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Short communication

Microbial contamination of traditional medicinal plants sold at the Faraday *muthi* market, Johannesburg, South Africa

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ABSTRACT

The Faraday traditional healers' trading market is the hub of the medicinal plant trade in Johannesburg, South Africa. Modes of harvesting, transporting, storage and distribution of medicinal plants render them susceptible to microbial attack, and thereby make customers, especially patients with compromised immune systems, vulnerable to infections that could increase morbidity and mortality. This study evaluated the microbial contamination on five frequently used traditional medicinal plant species sold by traders in the Faraday market. Bacterial contamination was determined using serial macro dilutions, spread plate and streak plate techniques. Fifteen bacterial contaminants were identified, the most recurrent being *Pantoea* sp. and five strains of *Bacillus* spp. (non-pathogenic). There was little variation between contamination levels of the five different traders, and the mean CFU/g per species ranged from 3.03×10^4 (*Hypoxis* sp.) to 4.22×10^5 (*Hydnora abyssinica*). While there was no overall significant levels of contamination, the CFU counts for two plant species purchased from one specific trader (viz. *H. abyssinica* and *Acacia xanthophloea*) exceeded maximum acceptable contamination limits set by the World Health Organisation (i.e. $\leq 10^5$ to $\leq 10^7$ CFU/g). The levels of contamination varied greatly between the commercially available over the counter product and the plant samples investigated. The microbial types are predominantly opportunistic pathogens. The implementation of good processing practices therefore clearly influences the quality and safety of medicinal products, especially regarding microbial contamination. It is evident that policies and regulations need to be developed and implemented in order to address possible contamination by opportunistic pathogens.

1. Introduction

The use of traditional medicine, in particular medicines of plant origin, has increased worldwide since the 1990s due to its affordability, availability, accessibility and the role in meeting primary healthcare demands in many developing African and Asian countries (WHO, 2008). The number of practising traditional healers in South Africa is estimated to be around 200,000, and up to 60% of the population reportedly consult healers and use traditional medicines (*umuthi*) as their primary source of healthcare (Van Wyk et al., 2009). The use of *umuthi* is firmly rooted in cultural and religious beliefs and is not exclusively a rural phenomenon. These herbal medicines are also widely used for therapeutic purposes in urban areas by more formally educated South Africans in higher income brackets (Dold and Cocks, 2002; Mander et al., 2008; Makunga et al., 2008; Ndhlala et al., 2011). Once described as a *"multi-million rand hidden economy"* (Cunningham, 1991), the South African trade in traditional medicines was valued at R2.9 - billion per annum in 2008 (Mander et al., 2008).

A host of environmental, agricultural, urban and industrial factors, coupled with less than adequate harvesting, storage and processing techniques, are causes of contamination in natural plant products that compromises the quality and efficacy of traditional medicines (Kneifel et al., 2002; Street et al., 2008). Thus, in order to ensure the safety of consumers, the fallacy that traditional medicinal plants are safe, 'pure and natural' and pose no harm to users (Govender et al., 2006; Street et al., 2008) must be challenged. To date, most of the plant research in the related field has focussed on isolating and evaluating the active and/or toxic compounds of herbal medicines in efforts to establish and validate their safety and efficacy. However, less research has been conducted into assessing the occurrence of toxic substances and other contaminants that leave potentially detrimental residues and micro-organisms on the plants (Street et al., 2008). Contaminating micro-organisms and their toxins may lead to diseases, making them hazardous for consumption (Govender et al., 2006). One of the major contributing factors to their growth is increased temperatures and moisture (Hell and Mutegi, 2011). Previous studies on herbal medicines have found highly

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pathogenic micro-organisms such as methicillin and vancomycin resistant *Staphylococcus aureus*, various *Bacillus* species as well as multiple fungal species such as *Aspergillus flavus*, *Penicillium viridicatum* and *Fusarium oxysporum* (Aziz et al., 1998; Govender et al., 2006; Stickel et al., 2009; Kaume et al., 2012). Thus, caution is warranted regarding the safety of contaminated plants in the outdoor informal *umuthi* market trade where conditions are conducive to microbial exposure and proliferation.

Much of the previous research on medicinal plant contamination by microbes has assessed fungal species and the aflatoxins that they produce (e.g. Katerere et al., 2008; Hell and Mutegi, 2011). This study, however, focuses on bacterial contamination by enumerating the types and levels of bacteria found on selected plants purchased from the Faraday *umuthi* market in Johannesburg, which has, to the best of our knowledge, not been studied before. Faraday is a lively trading hub and an important source of medicinal plants for the region. The more than 200 vendors sell species harvested from across southern Africa, but mainly from the provinces of Kwazulu-Natal, Eastern Cape, Gauteng and Mpumalanga (Williams, 2007; Williams et al., 2011). The majority of vendors sell plants from stalls that are mostly outdoors and/or semi-exposed but protected from the rain by canopy roofs. The remainder of the traders sell from within naturally ventilated buildings.

Five commonly traded and identifiable plants were selected for the study to assess levels of microbial contamination, namely: *Helichrysum* sp. (iMphepho, leaves/stems), *Drimia sanguinea* (isiKlenama/Skanama, bulb), *Hypoxis* sp. (iLabatheka, tuber), *Hydnora abyssinica* (uMavumbuka, rhizome and root holoparasite) and *Acacia xanthophloea* (umKhanyakude, bark) (Williams, 2007). The underground plant parts of *D. sanguinea*, *Hypoxis* sp. and *H. abyssinica* are three of South Africa's most regularly used traditional medicines, and are administered both orally and externally for a wide variety of uses that may include ritual cleansing and washing (e.g. Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Van Wyk et al., 2009; Williams et al., 2011). In addition, the bulbs of *Drimia* spp. contain a stinging sap that causes skin irritations (Hutchings et al., 1996; Von Ahlefeldt et al., 2003).

The aromatic leaves and stems of *Helichrysum* sp. are generally not administered orally, but are used in a 'magical sense' as an incense to invoke the goodwill of the ancestors in rituals to cleanse a consecrated place (Hutchings et al., 1996; Arnold et al., 2002; Street et al., 2012). Thus, microbial contaminants present on plants are not necessarily of major consumptive concern to users *per se*, but cross-contamination with other plants is. The fluorescent yellow bark of *A. xanthophloea* is also used in a magical sense as a good luck charm, but powdered bark decoctions and infusions are reported to be taken orally for malaria and applied externally to treat eye conditions (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Arnold et al., 2002; Hankey and Stern, 2002).

2. Materials and methods

2.1. Collection of samples

Three samples for each of the five plant species (*Helichrysum* sp., *Drimia sanguinea*, *Hypoxis* sp., *Hydnora abyssinica* and *Acacia xanthophloea*) (Fig. 1) were purchased from five different vendors at the Faraday market ($n = 75$ samples). The five vendors (F1 to F5) were randomly selected on the basis of plant availability and

Fig. 1. A) *Helichrysum* sp. (iMphepho), B) *Drimia sanguinea* (isiKlenama/Skanama), C) *Hypoxis* sp. (iLabatheka), D) *Hydnora abyssinica* (uMavumbuka) and E) *Acacia xanthophloea* (umKhanyakude) as sold at the Faraday market and after processing.

were evenly distributed throughout the market in distinctly different areas that had varying levels of exposure to the outdoors. Vendor F1 was situated outdoors on the eastern side of the market, whereas vendors F2–F5 were distributed from east to west and were under a canopy roof. The plant samples were collected in the plastic packaging provided by the vendors. After collection, the samples were placed in sterile autoclaved bags using sterile gloves. All the samples were stored in the dark at room temperature until processing, which commenced within 48 h of purchasing the samples.

For comparative purposes, three commercial samples of Panado® with three different batch numbers were purchased at randomly selected pharmacies and contained in their primary packaging until aseptic processing commenced. Selection of the commercial sample with regulated manufacturing procedures was based on consumer popularity and was included in the study as a control, whereby comparison of commercial pharmaceuticals could be compared to the samples from the informal trade.

2.2. Determining the presence of bacterial contamination

Each selected plant sample was weighed and placed in a sterile beaker containing a measured volume of sterile water. The ratio of plant weight to water volume was kept constant for all samples. The plant samples were then agitated using a shaking incubator at 37 °C at 104 rpm for 1 h. After agitation, four 1:10 serial dilutions were performed on the water collected from the samples. Each dilution was homogenised using a vortex mixer and 1 ml sample plated onto to a 5% sheep blood agar plate using the spread plate method. The agar plates were incubated at 37 °C for 24 h to monitor detection of bacterial contamination. After incubation, colony counts were performed on all dilutions and colony forming units (CFU) per gram determined for each sample. The study was undertaken in triplicate and expressed as a mean of three replicate samples (Singh et al., 2008). The levels of bacterial contamination were compared to the limits set for medicinal plant materials by the International Pharmacopoeia of the World Health Organisation (WHO, 2007). Acceptable colony counts range from $\leq 10^5$ to $\leq 10^7$ CFU/g depending on the method of plant processing and product administration to the user (WHO, 2007).

The commercial Panado® samples (one tablet in triplicate) were weighed and placed in a sterile test tube containing a measured volume of sterile water in the same ratio as represented for the plant samples. The samples were then further processed as for medicinal plant samples.

2.3. Identification of microbial species isolated

The different isolated colonies were streaked onto slanted transport media and incubated at 37 °C for 24 h, after which they were immediately transported to the National Institute of Communicable Diseases for identification. Isolates were then streaked out onto 5% Columbia blood agar and MacConkey agar, and incubated at 37 °C overnight to confirm purity of isolates. A Gram stain was performed on each culture and depending on the outcome, isolates were biochemically identified using the VITEK Compact 2 automated system (BioMerieux, Marcy l'Etoile, France) whereby 3 ml of sterile 0.5% saline was dispensed into clear plastic tubes. Well isolated colonies were selected using a sterile swab and transferred to the saline and then emulsified to obtain a homogeneous suspension with an opacity for Gram-negative isolates equivalent to 0.50–0.63 McFarland standard and for Gram-positive isolates, equivalent to 1.8–2.2 McFarland standard. For all Gram-negative isolates a VITEK GN card was used and for all Gram-positive isolates, VITEK BCL cards were used. These were placed in tubes with the bacterial suspension and identification was provided after 5–8 h.

3. Results and discussion

The mean CFU for all samples purchased from five different traders in the different areas of Faraday ranged from 3.03×10^4 CFU/g (*Hypoxis* sp.) to 4.22×10^5 CFU/g (*H. abyssinica*) (Table 1). Samples from trader F1 had the highest contamination levels (2.81×10^6 CFU/g), while samples from trader F5 were the least contaminated (4.16×10^3 CFU/g). Since microbial contamination counts for species purchased from trader F1 were generally higher than samples purchased anywhere else in the market, especially for *H. abyssinica* and *A. xanthophloea*, it is probable that plant contamination levels may be (a) trader-specific (rather than species-specific), (b) related to the location of the vendors' stalls in the market, (c) influenced by the degree of exposure (including airborne) and handling, and (d) the amount of cross-contamination. Since area F1 was more exposed to environmental factors, with higher numbers of human traffic in the vicinity, this may have contributed towards the higher CFUs encountered.

In terms of the World Health Organisation's microbial contamination limits for medicinal plant materials intended for internal use, the colony counts should not exceed 10^5 CFU/g (WHO, 2007). Furthermore, for plant products intended for topical use or those that are pre-treated with boiling water, the colony count should not exceed 10^7 CFU/g. Thus, bacterial contamination on samples of *H. abyssinica* and *A. xanthophloea* purchased from trader F1 exceeds the maximum acceptable levels for both internal and external use (Table 1). The results for *Drimia sanguinea* and *Hypoxis* sp. indicate that CFU counts are within limits acceptable for internal and topical use. Since *Helichrysum* sp. is usually burned and not ingested, the CFU counts are also acceptable, but the species could be a vector for the broader transmission of bacteria given its popularity in the market.

The control samples (Pando® tablets), showed bacterial contamination but only on one of the three control samples of Panado® where 1.00×10^2 CFU/g was observed. The level of contamination was notably much lower than that found in the plant samples. The implementation of good manufacturing and processing practices therefore clearly influences the quality and safety of medicinal products, especially with regards to microbial contamination.

Table 1
Average number of colony forming units per gram (CFU/g) of sampled plant material ($n = 3$).

Plant sample	No. of colonies in original inoculum (CFU/g plant material) per sampled area					
	Trader F1	Trader F2	Trader F3	Trader F4	Trader F5	Average CFU per species
Helichrysum sp.	3.89×10^4	8.66×10^4	8.66×10^4	7.91×10^5 [b]	1.60×10^4	5.82×10^4
Drimia sanguinea	4.67×10^4	3.11×10^4	3.11×10^4	6.44×10^4	8.89×10^3	5.24×10^4
Hypoxis sp.	1.61×10^5 [a]	2.00×10^4	2.00×10^4	7.78×10^3	1.78×10^3	3.03×10^4
Hydnora abyssinica	2.89×10^9 [a,b]	5.55×10^4	5.55×10^4	1.02×10^6 [b]	6.11×10^4	4.22×10^5 [b]
Acacia xanthophloea	1.00×10^8 [a,b]	1.11×10^4	1.11×10^4	9.89×10^4	2.44×10^3	3.11×10^5 [b]
Average CFU per trader	2.81×10^6 [b]	4.09×10^4	4.09×10^4	6.61×10^4	4.16×10^3	

[a] CFU counts above those recommended for plant materials that have been pre-treated (e.g. as for infusions or decoctions using boiling water) or that are used as topical dosage forms (allowable limit 10^7 CFU/g) (WHO, 2007).
[b] CFU counts above those recommended for other plant materials for internal use (allowable limit 10^5 CFU/g) (WHO, 2007).

Table 2
Identification of contaminants from samples of five plant species purchased at the Faraday *umuthi* market.

Micro-organism identified	*Helichrysum* sp.	*Drimia sanguinea*	*Hypoxis* sp.	*Hydnora abyssinica*	*Acacia xanthophloea*	Incidence of micro-organism	Associated pathogenesis
Acinetobacter baumannii		F4				1	Opportunistic pathogen that may cause bacteremia, pneumonia, meningitis, urinary tract infection and wound infections (Maragakis and Perl, 2008)
Acinetobacter lwoffii			F3			1	Cause of infections in patients with impaired immune systems; cause of nosocomial infections e.g. septicaemia, pneumonia, meningitis, urinary tract infections, skin and wound infections (Regalado et al., 2009)
Bacillus amyloliquefaciens		F4				1	None
Bacillus lentus		F5				1	None
Bacillus megaterium			F3		F4	2	None
Bacillus subtilis		F5				1	None
Bacillus vallismortis		F4				1	None
Enterobacter cloacae		F5		F4		2	Opportunistic pathogen that can be acquired through the skin, gastrointestinal tract and urinary tract; cause of nosocomial bloodstream infections (Mezzatesta et al., 2012)
Klebsiella oxytoca				F2		1	Opportunistic pathogen most often involving immunocompromised patients (Lowe et al., 2012)
Leclercia adecarboxylata	F1					1	Opportunistic pathogen (Stock et al., 2004)
Pantoea species	F1, F3	F1	F1, F3, F4	F3	F3	8	Some species are opportunistic pathogens causing wound, blood, and urinary tract infections
Pseudomonas oryzihabitans	F3, F5					2	Infections in immuno-compromised patients (Iglesias and Martínez, 2004; Decker et al., 1991)
Sphingomonas paucimobilis					F3	1	Opportunistic pathogen (Ryan and Adley, 2010)
Streptococcus mitis				F2		1	Infections associated with immuno-compromised patients (Bannister et al., 2000)
Staphylococcus hominis						1[a]	Harmless commensal on humans and animals but may cause infection in patients whose immune systems are compromised
Type no's of micro-organisms identified per plant species	3	7	3	4	3	15	
Incidence of micro-organisms identified per species	5	7	5	4	3	24	

[a] Panado® control.

When assessing bacterial contamination, an indication of the type/identity of colonies found is imperative, since low levels of pathogenic strains may have more adverse effects than higher levels of non-pathogenic organisms. Fifteen types of micro-organisms were identified, the most recurrent of which were *Pantoea* species (present on eight samples between all five plant species purchased from three traders) and *Bacillus* spp. (present on six samples of three species; four types identified on *D. sanguinea*) (Table 2). Many of these organisms are residents of the soil, water and natural environment and hence it is conceivable that they were already residing on the plant material and not introduced from a secondary source. With the exception of the *Bacillus* species, which are non-pathogenic, most of the organisms are capable of causing infections in immuno-compromised patients (especially *Pseudomonas oryzihabitans* in patients with HIV). The prevalence of *Bacillus* spp. is possibly due to its spore forming nature. Four of the seven micro-organisms identified on *D. sanguinea* tended to be non-pathogenic, whereas all four types identified on *H. abyssinica* were usually opportunistic, pathogenic and capable of causing infections (Table 2). No *Staphylococcus* strains were recovered in this study.

It is important for researchers to be cognisant of environmental contaminants and the effects thereof when reporting on the activity and efficacy of crude plant extracts (Street et al., 2008). Bacterial and fungal contamination can occur at different entry points along the medicinal plant trade chain, from harvesting to the loci of sale in the *umuthi* market and can critically influence the quality of the product that the consumer receives (Zhang et al., 2012). The risk of contamination often increases when harvesting is done in areas with high temperatures and humidity or when plants are collected during the rainy season (WHO, 2003). KwaZulu-Natal, the main source of supply for plants sold in the Faraday market (Williams, 2004), has a sub-tropical climate with high ambient humidity throughout the year. This increases the risks of microbial growth.

The mode by which plants are conveyed to markets further increases the risk of microbial cross-contamination (Katerere et al., 2008). Most species are packaged in reused plastic or hessian bags that previously contained maize products and which, if contaminated, can act as vectors for micro-organisms such as *Aspergillus* species (a producer of various carcinogenic aflatoxins) (Katerere et al., 2008). Similarly, packaging infected with bacterial contaminants would enable opportunistic transmission and cross-contamination. Once plants reach the markets they are stored uncovered, exposed to air contaminants and hence are rendered susceptible to further adverse environmental conditions and microbial attack. Plants sold by trader F1, for example, and to a reduced degree trader F4, had CFU/g counts higher than plants sold by the other traders. F1 was in an area of the market that was more exposed and had a greater volume of human traffic, whereas the other traders were in more enclosed sections of the market. While the manner in which the bacteria were introduced on the plant samples remains unknown, the most frequently occurring contaminants appear to be harmless and may have originated at the sites where the plants were harvested. Some of the potentially harmful pathogenic bacteria identified on the samples may, however, be indicative of unsanitary and unhygienic conditions and methods of product handling and processing.

The potential for the growth of micro-organisms can by limited by drying the herbal medicines after harvesting (Jordan et al., 2010), and underground and wetter plant parts may be more susceptible than aerial parts to contamination (Katerere et al., 2008). However, the relatively dry bark of *A. xanthophloea* and the dried leaves and stems of

Helichrysum sp. had the second and third highest CFU counts respectively when compared to the wetter material of the remaining three species (Table 2). These results are indicative of the complexity of the contamination processes within open informal markets such as Faraday and the challenges for regulating, controlling and preventing potential bacterial infections. These challenges are further compounded by a lack of concern amongst some consumers who believe that the more unhygienic some specific products look, the more efficacious they are (Katerere et al., 2008).

Although the cultural significance, beliefs, plants and other raw materials used with regards to traditional medicines may differ from one market and one country to the next, concerns related to the contamination of these plants are universal. A study on the microbial quality of herbal medicines from shops in the Nelson Mandela Metropole (Port Elizabeth), South Africa (Govender et al., 2006) found significant contamination by bacteria and fungi. Of particular concern was the evidence of resistant strains of *Staphylococcus aureus*. It was interesting and surprising to note that no *Escherichia coli* or *S. aureus* contaminants were evident on the samples from this study. The study by Govender et al. (2006), together with concerns raised by Fennell et al. (2004) and Street et al. (2008) regarding the informal medicinal plant trade of South Africa, confirm the need to continuously monitor contamination levels. Similarly, contamination of herbal medicines by bacteria and fungi have been found in studies conducted in China, Indonesia, Brazil, Malaysia and India (Zhang et al., 2012). This evidence supports the notion that the problems associated with the contamination of herbal medicinal plants are a global phenomenon for which appropriate regulation could mitigate against factors that have negative consequences for human health and safety.

4. Conclusion

Even though the levels of contamination present on the samples in question are generally below the levels recommended by the World Health Organisation and together with the fact that the organisms are likely to be merely commensals, immuno-compromised patients are nevertheless at risk of developing disease due to such contamination. It is therefore pertinent that the safety of these products is of primary concern to healthcare providers in South Africa. Faraday, or any other similar trading market like it, is a market for traditional medicine and therefore attracts sick patients. Microbial contamination could additionally be attributed to these customers and is not necessarily caused by any unhygienic practises by the vendors and harvesters from the market. To control any contamination from this source, one would have to regulate the contact with customers and the plants. This may add even further complexity to finding a solution to contamination.

This study therefore brings the problems associated with microbial contamination to the forefront and aims to influence policy makers, healthcare workers, traditional herbalists and healers, traders and consumers, to consider prevention strategies that ensure the safe and effective use of these medicinal plants.

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