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Bacteriological analysis and efficacy assessment of liquid herbal mixtures sold in Anyigba, Kogi state, Nigeria

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ABSTRACT

Objectives: The use of herbal treatment is an old practice usually employed in diseases treatments. It is sometimes referred to as complementary and alternative medicine (CAM). The aim of this study was to bacteriologically assess some liquid herbal mixtures sold in Anyigba, Kogi State, Nigeria.

Materials and method: Eight (8) liquid herbal mixtures (4 NAFDAC registered and four unregistered) were purchased from different vendors in Anyigba and analyzed for their bacteriological quality by determining the levels of bacteria in them. Aliquots of the various serially diluted herbal products were cultured on nutrient agar media plates.

Results: Total bacterial count of $4.0*10^3$ – $5.2*10^4$ CFU/ml, total coliform count of 2.0 x 102 to 4.4 x 105CFU/ml. The result showed that 75% of the registered herbal mixtures met the World Health Organization standard of liquid herbal drugs while 28% of the unregistered ones met the WHO standard. There was a significant difference between the total heterotrophic bacterial count and total coliform of registered and unregistered drugs. Isolates gotten from both samples includes Enterobacter spp, Ancinetobacter spp, Staphylococcus spp, Bacillus spp. The antimicrobial susceptibility test by agar well diffusion methods showed that Escherichia coli and Pseudomonas spp was the most susceptible.

Conclusion: The findings from this research work emphasized the need for constant quality assessment of herbal drugs on sale in order to ensure the production of therapeutic products of human consumption. The findings of this study showed that registered herbal mixtures have better bacteriological quality than the herbal mixtures have better bacteriological quality than the unregistered ones. It also showed that the herbal mixtures tested had high level of activity against some of the tested organisms. This implies that the herbal mixtures are safe for consumption but might not necessarily treat target infection.

Keywords: *Enterobacter* spp, *Ancinetobacter* spp, *Staphylococcus* spp, *Bacillus* spp

INTRODUCTION

The use of herbal treatment is an old practice usually employed in diseases treatments. It is sometimes referred to as complementary and alternative medicine. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing diseases. The World Health Organization (WHO) estimates that 4 billion people (about 80% of the world population) use herbal medicine for some aspect of primary health care.[1] The term "herbal drugs" denotes

plants and plant parts that have been converted into phytopharmaceuticals by means of some simple processed state as active ingredients and may contain excipients (foreign substance).[2] Similarly, the European Medicine Evaluation Agency defines herbal preparations as medicinal products containing exclusive herbal drugs or herbal drug preparations as active substances.[3]

Herbal medicine has been known to be used to treat many conditions, such as asthma, eczema, irritable bowel syndrome, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, and cancer among others.[4] Widespread use of herbal medicine to ensure continued access especially for rural communities, without compromising patient's safety.[5] Interest in medical plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being. In Nigeria, even though, there is proliferation of herbal preparations in Nigeria that do not have the required expertise to perform quality control on the preparation that they produce. This brings about the problem of inconsistency on the quality of the herbal preparation in the country.

With the ever increasing use of herbal medicines and the global expansion of the medicines, market safety has become a concern for both health authorities and the public in many countries. This is because many contaminants and residues that may cause harm to the consumers have been reported.[6] The quality assessment of herbal medications is therefore very important to justify their acceptance in modern system of medicine. It is thus necessary that the microbiological limit test of herbal medicinal preparations be done to ensure that the product is free from risk. Some of the formulations are not subject to aseptic conditions during various stages of preparation, packaging, storage, and transportation, as required by regulatory norms of National Agency for Food and Drug Administration and Control (NAFDAC) which aims at achieving high standards of quality of food and drugs, including herbal preparation in Nigeria. Plants and plant materials also carry huge number of organisms mainly originating from the soil. Aerobic sporulating bacteria frequently predominate in this to which additional contamination and microbial growth occur during harvesting, handling, and production.[7] It is important that physicians and herbalists have the knowledge about the microbiological safety of these preparations.[8]

MATERIAL AND METHODS

Sample collection

Eight different brands of liquid herbal mixtures (four NAFDAC registered ones and four unregistered ones) were purchased from different sellers in Anyigba, Kogi State. The registered herbal mixtures were bought from shops, while the unregistered herbal mixtures were bought from vendors and market. It was then transported immediately to Microbiology laboratory of Kogi State University, Anyigba for analysis.

Preparation of culture media

All media used were prepared according to manufacturer's stipulation following standard laboratory ethics.

Source of test isolate

The test organisms were collected from the microbiological laboratory Kogi State University, Anyigba Nigeria. The organism includes *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa*.

Characterization identification of bacterial isolate

The bacterial isolates were identified by Gram staining techniques and other biochemical tests. Pure colony of bacteria isolates was characterized and identified based on, colonial morphology, cellular morphology, and biochemical characteristics (indole test, methyl red test, citrate utilization test, catalase test, coagulase test, oxidase test, and motility test).

Method of Gram staining

A clear grease free slide was prepared, air dry, and heat fixed smear on staining tray. Smear was gently flooded with crystal violet for 1 min. The slide was slightly tilt and gently rinse with tap water or distilled water using a wash bottle. The smear was gently flooded with gram's iodine and was left for 1 min. The slide was slightly gently rinse with distilled water using a wash bottle. The smear appeared as a purple circle on the slide. It was decolorized using 95% ethyl alcohol for 10–30 s. The slide was slightly rinsed safranin was added and allowed to stand for 1 min. The slide was slightly and gently rinsed with distilled water. The slides are air dry, blot dry, and observed under the microscope.[9]

Microbiological analysis

The sealed bottle of herbal preparations was cleaned with 70% ethanol before opening to prevent contamination. Microbial quality was determined by standard plate count method as described by Zakari *et al*.[10] Total viable bacterial count and total coliform count were determined as described.[7] Each liquid preparation was serially diluted to get a ten-fold dilution before being plate out in duplicate using concentrations of 10^{-2} and 10^{-3} for bacterial count; the plates will then be incubated at 37°C for 24 h. Nutrient agar (NA) was used to count for total viable bacteria.

Test for coliforms

Coliform agar was used to test for the presence of coliforms and total coliform count.

About 100 ml of herbal mixture sample were poured through the filter and placed on the plate agar (on MI agar) and also incubated for about 24 h at 35°C.

For this technique, the filter membrane is used to filter and thus retain any coliform bacteria and total coliform count that may be present in the samples.

From each sample, 0.1 ml was taken from the dilutions 10^{-1} CFU/ml to 10^{-2} CFU/ml, it was introduced into the NA by means of spread plate technique. The plates were, then, incubated at 37°C for 24 h. Moreover, the colonies were counted.

Antimicrobial activity assay of the herbs

Agar well diffusion method was performed to determine the antimicrobial activity of the herbal samples. Test organisms (*Pseudomonas* spp., *Staphylococcus* spp., *Escherichia* spp., *and Salmonella* spp.) were spread properly to evenly distribute the bacteria culture over the surface of the sterile Mueller–Hinton Agar plates and allowed to dry for 15 min. Four wells were made in the agar using sterile cork borer. Sample solutions from the dilutions 10^{-1} CFU/ml were introduced into the wells, along with a positive control (streptomycin) and a negative control (Water). The presence of antimicrobial activities was determined by the presence of clear zones around the bored wells and the diameter of these zones was measured using a meter rule.[11]

Table 1: Microbial counts of the registered liquid herbal mixtures (CFU/ml).

S. No.	Product code	Bacteria	Coliform	Probable organism
1.	IB	-	-	-
2.	BE	-	-	-
3.	GO	$5.2*10^4$	$1.0*10^3$	*Staphylococcus* spp.
4.	7K	$4.0*10^3$	-	*Bacillus* spp.

-: No growth, IB: Iba Africa, BE: Beta, Go: Goko, 7K: 7 Key

Table 2: Microbial count of the unregistered liquid herbal mixtures (CFU/ml).

S. No.	Product code	Bacteria	Coliforms	Probable organism
1.	AL	$4.8*10^4$	$1.0*10^5$	*Bacillus* spp.
2.	TH	$6.8*10^4$	$1.0*10^4$	*Bacillus* spp.
3.	HM	$4.0*10^5$	-	*Bacillus* spp.
4.	EN	$1.48*10^3$	$3.4*10^4$	*Bacillus* spp.

-: No growth, AL: Al'madinah herbal mixture, TH: Traditional herbal medicine, HM: Healer mixture, EN: Enyo-ojo herbal mixtures

RESULTS

The result of the microbial load of the different herbal mixtures is shown in [Tables 1 and 2]. The mean heterotrophic count of the different herbal samples ranged from $1.48*10^5$ CFU/ml to $6.8*10^4$CFU/ml**.**

DISCUSSION

The samples were contaminated to varying degrees with bacteria which are in agreement with previous works on microbial quality by Czech *et al.*[12] Two of the four NAFDAC registered herbal preparations were free from microbial contamination. This may be due to natural barriers and antimicrobial substances of different chemical nature as oils, peptides, liquid, and organic extracts contained by certain plants which exert typical inhibitory effects on microbial growth and stability as observed by Idu *et al.*[13] According to the WHO standards, values of the microbial limits should not exceed 10^5 CFU/ml for total aerobic bacteria and 10^3 CFU/ml for Enterobacteria, whereas *Salmonella* and *E. coli* should totally be absent.[13] Seventy-five percentages of registered products met these specifications.

All the unregistered samples were not sterile as they had bacterial contamination. This is unlike the registered samples, where two samples were sterile even after repeated tests. This observation is similar to the observation of Onyambu *et al.*[14] who observed that unregulated herbal drugs were all contaminated beyond limits.

The characteristics of the isolated organisms as shown in Table 3 in this study are in agreement with the previous

Table 3: Morphological and Biochemical Characteristics of Bacterial isolates from all the liquid herbal mixtures and their gram reaction.

Indole	Methyl red	Motility	Morphology	Motility	Citrate	Glucose	Oxidase	Catalase	Probable organism	Methyl red
Rods	+	−	+	+	+	+	−	+	+	*Bacillus* spp.
Coccus	+	−	−	−	−	−	−	+	+	*Staphylococcus* spp.
Rods	+	−	−	−	+	+	−	+	+	*Bacillus* spp.
Rods	−	+	+	−	−	+	−	−	+	*Acinetobacter* spp.
Rods	−	−	−	−	+	+	−	−	−	*Enterobacter* spp.

Keys: = negative + = positive

works on characterization (*Bacillus* spp., *Staphylococcus* spp., *Acinetobacter* spp., and *Enterbacter* spp.) as shown in Table 4; all the named pathogens isolated from the indigenous herbal samples in this study have been implicated in the previous studies on gastroenteritis and other transmissible diseases.[14]

Rajapandiyan *et al.*[15] also implemented *Enterobacter asburiae* as an opportunistic pathogen in extraintestinal infections associated with diarrhea in children and also in nosocomial infections. Therefore, the high recovery rates of these suspected perilous bacteria from indigenous orally consumed herbal medications could be of clinical relevance as shown in Table 5. *Acinetobacter baumannii* is also an opportunistic pathogen and is becoming increasingly important in nosocomial infections. They are equally known to be multidrug resistant.[13] *Bacillus* species are known to cause gastrointestinal infection which is characterized by diarrhea. It has been reported by Rajapandiyan *et al.*[15] that *Bacillus subtilis* is the most predominantly isolated from herbal preparation. This could be due to the fact that they are spore producing organisms.

Table 4: Colonial and Microscopic Characteristics of microorganism from the Herbal Mixtures

Colony Characteristics	Microscopic View	Probable Organism
Colonies are rod-shaped circular colony of these bacteria is rough, opaque, whitish or slightly yellow, flat.	The rods are Gram-positive	*Bacillus* spp
Colonies are round, convex, grow in clusters, pairs, and occasionally in short chains	Gram-positive cocci about 0.5–1.0 μm in diameter	*Staphylococcus* spp.
Colonies are 1–2 mm, Domed and nonpigmented	Gram-negative, non-fermenting coccobacilli	*Acinetobacter* spp.
Smooth, irregular, round to rough, "cauliflower" type colonies	Rod-shaped bacilli, gram-negative	*Enterobacter* spp.

The high level of microbial contamination observed from the unregistered samples in this study may be attributed to the methods of their preparation as observed by Rocha *et al.*[16] The soil, harvesting, drying, improper handling, and storage conditions influence the microbial quality of herbal drugs. The presence of microbial contaminant in the mixtures can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect patients.

CONCLUSION

This result has shown that unregistered herbal mixtures marketed in Anyigba are highly contaminated with microorganisms, some of which are pathogenic compared to the regulated ones. Such unregistered herbal mixtures products may facilitate transmission of communicable disease in the population and therefore present a public

Table 5: Antimicrobial Activity Assay of the Liquid Herbal mixtures

	Registered Herbal Mixture Zone of inhibition (mm)					Unregistered Herbal Mixture Zone of inhibition (mm)				
Test Organism	**Sample**	**10^1 (cfu/ml)**	**10^2 (cfu/ml)**	**Streptomycin (+ve)**	**Water (-ve)**	**Sample**	**10^1 (cfu/ml)**	**10^2 (cfu/ml)**	**Streptomycin (+ve)**	**Water (-ve)**
Staphylococcus Aureus	IB	-	-	-	-	AL	10	10	35	-
	BE	-	-	-	-	TH	10.5	10.2	-	-
	7K	20	10.5	20	-	HM	-	-	40	-
	GO	-	15	20	-	EN	-	-	-	-
Escherichia coli	IB	-	-	20.6	-	AL	10.4	10	20.9	-
	BE	20	20	30.5	-	TH	-	-	20.6	-
	7K	-	-	30	-	HM	-	10.2	20	-
	GO	-	-	20.5	-	EN	20	10	20.7	-
Salmonellatyphi	IB	-	-	-	-	AL	10.6	10	35	-
	BE	20.5	20	-	-	TH	10	-	20.4	-
	7K	-	10	-	-	HM	20	10.6	25	-
	GO	-	-	-	-	EN	-	20	23	-
Pseudomonas aeruginosa	IB	-	-	20	-	AL	-	10	20.4	-
	BE	-	10.5	-	-	TH	10.1	9.5	20.9	-
	7K	-	-	-	-	HM	-	-	NG	-
	GO	-	-	-	-	EN	10.8	10.5	NG	-

KEY: According to Kirby Bauer agar well diffusion method: 15mm = highly active (resistant), 11-14mm= moderately active, 0-10mm= no activity, - = no growth

health problem. Most of the herbal medicines analyzed in this study would be consumed at room temperature and do not get heated to above 60°C before consumption, thereby increasing risk of food-borne infections.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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