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MICROBIAL CHARACTERIZATION OF SPOILAGE MICROBES OF DRY HERBAL MEDICINAL POWDERS/TEAS

*George Osei-Adjei[1], FC Mills-Robertson[2], SC K Tay[3], FB Apea-Bah[4] and A Adu-Gyamfi[4]
[1]Accra Polytechnic, Science Laboratory Technology Department, PO Box 561, Accra
[2]Biochemistry Department, Kwame Nkrumah University of Science and Technology, Ghana
[3]Department of Clinical Microbiology, School of Medical Sciences
Kwame Nkrumah University of Science and Technology
[4]Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission
Box LG 80, Legon, Accra, Ghana

ABSTRACT

The study investigated and characterized the microbes that contaminate and cause spoilage of most dry herbal medicinal powders/teas sold on the Ghanaian Market. Also investigated was the microbial load of these herbal powders/teas using pour plate and/or spread plate methods. Ten herbal medicinal powders were analyzed. The powdered herbal medicinal powders were taken through irradiation processes at the Ghana Atomic Energy Commission. The results showed that all the dry powders were contaminated. The microbial load count of the samples indicated thirty percent (3 out of 10) of the dry powders achieved the specifications for both aerobic bacteria and fungal growth whilst one hundred percent (10 out of 10) of the dry powders irradiated were free from microbial contamination. The characterization of the isolated microbes confirmed the dominance of members of the genus *Bacillus*, *Rhizopus*, *Aspergillus* and *Candida* in the dry powders included. In conclusion, the study confirmed that most herbal medicinal products on the Ghanaian market are contaminated with pathogenic bacteria, yeasts and moulds. Therefore, there is the need for constant monitoring and control of the standards of herbal medicines.

Keywords: Herbal powders, microbial load, contaminants, spoilage, irradiation.

INTRODUCTION

Herbal medicine/phytomedicine/botanical medicine refers to the use of seeds, berries, roots, leaves, stem bark, or flowers of plants for medicinal purposes. According to WHO (2006), about 70 to 80% of the populations of most countries of the developing world rely on herbal or indigenous forms of medicine. Herbal medicines are often easy to prepare, affordable and accessible to the vast rural populace (Abbiw, 1990). It can therefore serve as a forerunner in the primary medical care of the population. Calixto (2000) proposed that phytomedicines, if combined with the preventive model of medical practices, could be among the most cost-effective, practical ways to shift the focus of modern health care from disease treatment to prevention. Due to the relatively high cost of the conventional pharmaceutical dosage forms, the increased resistance of bacteria to most antibiotics and the reduced side effects, the search for natural remedies has become necessary (Okeke *et al*., 1999; Hack, 2005). However, medicinal plant materials normally carry a large number of microbes originating from the soil (Adeleye *et al*., 2005). Additionally, contaminants may be introduced during harvesting, handling, and production of various herbal remedies (Adeleye *et al*., 2005; WHO, 1998; Sofowora, 1982). Herbal and natural products can be safer than synthetic medicines by imposing regulatory standards to improve shelf life of products and reduce risk on consumers` life. This study was designed to investigate the microbial contaminants that cause spoilage of dry herbal medicinal powders/teas produced by herbal manufacturing outlets in Ghana.

MATERIALS AND METHODS

Materials

The microbial quality of 10 dry herbal medicinal powders/teas sold by the manufacturers were examined for their microbial profiles.

Method

1g of the dry powders/teas were aseptically transferred separately and mixed in 9ml of sterile peptone water in Stomacher bags using the Stomacher machine for 10s.
Ten-fold serial dilutions were made and viability assessed using the pour plate and/or spread plate method in triplicates and plates incubated at 30°C for 3 days in the case of bacteria and at 25°C for 3-5 days for yeasts and moulds.

Microbial load count

Bacteria were enumerated on Plate Count Agar, Violet

*Corresponding author email: geoadjei@hotmail.com

Red Bile Agar, deMann-Rogosa-Sharpe Agar and Glucose Yeast extract Calcium Carbonate Agar. Yeasts and moulds were enumerated on Malt Extract Agar containing specific antibiotics. The viable aerobic bacterial count were assessed using well established methods

Microbial identification

The pure isolates were examined by their colonial and cell morphology, Gram reaction and other biochemical tests. Identification of species was carried out by assaying cultures in the Analytical Profile Index (API) galleries (Biomerieux, France).

RESULTS AND DISCUSSION

The results of the mean microbial load counts of the control and irradiated powders. The values for 6 of the powders were within the acceptable range for APC whilst on the malt extract agar only 4 were within the acceptable range (EP, 2007). In combination counts 3 out of the 10 herbal powders did not exceed the limits for microbial load. All the 10 powders were irradiated at different doses of 5.0 kGy, 7.5 kGy, 10.0 kGy and 24.0 kGy. The results from table 1 confirmed total elimination/inhibition of microbes in all the 10 products analyzed after irradiation.

Microbial isolates

Table 2 shows the microbial content of the dry powders analyzed. A total of six bacterial and six fungal species were isolated from the dry powders. *Bacillus subtilis* (47.1%) were the most prevalent bacteria isolated from the dry powders. Of the fungal isolates, the most common were the moulds, *Rhizopus stolonifer*, *Aspergillus niger* and a yeast called *Candida silvicola*, representing 35.7, 28.6 and 14.3% of the total fungal population respectively.

Ghana today has an increased public awareness and usage of herbal medicinal products in the treatment and/or prevention of diseases (Abbiw, 1990). The relatively high cost of the conventional pharmaceutical dosage forms and inaccessibility of the orthodox medical services to most people particularly in the rural areas are contributing factors (Krogsgaard *et al*., 1984; Abbiw, 1990). Since there have been increased usage, health authorities and health professionals are concerned about the safety, efficacy and quality of these medicines (WHO, 2004). The methods used in harvesting, handling, processing, storage and distribution of herbal medicines subject them to contamination by various microorganisms, some of which may be responsible for spoilage (WHO, 2004).

The results of this study showed that the samples analyzed were contaminated to various extents with

Table 1. Mean microbial load count of control and irradiated powders.

Product/Media		Mean Microbial Load of Irradiated Powders (cfu/g)				
		Control (0.0kGy)	5.0kGy	7.5kGy	10.0kGy	24.0kGy
	PCA	$8.0 \times 10^3 \pm 1.00$	No growth	No growth	No growth	No growth
XLV	MEA	$2.0 \times 10^3 \pm 1.00$	"	"	"	"
	PCA	$2.3 \times 10^3 \pm 2.00$	"	"	"	"
FEF	MEA	$4.0 \times 10^2 \pm 1.00$	"	"	"	"
	PCA	$8.8 \times 10^2 \pm 2.08$	"	"	"	"
KPN	MEA	No growth	"	"	"	"
	PCA	$1.6 \times 10^3 \pm 2.00$	"	"	"	"
BLG	MEA	$5.0 \times 10^2 \pm 2.00$	"	"	"	"
	PCA	$3.2 \times 10^5 \pm 2.65$	"	"	"	"
LPT	MEA	TNTC	"	"	"	"
	PCA	TNTC	"	"	"	"
APS	MEA	$3.1 \times 10^5 \pm 2.65$	"	"	"	"
	PCA	$4.2 \times 10^4 \pm 2.65$	"	"	"	"
TAA	MEA	$2.1 \times 10^3 \pm 2.00$	"	"	"	"
	PCA	$3.4 \times 10^5 \pm 4.00$	"	"	"	"
CHD	MEA	$3.0 \times 10^2 \pm 3.61$	"	"	"	"
	PCA	$2.6 \times 10^4 \pm 3.04$	"	"	"	"
SPP	MEA	$5.3 \times 10^3 \pm 4.20$	"	"	"	"
	PCA	$1.35 \times 10^5 \pm 3.61$	"	"	"	"
CND	MEA	$1.9 \times 10^4 \pm 2.00$	"	"	"	"

Key: kGy - kiloGray

Table 2. Microbial content of dry powders.

Product	Microbial isolate	
	Bacteria	Fungi
XLV	*Staphylococcus aureus* *Bacillus subtilis*	*Aspergillus niger*
FEF	*Bacillus licheniformis* *Escherichia vulneris*	*Aspergillus niger* *Penicillium digitatum*
KPN	*Bacillus subtilis*	-
BLG	*Bacillus subtilis*	*Rhizopus stolonifer* *Aspergillus niger*
LPT	*Bacillus subtilis* *Serratia ficaria*	*Rhizopus stolonifer*
APS	*Bacillus megaterium* *Bacillus subtilis* *Enterobacter aerogenes* *Klebsiella oxytoca*	*Rhizopus stolonifer* *Candida silvicola,* *Geotrichum spp*
TAA	*Bacillus subtilis* *Serratia ficaria*	*Rhizopus stolonifer*
CHD	*Bacillus circulans*	*Aspergillus niger*
SPP	*Bacillus subtilis*	*Aspergillus flavus*
CND	*Bacillus subtilis*	*Rhizopus stolonifer* *Candida silvicola*

bacteria and fungi. The limits of microbial contamination for raw materials are: total aerobic bacteria, maximum 10^7 cfu/g, moulds and yeast, maximum 10^5 cfu/g, *Escherichia coli*, maximum 10^4 cfu/g, Enterobacteria and other Gram negative bacteria, maximum 10^4 cfu/g. *Shigella* and *Salmonella* must be absent per gram according to the World Health Organization (WHO, 2004). The microbial load count of the isolated and characterized microbes from the raw materials indicated the presence of the following bacteria species: *Escherichia coli, Proteus vulgaris, Serratia ficaria, Bacillus cereus, Staphylococcus aureus, Bacillus subtilis and Bacillus licheniformis*. The isolation of pathogenic strains, including *B. cereus, S. aureus* and *E. coli* is very important, because of health concern. They are known to cause human illness (Dohmai *et al*., 2008). Fungal isolates encountered included *Aspergillus niger, Aspergillus flavus, Aspergillus sulphureus, Penicillium digitatum, Fusarium oxysporum, Mycelia sterilia* and *Cladosporon herbarum* whilst the yeasts strains isolated were *Trichosporon mucoides, Candida membranifasciens* and *Candida krusei*. Research work by Efuntoye (1996), revealed the presence of *A. parasiticus, A. flavus and A. ochraceus* on dried medicinal herbs from the Nigerian market, whilst Czech *et al.* (2001) reported bacterial and fungal contamination of medicinal herbs in Austria. Martins *et al*. (2001a) isolated several *Aspergillus* species including *A. niger* from orange tree leaves. The isolation of various aspergilli, especially *A. flavus* is of highest concern because it is known to produce aflatoxins. *A. niger, A. versicolor* and *Penicillium* spp. also have the potential for toxigenesis (El-Kady *et al*., 1994; Perone *et al*., 2006). Their presence in the raw materials should be kept as low as possible and the moisture content of the product maintained at levels that do not allow fungal growth (Lugauskas *et al*., 2006).

The results of the microbial load count of the dry powders (Table 2) was one hundred percent (10 out of 10) contaminated by aerobic bacteria and ninety percent (9 out of 10) contaminated by fungi. However, forty percent (4 out of 10) of the powders were found to have exceeded the acceptable limit for APC whilst sixty percent (6 out of 10) of the powders were found to have exceeded the acceptable limit for moulds and yeasts count (EP, 2007). KPN was found to be free from fungal contamination. A total of nine bacterial and six fungal species (Table 2) were isolated from the powders with *Bacillus* species found to be contaminating all the ten powders. The presence of *Bacillus* species in the powders may be as a result of inadequate heat processing, improper handling of products and contaminated processing equipment (Frazier and Westhoff, 2003) although these stages of processing were not investigated but are known to be predisposing factors. The isolation of *Staphylococcus aureus* from XLV makes it a health risk since this organism is capable of causing human infection such as boils, skin sepsis, toxic shock syndrome, scalded skin syndrome, pneumonia, osteomyelitis and food poisoning (Jawetz *et al*., 2004). The dominating fungal contaminants were *Rhizopus stolonifer* (35.7%), *Aspergillus niger* (28.6%) and the yeast, *Candida silvicola* (14.3%). Apart from *R. stolonifer*,

A. niger and *C. silvicola*, *A. flavus*, *P. digitatum* and *Geotrichum* species were confirmed to contaminate the powders. Romagnoli *et al*. (2007) reported that dried material from plant origin such as spices are commonly heavily contaminated with xerophilic storage moulds and bacteria. The level of mould contamination in powders depends largely on the environmental conditions during cultivation, harvesting, storage and processing (Romagnoli *et al*., 2007). Moulds normally proliferate and spoil the product and possibly produce mycotoxins if the moisture of the product increases during storage (Romagnoli *et al*., 2007). This might have been responsible for the organisms isolated from the medicinal powders of plant origin investigated in the present study.

The results of the irradiated powders (Table 2) confirmed that the ionizing radiation destroyed/inhibited all the spoilage and pathogenic bacteria and fungi present in the powders at all the doses used. The destruction/inhibition of microbial contaminants can prolong the shelf-life of products in cases where microbial spoilage is the limiting factor (Satin, 1996). As a result, irradiation is used for purposes such as reducing or eliminating food borne pathogens, disinfecting food, and extending product shelf-life. The result of the irradiated medicinal powders of plant origin is not different from this practice in USA by application (Marsden, 1994).

The volume of irradiated spices and dried vegetable seasonings globally has increased significantly from about 5,000 tonnes in 1990 to over 60,000 tonnes in 1997 (WHO, 1997). Irradiation will therefore be useful in preventing contamination of herbal medicinal products.

CONCLUSION

The study demonstrated the presence of microbial contaminants in the products at levels most times exceeding the acceptable limits of microbial load count. The presence of *Aspergillus* species isolated from the products has the potential for toxin production in the products. This study also confirmed that exposure of the powders to ionizing radiations resulted in the elimination/inhibition of spoilage and pathogenic bacteria and fungi. Irradiation can therefore be the best and effective method for reducing and eliminating product pathogens and thereby extending product shelf-life.

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