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Microbial and Heavy Metals Contamination of Herbal Medicines

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Abstract: This study was conducted to evaluate the microbial contaminants and presence of toxic heavy metals on some herbal medicines. Twenty-seven samples (3 kg each) of well-known herbs and 5 kinds of henna available in herb markets around Riyadh, Saudi Arabia were collected for microbial and toxic metal contamination. Twenty-one (60%) of the samples showed the presence of fungi. *Aspergillus flavus* and *Aspergillus fumigatus* were the most common isolates (9/35, 35%). *Astralagus sarcocolla* had the highest TPC count; *Matricaria chamomilia* had the highest total coliform count and fecal coliform count. Two henna samples showed more than 1 ppm lead content. Mercury was the highest in *Lepidium sativum,* aluminum in *Zingiber officinale*, calcium in *Artemisia herba alba*, cadmium in *Lepidium sativum*, *Vigna radiata* and *Zingiber officinale*. Copper was the highest in *Cinnamomum zeylanicum*, iron in *Zingiber officinale*, zinc in *Salvia officinalis*, potassium in *Matricaria chamomilia*, whereas sodium was the highest in *Pimpinella anisum*. Microbial determination showed that *Bacillus* species was seen in 3 (9.7%) of the isolated microorganisms with the predominance of *Bacillus cereus* (14/31, 45.2%). Other microbial isolates were *Aeromonas hydrophilia*, *Shigella* spp., *Enterobacter agglomerans*, *Enterobacter* spp., *Vibrio fluvialis*, *Escherichia coli*, *Pasteurella multocida*, *Enterobacter cloacae*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, *Acinetobacter iwoffii* and *Klebsiella*. Tests showed the sensitivity of most isolated bacteria to amoxicillin, gentamicin, imipinem, tobramycin and trimethoprim-sulfamethoxazole.

Key words: Heavy metals, contamination, microbial, herbal medicines

INTRODUCTION

Herbal drugs have increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs. According to WHO estimates, the present demand for medicinal plants is about US $14 billion a year and by the year 2050 it would be about US $5 trillion. Now people rely more on herbal drugs because of the high prices and harmful side effects of synthetic drugs and this trend is growing, not only in developing countries but in developed countries too. Unfortunately, the number of reports of people experiencing negative effects, caused by the use of herbal drugs, has also been increasing. There may be various reasons for such problems and one of the major causes of adverse effects is directly linked to the poor quality of herbal medicines. Therefore, it is realised that insufficient attention was being given to the quality assurance and control of herbal medicines. Although WHO has developed guidelines to maintain the quality of herbal drugs which includes a detailed description of the techniques and measures required for the appropriate cultivation and collection of medicinal plants. Despite such guidelines, there is still a lacuna between this available knowledge and implementation, because farmers and other relevant persons like producers, handlers and processors of herbal drugs are not very aware of WHO's guidelines.

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They continue their work, as before without any quality control measures which results in inferior quality of herbal drugs with lots of contaminants like heavy metals, pesticides and microbes.

Several studies showed that herbal plants are associated with a broad variety of microbial contaminants (Kneifel *et al*., 2002; Ichinoe *et al*., 1988). Recently, Ang (2003) showed that 22% of herbal plant samples studied failed to comply with quality requirement for traditional medicines. On the other hand, many studies have investigated the presence of toxic contaminants in herbal plants. Trace elements such as zinc, manganese, chromium, copper, iron, lead, nickel and vanadium were found in a traditional Chinese herb Jinqi (Han *et al*., 2008). In 2007, a 93 year old hypertensive woman was reported with severe hypokalemia due to consumption of licorice-containing herbal medicine for 7 years (Yasue, 2007). Another study showed that 26% of the available herbal plants in Malaysia possessed 0.53-2.35 ppm of mercury and therefore, do not comply with the quality requirement for traditional medicine (Ang and Lee, 2006). A study in Brazil (Caldas and Machado, 2004) showed that samples of herbal medicines had cadmium up to 0.74 mcg g^{-1} and mercury up to 0.087 mg g^{-1} and lead estimated intake through consumption of these herbs reached 440% of the tolerable intake. Metal toxicity has been associated with pathophysiological effects which included neurological behavioral effects (Boyd *et al*., 1991; Echeverria *et al*., 1998), cardiac dysfunction (Frustaci *et al*., 1999), fetal malformations (Vimy *et al*., 1990), Alzheimer's disease (Cornett *et al*., 1998) and Parkinson's disease (Ngim, 1989). The concentration of heavy metals is increasing in the environment and many hazardous effects are caused in the inhabitants of that environment. Modern detection methods have revealed trace amounts of lead in plants and water. However, these herbs have never been shown, or even suspected, of causing any disease associated with lead poisoning. For such reasons, we opted to investigate the microbial and toxic metal contamination of the most common herbal products available from random markets in Saudi Arabia's capital city of Riyadh.

MATERIALS AND METHODS

Sampling

Twenty-seven samples of well-known plant herbs and 5 different types of henna were collected at random in February 2008 from random herbal markets in the city of Riyadh, Saudi Arabia to determine the predominant microorganism and mycoflora and to assess the toxic heavy metal content. The herbal products were chosen on the basis of their commercial availability and popularity of use (Table 1). Every sample weighed 3 kg and was stored inside clean plastic bags at a temperature ranging from 4-5°C.

Evaluation of Fungal Contamination

Every sample was examined for Total Fungal Count (TFC) according to Nordic Committee on Food Analysis (2005), Total Coliform Count (TCC) according to Nordic Committee on Food Analysis (2004), Fecal Coliform Count (FCC) according to Nordic Committee on Food Analysis (2005), staphylococcus count according to Nordic Committee on Food Analysis (1999) and *Bacillus cereus* count according to Nordic Committee on Food Analysis (2003). Five grams of each sample were mixed with 45 mL of diluent from which tenfold serial dilution was made. Three milliliter from each dilution was inoculated each in sterile petri dishes in which sterile media was poured. After solidification plates were incubated. Plates were incubated upside down at 26±1°C for 7 days. After incubation, the fungal colonies were counted, recorded and the Colony of Forming Units (CFU) per gram were calculated. Identification was performed by cultural and morphological characteristics and followed taxonomic schemes for *Aspergillus* and *Penicillium* (Samson and Pitt, 2000).

Evaluation of Bacterial Contamination

Every sample was examined for bacterial contamination. 0.2 g of each sample was placed on blood agar plates and incubated for 24 h. Bacterial growth was identified using the API method. Pure colonies were isolated and transferred to blood agar plates for antibiotic susceptibility testing.

Table 1: Herbal plants analysed

Common name	Scientific name	Known use (*)
Aloe	*Aloe vera*	For wound and burns
Anise	*Pimpinella anisum*	For digestion, anti-bloating
Caraway	*Carum carvi*	For digestive disorders
Chamomile	*Matricaria chamomilia*	For digestive ailments
Cinnamon	*Cinnamomum zeylanicum*	Food flavoring, spice
Cumin	*Cuminum cyminum*	Stimulant, antimicrobial
Dill	*Anethum graveolens*	For insomnia
Ducrosia	*Ducrosia ismaelis*	CNS depressant
Dymock	*Astralagus sarcocolla*	To boost immune system
Fennel	*Foeniculum vulgare*	Analgesic, anti-inflammatory
Fennel flower plant	*Nigella sativa*	For cancer prevention
Garden cress	*Lepidium sativum*	Prevent post-natal complications
Garden sage	*Salvia officinalis*	Antiseptic, estogenic
Ginger	*Zingiber officinale*	Arthritis, rheumatism
Green mist	*Ammi visnaga*	For kidney stones, antispasmodic
Hassaniya (in Arabic)	*Calligonum comosum*	Anti-inflammatory, anti-ulcer
Hulls	*Coffea arabica*	Coffee drink
Lavender cotton	*Achillea fragrantissima*	For diabetes, kidney stones, UTI
Mung beans	*Vigna radiata*	Cuisine
Myrrh	*Commiphora myrrah*	Incense
Oliban	*Boswelia carterii*	Antibiotic
Fenugreek	*Trigonella foenum*	Lowers blood pressure, carminative, increases milk production and flow, emollient, rids the body of impurities and toxins.
Thyme	*Thymus serpyllum*	Aphrodisiac
White wormwood	*Artemisia herba alba*	Anti-oxidant
Rose of Jericho	*Anastatica hierochuntica*	As a representation of the cervix during labor.
Mixed herbs of chamomile		
Cumin, cinnamon, ammi, anise, thyme, mung beans, caraway seeds Grained herbs (as marketed)		
Henna (5 types)	*Lawsonia inermie*	Hair coloring/dye

*www.wikipedia.org/wiki/list_of_plants_used_as_medicine
http://www.impgc.com/index.php (Indian Medicinal Plants Growers' Consortium)

Evaluation of Toxic Heavy Metal Contamination

All 32 samples were analyzed for toxic heavy metal contamination. Microwave-assisted acid digestion was used for all of the samples using Multiwave 3000 Platform system (Anton Paar, Graz, Austria) and the elemental contents and their infusions were determined by FAAS and ICP-AES. Microwave digestion procedure was applied under optimized conditions for dissolution of herbal plants.

Microwave Acid Digestion and FAAS

In 400 mL cylinder 300 mL concentrated HCl and 100 mL of concentrated HNO_3 were added. Mixture was transferred to 1000 mL volumetric flask and volume was filled with diH_2O. Mixture was inverted to mix and allowed to stand. Samples were dried at 70°C for 24 h after which they were grinded in Spex mill. Crucibles and caps were prepared by washing in 10% HNO_3 and muffle at 750°C for 2 h. Ground samples of 0.5 g were placed into each crucible using Mettler (four-deck) balance and placed in muffle furnace to bring to ashing temperature (450°C) slowly for 90 min and ashed for 4 h. Crucibles were allowed to cool. Linear range of concentrations was determined for the wavelength to be used and an appropriate sample dilution scheme was devised.

Diluted extract was measured for metal content by atomic absorption spectrophotometry (Liberty spectrometer ICP-AES Series II, Varian Australia Pvt. Ltd., Mulgrave, Australia). Using FAAS for the determination of metals, the samples are mineralized in order to avoid possible matrix-related interferences. Iron, copper, zinc, manganese, nickel, cobalt, cadmium and lead content was

determined by analyzing the sample solutions undiluted due to low concentration of these metals. Cadmium and lead are complexed by ammonium pyrrolidine dithiocarbamate (APDC) and extracted into methyl isobutyl ketone before they were measured. Ionization of calcium and magnesium was avoided by adding NaCl solution to sample solutions. Chemical interference form phosphates and other anions in measurements of Ca, Mg, Fe, Cu, Zn and Mn are minimized by adding $La(NO_3)_3$ solutions to sample solutions.

RESULTS

Table 2 shows the total fungal count, total plate count, fecal coliform count and specific bacterial counts in 32 herbal plants analyzed. Of the 32 herbs analyzed, *Astralagus sarcocolla* had the highest TPC count (12×10^5 cfu g^{-1}) followed by *Pimpinella anisum* (16×10^4 cfu g^{-1}), *Matricaria chamomilia* (1×10^4 cfu g^{-1}), *Carum carvi* (76×10^3 cfu g^{-1}) and *Nigella sativa* (46×10^3 cfu g^{-1}). *Matricaria chamomilia* had the highest total coliform count (1×10^4 cfu g^{-1}) followed by mixed grained herbs (32×10^3 cfu g^{-1}), *Astralagus sarcocolla* (30×10^3 cfu g^{-1}) and *Ducrosia ismaelis* (29×10^3 cfu g^{-1}). *Matricaria chamomilia* also had the highest fecal coliform count (70×10^4 cfu g^{-1}) followed by *Astralagus sarcocolla* (15×10^3 cfu g^{-1}), mixed grained herbs (70×10^2 cfu g^{-1}), *Ducrosia ismaelis* (12×10^2 cfu g^{-1}) and mixed herbs (10×10^2 cfu g^{-1}). *Zingiber officinale* had the highest Staphylococcal content with 77×10^3 cfu g^{-1}, followed by grained mixed herbs (37×103 cfu g^{-1}) and *Cinnamomum zeylanicum* (35×10^3 cfu g^{-1}). Grained mixed herbs had the highest *Bacillus cereus*

Table 2: Total fungal count, total plate count, fecal coliform count and specific bacterial counts in 32 herbal plants analyzed

Scientific name	TPC	TCC	FCC	*Staphylococcus* (cfu g^{-1})	*B. cereus*	TFC	Fungi
Achillea fragrantissima	15×10^2	-	-	17×10	-	-	*A. flavus* and *A. fumig*
Aloe vera	-	-	-	-	-	3×10	-
Ammi visnaga	23×10^2	-	-	14×10	4×10	5×10	-
Anastatica hierochuntica	17×10^3	-	-	16×10	17×10^2	57×10^2	*A. fumigatus*
Anethum graveolens	16×10	-	-	-	-	64×10^2	*A. flavus* and *A. fumig*
Artemisia herba alba	55×10^2	-	-	56×10	-	12×10	*A. ochraceus*
Astralagus sarcocolla	12×10^5	30×10^3	15×10^3	18×10^2	2×10^3	21×10^3	*A. flavus* and *A. fumig*
Boswelia carterii	23×10^2	-	-	67×10	-	12×10	*A. ochraceus*
Calligonum comosum	37×10	-	-	20×10	-	10^5	*A. fumigatus*
Carum carvi	76×10^3	-	-	4×10^2	35×10	18×10	-
Cinnamomum zeylanicum	10^3	-	-	35×10^3	-	9×10	-
Coffea arabica	23×10^2	-	-	-	7×10	50×10	*A. flavus*
Commiphora myrrah	34×10	-	-	-	-	-	-
Cuminum cyminum	43×10	-	-	-	-	4×10	*A. fumigatus*
Ducrosia ismaelis	10^1	29×10^3	12×10^2	20×10^2	-	25×10	-
Foeniculum vulgare	11×10^2	-	-	14×10	-	13×10	*A. fumigatus*
Lepidium sativum	15×10	-	-	-	-	-	-
Matricaria chamomilia	10^4	10^4	70×10^4	30×10^2	31×10	17×10^2	*A. flavus*
Nigella sativa	46×10^3	-	-	-	-	-	-
Pimpinella anisum	16×10^4	-	-	8×10	-	9×10^4	-
Salvia officinalis	12×10^2	-	-	6×10	3x 10	23×10^3	*A. ochraceus*
Thymus serpyllum	48×10^2	45×10	48×10	-	-	18×10^2	*A. ochraceus*
Trigonella foenum	56×10^2	90×10	-	3×10	-	21×10	*A. ochraceus*
Vigna radiata	27×10^2	17×10	2×10	-	2×10	13×10	*A. ochraceus*
Zingiber officinale	10^3	-	-	77×10^3	5×10^2	23×10	-
Mixed herbs	13×10^3	20×10^2	10×10^2	-	-	20×10^2	*A. flavus* and *A. fumig*
Grained herbs (mixed)	14×10^3	32×10^3	70×10^2	37×10^3	10×10^3	24×10^3	-
Henna 1	33×10^2	48×10	3×10	-	13×10	13×10	*A. ochraceus*
Henna 2	56×10^2	-	-	56×10	-	2×10	*A. flavus*
Henna 3	27×10^2	17×10	17×10	-	34×10	23×10	*A. flavus*
Henna 4	46×10^2	-	-	34×10	-	15×10	*A. ochraceus*
Henna 5	53×10^2	46×10	27×10	-	25×10	19×10	*A. flavus*

TPC: Total (bacterial) Plate Count, TCC: Total Coliform Count, FCC: Fecal Coliform Count, TFC: Total Fungal Count, cfu: Colony forming units

Table 3: Heavy metal contents (in ppm) of 32 herbal plants analyzed

Scientific name	Pb	Hg	Al	Ca	Cd	Cu	Fe	Zn	K	Na
Achillea fragrantissima	0.131	0.0420	-	0.508	0.016	0.244	-	0.584	451.8	89.70
Aloe vera	0.094	0.0520	2.876	1.287	0.017	0.081	3.122	0.126	72.6	14.10
Ammi visnaga	0.090	0.0750	5.925	0.554	0.018	0.016	7.377	0.728	418.3	6.00
Anastatica hierochuntica	0.060	0.0660	17.950	0.065	0.017	0.159	16.720	0.135	37.2	4.10
Anethum graveolens	0.109	0.0810	1.970	1.288	0.016	0.074	2.631	0.073	165.1	3.30
Artemisia herba alba	0.123	0.1020	0.091	1.319	0.017	0.128	2.294	0.451	300.2	1.30
Astralagus sarcocolla	0.109	0.0390	4.980	1.164	0.016	0.026	5.157	0.029	52.4	5.60
Boswelia carterii	0.706	0.0720	0.718	1.162	0.016	0.104	1.138	0.023	6.8	20.20
Calligonum comosum	0.060	0.0700	5.041	0.419	0.017	0.023	3.040	0.124	103.4	12.30
Carum carvi	0.078	0.0740	8.162	0.593	0.017	0.274	9.069	0.775	415.1	16.20
Cinnamomum zeylanicum	0.124	0.0450	-	0.546	0.017	0.284	-	0.817	345.4	37.40
Coffea arabica	0.103	0.0790	3.360	0.881	0.016	0.131	4.425	0.081	555.4	8.50
Commiphora myrrah	0.080	0.0780	6.513	0.428	0.016	0.207	5.901	0.029	58.8	1.90
Cuminum cyminum	0.111	0.0830	6.456	1.055	0.016	0.158	7.012	0.401	515.7	64.30
Ducrosia ismaelis	0.067	0.0820	2.519	0.545	0.017	0.110	3.713	0.259	360.8	155.40
Foeniculum vulgare	0.091	0.0600	1.850	0.559	0.017	0.267	3.275	0.629	460.9	100.50
Lepidium sativum	0.099	0.6300	1.559	1.069	0.025	0.118	3.752	0.877	393.9	18.40
Matricaria chamomilia	0.128	0.0830	1.697	0.582	0.017	0.282	18.560	0.641	943.4	227.70
Nigella sativa	0.099	0.0620	2.170	0.788	0.018	0.271	3.530	0.956	201.9	2.30
Pimpinella anisum	0.104	0.0870	3.145	0.855	0.017	0.186	4.975	0.843	455.9	307.40
Salvia officinalis	0.134	0.0650	14.280	0.534	0.017	0.115	13.250	0.962	331.1	40.50
Thymus serpyllum	0.105	0.0470	-	0.479	0.016	0.128	-	0.346	208.9	16.40
Trigonella foenum	0.091	0.0821	0.757	1.281	0.017	0.104	2.735	0.375	276.1	20.30
Vigna radiata	0.214	0.0620	4.479	0.588	0.025	0.089	4.345	0.183	142.2	7.80
Zingiber officinale	0.094	0.0840	19.830	1.179	0.025	0.102	19.440	0.474	635.1	8.90
Mixed herbs	0.099	0.0870	6.305	0.057	0.017	0.198	8.002	0.586	500.8	32.30
Henna 1	0.120	0.0660	-	0.447	0.017	0.153	-	0.307	331.8	17.00
Henna 2	1.528	0.0260	-	0.552	0.018	0.142	-	0.458	317.3	28.60
Henna 3	0.151	0.0480	-	0.534	0.019	0.143	-	0.565	308.9	23.80
Henna 4	1.214	0.0690	-	0.514	0.018	0.088	-	0.375	183.4	102.10
Henna 5	0.112	0.0920	-	0.463	0.016	0.178	-	0.607	329.6	46.20

*values are expressed as ppm of samples analyzed

content with 10×10^3 cfu g^{-1}, followed by *Astralagus sarcocolla* (2×10^3 cfu g^{-1}), *Anastatica hierochuntica* (17×10^2 cfu g^{-1}) and *Zingiber officinale* (5×10^2 cfu g^{-1}). *Aspergillus flavus* and *Aspergillus fumigatus* dominated the picture in twenty-one (60%) of the analyzed herbal plants with 9 isolates of 35 (25.7%) for each. Whereas *Aspergillus ochraceus* was isolated from 8 of 35 samples (22.9%).

Table 3 shows the heavy metal contents of 32 herbal plants analyzed. Two henna samples showed a lead content of more than 1 ppm (1.528 and 1.214 ppm, respectively). The rest of the samples showed a lead content of less than 1.0 ppm. Mercury content was the highest in *Lepidium sativum* with 0.630 ppm of lead, followed by *Artemisia herba alba* (0.102 ppm), henna (0.092 ppm), *Pimpinella anisum* and mixed herbs (both with 0.087 ppm each). The rest of the samples had mercury content less than 0.08 ppm. Higher contents of aluminum were recorded in *Zingiber officinale* (19.83 ppm) followed by *Anastatica hierochuntica* (17.95 ppm) and *Pimpinella anisum* (14.28 ppm) the rest of the samples had aluminum content less than 10 ppm. Calcium content was the highest in *Artemisia herba alba* (1.319 ppm) followed by *Aloe vera* (1.287 ppm), *Trigonella foenum* (1.281 ppm), *Zingiber officinale* (1.179 ppm) and *Astralagus sarcocolla* (1.164 ppm), *Boswelia carterii* (1.162 ppm), *Anethum graveolens* (1.288 ppm), *Lepidium sativum* (1.069 ppm) and *Cuminum cyminum* (1.055 ppm). The rest of the samples had calcium content less than 1.0 ppm. Cadmium content was highest with *Lepidium sativum*, *Vigna radiata* and *Zingiber officinale* (all had 0.025 ppm of cadmium). The rest of the samples had cadmium content less than 0.02 ppm. Copper was highest with *Cinnamomum zeylanicum* (0.284 ppm) followed by *Matricaria chamomilia* (0.282 ppm), *Carum carvi* (0.274 ppm), *Nigella sativa* (0.271 ppm), *Foeniculum vulgare*

(0.267 ppm), *Achillea fragrantissima* (0.244 ppm) and *Commiphora myrrah* (0.207 ppm). The rest of the samples had copper content less than 0.2 ppm. Iron was highest in *Zingiber officinale* with 19.44 ppm followed by *Matricaria chamomilia* (18.56 ppm), *Anastatica hierochuntica* (16.72 ppm) and *Salvia officinalis* (13.25 ppm). The rest of the samples had iron content less than 10 ppm. Zinc was the highest in *Salvia officinalis* (0.962 ppm) followed by *Nigella sativa* (0.956 ppm), *Lepidium sativum* (0.877 ppm) and *Cinnamomum zeylanicum* (0.817 ppm). The rest of the samples had zinc content less than 0.8 ppm. Potassium was the highest in *Matricaria chamomilia* (943.4 ppm) followed by Zingiber officinale (635.1 ppm), *Coffea arabica* (555.4 ppm), *Cuminum cyminum* (515.7 ppm) and mixed herbs (500.8 ppm). The rest of the samples had potassium content less than 500 ppm. *Pimpinella anisum* showed the highest content of sodium (307.4 ppm) followed by *Matricaria chamomilia* (227.7 ppm), *Ducrosia ismaelis* (155.4 ppm), henna (102.1 ppm) and *Foeniculum vulgare* (100.5 ppm). The rest of the samples had sodium content less than 100 ppm.

Microbial analysis of 32 herbal plants showed that Bacillus species was seen in 3 (9.7%) of the isolated microorganisms of which *Bacillus cereus* dominated with 14 isolates (45.2%). Other microbial isolates were *Aeromonas hydrophilia*, *Shigella* spp., *Enterobacter agglomerans*, *Enterobacter* spp., *Vibrio fluvialis*, *Escherichia coli*, *Pasteurella multocida*, *Enterobacter cloacae*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, *Acinetobacter iwoffii* and *Klebsiella* (Table 4). Sensitivity testing for antibiotics showed that most of these isolated microorganisms were sensitive to amoxicillin, gentamicin, imipinem, tobramycin and trimethoprim-sulfamethoxazole. *Enterobacter cloacae* showed resistance to ampicillin and cefazolin, whereas *Aeromonas hydrophilia* showed resistance to cefotaxime and ceftazidime. *Shigella* spp. showed resistance to cefazolin and *Escherichia coli* showed resistance to ciprofloxacin (Table 5).

Table 4: Microbial isolates from 31 herbal plants analyzed

Herbal plants	Microorganism isolated
Achillea fragrantissima	*Aeromonas hydrophila*
Aloe vera	-
Ammi visnaga	*Enterobacter* spp.
Anastatica hierochuntica	*Vibrio fluvialis*
Anethum graveolens	*Bacillus* spp.
Artemisia herba alba	*Bacillus cereus*
Astralagus sarcocolla	*Bacillus* spp.
Boswelia carterii	*Aeromonas hydrophila*
Calligonum comosum	*Bacillus cereus*
Carum carvi	*Enterobacter agglomerans*
Cinnamomum zeylanicum	*Corynebacterium* spp.
Coffea arabica	*Bacillus cereus*
Commiphora myrrah	*Bacillus cereus*
Cuminum cyminum	*Bacillus* spp.
Ducrosia ismaelis	*Shigella* spp.
Foeniculum vulgare	*Bacillus cereus*
Lepidium sativum	*E. coli*, *Shigella* spp., *Pasteurella multocida*
Matricaria chamomilia	*Enterobacter cloacae*
Nigella sativa	*Enterobacter agglomerans*
Pimpinella anisum	*Staphylococcus hyicus*
Salvia officinalis	*Bacillus cereus*
Thymus serpyllum	*Klebsiella rhinoscleomatis*
Trigonella foenum	*S. epidermidis*, *Acinetobacter iwoffii*
Vigna radiata	*Bacillus cereus*
Zingiber officinale	*Bacillus cereus*
Mixed herbs	*Bacillus cereus*
Henna 1	*Bacillus cereus*
Henna 2	*Bacillus cereus*
Henna 3	*Bacillus cereus*, *S. epidermidis*
Henna 4	*Bacillus cereus*
Henna 5	*Bacillus cereus*

Table 5: Bacterial sensitivity test results of microbial isolates from analyzed herbal plants

Microorganism	Amox	Ampi	Cefaz	Cefot	Cefta	Cipro	Genta	Imipi	Tobra	Trim
Enterobacter cloacae	S	R	R	S	S	S	S	S	S	S
Enterobacter agglomerans	S	S	S	S	S	S	S	S	S	S
Aeromonas hydrophila	S	S	S	R	R	S	S	S	S	S
Shigella spp.	S	S	R	S	S	S	S	S	S	S
K. rhinoscleromatis	S	S	S	S	S	S	S	S	S	S
Escherichia coli	S	S	S	S	S	R	S	S	S	S
Acinetobacter iwoffii	S	S	S	S	S	S	S	S	S	S
Bacillus cereus	S	S	S	S	S	S	S	S	S	S

S = Sensitive, R = Resistant, Amox: Amoxicillin, Ampi: Ampicillin, Cefaz: Cefazolin, Cefta: Ceftazidime, Cipro: Ciprofloxacin, Genta: Gentamicin, Imipi: Imipinem, Tobra: Tobramycin, Trim: Trimethoprim/sulfamethoxazole

DISCUSSION

In our study, *Aspergillus* spp. was the predominant fungi recovered and as such the major toxigenic species. Twenty-one (65.6%) of our samples analyzed had *Aspergillus flavus* and *Aspergillus fumigatus* dominating the picture. Five (15.6%) had enumeration limits of more than 2×10^2, the enumeration limit for total fungal count as set by the US Pharmacopoeia. This high percentage of fungal isolation form herbal plants as revealed by our results may indicate the inherent capacity of these moulds to instigate deleterious effects on humans when consumed. Aside from these, microorganisms have been isolated from our samples with *Bacillus cereus* isolated in 14/31(45.2%) of samples. *Bacillus cereus* is an endemic, soil dwelling bacteria that causes food borne illness (Ryan and Ray, 2004). When ingested, this microbe causes severe nausea, vomiting and diarrhea. Generally speaking, Bacillus foodborne illnesses occur due to survival of the bacterial spores when food is improperly cooked. *Escherichia coli* can also cause serious food poisoning in humans. In fact, this organism has been used widely as an indicator of fecal contamination in water. *Shigellla* cause dysentery by invading the colonic mucosa. They cause cell death and spread laterally causing mucosal ulceration, inflammation and bleeding. Severe infection with *Shigella* and its subspecies include hemolytic-uremic syndrome, seizures, sepsis and toxic megacolon in humans. *Vibrio fluvialis* has been associated with diarrhea although they have been rarely isolated (Hickman-Brenner *et al*., 1984). *Acinetobacter iwoffii* can cause severe respiratory disease (Robino *et al*., 2005). *Pasteurella multocida* has been associated with zoonotic infection in humans and Klebsiella with chronic diseases of the upper airways (Botelho-Nevers *et al*., 2006). *Enterobacter agglomerans* and *Staphylococcus epidermidis* are generally prevalent in the environment and usually relatively benign, it does have a potential for nosocomial infection (Geere, 1977). Furthermore, the sensitivity testing results in this study showed that most of the organisms isolated are sensitive to the more common and available antibiotics, thus, available treatment protocols are in place for such bacterial colonization in humans.

Present study also showed contamination with heavy metals, some of them toxic to humans. In general, plants do not absorb or accumulate lead however, in soils with high lead content; it is possible for some lead to be taken up. Soils with lead levels exceeding 100 ppm should not be used for gardening and plants can be safely eaten from soils with soil lead levels up to 300 ppm for leafy and root type vegetables (www.grayenvironmental.com). Limit of quantification for lead in herbal medicine should not exceed 2.0 ppm. Lead was found in all of our samples, of which 2 henna samples showed 1.2-1.5 ppm lead content. This level however was not considerably high to alarm for human use or consumption. Similarly, trace levels of mercury and cadmium were present in our samples of up to 0.1 ppm. Limit of quantification for mercury is up to 0.5 and 0.20 ppm for cadmium. The highest recorded amount of mercury was 0.102 in *Artemisia herba alba* Whereas the highest level of cadmium was 0.025 ppm recorded in 3 samples. These levels of mercury and cadmium do not appear to be of health concern. Aluminum level was high in three herbs (*Anastatica hierochuntica*, *Salvia officinalis* and *Zingiber officinale*) of levels 14.28-19.83 ppm. This is way above the maximum allowed level of

0.2 ppm. Aluminum, as a metal when present in our food, water supply and soil can induce individuals to suffer from aluminum toxicity. After years of accumulated exposure and storage in our body, it can result to brain degeneration and skeletal deformities (www.drpepi.com). It is believed that Alzheimer's disease is related to aluminum toxicity (Derouesne, 2004).

Minerals such as magnesium and zinc are just as critical to maintaining optimal health, or that, taken in excess, these minerals can be toxic. Iron is essential for blood cells; potassium is needed for a healthy nervous system and zinc to enhance immunity and for reproductive function. However, when taken in amounts over the recommended maximum allowable range, they can be toxic to health. These effects occur in nervous system. However, our study showed that none of our samples exceeded the maximum allowable level of 5 ppm for zinc. Although 4 of samples had iron levels more than the maximum allowed level of 15 ppm, these levels were not significantly high. Similarly, most of our samples have high levels of potassium and sodium. Although potassium and sodium are essential to health, excess of potassium can cause cardiac dysfunction and excess sodium can cause metabolic problems and hypertension.

The results of this study especially on the heavy metal contents of herbal plants implicate an impending danger for consumers. Significant serious consequences may appear due to accumulation of heavy metal contents through years of frequent use of these herbal medicines. Several degenerative and life-threatening conditions were linked to accumulation of toxic metals in the body. When our body is compounded with microbial infection to an already toxin-filled system, the capacity of our immune system to defend itself is exhausted. Although this study showed heavy metal levels within the allowable limits, it is possible that some amounts can be taken up by the system and accumulate for years of use, thus cause serious consequences. Even if these metals found in herbal medicines are less likely than free to bind with molecules in our body and thus slower to be absorbed, the issue of safety and vigilance on its serious adverse effects be of a concern. Furthermore, the continued practice of safety and precautionary measures from the harvest area (e.g., minimization of pesticide use) to the household (e.g., thorough cleaning and washing of herbal plants prior to use) should be practiced.

CONCLUSION

There are considerable amounts of microbial contamination and heavy metal concentrations in commonly used herbal plants. Consumers have the right to be informed and must be warned about health hazards through proper signs, labels and brochures indicating possible dangers lurking in their food and household products.

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