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Short Communication **Open Access**

Comparative distribution of bacterial contaminants of packaged and unpackaged polyherbal products sold in Nnewi, Nigeria

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Abstract:

Background: The use of herbal medicine continues to remain popular despite advances in orthodox medicine largely as a result of affordability and availability. However, contaminated and potentially toxic polyherbal preparations remain a public health challenge despite regulations instituted by concerned agencies in Nigeria. The objective of this study was to determine and compare the bacterial contaminants of different polyherbal products sold in Nnewi, Nigeria
Methodology: This study evaluated the bacteriological profile of 22 packaged and 22 unpackaged polyherbal preparations sold in Nnewi, Nigeria. The samples were collected from different herbal medicine shops in Nnewi by simple random sampling and were assayed for comparative bacterial loads with chromogenic media and their total viable counts evaluated following standard method for microbial load analysis.
Results: Bacterial contaminants were isolated from 9 of 22 (40.9%) packaged polyherbal samples while 13 of 22 (59.1%) samples were bacteriologically sterile. For the unpackaged polyherbal, bacterial contaminants were isolated from 18 of 22 (81.8%) samples while 4 of 22 (18.2%) were bacteriologically sterile (OR 0.1538, p=0.0122). The most frequently isolated bacterial contaminant in the packaged polyherbal samples was *Enterococcus faecalis* with 33.3% (6/18) while *Salmonella* sp was the least frequently isolated with 5.6% (1/18). For the unpackaged polyherbals, the most frequently isolated bacterial contaminant was *Staphylococcus aureus* with 25% (7/28) while *Salmonella* sp and *E. faecalis* were the least frequently isolated with 10.7% (3/28) each. The median total viable count of the packaged group of the polyherbal products was $1.48x10^6$ CFU/ml, while the median total viable count for unpackaged group of polyherbals was $1.95x10^6$ CFU/ml.
Conclusion: This study shows that many polyherbal products sold in Nnewi are potentially contaminated with bacterial agents. It is therefore imperative that herbal medicine practitioners be enlightened on hygienic ways of preventing microbial contamination during polyherbal production.

Keywords: Bacterial contaminants, herbal products, Nnewi, Nigeria

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Distribution comparative des contaminants bactériens des produits polyherbal emballés et non emballés vendu à Nnewi, Nigeria

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Abstrait:

Contexte: L'utilisation de la phytothérapie continue de rester populaire malgré les progrès de la médecine orthodoxe en grande partie en raison de l'abordabilité et de la disponibilité. Cependant, les préparations à base de plantes contaminées et potentiellement toxiques restent un problème de santé publique malgré les réglementations mises en place par les agences concernées au Nigeria. L'objectif de cette étude était de déterminer et de comparer les

contaminants bactériens de différents produits polyherbal vendus à Nnewi, Nigeria
Méthodologie: Cette étude a évalué le profil bactériologique de 22 préparations polyherbal emballées et 22 non emballées vendues à Nnewi, Nigeria. Les échantillons ont été collectés dans différents magasins de plantes médicinales de Nnewi par simple échantillonnage aléatoire et ont été analysés pour les charges bactériennes comparatives avec les milieux chromogènes et leurs dénombrements viables totaux évalués selon la méthode standard pour l'analyse de la charge microbienne.
Résultats: Des contaminants bactériens ont été isolés dans 9 des 22 échantillons polyherbal emballés (40,9%) tandis que 13 des 22 échantillons (59,1%) étaient bactériologiquement stériles. Pour le polyherbal non emballé, des contaminants bactériens ont été isolés de 18 des 22 échantillons (81,8%) tandis que 4 des 22 (18,2%) étaient bactériologiquement stériles (OR 0,1538, *p*=0,0122). Le contaminant bactérien le plus fréquemment isolé dans les échantillons polyherbal emballés était *Enterococcus faecalis* avec 33,3% (6/18) tandis que *Salmonella* sp était le moins fréquemment isolé avec 5,6% (1/18). Pour les polyherbals non emballés, le contaminant bactérien le plus fréquemment isolé était *Staphylococcus aureus* avec 25% (7/28) tandis que *Salmonella* sp et *E. faecalis* étaient les moins fréquemment isolés avec 10,7% (3/28) chacun. Le nombre total viable médian du groupe emballé des produits polyherbal était de $1,48x10^6$ UFC/ml, tandis que le nombre total viable médian pour le groupe non emballé de polyherbales était $1,95x10^6$ UFC/ml.
Conclusion: Cette étude montre que de nombreux produits polyherbal vendus à Nnewi sont potentiellement contaminés par des agents bactériens. Il est donc impératif que les praticiens en phytothérapie soient éclairés sur les moyens hygiéniques de prévenir la contamination microbienne pendant la production de polyherbes.

Mots-clés: Contaminants bactériens, produits à base de plantes, Nnewi, Nigéria

Introduction:

Herbal medicine is a medication made from herbs and has long been used as a source of alternative medicines in developed, developping and underdeveloped countries. Throughout the ages, humans have turned to herbal medicine for healing. All societies have folk medicine traditions that include the use of plants and plant products. Many licensed drugs used today in conventional medicine originated from herbal products.

The World Health Organization (WHO) estimates that about 4 billion or at least 80% of the world's population use herbal preparations for some aspects of primary health care (10). In Nigeria, herbal therapy remains a popular alternative in many traditional communities where orthodox medicine is not affordable (2). Herbal medicine practitioners in Nigeria use various herbal preparations to treat various types of ailments including diarrhoea, urinary tract infections, typhoid fever and skin diseases (8). Unfortunately, many of these herbal medicine practitioners do not follow hygienic procedures in preventing microbial contaminants during production of their polyherbal products. The study is designed to comparatively evaluate bacteriological contaminants of packaged and unpackaged polyherbals sold in Nnewi, Nigeria.

Materials and method:

Collection of polyherbal samples

A total of 44 samples of liquid formulations of the polyherbals produced in Nigeria were purchased from 16 different herbal shops and trado-medical hawkers, who were selected by simple random sampling within Nnewi town. The samples of the packaged polyherbal preparations (n=22) were purchased while samples of the unpackaged extemporaneous polyherbal preparations (n=22) were collected in polythene bags that are used to dispense products to customers by the herbal medicine practitioners. All samples were labeled and immediately transported to the Faculty of Health Science Laboratory of the Nnamdi Azikiwe University, Okofia, Nnewi.

Estimation of total viable count of bacteria

A tenfold dilution of each sample of polyherbal was achieved by adding 1ml of each sample to 9ml of sterile normal saline in the first test tube of a row of 10 sterile tubes. One (1) ml from the first tubes on each row was then transferred to the 2nd test tube after proper mixing continuing up to the 10th tube where one (1) ml of the mixture was discarded to achieve a $1/10^1$ to $1/10^{10}$ dilutions. One (1) ml of the dilution from each test tube was then transferred into a sterile Petri dish and molten nutrient agar was added, the constituents were well mixed and incubated aerobically at 37°C for 24 hours. The number of colonies on each plate was counted and the mean for each sample was established and recorded as the mean colony forming units (CFU) per ml.

Isolation and identification of bacterial contaminants in the polyherbal preparations.

The isolation and identification of the bacterial agents was done by culture on two commercial chromogenic media; CHROMagar™ Orientation and HARDYCHROM™ SS NOPRO agar, which have been validated to have positive and negative predictive values of 99.3% and 100% respectively for the isolation and identification of the bacterial organisms such as *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli, Proteus* sp, *Salmonella* sp, *Citrobacter* sp, *Serratia marcescens*, *Providencia* sp, *Acinetobacter* sp, and

Pseudomonas aeruginosa which are common contaminants in polyherbal preparations (1,5). The media were prepared according to the manufacturer's instructions.

Briefly, labeled individual samples of both the packaged and unpackaged herbals were well mixed to ensure complete homogenization before culture. A loopful of each of the samples was streaked on the prepared agar plates using sterile wire loop. Incubation was done aerobically at 37ºC for 24 hours. A sterile non-inoculated plate was also placed in the incubator for quality control during incubation (3). Plates were read after 24 hours and bacteria were identified by their peculiar and different colony colours and chromogenic attributes using the colour charts as a guide (7), in determining the bacterial isolates (1). The identification parameters of bacterial colonies on the media based on colour are; *Klebsiella* sp colonies appeared metallic blue, *Enterococcus faecalis* colonies as red, *Proteus mirabilis* colonies as clear and brown halo, and *Salmonella* spp as pink.

Analysis of data

The data were presented in frequency tables and statistical analysis performed with IBM SPSS 20.0 version. Chi square test was used to measure association of bacterial contamination with packed and unpackaged polyherbals, and *p* value less than 0.05 was considered to be statistically significant.

Results:

The frequency of distribution of bacterial contaminants in the packaged and unpackaged polyherbal samples is shown in Table 1. Bacterial contaminants were isolated from 9 of 22 (40.9%) packaged polyherbal samples while 13 of 22 (59.1%) samples were bacteriologically sterile. For the unpackaged polyherbals, bacterial contaminants were isolated in 18/22 (81.8%) samples while 4/22 (18.2%) were bacteriologically sterile (OR 0.1538, *p* =0.0122). Of the 9 positive samples in the packaged polyherbals, a total of 18 bacterial isolates were recovered, *E. faecalis* was the most frequent with 33.3% (6/18), followed by *S. aureus* 16.7%, *E. coli* 16.7%, *Proteus* sp 16.7%, *K. pneumoniae* 11.1% and *Salmonella* sp 5.6%. Of the 18 positive samples in the unpackaged polyherbals, a total of 28 bacterial isolates were recovered, *S. aureus* was the most frequent with 25% (7/28), followed by *K. pneumoniae* 17.9%, *E. coli* 17.9%, *Proteus* sp 17.9%, *E. faecalis* 10.7% and *Salmonella* sp 10.7% (Table 1).

Table 2 shows the distribution of the polyherbal products from the 16 herbal shops and trado-medical hawkers, and the bacteria isolates recovered from those positive. Table 3 shows the total viability count (TVC) for each of the packaged herbal product that cultured positive for bacteria with a mean TVC of 1.48 x 10^6 CFU/ml, while Table 4 shows the TVC for each of the unpackaged herbal product that cultured positive for bacteria, with a mean TVC of 1.85 x 10^6 CFU/ml.

Discussion:

Data obtained from this study showed that bacterial agents such as *K. pneumoniae*, *E. faecalis, S. aureus, E. coli, P. mirabilis* and *Salmonella* sp were isolated at varying frequencies in both groups of polyherbals. These

Table 1: Frequency distribution of bacterial isolates in packaged and unpackaged polyherbal samples

No of polyherbals/ bacterial isolates	Packaged (%) (n=22)	Unpackaged (%) (n=22)	X^2	*P*
No bacteria isolate	13 (59.1)	4 (18.8)	7.8	0.01*
No positive for bacterial isolate	9 (40.9)	18 (81.2)		
Klebsiella pneumoniae	2 (11.1)	5 (17.9)		
Enterococcus faecalis	6 (33.3)	3 (10.7)		
Staphylococcus aureus	3 (16.7)	7 (25)		
Escherichia coli	3 (16.7)	5 (17.9)		
Proteus sp	3 (16.7)	5 (17.9)		
Salmonella sp	1 (5.6)	3 (10.7)		
Total isolates	**18 (100)**	**28 (100)**		

X^2= Chi square; *statistically significant

Table 2: Distribution of bacterial contaminants in polyherbals purchased at the herbal shops

Herbal shops	Polyherbals	Bacterial isolates
Dan-Iyke	Katoka, Ruzu bitters, Yoyo bitters	No growth
Blessed Mother	Blood purifier, Super bitters	No growth
Dr Chiagozie	Nando mixture, Super bitters, Mako cleanser, Super 7, Ruzu bitters	*Enterococcus faecalis*
Panx	Goko cleanser, dukun care, Dr Igah cleanser	*E. coli*, *Klebsiella* sp, *E. faecalis, S. aureus*
Fesco	Deep root, Bitterkinga	No growth
Dr Agnes	J.M.I herbal, Museya, Jalin herbal	*E. faecalis*, *Klebsiella* sp, *E. coli*, *Proteus* sp
Eze	Eze herbal mixture, new beta cleanser	*E. coli*, *Proteus* sp
Dr Benbella	Weifa body defense	No growth
Baba Oyo	Olori herbal mixture	*E. faecalis, S. aureus, Proteus* sp
Yemi	Anti-pile, Anti-diabetic, fibroid	*E. coli, Klebsiella* sp, *E. faecalis Salmonella* sp
Dan Obitube	Convulsion formula, general well-being, blood booster, energy booster	*E. coli, E. faecalis*
Titi	Fertility preparation, Laxative preparation	*S. aureus*, *Proteus* sp
Barakat	Anti-ulcer, STI preparation, Abdominal preparation	*S. aureus, Proteus* sp
Laide	Skin infection, menstruation prep	*Proteus* sp
Baba Osun	Male fertility, sexual health, anti-pile, back pain	*E. coli, S. aureus, Klebsiella* sp, *Salmonella* sp
Sunny	Anti-gonorrhoea, STD preparation, anti-malaria	*E. coli, Klebsiella* sp

Table 3: Total Viable Counts in packaged polyherbal samples and their bacteriological safety

Packaged polyherbals	Total Viable Count (CFU/ml)	Bacteriological Safety Level (10^5)
Deep Root	No Isolate	Safe
Blood purifier	No Isolate	Safe
Jalin herbal mixture	$1.5x10^6$	Unsafe
J.M.I herbal mixture	$3.6x10^6$	Unsafe
Mako Cleanser	No Isolate	Safe
Super 7	$1.4x10^6$	Unsafe
Museya bitters	No Isolate	Safe
Dr Igah Bitter cleanser	$0.7x10^6$	Unsafe
Goko Cleanser	No Isolate	Safe
New Beta cleanser	No Isolate	Safe
Infection destroyer	$1.3x10^6$	Unsafe
Dr sunny Gonorrhea herbal	$1.8x10^6$	Unsafe
Dukun Care	$1.7x10^6$	Unsafe
Katoka Mixture	No Isolate	Safe
Eze herbal	$3.2x10^6$	Unsafe
Dr Nando	No Isolate	Safe
Super bitters	No Isolate	Safe
Ruzu bitters	No Isolate	Safe
Weifa body defense	No Isolate	Safe
Bitterkinga	No Isolate	Safe
Yoyo bitters	No Isolate	Safe
Mean Total Viable Count (TVC) = $1.4845 \pm 122.17x10^6$		

Table 4: Total viable counts in unpackaged polyherbal samples and their bacteriological safety

Unpackaged Polyherbals	Total Viable Counts (CFU/ml)	Bacteriological safety Level 10^5
Anti-malaria preparation	$2.4x\ 10^6$	Unsafe
Convulsion formula	$3.3x10^6$	Unsafe
General Well-being formula	No Isolate	Safe
Anti-Pile preparation	$2.3x10^6$	Unsafe
Anti-Diabetic preparation	No Isolate	Safe
Fertility preparation	$2.1x10^6$	Unsafe
Blood booster	$1.9x10^6$	Unsafe
Back pain preparation	$3.1x10^6$	Unsafe
Laxative preparation	$8.9x10^6$	Unsafe
General well-being	$1.8x10^6$	Unsafe
Anti-Gonorrhoea preparation	$2.9x10^6$	Unsafe
Anti-ulcer preparation	$1.6x\ 10^6$	Unsafe
Anti-malaria preparation	$2.0x\ 10^6$	Unsafe
Male fertility preparation	$1.8x10^6$	Unsafe
Sexual health preparation	$3.7x10^6$	Unsafe
General wellness	$3.0x10^6$	Unsafe
STI preparation	$3.2x10^6$	Unsafe
Abdominal disturbance preparation	No Isolate	Safe
Skin infection preparation	No Isolate	Safe
Fibroid preparation	$1.2x10^6$	Unsafe
Menstruation preparation	$1.9x10^6$	Unsafe
Energy booster	$1.7x10^6$	Unsafe
Mean Total Viable Count (TVC) = $1.856\pm113.27x10^6$		

results are similar with the findings of Esimone et al., (4) and Tatfeng et al., (9), both of whom detected bacterial agents at varying frequencies in herbals and polyherbals samples in Nigeria. The predominance of *E. faecalis* and *S. aureus* in the packaged and unpackaged herbals respectively is in consonance with the study by Esimone et al., (4) who investigated the microbiological quality of liquid herbal preparations in south-eastern Nigeria and isolated arrays of microbial contaminants including *S. aureus* and *E. faecalis* as the most predominant bacterial contaminants of herbal medicines.

The higher frequency of contamination observed in the unpackaged group of polyherbals (81.8%) and higher median total viable count (TVC) compared to those of the packaged group may be attributable to contamination due to lack of standardization and quality control, poor personnel hygiene and handling, and use of contaminated water and raw materials (4). These findings could also be due to the fact that the packaged polyherbal products are comparatively better regulated by government agencies such as the National Agency for Food and Drug Administration and Control (NAFDAC) and the State Ministries of Health who usually mandate the manufacturers of packaged polyherbals to adopt some level of good manufacturing procedure, safe handling measures during production and quality control (6).

This study shows that many polyherbal products sold in Nnewi are potentially contaminated by bacterial agents, some of which are potential pathogens of man. It is imperative that herbal medicine practitioners be enlightened on hygienic ways of preventing microbial contamination during polyherbal production.

References:

1. Berger, D., Clasen, R., Cuna, A., Knox, J., and Hardy, J. Evaluation of Novel Chromogenic medium for the isolation and differentiation of *Salmonella* and *Shigella* spp. Presented at the 111th General Meeting of American society for Microbiology, New Orleans, LA. 2011**:** 3601
2. Burkill H. M. The Useful Plants of West Africa 3rd ed. London. Royal Botanical Gardens; Kew, Richmond, United Kingdom. 1997: 377.
3. Cowan, S. T., and Steel, K. J. Manual for identification of medical bacteria. Vol 2, 2nd edn; Cambridge University Press; London. 1974: 15-47.
4. Esimone, C. O., Shah, K. F., and Ikejide, F. C. Microbial quality of herbal preparations marketed in South Eastern Nigeria. Journal of Natural Remedies. 2001: 2 (1): 42-48.
5. Filius, P., Van Netten., D., Roovers, J., Vulto, G., Gyssens, C., Verbrugh, A., and Endtz, P. Comparative evaluation of three chromogenic agars for detection and rapid identification of aerobic gram-negative bacteria in the normal intestinal microflora. Clinical Microbiology and Infection. 2003: 9 (9): 912-918.
6. National Agency for Food and Drug Administration and Control. Standard Operating Procedures (NAFDAC SOP). Determination of moisture contents. Yaba, Lagos: Central Drugs and Vaccine Control Laboratory (CDVCL); 2000: 1–2.

7. Singh, A. K., and Bhunia, A. Optical scatter patterns facilitate rapid differentiation of Enterobacteriaceae on CHROMagar™ Orientation media. Microb Biotechnol. 2016; 9 (1): 127 - 135.
8. Sofowora, A. Medicinal plants and traditional medicine in Africa. Ibadan: Spectrum books Ltd (pub); 1993: 50–195.
9. Tatfeng, M., Ollama, H., and Ojo, O. Microbial burden of some herbal antimalarial marketed at Elele, Rivers State. J Afr Trad Compl Altern Med. 2010: 7(2): 149–152.
10. World Health Organization (WHO). Regulatory situation of herbal medicines: A worldwide review. Geneva: World Health Organization, 1998.