

Original article

# Bacterial contamination of orally-consumed crude herbal remedies: A potential source for multi-drug resistant pathogens in man

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## Abstract

**Objective:** The acceptability of herbal remedies for alleviating discomforts and ill-health has become very popular, on the account of the increasing cost of allopathic medicine for personal health maintenance. The observable non-adherence of herbalists to the established World Health Organization (WHO) / National Agency for Food and Drug Administration Control (NAFDAC) regulations for the quality control of herbal medicines is an issue for concern. In view of this, 34 popular and widely consumed crude herbal remedies in southwestern, Nigeria were screened for compliance with standard limits for bacterial contamination, bacteria flora and their antibiotic susceptibility pattern. **Methods:** Isolates recovered from samples were identified using the cultural, morphological and biochemical characteristics. They were also tested for drug sensitivity using standard procedures. **Results:** A heavy bacteria load ranging from  $3.00 \times 10^3 - 9.58 \times 10^5$  CFU/ML and  $1.20 \times 10^5 - 5.41 \times 10^5$  CFU/ML was observed for water and spirit extracted preparations respectively. The bacteria flora cum contaminants were: *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Lactobacillus plantarum*, *Klebsiella pneumoniae*, *Escherichia coli*, *streptococcus*, *Shigella*, *Neisseria*, *Arthrobacter*, *Kurthia* and *Clostridium* species. All the isolates were multi-drug resistant (MDR) strains. **Conclusion:** The crude herbal preparations consumed in Nigeria failed to comply with the internationally recognized standards regarding bacteria load and flora. The presence of MDR pathogens is of greatest concern. It poses a great risk to consumer's health and could be a source of introducing MDR organisms into the human population. There is the need for the enforcement of established guidelines to ensure the safety of these preparations.

**Keywords:** Herbal remedies; Bacterial contamination; Multi-drug resistant bacteria; Antibiotic susceptibility; Quality control

## INTRODUCTION

Infectious diseases are major causes of death disability, social and economic disruption for millions a-

round the World<sup>[1-3]</sup>. It has accounted for 14 to 17 million of deaths in the developing countries<sup>[1, 3, 4]</sup>. The initial discovery of antibiotics brought lots of relief to sufferers. However, the realization that chemical medicines are not always magic bullets is making herbalism and traditional medicine to make a comeback.

Recent surveys have shown that traditional and alternative medicines have become increasingly popu-

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lar in Africa. The rationale for this seems to be due to their holistic belief that health of body, mind and spirit are related and should be taken into account by the health care provider<sup>[5]</sup>. The broad use of traditional medicine is also attributable to its accessibility and affordability. A 1991 survey by the United States Agency for International Development (USAID) found that in sub-Saharan Africa, traditional practitioners outnumber allopathic practitioners by 100 to 1<sup>[6]</sup>. Moreover allopathic practitioners are located primarily in cities or other urban areas, so majority of the rural dwellers depend mostly on traditional medicines. This problem of lack of access to allopathic health care is further exacerbated by severe financial constraints, the high debt burden, a rapidly growing population, political instability, high inflation rates, declining real income and deteriorating growth rates<sup>[5]</sup>. In Africa "Herbalism" is based on the use of plants and plant extracts and the practice predates the advent of modern medicine<sup>[7]</sup>. This has become increasingly acceptable worldwide during the last few decades as evidenced by the rapidly growing global and national markets of herbal drugs. In a WHO estimates, the present demand for medicinal medicine is about US \$ 60 billion - US \$ 80 billion a year<sup>[6]</sup>. Almost half the population in many industrialized countries now regularly use some form of traditional medicine. This represents about 42% in the United States<sup>[8]</sup>, Australia 48%<sup>[9]</sup>, France 49%<sup>[10]</sup>, and Canada 70%<sup>[11]</sup>. However, considerable use exists in many developing countries; China 40%, Chile 71%, Colombia 40% and up to 80% in African countries<sup>[6]</sup>.

Traditional medicine has provided therapeutic intervention to restore balance and facilitate the body's own healing responses as documented by several workers<sup>[12-15]</sup>. In spite of the great success associated with the use of herbal medicines, the sale of products of assured quality control could do much to reduce risk. The presence of pathogenic organisms in traditional medicine has been documented<sup>[16, 17]</sup>. In Nigeria however, same has been reported in samples sold in the South-Eastern Nigeria and Lagos metropolis<sup>[18, 19]</sup>. The present study deals with crude herbal medicines prepared by the indigenous people and are hawked indiscriminately in public places in four states of South-Western, Nigeria. This increasing concern, over the perceived lack of adequate regulation of herbal medicines and the sale of unreg-

istered products amount to a safety issue. Although the WHO has guidelines for the quality control of herbal drugs, there is still a big gap between this available knowledge and implementation which results in inferior quality of herbal preparations and drugs. The study therefore assessed the bacterial quality of popular traditional herbal medicines, hawked and consumed in South-Western parts of Nigeria.

## MATERIALS AND METHODS

### Sample collection

Thirty-four herbal remedies sold for health conditions like fever, fistula, rashes, convulsion, cough, sore throat, typhoid fever, backache, arthritis, yellow fever, jaundice, erectile dysfunction and general health maintenance were used in this study. Twenty of these were water extracted while the remaining 14 were extracted with spirits e. g. dry gin. The samples were collected based on their popularity and use by the local population in four states (Oyo, Ogun, Osun and Lagos), Southwestern, Nigeria. Medicines sold in re-used plastic containers were kept at room temperature in different shops. These were purchased and taken to the laboratory where they were processed under aseptic conditions.

### Isolation, enumeration and identification

Pretreated samples were diluted using phosphate buffer and the  $10^{-3}$  dilution was used for plate count<sup>[20]</sup>. Subsequently isolates were purified and identification was achieved using the cultural, morphological and biochemical characteristics. The tentative identification was obtained using the Bergey's manual<sup>[21]</sup>. The enumeration data obtained was compared to the officially prescribed Indonesian<sup>[22]</sup>, Malaysian<sup>[23]</sup>, German<sup>[24]</sup> and World Health Organization<sup>[20]</sup> limits for herbal medicines.

### Antibiotic susceptibility profiles

Drug susceptibility tests were performed with the commercially available Fondoz multodisks (Fondoz Laboratories) on the Mueller Hinton agar. The method of Bauer<sup>[25]</sup> was used. The antibiotics include Amoxicillin, Ofloxacin, Streptomycin Chloramphenicol, Ceftriazone, Gentamycin, Pefloxacin, Cotrimoxazole, Ciprofloxacin and Erythromycin for the gram positive isolates. However, Tetracycline,

Ofloxacin, Pefloxacin, Nitrofuratoin, Gentamycin, Erythromycin, Ciprofloxacin, Ceftriazone, Cotrimoxazole and Augmentin were used on the gram negative isolates. Zones of inhibition lower than 11mm were regarded as showing resistance. The percentage resistance to each drug was calculated by dividing the total number of resistant organisms by the overall total of organisms assayed, and then multiplied by 100%.

## RESULTS

The total bacterial count of crude medicinal samples revealed a microbial load ranging from  $3.00 \times 10^3$  –  $9.58 \times 10^5$  CFU/ML and  $1.40 \times 10^4$  –  $5.41 \times 10^5$  for water and spirit extracted medicines respectively. The enumeration data obtained were compared to the officially prescribed Indonesian, Malaysian, German and WHO<sup>27</sup> limits for herbal medicines. On the overall, the German limit which permitted a higher bacteria level revealed that the extracts were acceptable for human consumption, while the contrary was observed on comparison with the Indonesian standard. Also 70 % and 64 % of water and spirit extracted concoctions were safe by the Malaysian quality control standard, while the WHO limit had 50 % and 79% acceptability for the two extracts respectively. Also the type of solvent used did not significantly affect the total load and survival of bacterial contaminants. Eight-one (81) isolates were recovered, with forty-one (50.6 %) being gram positive while the remaining 40 (49.4 %) were gram negative. The identification procedures grouped the contaminants into: *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Escherichia coli*, *Lactobacillus plantarum*, *Klebsiella pneumoniae*, *Streptococcus*, *Shigella*, *Neisseria*, *Arthrobacter*, *Kurthia* and *Clostridium* species. The *S. aureus* and *E. coli* were the predominant bacteria flora, each accounting for 17.3 % (14) of total isolate. This was followed by *K. Pneumoniae*, *P. aeruginosa*, *L. plantarum*, *Kurthia* sp, *M. luteus*, *Neisseria* sp, *Clostridium* sp, *B. cereus* and *B. subtilis* with 12,9,6,5,5,4,4,3,2 isolates respectively. However, the *Streptococcus*, *Shigella* and *Arthrobacter* species had only one isolate each. The result obtained for the antibiotic susceptibility was quite revealing. The isolates were all found to be multi-drug resistant

(MDR) strains. They were resistant to both the first, second and third generation of antibiotics. The resistance was however more pronounced in the gram negative organisms. The gram positive isolates exhibited resistance of 0 % to 100 %. The newer drugs (Ciprofloxacin, Pefloxacin, Ofloxacin and Cotrimoxazole) had some inhibitory effects while 100 % resistance was found to the earlier first and second generation drugs like Gentamycin, Amoxicillin, Streptomycin, Erythromycin, Ceftriazone and Chloramphenicol (Table 1). The antibiotics resistance pattern was highly alarming among the gram negative isolates. They exhibited resistance of 64 % to 100 %. None of the isolate was found susceptible to Nitrofuratoin, Gentamycin, Amoxycillin, Ceftriazone, Cotrimoxazole and Augmentin. Generally Ofloxacin and Pefloxacin exhibited some degree of inhibition on all the organisms screened with the exception of the *Shigella* and *Neisseria* species (Table 2). The MDR nature of the isolated bacterial could pose a great health risk to infections resulting from these contaminants.

## DISCUSSION

The high bacterial load observed in the herbal sample suggests the absence of good manufacturing practices during the traditional medicine preparation. The medicines were found to be unfit for human consumption using the Indonesian and Malaysian standards. However, the bacterial load of some of the herbal medicines was satisfactory using the German and WHO limits. This high bacterial load according to Govender et al<sup>[17]</sup>, is an indication of low environmental sanitation and low standard of processing during preparation of the medicines. Herbal preparations normally carry a number of bacteria originating in the soil, the poor methods of harvesting, cleaning, drying, handling and storage may also cause additional contamination. Roy<sup>[26]</sup> also observed that the traditional, unscientific methods of preparing, storing and marketing herbal preparations have promoted their association with microbial contaminants. The presence of intestinal organisms like *E. coli* and *Shigella* sp is indicative of faecal contamination. Also the recovery of *S. aureus*, *M luteus*, *P. aeruginosa*, *B. subtilis*, *Arthrobacter* sp. and *Kurthia* sp from the herbal medicines screened may not be unconnected with the lack of good aseptic practices dur-

ing the drug preparation. This may not be far-fetched because majority of the people involved at all stages of herbalism in Nigeria are usually aged, of low socio-economic group with no formal education. The earlier report of Govender *et al.*<sup>[17]</sup>, that tradi-

tional remedies sold in South Africa were produced in crowded living conditions with low environmental sanitation and hygiene by herbalists who had not received any formal training corroborated this opinion.

**Table 1** Percentage resistance of gram positive isolates to antibiotics .

Isolates	CPX 10µg	GEN 10µg	COT 25µg	CHL 30µg	OFL 5µg	PFX 5µg	ERY 5µg	AMX 25µg	CRO 30µg	STR 10µg
<i>S. aureus</i>	79	100	100	100	71	93	100	100	100	100
<i>M. luteus</i>	100	100	100	100	80	60	100	100	100	100
<i>Streptococcus</i> sp.	100	100	100	100	0	0	100	100	100	100
<i>Clostridium</i> sp.	25	100	100	75	25	25	75	100	100	100
<i>Kurthia</i> sp.	60	100	80	100	80	80	100	100	100	100
<i>L. plantarum</i>	67	100	100	100	67	67	83	100	100	100
<i>B. cereus</i>	33	100	67	100	0	33	100	100	67	100
<i>B. subtilis</i>	50	100	50	100	50	50	100	100	100	100
<i>Arthrobacter</i> sp	100	100	100	100	0	100	100	100	100	100

Legend:

AMX: Amoxicillin; COT: Cotrimoxazole; OFL: Ofloxacin; CPX: Ciprofloxacin; STR: Streptomycin; ERY: Erythromycin; CHL: Chloramphenicol; PFX: Pefloxacin; GEN: Gentamycin; CRO: Ceftriazone

**Table 2** Percentage resistance of gram negative isolates to antibiotics.

Isolates	TET 30µg	OFL 5µg	PFX 5µg	NIT 200µg	GEN 10µg	AMX 25µg	CPX 10µg	CRO 30µg	COT 25µg	AUG 30µg
<i>P. aeruginosa</i>	100	89	89	100	100	100	89	100	100	100
<i>K. pneumoniae</i>	100	75	92	100	100	100	100	100	100	100
<i>Neisseria</i> sp.	100	100	100	100	100	100	75	100	100	100
<i>E. coli</i>	93	64	79	100	100	100	100	100	100	100
<i>Shigella</i> sp.	100	100	100	100	100	100	100	100	100	100

Legend: AUG: Augmentin; NIT: Nitrofurantoin; CRO: Ceftriazone; GEN: Gentomycin; OFL: Ofloxacin; TET: Tetracycline; AMX: Amoxicillin; PFX: Pefloxacin; COT: Cotrimoxazole; CPX: Ciprofloxacin

The WHO recommended the absence of *E. coli*, *Clostridia*, *Salmonella* and *Shigella* in herbal medicines. The presence of these and other pathogens in the traditional medicine screened, make them unsafe, and their ingestion can lead to a variety of diseases, especially gastroenteritis when the contaminant produces enterotoxins or neurotoxins. The herbal medicine sellers believe that the efficacy of the medicines must be attributed to the purging ability of the drugs. This is with their belief that violent purging cleanses and violently expels the problem or disease from the system of the consumer (oral com-

munication). Although some preparations contain plants with purgative function, this is always uncommon especially in the herbal medicines screened. Based on the present study the purgative nature of herbal medicines hawked at popular markets in southwestern Nigeria may not be unconnected with the presence of enteric pathogenic bacterial contamination. The MDR nature of the isolated organisms is of greatest concern, they could pose a challenge to treatment. Although the present study did not undertake a survey of percentage of illnesses and death arising from the ingestion of herbal medicines, it was



gathered from users that several acute conditions have resulted in patients following the prolonged usage of the herbal preparations. This has often led to the development of complications, some of which have resulted into death. There is the need for compliance to the quality control guidelines for low microbial load and the absence of pathogenic microorganisms, if crude herbal remedies are not to be death traps and potential health risks to consumers and immuno-compromised patients. The herbal medicines screened may also be an important source of introducing MDR organisms into the human population. It is therefore imperative that these guidelines be strictly enforced especially now that many unregistered cheap products are on sale. Also education of traditional healers/herbalists on the importance of satisfactory environmental sanitation and hygiene must be undertaken from time to time.

## REFERENCES

- 1 World Health Organization. WHO global burden of disease (GBD) estimates. 2002a. Available from: <http://www.who.int/healthinfo/bodestimates/en/>.
- 2 **Breman J**, Alilio M, Mills A. Conquering the intolerable burden of malaria: What's new, what's needed: A summary. *Am J Trop Med Hyg* 2004; 71:1-5.
- 3 UNAIDS, WHO. Aids epidemic update. 2006. Available from: <http://data.unaids.org/pub/Epireport/2006/200>.
- 4 UNAIDS, WHO. Aids epidemic update. 2007. Available from: <http://data.unaids.org/pub/EPISlides/2007/2007>.
- 5 The Botanical Source. Overview on medicinal plants and traditional medicine in Africa. Available from: <http://www.thebotanicalsource.com/id274.htm>.
- 6 World Health Organization. WHO traditional medicine strategy 2002-2005. 2002b. Available from: <http://www.who.int/medicines/organization/trm/orgtrmmain.shtm/>.
- 7 **Tapsell LC**, Hemphill I, Cobiac L, Sullivan DR, Fenech M, Patch CS, et al. Health benefits of herbs and spices: the past, the present, the future. *MJA* 2006;185 (4): S1 - S24.
- 8 **Eisenberg DM**, Daris B, Ettner SL, Appel S, Wilkey S y col. Trends in alternative medicine use in the United States, 1990-1997. Results of a follow-up national survey. *J Am Med Assoc* 1998; 280: 1569 -1575.
- 9 **MacLennan AH**, Wilson DH, Taylor AW. Prevalence and cost of alternative medicine in Australia. *Lancet* 1996; 347: 569 - 573.
- 10 **Fisher PA**. Ward Medicine in Europe. *Br Med J* 1994; 309: 107 -111.
- 11 Health Canada. Complementary and alternative health care: The other mainstream? Health Policy Research Bulletin. 2003; (1)7. Available from: [www.hc-sc.gc.ca/sr-sr/pubs/hpr-rpms/bull/2003-7-complement/intro-eng.php](http://www.hc-sc.gc.ca/sr-sr/pubs/hpr-rpms/bull/2003-7-complement/intro-eng.php).
- 12 **Kurokawa M**, Nakano M, Ohyama H, Hozumi T, Kageyama S, T. Namba T, et al. Prophylactic efficacy of traditional herbal medicines against recurrent herpes simplex virus type 1 infection from latently infected ganglia in mice. *J Derma Sci* 1997; 14 (1): 76 -84.
- 13 **Brinckmann L**, Sigwart H, Taylor LH. Safety and efficiency of a traditional medicine (throat coat) in symptomatic temporary relief of pain in patient with acute pharyngitis: A multicenter, prospective, randomized, double-blinded, placebo-controlled study. *J Comp Med* 2003; 9 (2): 285-298.
- 14 **Wilcox MT**, Bodeker G. Traditional herbal medicines for malaria. *Bri Med J* 2004; 329: 1156-1159.
- 15 **Hyikata Y**, Makiura N, Kano T, Higasa K, Shimizu M, Kawata K, et al. Kampo medicine, based on traditional medicine theory in treating uncured glossodynia, efficacy in five clinical cases. *Am J Clin Med* 2008; 36 (5): 835-847.
- 16 **Czech EW**, Kneifel W, Kopp B. Microbiological status of commercially available medicinal herbal drugs - a screening study. *Planta Medica* 2001; 67 (3): 263 - 67.
- 17 **Govender S**, Du Pleissis - Stoman D, Downing TG, van de Venter M. Traditional herbal medicines; microbial contamination, consumer safety and the need for standards. *South Afr J Sci* 2006; 102; 253 - 255.
- 18 **Esimone CO**, Chah KF, Ikejude SC. Microbiological quality of herbal preparations marketed in south east Nigeria. *J Nat Rem* 2002; 2; 42-48.
- 19 **Adeleye IA**, Okogi G, Ojo EO. The microbial contamination of herbal preparations in Lagos, Nigeria. *J Health Popln Nutri* 2005; 23 (3): 296 -297.
- 20 World Health Organization. Quality control methods for medicinal plant materials. WHO working document of 2005, QAS/05.131/Rev/. Available from: [www.who.int/entity/medicine/services/expertcommittees/QAS05\\_131Rev1](http://www.who.int/entity/medicine/services/expertcommittees/QAS05_131Rev1).
- 21 **Buchanan RE**, Gibbons NE. Bergey's manual of determinative bacteriology. Baltimore Md; Waverly Press Inc; 1999.
- 22 **Prescott LM**, Harley JP, Klein DA. *Microbiology*. 6th ed. London; Mc Graw-Hill; 2002: 845-7.
- 23 **Bisset NG**, Wichtl M. *Herbal drugs and phytopharmaceuticals*. 2nd ed. Stuttgart (Germany); Medpharm GmbH Scientific Publishers; 2001.
- 24 Department of Health. Regulations governing microbiological standards for foodstuffs and related matters. Government notice No. R. 490 of 8th June 2001. Pretoria.
- 25 **Bauer AW**, Kirby WM, Sherris JC, Turch M. Antibiotic susceptibility testing by a standard disk method. *Am J Clin Pathol* 1966; 45: 493-496.
- 26 **Roy AK**, Sinha KK, Chouasia HR. Aflatoxin contamination of some common drug plants. *Appl Environ Microbiol* 1988; 54 (3): 842-843.