim of this study was to assess the level of microbial contamination of herbal medicines commonly sold in Calabar, Nigeria.
Methodology and results: A total of 28 herbal medicines comprising 16 liquid and 12 solid dosage forms were evaluated for microbial contaminants. The isolation of microbial contaminants was carried out using standard plate techniques, while specific microbial genera were enumerated on appropriate selective media. The microorganisms were identified using standard identification protocols with the aid of identification and taxonomic manuals. The results showed that 71.43% of the samples yielded high counts of viable microorganisms, which exceeded the tolerable limit stated in United State Pharmacopoeia. The total viable microbial count of all the solid dosage forms ranged from 2.05 x 10^4 to 5.6 x 10^4 CFU/g. Of the 28 samples studied, 18 samples (64.29%) contained heterotrophic bacteria and molds, respectively, while coliforms and yeast were present in 15(53.57%) and 12(42.86%) of the samples. The microorganisms recovered and their percentage occurrence in the solid and liquid dosage forms were *Pseudomonas aeruginosa* (11.54, 18.31%); *Bacillus subtilis* (17.31, 11.27%); *Klebsiella* sp. (5.77, 9.86%); *Staphylococcus aureus* (15.38, 14.08%); *Escherichia coli* (3.85,12.60%);*Salmonella* sp.(3.85,9.86%); *Enterobacter* sp. (5.77, 7.04%); *Aspergillus flavus* (5.77,2.82%) *Aspergillus niger* (11.54, 4.23%); *Fusarium* sp. (5.77,2.82%) and *Candida tropicalis* (3.85, 1.41%).
Conclusion, significance and impact of study: Microbial contaminants may cause spoilage of the herbal drugs and hence pose serious health risk to consumers. Therefore, adequate quality control measures and good manufacturing practice must be employed by herbal drugs producers, while relevant regulatory agencies should closely monitor the production processes, to ensure that microbiologically safe herbal drugs are marketed.

Keywords: Herbal medicines, microbial contamination, *Aspergillus flavus*, public health, quality control

INTRODUCTION

The use of herbal drugs in Nigeria is becoming increasingly popular and gaining momentum especially among the rural dwellers and low income earners, which form about 70% of the population (Esimone *et al*., 2007). Some of the reasons for the high patronage of herbal medicines include inaccessibility to orthodox medicines, high cost of orthodox medicines and services, and fear of side effects of/addiction to some synthetic drugs (Okunlola *et al*., 2007). Hence people use herbal medicines and traditional remedies as alternative to the modern medical interventions to improve and maintain health naturally. Also, most traditional healers do not request for payment until after the treatment is given. Hence patients may prefer traditional healers to orthodox health providers who often require payment before service, even when the treatment is ineffective.

Herbal drugs are concoctions from plant parts such as the roots, stems, leaves, flowers and seeds, or a combination of these parts. These concoctions are usually prepared in alcohol or water and taken by patients without much consideration to the toxicity level of the bioactive components and microbiological quality. Hence, some herbal products may cause severe toxic effects in patients or become “vehicles” for transmission of pathogenic microorganisms that may cause serious illnesses in human when ingested with the concoctions (Gupta *et al.*, 2012). In order to address the safety issues related to herbal drugs, the World Health Organization, in 1998 advocated for the integration of herbal products into the primary health care system of developing countries (WHO, 1998). However, these issues have been

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constantly ignored by the herbalists. Their herbal medicines are usually prepared under very doubtful hygienic conditions, which may result in microbial contamination of the final product. Gupta *et al.* (2012) reported that since plants, from which herbal medicines are made, are natural products, they may undergo some form of spoilage. Such spoiled plant materials may harbour some pathogenic microorganisms which can contaminate the herbal medicines produced from the spoiled plant materials. They also identified unscientific methods of cultivation and collection, inappropriate harvesting and cleaning, unsuitable transportation, prolonged drying and storage, inadequate hygiene of producers and congenital climatic conditions as factors which predispose herbal drugs to microbial contamination.

Although some people believe that herbal medicines are safer than synthetic drugs, the number of reports of people experiencing negative effects, caused by the use of herbal medicines has been increasing. One of the major causes of adverse effects is directly linked to the poor quality of herbal medicines, especially contamination with pathogenic microorganisms, and this is of public health concern.

Few published works exist on microbial quality of herbal medicines sold in some parts of Nigeria (Aziz *et al.*, 1998; Esimone *et al.*, 2007; Okunlola *et al.*, 2007; Oluyege and Adelabu, 2010). The outcome of the various investigations reveal microbial contaminants in crude herbal medicines and in some cases beyond the WHO's permissible limit. However, there is no published work on microbiological status of herbal medicines sold in Calabar, Nigeria, despite the high number of the product marketed in the city, with attendant increase in the number of reported cases of adverse reactions as a result of the use of the herbal drugs. It appears that insufficient attention is being paid to the quality assurance and control of herbal medicines marketed in Calabar. The upsurge in the use of herbal medicines in Calabar despite the aforementioned drawbacks, has generated much concern about their safety, quality and effectiveness. Therefore, it is justifiable to assess the microbiological status of some herbal medicines sold in Calabar metropolis. The aim of this study is to determine the microbiological status of the herbal medicines sold in Calabar, based on WHO's guidelines and the United States Pharmacopeoial standards for microbial contaminants in herbal medicines.

MATERIALS AND METHODS

Culture media

The culture media used in this study were the Oxoid brand of cetrimide agar, Sabouraud dextrose agar, mannitol salt agar, MacConkey agar, nutrient agar, brilliant green agar. The chemicals (brilliant green, potassium iodide and iodine) were analytical grades obtained from Sigma-Aldrich Corporation, Bangalore, India.

Collection of samples

Twenty eight different herbal dosage forms were randomly purchased from four herbal medicine shops in Calabar metropolis, Nigeria. The samples comprised 16 liquid and 12 solid forms. The solids were in the form of powder, capsules and tablets, while the liquids were in the form of solutions and suspensions as shown in Table 1.

Preparation of samples

All the samples were prepared under aseptic conditions using standard procedures. All the solid dosage forms were aseptically ground to produce powder. Exactly 10 g of each powdered sample was dispersed in sterile peptone water and made up to 100 mL with the same medium. Similarly, 10 mL of each liquid dosage form was aseptically transferred into separate sterile 100 mL flask and sterile peptone water was added to the 100 mL mark. Ten-fold serial dilutions, up to 10^{-4} were carried out on the samples and 1.0 mL of the last two dilutions were plated in duplicate and incubated under appropriate conditions.

Isolation and identification of microbial contaminants in the herbal medicines studied

The methods of Gupta *et al.* (2012) were employed, with some modifications, in the isolation and identification of microbial contaminants of public health interest from the herbal preparations. One milliliter of aliquot of each of the last two dilutions (10^{-3} and 10^{-4}) of the samples were mixed in duplicate with 15ml of appropriate molten plate count agar previously cooled to 45 °C , and allowed to solidify. Molds and yeasts were detected on Sabouraud dextrose agar while aerobic bacteria were detected on soybean casein digest agar. Specific bacterial genera were enumerated using appropriate selective media. For example, mannitol salt agar was used for enumeration of *Staphylococcus aureus*, cetrimide agar for *Pseudomonas aeruginosa*, differential MacConkey agar eosin methylene blue agar for *Escherichia coli*, *Salmonella-Shigella* medium for *Salmonella* sp. Sabouraud dextrose agar impregnated with 30 µg of chloramphenicol was used to enumerate yeasts in conjunction with cultural and morphological characteristics, and germ tube test. The identities of the microorganisms were confirmed using Bergey's Manual for Determinative Bacteriology (Holt *et al.*, 1994), Barnett and Hunter (1982) and Kreger-van's Manual for Yeast Taxonomy (Kreger-van, 1984).

RESULTS

The results of the mean viable counts of microorganisms isolated from the herbal medicines are presented in Table 2. The total viable bacterial counts (TVBC) of the solid dosage forms ranged from 2.2×10^4 to 5.6×10^4 CFU/g, while the TVBC of the liquid samples ranged from 3.8×10^4 to 6.8×10^4 CFU/mL. A total of 36 bacterial and 14 fungal isolates were recovered from the herbal medicines

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Table 1: Description of the herbal drugs studied.

Code	Dosage form	Packaging	Date of manufacture	Expiry date	Country of manufacture	NAFDAC number	Therapeutic claim
1.	Capsule	Blister	Oct 2012	Oct 2015	Nigeria	No	Immune booster, Energizer
2.	Solution	Bottle	Jul 2011	Jul 2014	Nigeria	Yes	Antimalarial
3.	Powder	Sachet	–	–	Nigeria	No	Pile
4.	Suspension	Bottle	Sep 2012	Sep 2015	Nigeria	No	Diabetes, yellow fever
5.	Solution	Bottle	Jun 2011	Jun 2014	Nigeria	Yes	Ezcema, body rashes
6.	Capsule	Blister	Aug 2012	Aug 2015	Nigeria	No	Hypertension
7.	Solution	Bottle	–	–	Nigeria	No	Puritis, allergies
8.	Tablet	Bulk	Nov 2011	Nov 2014	Nigeria	Yes	Cough, chronic fever
9.	Powder	Sachet	Oct 2012	Oct 2015	Nigeria	No	Typhoid fever
10.	Powder	Sachet	–	–	Nigeria	No	Haemorrhoids
11.	Solution	Bottle	Jan 2013	Jan2016	Nigeria	No	Ringworm, Acne
12.	Solution	Bottle	May2011	May 2014	Nigeria	Yes	Measles
13.	Solution	Bottle	–	–	Nigeria	No	Waist pain, menstrual pain
14.	Powder	Sachet	Dec 2010	Dec 2013	Nigeria	No	Pile, haemorrhoids
15.	Solution	Bottle	Feb 2011	Feb 2014	Nigeria	No	Skin rashes
16.	Solution	Bottle	Apr 2012	Apr 2015	Nigeria	Yes	Toothache
17.	Powder	Sachet	–	–	Nigeria	No	Teething problem
18.	Powder	Sachet	Oct 2010	Oct 2013	Nigeria	Yes	Fever, cough
19.	Solution	Bottle	Jun 2009	Jun 2012	Nigeria	Yes	Antimalarial
20.	Solution	Bottle	–	–	Nigeria	No	Fever, measles
21.	Solution	Bottle	May 2011	May 2014	Nigeria	Yes	Menstrual disorder
22	Suspension	Bottle	Mar 2012	Mar 2015	Nigeria	Yes	Energizer, immune booster
23	Powder	Sachet	–	–	Nigeria	No	Dysentery, stomachache
24	Solution	Bottle	Feb 2010	Feb 2013	Nigeria	No	Skin rashes
25	Capsule	Blister	Aug 2011	Aug 2014	Nigeria	No	Hypertension, diabetes
26	Tablet	Bulk	Jul 2011	Jul 2014	Nigeria	No	Brain fatigue
27.	Solution	Bottle	Oct 2010	Oct 2013	Nigeria	No	Eye ache, stomachulcer
28.	Solution	Bottle	Mar 2011	Mar 2014	Nigeria	Yes	Menstrual disorder, painful menstruation, internal heat.

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studied. Based on standard identification protocols employed in this investigation, the organisms isolated from the herbal products were *Salmonella* sp., *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *Klebsiella* sp., *Enterobacter* sp., *Aspergillus flavus*, *A. niger*, *Rhizopus* sp.,*Mucor* sp., *Fusarium* sp. and *Candida tropicalis.* Of the 28 herbal preparations studied, 20 samples, representing 71.43%, were contaminated. Nineteen samples, representing 67.86% contained heterotrophic bacteria, molds and coliforms were present in 15 (53.57%) and 12 (42.86%) of the samples respectively, while yeasts were present in 8 (28.57%) of the samples as shown in Table 3. The prevalence of the different microorganisms in the herbal medicines is depicted in Table 4. The most prevalent organism in solid dosage form was *B. subtilis*(48.7%), while *P. aeruginosa* occurred in most liquid dosage forms with 52.6% prevalence. Table 5 shows the distribution of the different microbial contaminants in the herbal products analyzed. Sample 10 was the most contaminated product, containing all the microbial groups shown in Table 4. This sample was claimed to be effective against hemorrhoids, even though it was not registered by the National Agency for Food and Drug Administration and Control (NAFDAC). However, samples no. 3,14,16,20,21,24,27 and 28 did not contain any microbial contaminant.

DISCUSSION

The outcome of this study reveals that 71.43% of the herbal drugs analyzed were contaminated with different kinds of microorganisms. The level of microbial contamination of the solid and liquid dosage forms clearly exceeded the tolerable limit listed in United State Pharmacopoeia[30] (2007). The sources of contamination might be from the natural raw materials used in the formulation of the herbal products, low processing standard, untreated water used in mixing the excipients or poor handling of the final products by personnel.A similar report has been made by Westwood (1971) and Enayatifard (2009) during their studies on microbiological

Table 2: Mean viable counts of microorganisms isolated from herbal medicines.

Code	Dosage form	Mean viable counts of microorganisms (x10^{4}CFU/g or /mL)*			
		Heterotrophic bacteria	Coliforms	Molds	Yeast
1	Capsule	2.72 ± 0.16	0	2.17 ± 0.73	0
2	Solution	4.20 ± 0.11	3.81 ± 0.03	0	0
3	Powder	0	0	0	0
4	Suspension	5.76 ± 0.22	2.34 ± 0.28	2.46 ± 0.08	0
5	Solution	0	4.24 ± 0.16	3.21 ± 0.18	0
6	Capsule	0	0	5.23 ± 0.17	0
7	Solution	2.68 ± 0.68	3.32±0.03	2.94±0.48	0
8	Tablet	3.85±0.14	3.60±0.74	2.53±0.27	0
9	Powder	2.81±0.02	2.70±0.13	0	2.84±0.18
10	Powder	3.72±0.26	2.49±0.64	2.25±0.06	2.34±0.24
11	Solution	6.36±0.17	3.71±0.48	3.15±0.02	0
12	Solution	4.65±0.02	2.88±0.05	0	0
13	Solution	6.80±0.15	0	0	0
14	Powder	0	0	0	0
15	Solution	4.26±0.04	0	2.75±0.01	0
16	Solution	4.81±0.06	2.78±0.13	2.62±0.27	2.26±0.08
17	Powder	3.65±0.12	2.46±0.48	0	0
18	Powder	2.61±0.07	2.34±0.01	0	0
19	Solution	3.80±0.62	0	2.98±0.24	2.50±0.16
20	Solution	0	0	0	0
21	Solution	0	0	0	0
22	Suspension	3.61±0.63	2.46±0.74	2.12±0.27	0
23	Powder	0	0	2.73±0.19	0
24	Solution	0	0	0	0
25	Capsule	4.65±0.44	0	2.40±0.16	0
26	Tablet	5.18±0.53	0	4.74±0.36	0
27	Solution	0	0	0	0
28	Solution	0	0	0	0

*Values are mean ± standard deviation from three replications

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quality of some pharmaceutical raw materials and some herbal products respectively.

In this study, some pathogenic microorganisms were recovered from the herbal products. The presence of these pathogens in numbers far above the acceptable limits constitutes a serious health hazard. Of particular public health concern is the presence of *Salmonella* sp*., Staphylococcus aureus*, *Escherichia coli and Aspergillus flavus.* These organisms are the causative agents of harmful diseases. According to Abba *et al.* (2009), *Salmonella* is a leading cause of food poisoning, which is now a global problem. Of equal severity is typhoid fever, caused by some serotypes of *Salmonella*, which has increased in incidence, especially in developing countries including Nigeria.

The occurrence of toxigenic mold, *Aspergillus flavus*, in herbal drugs is of clinical significance as it can elaborate toxic aflatoxins into these products. When such contaminated products are consumed, they can cause liver cancer and systemic shock in susceptible patients notably geriatrics, children and other immunocompromised patients. The presence of other members of the family Enterobacteriaceae in the products might have been as a result of the use of animal manure, untreated sewage and chicken droppings to fertilize the farms from which the medicinal plants were cultivated and harvested. These sources may contain a wide variety of pathogenic organisms which may survive for a long period of time in the soil and thus, increase the chances of contaminating the medicinal plants. Previous studies by Goyal *et al.* (1977) and Gudva *et al.* (1998) showed that the only known source of plant contamination was plant contact with contaminated water. However, recent studies reveal that *Salmonella typhimurium* can infect plant cells and multiply inside the plant cells (Solomon *et al.*, 2002). They can also survive upto 900 days in contaminated soils and infect the tissues of plant materials(Gupta *et al.*, 2012). Therefore, ordinary washing of the surfaces of plant materials such as roots, barks and leaves is not sufficient to completely eradicate microbial contaminants. Oluyege and Adelabu (2010) in a similar study reported a 60% and 40% contamination by *E. coli* and *Salmonella* sp. respectively, which were 43.55% and 26.29% higher than the percentage contamination by similar organisms recorded in this study.

Table 3: Extent of microbial contamination of herbal medicines studied.

Dosage form	Number examined	Number contaminated	Number of samples contaminated with:			
			Heterotrophic bacteria	Coliforms	Molds	Yeast
Capsule	3	3	2	0	3*	0
Powder	7	5	5	4	3	3
Solution	14	8	8	5	5	5
Suspension	2	2	2	2	2	0
Tablet	2	2	2	1	2	0
Total	28	20(71.43%)	19(67.86%)	12(42.86%)	15(53.57%)	8(28.57%)

*Some samples yielded more than one microbial type.

Table 4: Prevalence of microbial contaminants in herbal medicines.

Microorganism	Number of isolates in:		Prevalence (%) in:	
	SDF	LDF	SDF	LDF
Pseudomonas aeruginosa	6	13	11.54	18.31
Bacillus subtilis	9	8	17.31	11.27
Klebsiella sp.	3	7	5.77	9.86
Staphylococcus aureus	8	10	15.38	14.08
Escherichia coli	2	9	3.85	12.68
Salmonella sp.	2	7	3.85	9.86
Enterobacter sp.	3	5	5.77	7.04
Aspergillus flavus	3	2	5.77	2.82
Aspergillus niger	6	3	11.54	4.23
Rhizopus sp.	3	2	5.77	2.82
Mucor sp.	2	2	3.85	2.82
Candida tropicalis	2	1	3.85	1.41
Fusarium sp.	3	2	5.77	2.82
Total	52	71	100	100

SDF, Solid dosage form; LDF, Liquid dosage form.

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Table 5: Distribution of microbial contaminants in herbal medicines.

Code	Dosage form	Microorganisms present												
		A	B	C	D	E	F	G	H	I	J	K	L	M
1	Capsule	-	+	-	+	-	-	-	-	-	+	-	-	-
2	Solution	+	-	+	-	+	+	-	+	-	+	-	-	-
3	Powder	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Suspension	+	+	-	+	+	-	+	-	-	-	+	-	-
5	Solution	+	-	+	-	+	-	+	-	-	+	-	-	-
6	Capsule	-	+	-	-	-	-	-	-	+	-	-	+	-
7	Solution	+	-	+	-	+	-	-	-	-	-	+	-	-
8	Tablet	+	+	+	-	+	+	+	+	-	-	-	-	-
9	Powder	-	+	+	+	-	+	-	+	-	-	-	-	+
10	Powder	+	+	-	+	-	-	+	-	-	+	-	-	-
11	Solution	+	+	-	+	+	-	+	-	-	-	-	-	-
12	Solution	+	-	+	-	+	-	+	-	-	-	-	-	+
13	Solution	+	-	-	+	-	+	-	-	-	-	-	+	-
14	Powder	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Solution	+	-	+	-	+	-	-	-	-	-	-	-	-
16	Solution	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Powder	-	+	+	+	-	-	+	-	-	+	-	-	+
18	Powder	+	-	-	-	+	-	-	-	+	-	-	+	-
19	Solution	-	+	-	+	-	+	-	-	-	-	-	-	-
20	Solution	-	-	-	-	-	-	-	-	-	-	-	-	-
21	Solution	-	-	-	-	-	-	-	-	-	-	-	-	-
22	Suspension	+	+	-	+	-	+	-	-	+	-	-	-	-
23	Powder	-	+	-	+	-	-	-	+	+	-	-	-	-
24	Solution	-	+	-	+	-	-	-	-	-	-	-	-	-
25	Capsule	-	-	-	-	-	-	-	-	-	-	-	-	-
26	Tablet	+	+	-	+	-	-	-	-	+	-	-	-	-
27	Solution	-	-	-	-	-	-	-	-	-	-	-	-	-
28	Solution	-	-	-	-	-	-	-	-	-	-	-	-	-

+, Present; -, Absent; A , *Pseudomonas aeruginosa*; B, *Bacillus subtilis*; C, *Klebsiell*a sp.; D, *Staphylococcus aureus;* E, *Escherichia coli*; F,*Salmonella* sp.; G, *Enterobacter* sp.; H, *Aspergillus flavus*; I, *Aspergillus niger*; J, *Rhizopus* sp.; K, *Mucor* sp; L, *Candida tropicalis*; M, *Fusarium* sp.

The total viable counts recorded in the liquid dosage form were higher than those recorded in the solid dosage form. Similarly, the total number of microbial isolates recovered from the liquid drugs was higher than those recovered from the solid dosage form. This increase is obvious and expected because moisture is an essential requirement for microbial growth and proliferation. In the absence of adequate moisture most microorganisms go into dormancy or die.

The extent of mold contamination of the herbal drugs was 53.57%, while *Bacillus* contaminated 60.71% of the samples tested. The level of contamination by these organisms recorded in this study is higher than those recorded by Esimone *et al.* (2007), who reported 15% and 28.4% mold and *Bacillus* contaminations respectively. The disparity in the two results may be due to differences in the type of herbal drugs evaluated, methods of preparation by the herbalists and the environment in which the drugs were prepared. Mold and *Bacillus* sp. formed the bulk of microbial contaminants in the solid dosage form. The high prevalence of these organisms in solid dosage forms could be explained by the fact that they produce spores which can withstand rigorous processing and low moisture conditions. Therefore, they can survive for a long time in the herbal product in a dormant form. A similar explanation was given by Stevic *et al.* (2012). In this study, some of the products with NAFDAC registration number were contaminated. It appears therefore that NAFDAC approved the sales of the herbal drugs based on their therapeutic efficacy, without much regards to their microbiological safety. More worrisome is the presence of toxigenic mold, *Aspergillus flavus*,in antimalaria solution with NAFDAC registration number. It is possible that the producers of herbal drugs might have used fake NAFDAC numbers on their products to deceive people and give their products wide acceptability. The contaminants might have originated from contaminated plant materials, untreated water source, improper processing, poor storage facilities, unhygienic handling by personnel and dirty production environment. Some of these microorganisms, example, *Salmonella* sp.,*Staphylococcus aureus*, *Escherichia coli* and *Aspergillus flavus*, are very harmful and adversely affect human health and drug quality.

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CONCLUSION

Based on the outcome of this study, it can be concluded that most of the herbal drugs sold in some Nigerian markets are contaminated with various kinds of microorganisms and thus, microbiologically unsafe for consumption. Microbial contamination often lead to reduced performance of the product owing to the disruption of the therapeutic effects of the dosage form, alteration of physical characteristics and appearance, and inactivation of the active ingredients in the formulation. Such therapeutically altered products can cause loss of confidence by the consumers. The methods of preparation of the herbal drugs, the equipment and plant materials used contribute to the contamination of herbal medicines, hence there is a need to improve the quality of plant materials and sanitize the production areas. There should be proper in-house microbiological controls at all stages of production of herbal medicines to the marketing of the final product. Also the products should be regularly monitored by relevant agencies such as NAFDAC, National Drug Law Enforcement Agency (NDLEA), and Ministry of Health, to ensure that only standard, high quality and microbiologically stable herbal drugs are sold for human consumption.

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