Determination of the Levels of selected Heavy Metals in Medicinal plants from Narok County, Kenya and variations in their levels due to hot water Infusion

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Abstract

The use of traditional medicine to majority of communities in developing countries is well accepted since they are affordable and readily available. Narok County, Kenya which is home to the Maasai Community commonly use Dovyalis abyssinica, Todalia asistica, Clutia abysssinica, Trimelia grandifolia, Rhamnus prinoides and Caylusea abyssinica plants for medicinal purposes. This study assessed the levels of selected heavy metals in leaves, stem, and roots of these medicinal plants and their infusions using AAS. The trend in heavy metal accumulation was Fe > Cu > Co > Cr. The levels significantly $(P \le 0.05)$ differed among the different plants and even further variation $(P \le 0.05)$ was observed in different plant parts. The levels in the infusions for all the metals studied were lower than 50%. Generally, the levels in the studied medicinal plants were low and safe for human consumption.

Keywords: Medicinal plants, heavy metals, infusion, acid digestion, traditional medicine, AAS.

Introduction

Medicinal plants have been used in all cultures as a source of medicine. The widespread use of herbal remedies obtained from commonly used traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties¹. There is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. Herbal medicines and supplements are considered less toxic than the synthetic compounds and are of low cost²⁻⁴. Many communities in Kenya including the Samburu, Luo and Maasai heavily depend on medicinal plants as first line treatment of several diseases⁵. Herbs may be contaminated with heavy metals during growing in the field, processing and handling. It is therefore important to have medicinal herbs free from heavy metal contamination in order to protect consumers from complications related to continuous exposure to heavy metals^{6,7}.

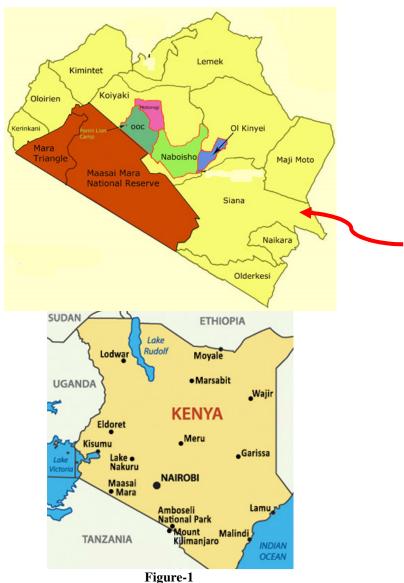
Plants primarily absorb heavy metals from soil water or air⁸. Usually soil is subjected to contamination through atmospheric deposition of heavy metals from point sources including metal mining, smelting and different industrial activities. Some other sources of soil contamination involve use of fertilizers, pesticides, sewage sludge and organic manures⁸. Plants readily assimilate such elements through the roots while other additional sources of these elements include rainfall, atmospheric dusts and plant protection agent which could be absorbed through leaf blades⁹. Plants are susceptible to heavy metal toxicity and respond to avoid detrimental effects in a

variety of different ways. The toxic dose depends on the type of ion concentration, plant species, stage of plant growth and plant part¹⁰.

It has been reported that whatever is taken as herbs could cause metabolic disturbance subject to the allowed upper and lower limits of the heavy metals. After collection and transformation of herbs into dosage form, the heavy metals confined in plants may enter the human body and disturb the normal functioning of central nervous system, liver, lungs, heart, kidney and brain leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancers ^{11,12}. The concentration of heavy metals in medicinal plants is of great concern to public safety as many people prefer herbal medicines due to the reduced side effects. Consumption of medicinal herbs is not regulated by quality assurance parameters nor drug regulatory bodies available thus the need to analyze their safety and advice the regulatory authorities, local communities in case the levels escalate beyond allowable limits.

Material and Methods

The samples of different herbs species *Dovyalis abyssinica*, *Todalia asiastica*, *Clutia abyssinica*, *Trimelia grandifolia*, *Rhamnus prinoides* and *Caylusea abyssinica* were collected from areas unaffected by pollution, species that grow in Maasai Mara game reserve, Narok County with geographical coordinates (1.15 °S, 35.76 °E and altitude 1827 m) in Kenya were sampled in a completely randomized design.



Maps showing Maasai Mara national game reserve where sampling was done

These samples were put in plastic bags and transported to the chemistry laboratory at Maasai Mara University for further treatment. The root, stem and leaves were thoroughly washed with de-ionized water to remove dust, dirt and other particles, dried at room temperature and ground to fine powder and oven dried at 105°C for 24 hours before being put in plastic bags read for analysis. The standard procedure described in AOAC¹³, was followed with slight modifications for the preparations of samples for analysis. For the determination of total heavy metal concentrations in the plant parts, the wet digestion was adopted where a homogenous solution of perchloric acid, nitric acid and sulphuric acid in the ratio 3:1:1 to prepare a digesting solution. 0.5 g of the powdered portion from each plant part was weighed and transferred to a 100 mL beaker, 20mL of tri-acid was added and heated until white fumes were observed and volume reduced to 5mL and the solution was filtered using a Whatman ashless filter paper number 42. The filtrate was then transferred to a 100 mL volumetric flask and the final volume made up to the mark using distilled de-ionized water. These solutions were then stored in plastic bottles ready for analysis using the Atomic Absorption Spectrophotometer (990 AAS, Flame and graphite, PG Instruments Ltd.).

Preparation of infusion: 2 g of the ground plant sample was added to a 100 mL of deionized water added. The mixture was heated on a hot plate fitted with a magnetic stirrer for 30 minutes and then left to cool. The cooled solution was filtered using a Whatman ashless filter paper number 42. The filtrate was acidified and analyzed using AAS-PG-500.

Recovery tests: Recovery test of analytical procedure used was performed by spiking a known concentrations of metal ions to

the prepared medicinal plant extracts (0.5 g) to get to concentration in range of 10-20 $\mu g/g^{14}$. These samples were analyzed by AAS to get to the recovery of the method.

Results and Discussions

The recovery values for the plant samples are given in table-1. These results show the validity of this method for medicinal plant material as the recovery was in the range of between 94.68-98.41% which is acceptable¹³.

Table-1
Recovery of heavy metals from medicinal plant materials by wet digestion

Metal	Recovery* (This study)	Recovery 14
Fe	98.41+1.41	102.31+13.33
Cu	96.32+3.68	93.00+3.85
Cr	97.46+2.13	97.04+4,68
Co	94.68+1.42	-

^{*}Mean recovery + SD of percent recoveries of triplicate analysis.

Cu concentrations in herbals plants in the current study ranged from 0.005 - 0.071 and these results were comparable by results reported by Intidhar *et al.*¹⁵, Laszlo and Artur, 16, Dzomba et

al.¹⁷, Ghulam *et al.*¹⁸ but the current results were lower compared the results reported by Sahito *et al.*,¹⁹, Ararso and Alemayehu²⁰, Maobe *et al.*²¹, Samira *et al.*²², Subramanian *et al.*²³ and Diaconu *et al.*¹⁴.

Generally, the concentrations of Cu in this study was low than the concentration of 3 μ g/g in edible plants as recommended by FAO/WHO²⁴ and this can be attributed to the low pollution and bed rock concentrations of Cu in the sampling sites.

The levels of iron in the selected medicinal plants ranged from 1.158 μ g/g to 9.522 μ g/g and this results were comparable by results reported by Ghulam *et al.*¹⁸, Ararso and Alemayehu, 20, Sahito *et al.*¹⁹, Maobe *et al.*²¹ and Dzomba *et al.*¹⁷ but the current concentrations were lower compared the results reported by Laszlo and Artur¹⁶ and Shad *et al.*¹¹. The concentration of Fe in this study were low than 20 μ g/g as set by FAO/WHO²⁴, in edible plants.

The levels of Co in the current study ranged from 0.014 - 0.042 µg/g and these concentrations were comparable to the results reported by Laszlo and Artur¹⁶, but these concentrations in current study were lower than that reported by Maobe *et al.*²¹, and the results of Co concentrations in this study were higher than that reported by Intidhar *et al.*¹⁵.

 $Table - 2 \\ Levels of heavy metals (\mu g/g) ingested by different medicinal plants in various plant parts$

		Type of plant						
Metal	Plant part	D. abssinica	T. asiastica	C.abyssinica	T.grandifolia	R. prinoides	C. abyssinica	Mean
Cu	Leaves	0.069±0.003	0.060±0.004	0.005±0.003	0.060±0.004	0.005±0.002	0.070±0.001	0.06
	Stem	0.063±0.006	0.061±0.005	0.057±0.001	0.040±0.002	0.055±0.004	0.049±0.003	0.054
	Root	0.049±0.005	0.061±0.009	0.071±0.001	0.049±0.006	0.008±0.002	0.046±0.003	0.047
	Mean	0.061	0.06	0.06	0.048	0.038	0.055	
	CV (%)	5.94						
	L.S.D(P≤0.05)	0.003					0.003	
Fe	Leaves	4.860±0.021	3.788±0.016	6.748±0.042	1.450±0.016	5.750±0.035	4.043±0.014	4.44
	Stem	5.226±0.014	1.158±0.043	3.505±0.008	2.749±0.024	2.368±0.029	2.628±0.008	2.939
	Root	4.549±0.008	5.829±0.016	5.087±0.000	9.522±0.056	4.113±0.014	8.330±0.037	6.238
	Mean	4.878	3.592	5.113	4.574	4.077	5	
	CV (%)	0.6						
	L.S.D(P≤0.005)	0.025					0.026	
	Leaves	0.017±0.001	0.018±0.006	0.038±0.008	0.042±0.012	0.024±0.003	0.030±0.004	0.470
	Stem	0.016±0.007	0.037±0.003	0.040±0.002	0.026±0.016	0.015±0.004	0.035±0.005	0.506
Со	Root	0.039±0.005	0.024±0.006	0.014±0.005	0.042±0.003	0.015±0.005	0.033±0.012	0.416
	Mean	0.02	0.026	0.031	0.034	0.021	0.023	
	CV (%)	21.5						
	L.S.D(P≤0.005)	0.007					0.007	
Cr	Leaves	0.016±0.004	0.013±0.005	0.006±0.003	0.010±0.000	0.007±0.003	0.008±0.002	0.010
	Stem	0.011±0.001	0.013±0.001	0.013±0.003	0.011±0.003	0.008±0.002	0.008±0.002	0.011
	Root	0.019±0.001	0.010±0.004	0.014±0.002	0.021±0.001	0.016±0.002	0.013±0.003	0.016
	Mean	0.015	0.012	0.011	0.014	0.01	0.01	
	CV (%)	20.72						
	L.S.D(P≤0.005)	0.002					0.002	

The concentrations of Cr in the current study ranged from $0.006 - 0.021 \,\mu\text{g/g}$ and were found to be comparable to that reported by Sahito *et al.*¹⁹, but the current results were lower compared to the results reported by Maobe *et al.*²¹, Samira *et al.*²² and Ararso and Alemayehu²⁰ but were found to be higher than that reported by Somnath_and Badal¹⁷. The concentrations of Cr in this study were comparable to the permissible limit of $0.02 \,\mu\text{g/g}$ set by FAO/WHO²⁴ in edible plants.

The medicinal plants around Maasai Mara game reserve, Narok region, Kenya seem to bioaccumulate Fe more than other studied metals. The trend of concentration levels was Fe > Cu > Co > Cr. These findings were similar to that of Diaconu *et al.*¹⁴ where the order was Fe > Zn > Mn > Cu > Cr while Pb and Cd were present in minor amounts. The levels differed significantly ($P \le 0.05$) from one medicinal plant to the other and this explains that plants accumulate heavy metals differently²⁵.

The differences can be explained in the sense that plants have varying abilities to absorb heavy metals from the soil^{26,27}, leading to variation in the plant and several parts of the plant.

The different parts of the plant also showed significant ($P \le 0.05$) variation in the levels accumulated and varied amongst the species.

Water extraction from medicinal plants allows passing only of a part of quantity present in plants and this differed from one plant to the other. Co extraction was higher compared to the studied metals which had 21-39% infused into hot water while Cu infusion was below the detection of the instrument and this could be attributed to the low concentration of Cu in these plants.

The different parts of the plants accumulated these metals differently and this can be seen from the fact that different parts of the plant are in different clusters (figure-1). *C. abyssinica* roots and leaves are in different cluster and *T. grandfolia* stem accumulated differently as compared to its leaves. Through this dendrogram it can be concluded that different parts of the studied plants accumulated these metals differently.

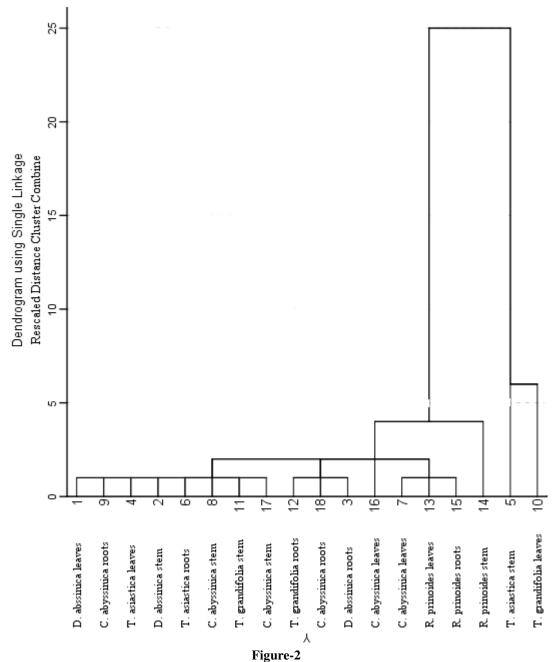
Table-3

Average levels of heavy metal in medicinal plants and the relative percentage in their infusions

Metal	Plant Species	Average metal content	In Infusion	% Infusion	Other studies (% infusion)	
Cu	D.abyssinica	0.061	BDL	0.00		
	T. asiastica	0.060	BDL	0.00	7-67.67	14
	C. byssinica	0.060	BDL	0.00	1 - 50	22
	T.grandifolia,	0.048	BDL	0.00		
	R. prinoides	0.048	BDL	0.00		
	C. abyssinica	0.055	BDL	0.00	6.9-32.7	25
	D.abyssinica	4.878	0.002	0.04		
	T. asiastica	3.592	0.024	0.68	14.94-81.95	14
Fe	C. byssinica	5.113	0.067	1.31		22
re	T.grandifolia,	4.574	0.016	0.35	2.5 - 20	
	R. prinoides	4.077	0.027	0.66		
	C. abyssinica	5	1.084	1.38	12.4-16.8	25
	D.abyssinica	0.02	0.008	28.33		
	T. asiastica	0.026	0.006	33.33		
Со	C. byssinica	0.031	0.008	25.81	-	
	T.grandifolia,	0.034	0.007	21.57		
	R. prinoides	0.021	0.008	41.27	-	
	C. abyssinica	0.023	0.009	39.13		
	D.abyssinica	0.015	0.002	13.33		
Cr	T. asiastica	0.012	0.003	25.00	12.50-83.56	
	C. byssinica	0.016	0.002	13.04	5 - 70	14
	T.grandifolia,	0.014	0.003	21.43	3 - 10	22
	R. prinoides	0.016	0.002	12.50	_	
	C. abyssinica	0.023	0.003	13.04	<u>-</u>	

BDL=Below Detection Limit

Machine Detection Limit for Cu = 0.004 mg/L



Dendrogram showing the different clusters of different medicinal plants and how they relate to each other

Conclusion

The metallic elements Cu, Cr, Fe, and Co contained in six medicinal plants showed a wide variability. Then analyzed metal concentration in the plants were in the order of Fe > Cu > Co > Cr. Analysis of plant infusions showed transfer of heavy metals during extraction procedure and extraction coefficients were less than 40% on all the plant species. Various plant parts were seen to have different concentrations of the heavy metals. In general, metal contents of whole medicinal plants were found to be higher than those of their infusion. The obtained results

showed that heavy metals were in minimal amounts and not very soluble in infusions, and thus do not pose any health risks in terms of toxicity in the diet.

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