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Heavy metal contamination in South African medicinal plants: A cause for concern

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ABSTRACT

The quality and safety of herbal medicines is becoming a major concern worldwide particularly due to contamination by heavy metals. The present study quantified the levels of heavy metals in frequently used South African medicinal plants and determined the variations in certain biological activities and phytochemical compositions. Eleven plant species were obtained from both *muthi* shops (MS) (commercial outlets) and from open street markets (OSM) for comparison. Samples were dried, powdered and digested using microwave acid-assisted digestion. The digested solutions were analysed for heavy metals using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Plants were classified based on their elemental composition using chemometric techniques. Powdered plant samples were extracted using 70% acetone and screened for antibacterial activity against *E. coli* and *S. aureus*. Phytochemical analyses were carried out to determine total phenolic and flavonoid content. Of the 22 samples analysed, *Bulbine natalensis* obtained from OSM and *Alepidea amatymbica* obtained from MS exhibited high levels of Al [5559 and 4392 mg/kg dry weight (DW)] and Fe (4164 and 4465 mg/kg DW) respectively. Levels of As and Hg were above the World Health Organization permissible limits in most of the samples analysed. Hierarchical cluster analysis classified the samples into four groups based on their metallic analyte concentrations. Group one having low metal content and group four having a high metal content. In general, plant samples with high levels of metals yielded greater antibacterial activity. However, antibacterial activity recorded in this study is not an indicator of high levels of heavy metal contamination as some samples despite the high levels of metal exhibited low antibacterial activity. The variations in the amounts of phenolics and flavonoids in the evaluated samples could have probably been that some of the plant samples may have been harvested from different localities or at different times of the year, perhaps plant age or degree of storage. The results highlighted the need for in-depth risk and quality assessments.

1. Introduction

The quality and safety of herbal medicines are major concerns worldwide due to increasing heavy metal contamination resulting from anthropogenic activity. Adverse effects of herbal medicines contaminated with heavy metals are well documented (Ernst, 2002; Steenkamp et al., 2006; Dargan et al., 2008). In South Africa, cases of metal poisoning associated with the use of traditional medicines are common, with arsenic (As), chromium (Cr) and magnesium (Mg), the most frequently implicated metals resulting in poisoning, morbidity and mortality (Steenkamp et al., 2002). Thus, the screening of traditional medicines for potentially harmful components has been recommended to protect consumers (Chang, 1995).

Some medicinal plants have the ability to accumulate heavy metals when grown in contaminated soils. The addition of heavy metals in herbal medicinal products through deceitful practices such as adulteration for alleged increase in therapeutic properties is also well documented (Ernst, 2002; Chan, 2003; Haider et al., 2004). Thus stringent legislation on the production and processing of herbal medicine as well as detailed information on herbal products is of paramount importance to protect consumers. One of the most common practices in South Africa is the collection of medicinal plants from wild populations (Zschocke et al., 2000). This not only threatens the ecological balance but also leads to safety concerns as a result of heavy metal contamination arising from industrial encroachment. In South Africa, medicinal plants are commonly sold at informal open street markets (OSM) or indoor *muthi* shops (MS). The OSM are usually positioned in the hub of the city centre to allow easy access to commuters. This exposes herbal material to various kinds of urban pollutions such as industrial and vehicular emissions. Numerous efforts have been made by the South African Medicines Regulatory Authority in terms of regularizing the practice of South African Traditional Medicine. However, issues regarding the procurement, processing and sale of herbal products are still major

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concerns. The source and quality of raw materials as described in good agricultural and collection practices (GACP) and good manufacturing processes (GMP) are essential steps for quality control of herbal medicine. These will play a pivotal role in guaranteeing the quality and stability of herbal products (WHO, 1998). According to WHO (2007), the concentration of trace elements must be controlled in medicinal plants in order to meet and improve quality assurance and safety.

Heavy metal contaminations in medicinal plants affect the phytochemical composition as well as the biological activities and thus influence the efficacy of medicinal plant products (Street, 2012). Zahid et al. (2011) reported that the presence of heavy metals in the medicinal plant *Marsilea minuta* reduced antimicrobial activity by two possible mechanisms either by changing the amounts of bioactive compounds in the plants or by deactivating the bioactive compounds through chelation of metal ions. There is insufficient information linking the quality, safety concerns and efficacy measures with regard to heavy metals in South African medicinal plants.

The aim of this study was to quantify heavy metals in some frequently used South African medicinal plants obtained from both herbal shops and outdoor markets and to classify the samples based on their elemental composition using chemometric techniques. Plant samples were also assessed for efficacy by screening the antimicrobial activity and quantifying phenolic and flavonoid contents.

2. Materials and methods

2.1. Sample collection and preparation

Eleven plant species were obtained from both MS and OSM in Pietermaritzburg, KwaZulu-Natal, South Africa. Voucher specimens were prepared and are deposited at the John Bews Herbarium, University of KwaZulu-Natal, Pietermaritzburg. Information with regards to the use of the selected medicinal plants is presented in Table 1. All plant materials were washed under running tap water, oven dried at 50 °C and ground into powders. The powdered samples were used for analysis.

2.2. Reagents and solutions

All reagents used were of analytical grade. Ultra-pure (UP) water was used for preparing the solutions and dilutions. Stock solutions of metals (1000 mg/L) were prepared from their nitrate salts.

2.3. Microwave acid-assisted digestion

A microwave acid-assisted system was used to digest plant material. Plant samples (0.5 g DW) were placed in Teflon vessels and 10 mL 55% HNO_3 added. The vessels were heated in a microwave system operating up to 1200 W. The temperature of the microwave system was programmed as follows: 1st stage, 2 min heating from ambient to 170 °C; 2nd stage, 3.30 min heating to 180 °C and 3rd stage, 9.30 min held at 180 °C. After complete digestion, the clear solutions were transferred to 50 mL volumetric flasks and made to volume with UP water. These samples were stored in high density polyethylene bottles until analysis. A blank containing 10 mL 55% HNO_3 was used.

2.4. Elemental analysis using ICP-OES

An Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Varian 720-ES, Varian Inc, Palo Alto, CA, USA) instrument was used for elemental analysis. ICP-OES provides a multi-elemental analysis and supports a broad linear calibration range. ICP-OES is a powerful tool that is used in determining metal concentrations in a variety of different sample matrices (Hou and Jones, 2000). The operating conditions for the ICP-OES instrument were as follows: RF power 1.0 kW; viewing geometry axial; Argon gas used as plasma gas flow at the rate of 15.0 L min^{-1}; auxiliary gas flow rate 1.50 L min^{-1}; nebulizer gas flow rate 0.75 L min^{-1}; and replicate reading time 9.0 s. All analyses were performed in triplicate.

2.5. Sample preparation for antibacterial and phytochemical screening

The ground material (1 g DW) was extracted with 70% acetone in a sonication bath (Julabo GmbH sonicator) for 1 h. The plant extracts were then filtered using Whatman No. 1 filter paper. The filtrates were concentrated by a rotary evaporator and then air-dried under a stream of cold air. The dried extracts were kept in the dark at 10 °C until ready for use. The dried extracts were resuspended in 50% methanol and used for the phytochemical analysis.

2.6. Antibacterial microdilution assay

Antibacterial activity of the plant extracts was determined using the minimum inhibitory concentration (MIC) technique (Eloff, 1998; Ndhlala et al., 2009). Overnight cultures of a Gram-negative (*Escherichia coli* ATCC 11775) and a Gram-positive (*Staphylococcus aureus* ATCC 12600) bacterial strains were used. Neomycin was used as the positive control and 70% acetone was used as the negative control. The MIC was recorded as the concentration of the last well in which there was no bacterial growth. The assay was repeated twice in duplicate for each extract.

Table 1
Selected South African medicinal plants investigated in this study and their uses in treating various ailments.
Information presented in this table was sourced from Van Wyk et al. (2008) and Hutchings et al. (1996).

Plant family	Species	Voucher number	Plant part	Traditional uses
Celastraceae	*Cassine transvaalensis* (Burtt Davy)	A Okem 10 NU	Stem bark	Infusion is used for stomach pain and haemorrhoids and as enemas
Hypoxidaceae	*Hypoxis hemerocallidea* Fisch. & C.A. Mey.	A Okem 21 NU	Tuber	Immune booster of HIV patients, treating prostate hypertrophy and urinary tract infections, treating cancer and as emetics
Myrsinaceae	*Rapeana melanophleos* (L.) Mez	A Okem 14 NU	Stem bark	Decoction used as expectorants and emetics, stomach disorders, muscular pains and to strengthen the heart
Asphodelaceae	*Bulbine natalensis* A. Rich	A Okem 18 NU	Root	Back pain and kidney diseases, venereal diseases, diabetes, rheumatism and to purify blood
Passifloraceae	*Adenia gummifera* (Harv.) Harms	A Okem 20 NU	Stem	Infusion used as emetics, decoction for malaria
Apiaceae	*Alepidea amatymbica* Eckl.	A Okem 16 NU	Root	Decoction used for colds and chest complaints, as well as for asthma, influenza and abdominal cramps
Hyacinthaceae	*Drimia elata* Jacq. Ex Willd.	A Okem 19 NU	Bulb	Bulb scales are rubbed on chest for stabbing pain, infusion used as emetic, expectorants and diuretic
Lycopodiaceae	*Lycopodium clavatum* L.	A Okem 8 NU	Whole plant	Kidney stones, urinary tract infections, gastric inflammations, for lung and bronchial disorders and fevers
Lauraceae	*Ocotea bullata* (Burch.) E. Mey.	A Okem 9 NU	Stem bark	Bark infusion for headache, nervous disorders, stomach pain and urinary disorders
Hyacinthaceae	*Schizocarphus nervosus* (Burch.) Jessop	A Okem 15 NU	Bulb	Infusion for enema and rheumatic fever
Cucurbitaceae	*Momordica balsamina* Schumach.	A Okem 13 NU	Root	High blood pressure, diabetes, stomach disorders, malaria and hepatitis B

2.7. Screening for phenolics and flavonoids

The amount of total phenolics was evaluated using the Folin Ciocalteu (Folin C) assay (Makkar, 1999). In triplicate, 50 μL of each plant extract was transferred into test tubes and made up to 1 mL by adding 950 μL of distilled water followed by 500 μL of 1 N Folin C phenol reagent and 2.5 mL of 2% sodium carbonate. A blank that contained aqueous methanol instead of plant extract was included. The test mixtures were incubated for 40 min at room temperature, thereafter the absorbance was read at 725 nm using a UV–vis spectrophotometer (Varian Cary 50, Australia). Total phenolic contents were expressed as gallic acid equivalents (GAE).

Flavonoid content was determined using the aluminium chloride colorimetric assay as described by Zhishen et al. (1999) and Marinova et al. (2005). In triplicate, 500 μL of plant extracts was pipetted into test tubes and 2 mL of distilled water was added to all test tubes, followed by 150 μL of 5% $NaNO_2$. After incubating for 5 min, 150 μL of 10% $AlCl_3$ was added to all the test tubes. At the 6th min of incubation, 1 mL of 1 M NaOH was transferred to all the test tubes and the volume was increased to 5 mL by adding 1.2 mL of distilled water. Thereafter, the mixture was vortexed and the absorbance was read at 510 nm using a UV–vis spectrophotometer against a reagent blank containing 50% methanol instead of the plant extract. Total flavonoid content was expressed as catechin equivalents (CTE).

2.8. Statistical analysis

The data sets were subjected to multivariate statistical analyses using SPSS statistical package version 21.0 for Windows.

2.9. Hierarchical cluster analysis (HCA)

This is an unsupervised technique that involves classification and measurement of similarity between samples to be clustered. The HCA technique was applied to the data sets using SPSS statistical package using Ward's method with Euclidean distance to calculate the sample interpoint distance.

3. Results and discussion

3.1. Elemental contents

Variable concentrations of heavy metals were recorded in all samples analysed (Table 2). Samples of *Bulbine natalensis* obtained from OSM and *Alepidea amatymbica* obtained from MS exhibited extremely high levels of Al (5559 and 4392 mg/kg DW) and Fe (4164 and 4465 mg/kg DW) respectively. This was contrary to a previous study which reported low levels of heavy metals in *A. amatymbica* (Mtunzi et al., 2012). *Hypoxis hemerocallidea* has been reported to have the ability to take up and accumulate high amounts of Al and Fe (Jonnalagadda et al., 2008). In the present study, high levels of Al and Fe were also recorded in corms of *H. hemerocallidea* obtained from MS. It is well known that over 80% of South Africans use medicinal plants for their day-to-day healthcare to manage various kinds of ailments. One example of a highly consumed medicinal plant is *H. hemerocallidea* which was acclaimed as a medicine for all kinds of diseases including HIV/AIDS (Ojewole, 2006). The consumption of these medicinal plants which have the tendency to accumulate high levels of various toxic metals could be a potential threat to consumer health. One of the most ubiquitous elements in the environment is aluminium and could be the reason for the high levels detected in the plant samples analysed. The daily allowance for Al ingestion in humans has been estimated to range from 10 to 50 mg (National Research Council, 1982). Ingestion of high levels of Al could impair cognitive and speech functions, and may lead to neurodegerative diseases (Bolla et al., 1992). The exact role of Al in the development of neurodegenerative disease is still a subject of intense research and debate (Shafer and Mundy, 1995). Intake of high levels of Fe may be toxic, causing severe damage in the stomach or haematemesis leading to gastric discomfort, nausea, vomiting and diarrhoea. It may also lead to necrosis of mucosal cells and perforation of the gut wall (Bourman and Rand, 1980). The high levels of Al and Fe in most of the samples analysed in the present study are a safety concern. It is therefore important that a comprehensive elemental profile of important medicinal plants is carried out to safeguard the health of consumers.

The concentrations of Cd and Pb were below the WHO permissible limits (0.3 and 10 mg/kg respectively) in all the samples analysed. Elements such as Cd and Pb are toxic at very low concentrations (Nies, 1999). The levels of As and Hg were high in most of the samples analysed, with concentrations above the safety limits of 0.2 mg/kg and 2 μg/kg (Codex Alimentarius Commission, 1991; WHO, 2010) respectively. Street et al. (2008) also reported high levels of As and Cd which exceeded the permissible limits recommended by the WHO (1998) in bulbs and tubers of some South African medicinal plants obtained from OSM in Pietermaritzburg (KwaZulu-Natal, South Africa). Arsenic at low concentrations causes clinical manifestations such as chronic obstructive pulmonary disease and bronchiectasis, liver disease, cancer of the skin, lung and bladder (Guha, 2008). In an earlier study, Steenkamp et al. (2006) reported that the levels of toxic heavy metals in South African medicinal plants are within the permissible limits except for Mn which was relatively high in most of the investigated plant samples. A similar pattern in Mn concentrations was found in the present study alongside Cr and other toxic heavy metals which are above the permissible limits (Table 2). Toxic levels of Cr can cause irritation of skin, damage to kidneys, liver, circulatory and nerve tissues, respiratory problems and nose bleeds (Friberg et al., 1986). The levels of metal concentrations found in some of the samples in the present study highlights the need for further investigation on the safety and quality assurance in South African herbal medicines which is largely based on collections from the wild. There is a need for sustainable cultivation of valued medicinal plants to prevent heavy metal accumulation as well as to enable consistency in terms of quality and efficacy.

Some of the elements determined in this study are known to play beneficial roles in plants such as macro- (Fe) and micro-elements (Mn, Zn and Cu) (Jeffrey, 1987). For example, Zn, Mn and Fe are important co-enzymes in antioxidant processes and deficiency in any of these essential elements may impair the overall function of the oxidation systems (Lemberkovics et al., 2002). However, extremely high levels of these essential elements can be toxic (Sandstead, 1995). The concentration of these essential elements in most of the samples analysed in the present study is of concern.

3.2. Hierarchical cluster analysis

The data on elemental compositions were subjected to hierarchical cluster analysis and the results obtained are presented as a dendrogram (Fig. 1). All the samples were separated into four main groups except for *Drimia elata* obtained from OSM and *Lycopodium clavatum* obtained from MS which did not belong to any of the clusters. These groups were:

Group 1 *Cassine transvaalensis* MS, *C. transvaalensis* OSM, *Ocotea bullata* OSM, *Adenia gummifera* MS, *A. gummifera* OSM, *Rapanea melanophloeos* OSM, and *R. melanophloeos* MS.
Group 2 *O. bullata* OSM, *Momordica foetida* OSM, *M. foetida* MS, *H. helmerocallidea* OSM, *Schizocarphus nervosus* MS, *D. elata* MS.
Group 3 *B. natalensis* MS, *S. nervosus* OSM, *H. helmerocallidea* MS, *L. clavatum* OSM.
Group 4 *A. amatymbica* MS, *A. amatymbica* OSM, *B. natalensis* OSM.

Cluster analysis show samples in each group having similar patterns in elemental concentrations which implies that they may either have been harvested from the same locality, or have similar strategies for heavy metal accumulation. The plant part used could have been the major means for the high levels of heavy metals. For instance, group 4

Table 2
Elemental composition in South African herbal medicinal material investigated (mg/kg DW). Results are presented as mean ± SE (n = 3).

Plant	Source	Metal element and the analytical wavelength (nm)											
		Zn 213.857	Cr 267.716	Mn 259.372	Fe 238.204	Ni 231.601	Cu 324.754	Cd 226.502	Hg 194.164	Al 237.312	Sn 283.998	Pb 220.353	As 193.696
Cassine transvaalensis	MS	3.78 ± 0.03	4.82 ± 0.23	11.29 ± 2.84	59 ± 11.06	2.58 ± 0.94	2.77 ± 0.10	0	2.39 ± 0.55	41.62 ± 1.76	40.15 ± 2.13	0	0.06
	OSM	4.36 ± 0.21	4.76 ± 0.64	12.73 ± 2.47	206 ± 1.66	1.78 ± 0.05	3.48 ± 0.55	0	8.15 ± 0.66	26.48 ± 5.90	42.08 ± 0.33	1.22 ± 0.08	0
Hypoxis helmerocallidea	MS	40.67 ± 2.90	6.54 ± 0.36	234.79 ± 8.50	698 ± 82.33	2.59 ± 0.36	7.83 ± 1.18	0.04	0.15	938 ± 109.16	37.99 ± 5.97	1.50 ± 0.35	0.55 ± 0.02
	OSM	27.15 ± 1.76	5.78 ± 0.27	182.25 ± 5.25	342 ± 54.64	2.54 ± 0.16	11.90 ± 0.49	0	14.25 ± 0.0	267 ± 13.32	86.21 ± 10.04	0.58 ± 0.07	0.47 ± 0.04
Rapenea melanophloeos	MS	3.99 ± 0.01	6.22 ± 1.09	55.14 ± 1.65	114 ± 3.75	2.52 ± 0.15	1.96 ± 0.38	0	0.74 ± 0.11	92.60 ± 0.99	38.72 ± 5.69	1.96 ± 0.03	5.19 ± 0.15
	OSM	5.16 ± 0.04	5.31 ± 0.12	163 ± 4.27	78.30 ± 8.23	2.47 ± 0.41	3.47 ± 0.17	0.04	0.84 ± 0.03	49.66 ± 5.66	83.08 ± 1.81	0.24 ± 0.03	0.40 ± 0.09
Bulbine natalensis	MS	107.33 ± 4.42	8.69 ± 0.26	169 ± 21.38	729.59 ± 26.56	3.82 ± 0.27	19.70 ± 1.39	0.24 ± 0.07	9.56 ± 0.18	772.56 ± 88.40	57.50 ± 18.68	5.16 ± 0.61	5.22 ± 0.12
	OSM	51.68 ± 1.60	237 ± 4.36	479 ± 20.28	4164 ± 513.24	6.28 ± 1.02	4.58 ± 0.50	0.28 ± 0.02	0.48 ± 0.08	5559 ± 220.85	3.67 ± 0.04	3.69 ± 0.16	2.50 ± 0.06
Adenia gummifera	MS	28.37 ± 10.65	6.30 ± 1.34	23.80 ± 2.06	165.50 ± 56.60	2.80 ± 0.19	4.10 ± 0.98	0.04	0.79 ± 0.07	41.58 ± 3.50	77.04 ± 1.36	0	0
	OSM	34.31 ± 4.95	5.76 ± 0.87	32.77 ± 2.06	70.34 ± 10.13	6.51 ± 0.83	4.12 ± 0.99	0	3.13 ± 0.46	21.68 ± 7.38	76.43 ± 0.44	0.72 ± 0.03	0
Alepidea amatymbica	MS	55.93 ± 4.06	17 ± 0.68	248.69 ± 2.51	4465 ± 1.08.50	12 ± 1.71	19.33 ± 0.81	0.27 ± 0.01	0	4392 ± 173.55	33.84 ± 6.19	0.37 ± 0.06	2.15 ± 0.41
	OSM	45.25 ± 3.97	33 ± 7.06	238 ± 36.48	3345 ± 390.54	30 ± 4.95	13.00 ± 1.31	0.39 ± 0.10	0.04	3954 ± 266.53	85.40 ± 6.88	4.54 ± 0.80	1.96 ± 0.03
Drimia elata	MS	34.07 ± 4.57	7.84 ± 0.09	60.69 ± 4.72	592.98 ± 50.05	4.24 ± 0.52	5.61 ± 0.12	0.01	0.76 ± 0.08	559.78 ± 71.31	79.81 ± 0.41	0.22 ± 0.06	0
	OSM	102.57 ± 5.33	12 ± 1.98	145.76 ± 4.18	1634 ± 78.61	10 ± 0.78	11.25 ± 0.43	0.06	0.04	1595 ± 73.63	31.40 ± 6.39	1.23 ± 0.37	1.75 ± 0.05
Lycopodium clavatum	MS	24.37 ± 5.21	21 ± 2.61	315 ± 18.26	230.57 ± 38.01	6.55 ± 0.64	7.15 ± 1.24	0.11 ± 0.03	0.41 ± 0.01	2496 ± 143.84	59.88 ± 2.29	3.93 ± 0.01	1.76 ± 0.03
	OSM	20.48 ± 5.07	12 ± 1.89	144 ± 44.03	640.88 ± 21.08	4.56 ± 0.71	6.25 ± 1.51	0.15 ± 0.01	1.27 ± 0.11	1253 ± 57.23	27.04 ± 1.43	0	2.04 ± 0.05
Ocotea bullata	MS	38.54 ± 4.65	10 ± 0.55	51.48 ± 1.35	399.61 ± 44.68	8.65 ± 0.22	18.51 ± 6.05	0.05	1.77 ± 0.03	222.95 ± 0.30	33.96 ± 5.61	0	1.80 ± 0.10
	OSM	5.53 ± 0.82	5.53 ± 0.18	21.88 ± 1.26	59.72 ± 4.67	3.17 ± 0.12	2.37 ± 0.55	0	1.29 ± 0.21	29.92 ± 2.92	24.63 ± 6.67	0	1.61 ± 0.38
Schizocarphus nervosus	MS	25.28 ± 3.39	9.99 ± 0.52	49.57 ± 1.04	434.89 ± 24.40	4.89 ± 0.14	3.71 ± 0.89	0	1.50 ± 0.02	459.01 ± 23.06	26.01 ± 5.41	0.1	2.52 ± 0.80
	OMS	19.79 ± 2.41	9.96 ± 1.33	41.86 ± 0.54	793 ± 154.92	3.98 ± 0.04	2.46 ± 0.48	0.01	0.90 ± 0.05	809 ± 111.56	26.40 ± 11.07	0.42 ± 0.09	1.68 ± 0.26
Momordica foetida	MS	34.56 ± 9.08	73 ± 4.02	44.77 ± 1.59	446.34 ± 20.32	5.57 ± 1.03	5.78 ± 0.08	0.01	2.46 ± 0.43	382.26 ± 24.45	182.20 ± 7.47	3.62 ± 0.80	1.77 ± 0.31
	OSM	36.11 ± 1.80	6.00 ± 0.18	55.28 ± 2.09	266.74 ± 21.40	3.31 ± 0.13	6.61 ± 0.29	0.01	1.49 ± 0.40	215.75 ± 26.78	25.27 ± 7.38	2.87 ± 1.02	2.30 ± 0.62

MS = *muithi* shops, OSM = open street markets.

is all rhizomes and bulbs; and group 3 includes bulbs, corms and whole plants, all of which are in direct contact with the soil when the plant is growing. Hence, there is likelihood that plants in this group will contain more of the elements, due to the fact that most plants tend to accumulate elements in the underground parts as compared to translocation to the above ground parts. Group 2 is a mixture of plant parts and group 1 includes mainly stems and bark. Samples of *D. elata* OSM and *L. clavatum* MS which did not belong to any of the clusters, indicate that these plant samples might have come from different localities or have been exposed to contaminants after harvesting.

This study shows that HCA can be used in preliminary screening to group medicinal plants based on their metal analyte content using software. The potential to develop HCA techniques as a useful tool to improve the safety of medicinal plants needs to be further investigated. By developing a large database of heavy metal content in medicinal plant species collected from known sites and comparison of their metal content, it should be possible to identify plant species that are relatively safe in terms of their metal content. For instance, all plant samples in group 1 have low concentrations of metals whereas plant samples in group four have extremely high levels of heavy metals. Such an analysis would highlight the potential hyperaccumulating species which could be more closely monitored to safeguard the consumer health.

3.3. Antibacterial activity and phytochemical compositions

Plant extracts that exhibited MIC values <1 mg/mL (highlighted in bold in Table 3), were considered as having good antibacterial activities (Gibbons, 2005). Of the 22 samples tested, only 8 showed good antibacterial activity against *E. coli* compared to 14 samples that have good activity against *S. aureus*. In general, plant samples with high levels of metals yielded greater antibacterial activity. Noori et al. (2012) reported that aqueous extracts of *Verbascum speciosum* Schard obtained from heavy metal contaminated land exhibited potent antibacterial activity against *Salmonella paratyphi* at different concentrations. When a plant is exposed to heavy metal stress it adjusts several biochemical and physiological processes for survival but these to some extent favour the production of potent secondary metabolites. This could be one of the reasons for the high antibacterial activity noted in this study for plants with high levels of heavy metals. The exceptions in this study were *D. elata* obtained from both MS and OSM; *B. natalensis* obtained from OSM, and *L. clavatum* obtained from OSM which exhibited poor antibacterial activity despite having high levels of metals. This implies that antibacterial activity is not necessarily an indicator of high levels of metal contamination. The use of bioassays in determining the quality of herbal products is a widely acceptable method for screening. The result of antibacterial activity in the present study indicates that the efficacy of the investigated plants has been compromised in one way or another due to heavy metal contamination. Street et al. (2009) reported increased antibacterial activity in *Merwilla plumbea* plants treated with high levels of Cd.

Phenolic concentrations are presented in Fig. 2. Samples of *C. transvaalensis*, *B. natalensis* and *S. nervosus* obtained from MS exhibited significantly higher phenolic and flavonoid contents than samples of the same species obtained from the OSM. The exception was *O. bullata* obtained from the OSM which had higher flavonoids (Fig. 3) than the MS. Surprisingly extracts of *B. natalensis* and *S. nervosus* obtained from the MS and *O. bullata* obtained from OSM exhibited poor antibacterial activity against *E. coli* and *S. aureus* despite their higher phenolic and flavonoid contents. The levels of phenolic in *A. amatymbica*, *R. melanophloeos*, *A. gumnifera*, *D. elata* and *M. foetida* in the samples obtained from the OSM were higher compared to samples obtained from the MS. These samples exhibited good antibacterial activity against either *E. coli* or *S. aureus.* Evaluating the quality and authenticity of herbal products using phytochemical screening is a widely accepted method in quality control of medicinal plant products. The variations in the

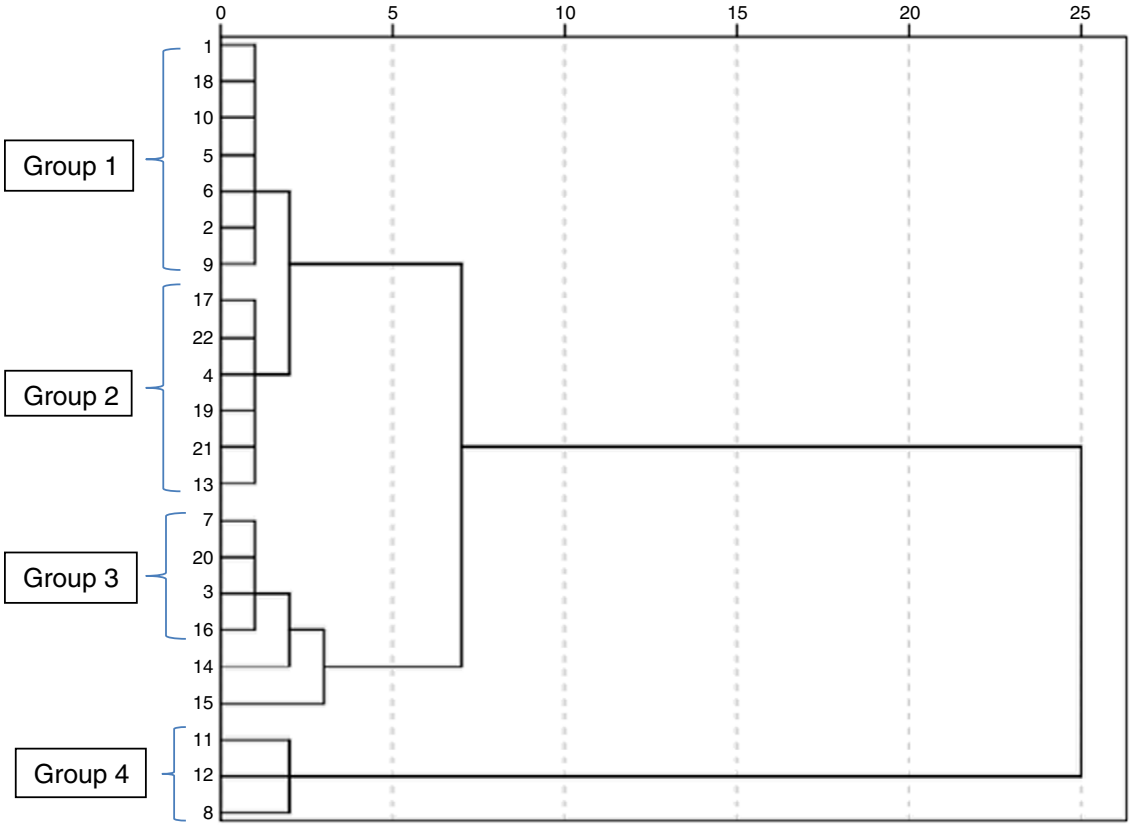

Fig. 1. Dendogram of some important South African medicinal plants based on their elemental compositions obtained by HCA using Ward's method. Group 1: 1 = *Cassine transvaalensis* MS, 2 = *Cassine transvaalensis* OSM, 18 = *Ocotea bullata* OSM, 9 = *Adenia gummifera* MS, 10 = *Adenia gummifera* OSM, 5 = *Rapanea melanophloeos* OSM, 6 = *Rapanea melanophloeos* MS. Group 2: 17 = *Ocotea bullata* MS, 21 = *Momordica foetida* MS, 22 = *Momordica foetida* OMS, 4 = *Hypoxis helmerocallidea* OSM, 19 = *Schizocarphus nervosus* MS, 13 = *Drimia elata* MS. Group 3: 7 = *Bulbine natalensis* MS, 20 = *Schizocarphus nervosus* OSM, 3 = *Hypoxis helmerocallidea* MS, 16 = *Lycopodium clavatum* OSM. Group 4: 11 = *Alepidea amatymbica* MS, 12 = *Alepidea amatymbica* OSM, 8 = *Bulbine natalensis* OSM. 14 = *Drimia elata* OSM, and 15 = *Lycopodium clavatum* MS.

amounts of phenolic and flavonoid in the present study highlighted the need for in-depth quality checks of medicinal plant material used in South African traditional medicine. In a previous study, an increase in phenolics was shown to be correlated with an increase in activities of enzymes involved in metabolism of phenolic compounds under heavy metal stress (Winkel-Shirley, 2002; Michalak, 2006). However, in the present study the levels of metal compositions in most of the plant samples analysed did not translate into high levels of phenolics. This indicates that the concentration of heavy metals in these plant species could be from post-harvest contamination.

Table 3
Antimicrobial activity of herbal samples obtained from MS and OSM (mg/mL).

Plant name	Source	*E. coli*	*S. aureus*
Cassine transvaalensis	MS	3.125	**0.78**
	OSM	3.125	1.56
Hypoxis helmerocallidea	MS	3.125	3.125
	OSM	**0.78**	1.56
Rapenea melanophloeos	MS	1.56	1.56
	OSM	6.25	**0.78**
Bulbine natalensis	MS	**0.78**	**0.39**
	OSM	3.125	1.56
Adenia gummifera	MS	6.25	**0.78**
	OSM	12.5	**0.39**
Alepidea amatymbica	MS	**0.195**	**0.39**
	OSM	**0.195**	**0.39**
Drimia elata	MS	12.5	6.25
	OSM	12.5	12.5
Lycopodium clavatum	MS	6.25	**0.78**
	OSM	6.25	1.56
Ocotea bullata	MS	1.56	**0.78**
	OSM	**0.195**	**0.39**
Schizocarphus nervosus	MS	**0.195**	**0.39**
	OMS	**0.78**	**0.39**
Momordica foetida	MS	1.56	**0.78**
	OSM	**0.195**	**0.39**

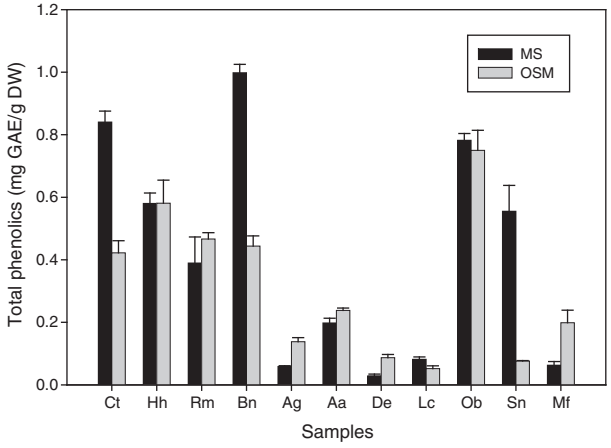

Fig. 2. Total phenolic composition expressed as gallic acid equivalents for South African medicinal plants collected from MS and OSM. DW = Dry weight; GAE = Gallic acid equivalents; Ct = *Cassine transvaalensis*; Hh = *Hypoxis hemerocallidea*; Rm = *Rapenea melanophloeos*; Bn = *Bulbine natalensis*; Ag = *Adenia gumnifera*; Aa = *Alepidea amatymbica*; De = *Drimia elata*; Lc = *Lycopodium clavatum*; Ob = *Ocotea bullata*; Sn = *Schizocarphus nervosus*; Mf = *Momordica foetida*.

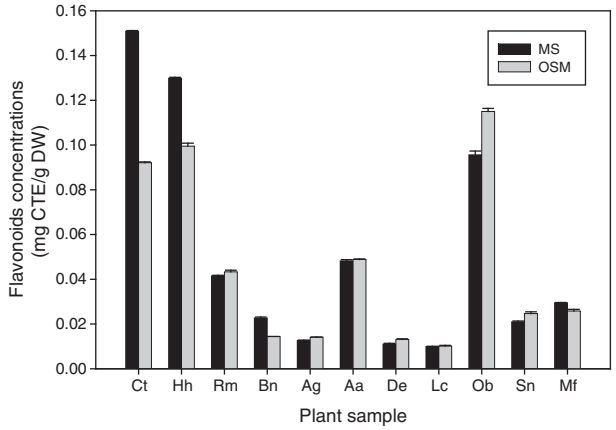

Fig. 3. Flavonoid concentration as catechin equivalents for some South African medicinal plants collected from MS and OSM. DW = Dry weight; CTE = Catechin equivalent; Ct = *Cassine transvaalensis*; Hh = *Hypoxis hemerocallidea*; Rm = *Rapenea melanophloeos*; Bn = *Bulbine natalensis*; Ag = *Adenia gumnifera*; Aa = *Alepidea amatymbica*; De = *Drimia elata*; Lc = *Lycopodium clavatum*; Ob = *Ocotea bullata*; Sn = *Schizocarphus nervosus*; Mf = *Momordica foetida*.

4. Conclusions

The levels of heavy metals in the present study highlight the need for an in-depth investigation into the safety and authenticity of medicinal plant material sold in South African traditional medicinal markets. The cluster analysis revealed four main clusters to account for all the species analysed, except for *D. elata* obtained from the OSM and *L. clavatum* obtained from the MS which did not fit into any of the clusters. Strict adherence to GACP of medicinal plant material is of paramount importance to safeguard the health of consumers. Variable antibacterial activity and phytochemical compositions recorded in the present study was not an indicator of high levels of heavy metal compositions. This variability could be a result of harvesting of plant materials at different times of the year or from different localities, age of the plants and storage periods. Hence, there is a need to encourage sustainable cultivation of important medicinal plants and GACP to ensure consistency in terms of safety and efficacy of medicinal products.

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References

Bolla, J.L., Ross, A.H., Spector, D., Schwartz, B.S., Wieler, L., Herron, J., Gimenez, L., 1992. Neurocognitive effects of aluminum. Archives of Neurology 4, 1021–1026.
Bourman, W.C., Rand, M.J., 1980. Textbook of Pharmacology, 2nd ed. Blackwell Scientific Publications, London.
Chan, K., 2003. Some aspects of toxic contaminants in herbal medicines. Chemosphere 52, 1361–1371.
Chang, R., 1995. Functional properties of edible mushrooms. Nutrition Reviews 54, 91–93.
Codex Alimentarius Commission, 1991. Joint FAO/WHO food standards program. Nineteenth Session. Rome, 1–10 July 1991.
Dargan, P.I., Gawarammana, I.B., Archer, J.R.H., House, I.V., Shaw, D., Wood, D., 2008. Heavy metal poisoning from Ayurvedic traditional medicines: an emerging problem? International Journal of Environmental Health 2, 463–472.
Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extract for bacteria. Planta Medica 64, 711–714.
Ernst, E., 2002. Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends in Pharmacological Sciences 23, 136–139.
Friberg, L., Nordbergs, G.F., Vouk, V.B., 1986. Hand Book on the Toxicology of Metals, 2nd ed., vol. 2. Elsevier Science Publishers BV, New York.
Gibbons, S., 2005. Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochemistry Reviews 4, 63–78.
Guha, M.D.N., 2008. Chronic arsenic toxicity and human health. Indian Journal of Medical Research 128, 436–447.
Haider, S., Naithani, V., Barthwal, J., Kakkar, P., 2004. Heavy metal content in some therapeutically important medicinal plants. Bulletin of Environmental Contamination and Toxicology 72, 119–127.
Hou, X., Jones, B.T., 2000. Inductively coupled plasma/optical emission spectrometry. In: Meyers, R.A. (Ed.), Encyclopedia of Analytical Chemistry. John Wiley and Sons Ltd, Chichester.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants: An Inventory. Natal University Press, Pietermaritzburg.
Jeffrey, D.W., 1987. Soil Plant Relationships. Croom Helm Ltd, Kent.
Jonnalagadda, S.B., Kindness, A., Kubayi, S., Cele, M., 2008. Macro, minor and toxic elemental uptake and distribution in *Hypoxis hemerocallidea*, "the African Potato" — an edible medicinal plant. Journal of Environmental Science and Health. Part. B 43, 271–280.
Lemberkovics, E., Czinner, E., Szentmihalyi, K., Balazs, A., Szoke, E., 2002. Comparative evaluation of *Helichrysi flos* herbal extracts as dietary sources of plant polyphenols, and macro- and microelements. Food Chemistry 78, 117–127.
Makkar, H.P.S., 1999. Quantification of tannins in tree foliage: a laboratory manual for the FAO/IAEA co-ordinated research project on "Use nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on the tanniferous tree foliage". Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.
Marinova, D., Ribarova, F., Atanassova, M., 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy 40, 255–260.
Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies 15, 523–530.
Mtunzi, F., Muleya, E., Modise, J., SipamLa, A., Dikio, E., 2012. Heavy metals contents of some medicinal plants from KwaZulu-Natal, South Africa. Pakistan Journal of Nutrition 11, 757–761.
National Research Council, 1982. Drinking Water and Health, vol. 4. National Academy Press, Washington, D.C.
Ndhlala, A.R., Stafford, G.I., Finnie, J.F., Van Staden, J., 2009. In vitro pharmacological effects of manufactured herbal concoctions used in KwaZulu-Natal South Africa. Journal of Ethnopharmacology 122, 117–122.
Nies, D.H., 1999. Microbial heavy metal resistance. Applied Microbiology and Biotechnology 51, 730–750.
Noori, M., Malayeri, B., Moosaei, M., Pakzad, R., Piriye, M.H., 2012. Effects of heavy metals on the antibacterial properties of *Verbascum speciosum* Schard. Revista Cientifica UDO Agricola 12, 463–471.
Ojewole, J.A.O., 2006. Antinociceptive, anti-inflammatory and antidiabetic properties of *Hypoxis hermerocallidea* Fisch. & C.A. Mey (Hypoxidaceae) corm ['African Potato'] aqueous extract in mice and rats. Journal of Ethnopharmacology 103, 126–134.
Sandstead, H.H., 1995. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. The American Journal of Clinical Nutrition 61, 6215–6245.
Shafer, T.J., Mundy, W.R., 1995. Effect of Aluminum on neural signal transduction: mechanisms underlying disruption of phosphoinositide hydrolysis. General Pharmacology 26, 889–895.
Steenkamp, V., Stewart, M.J., Curowska, E., Zuckerman, M., 2002. A severe case of multiple metal poisoning in a child treated with a traditional medicine. Forensic Science International 128, 123–126.
Steenkamp, V., Cukrowska, E., Stewart, M.J., 2006. Metal concentrations in South African traditional herbal remedies. Research Letters. South African Journal of Science 102, 256–258.
Street, R.A., 2012. Heavy metals in medicinal plant products — an African perspective. South African Journal of Botany 82, 67–74.
Street, R.A., Kulkarni, M.G., Stirk, W.A., Southway, C., Van Staden, J., 2008. Variation in heavy metals and microelements in South African medicinal plants obtained from street markets. Food Additives and Contaminants 25, 953–960.
Street, R.A., Kulkarni, M.G., Stirk, W.A., Southway, C., Abdillahi, H.S., Chinsamy, M., Van Staden, J., 2009. Effect of cadmium uptake and accumulation on growth and antibacterial activity of *Merwilla pumbea* — an extensively used medicinal plant in South Africa. South African Journal of Botany 75, 611–616.
Van Wyk, B.-E., De Wet, H., Van Heerden, F.R., 2008. An ethnobotanical survey of medicinal plants in the south eastern Karoo, South Africa. South African Journal of Botany 74, 696–704.
Winkel-Shirley, B., 2002. Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology 5, 218–223.
World Health Organization, 1998. Quality Control Methods for Medicinal Plants Materials. WHO Publishers, Geneva.
World Health Organization, 2007. WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues. WHO Publishers, Geneva.
World Health Organization, 2010. Preventing disease through healthy environments. Exposure to Mercury: A Major Public Health Concern. WHO Publishers, Geneva.
Zahid, H., Khalid, M.K., Shahnaz, P., Nida, A., Wajid, R., Ata, U., 2011. The effect of cadmium and chromium concentration, on biological activity of *Marsilea minuta*. Journal of the Chemical Society of Pakistan 33, 874–876.
Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64, 555–559.
Zschocke, S., Rabe, T., Taylor, J.L.S., Jäger, A.K., Van Staden, J., 2000. Plant part substitution — a way to conserve endangered medicinal plants? Journal of Ethnopharmacology 71, 281–292.