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Short Communication

Suitability of Four Anti-arthritic Plants of Sindh Pakistan in Terms of Major and Trace Toxic Metals Profile

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Abstract. Metals profiling of medicinal plants is obligatory to uncover the therapeutic use and safe dose judgment. Therefore, anti-arthritic plant *Moringa oleifera* (MO), *Smilax china* (SC), *Vernonia anthelmintica* (VA) and *Colchicum aitchisonii* (CA) were screened for heavy metals by Atomic Absorption Spectrophotometer. The study revealed Ca, Na, K and Mg as predominant metals. Pb was not detected in any plant and their extracts whereas, Fe, Zn, Co, Cr, Cd and Ni accumulated beyond the accepted limits set for medicinal plants according to WHO. Except Cr in *Colchicum aitchisonii*, toxic metals such as Ni (4.24-8.99), Cd (2.39-4.73) and Cr (3.52-19.39) mg/Kg were found higher in all powder samples with maximum in the *Vernonia anthelmintica*. All methanolic and aqueous extracts contained Cd and Cr, whereas only one out of four aqueous extracts contanied Ni. It is concluded from the result that these medicinal plants could be a significant source of major nutrients but their inappropriate use may cause noxious effects due to the accumulation of toxic metals. So, constant checking of medicinal plants is needed.

Keywords: anti-arthritic plants,aqueous extracts,methanolic extract, ASS, heavy metals.

Traditionally to treat and prevent alignments, the use of therapeutic plants has always been valued in both developing and developed countries (Maqsood *et al*., 2017; Nimenibo-Uadia *et al*., 2017). Medicinal plants are extensively used for several healthcare needs, based on the general perception that they are free from any harmful effects (Nasri and Shirzad, 2013; Philomena, 2011). But nowadays it's clear that plants can accumulate metals from their surrounding environment (Rehman *et al*., 2019; Khathi *et al*., 2016) as in last decade soil pollution has raised many folds in terms of metals and mainly toxic heavy metals. Particularly plants of the Asian origins which are still gathered from the wild habitat and face more environmental pollution than cultivated plants (Khattak *et al*., 2015). The alternative medicine users of Asian countries are at more risk of metal transmission that can cause serious health problems (Idrees *et al*., 2018; Khattak and Khattak, 2011). From around the world, numerous clinical cases of metal in toxications in infants and adults had been reported due to the consumption of different herbal medications (Chambial *et al*., 2017). Lead, zinc, iron, cadmium and nickel are predominantly reported to be found in medicinal plants that in case of prolonged exposure may reasons for acute or chronic effects on health, including cancer (Helal Uddin *et al*., 2013).

The problem is rather more severe in Pakistan, where neither any controlled nor properly checked mechanism exists to regulate and monitore quality assurance parameters of medicinal plants for public safety (Khan *et al*., 2007). Several studies have gained considerable attention and exposed the cytotoxic potential of normally consumed foods and medicinal herbs grown in polluted soils with heavy toxic metals (Arojojoye *et al*., 2015; Aslam *et al*., 2014). World Health Organization (2007) proposed that prior to approach in the living systems, herbs must be assessed for the extent of heavy metals level. In Pakistan, *Vernonia anthelmintica* L Wild, *Smilax china* Linn, *Colchicum aitchisonii* and *Moringa oleifera* Lam are used in the management of arthritis and in several others herbal remedies (Kamal *et al*., 2016; Choudhary *et al*., 2015). Except for *Vernonia anthelmintica,* no previous data on the metals contamination was available in the research area. Keeping in sight the importance of the mentioned anti-arthritic plants, the attempt was to figure out to ascertain the values of 13 metals; calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), lead (Pb), Iron (Fe),

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cadmium (Cd), cobalt (Co), zinc (Zn), manganese (Mn), chromium (Cr), copper (Cu) and nickel (Ni) by Atomic absorption spectrophotometer (AAS).The use of AAS for the assesment of the metals in the medicinal plants has been well reported (Khathi *et al*., 2016). This study can provide scientific data of heavy metals levels that will help to understand the nutritional values and safety concerns of selected anti-arthritic plants.

Anti-arthritic plants parts *vie Vernonia anthelmintica* seed (VAS), *Smilax china* rhizome *(SCR), Colchicum aitchisonii* corm (CAC) *and Moringa oleifera* gum (MOG) were purchased from market located in the Hyderabad, state of Sindh province, Pakistan to performe metals analysis. Collected material was identified and authenticated in herbarium Institutes of Plant Sciences, University of Sindh, Pakistan. After cleaning all parts of selected herbs were shade dried for a week at room temperature, followed by milled into a fine powder with an electric grinder (ANEX TS-630S, Germany) and packed in airtight plastic bottles. For extraction, plant powder was subjected to aqueous and 80% methanolic extractions check the quantity of analyzed metals that could be extracted as medicinal plants are mostly consumed as herbal formulation obtained by soaking in solvents. Withdrawn filtrates after 24 h were further concentrated under reduceed pressure using Buchi rotary evapourator (40 °C).

Standard solutions of Ca, Mg, Na, K, Mn, Pb, Fe, Cr, Cu, Cd, Co, Ni and Zn were prepared by dilution of stock solutions (1000 mg/L) and served as reference analytes. For all dilutions, deionized water was used. Prior to using, conical flask and glassware were sanitized by soaking in dilute nitric acid (10% v/v) and were rinsed with double distill H_2O

Samples for metals analysis was made through the acid digestion method (Saeed *et al*., 2010) 1.0 g of powdered and extracts were digested by adding 6.0 mL of concentrated HNO_3 (65% Sigma- Aldrich, USA) for overnight. On the next day, H_2O_2 (30% Sigma- Aldrich, USA) was added in 2.0 mL quantity, followed by heating using an electric hot plate at 60 °C, digested all samples until the volume was reduced and brown fumes disappeared. After cooling and filtration volume was makeup 25 mL with deionized water. The blank and standard solutions were initial runs, followed by the samples accurately analyzed through Atomic Absorption Spectrophotometer (AAS, Perkin Elmer, AAnalyst 800) equipped with acetylene flame.

Acquire values (mg/L) were converted in mg/Kg according to the following method (Alkherraz *et al*., 2013) mg/Kg = A (mg/L = metal value in sample) * B (final volume)/W (sample weight).

Obtained figures were further subjected to statistical analysis using statistical package (ANOVA 8.1) at levels of significance $P \leq = 0.01$.

In current work for comparison of metals contents, standard limits of metals in medicinal plants confines by world health organization was used (Table 1).

Data for quantification of major and trace toxic metals in crude powder, aqueous and methanolic extracts of plants parts are summarized in Table 2. Non significant ($P \leq 0.01$) differences, between the values of metals detected in the analyzed plants parts. All plants represent Ca in enough quantity with maximum in VAS (752.1-1390.2 mg/Kg) and MOG (723.5-1002.4 mg/Kg). Ca maintained cellular and neuro muscular functions in human, plays part in the formation of bones and teeth (Robertson and Marshall, 1981). People that suffer from inflammatory disorders like arthritic are suggested to be taking about 1500 mg of Ca on daily bases (Gbadamosi and Oloyede, 2014). Na present in the range of 126.9-163.8 mg/Kg in the plants, 54.7-86.1 mg/Kg in an aqueous extract and 110.9-165.2 mg/Kg in the methanolic extract. VAS contained higher (163.8 mg/Kg) concentration of Na, while lowest (126.9 mg/Kg) was recorded in the MOG. Sodium helps to maintain the fluid volume in the body, and any change either excess or scarcity of sodium can imbalance the mechanism and lead to fatal condition (Chavan *et al*., 2014). There is no described limit for sodium in plants However; daily recommended intake is 1-3.3 mg (Saeed *et al*., 2010). SCR had the highest value of K (390-1525.4 mg/Kg), while MOG had the lowest concentration (74-149.1 mg/Kg). Potassium performed multiple functions in cells under normal intake for an adult man and women are 3100 mg/day and 2300 mg/day, respectively (Saeed *et al*., 2010). Mg was the

Table 1. Heavy metals permitted limits (ppm) in medicinal plants and food by (WHO 2007; 1984)

Heavy metals	Pb	Cd	Cr	Ni	Zn	Cu	Fe	Mn
Medicinal plants	10	0.3	2.0	1.63	50	20	-	-
Food	-	-	-	-	-	-	20	2.0

lowest in the plant SCR (261.7 mg/Kg), while the highest amount (328.9 mg/Kg) was found in the VAS. In the extracts, the Mg was in the range from 80.3 to 291.3 mg/Kg. Mg along with Ca helps in transmitting of nerve impulses (Aslam *et al*., 2014). Fe and Mn having the array of Fe 101.2-477 mg/Kg in plants, while 25.4-1041.9 mg/Kg in extracts, followed by Mn that ranged in 47.1 to 285 mg/Kg and 42.2-322.8 mg/Kg in plants and their extracts, respectively. SCR and MOG showed maximum values of Fe and Mn in both extracts. Iron served as component of oxygen carrier protein hemoglobin and myglobin in human body, its deficiency results in anaemia (Fakankun *et al*., 2013), while excessive intake of iron induces free radicals that cause

Table 2. Metals analysis (mg/Kg) of CP, AE and 80% ME of medicinal plants

Metals	Samples	SCR	CAC	MOG	VAS
Ca	CP	285.5 ± 1.86	284.1 ± 4.3	723.5 ± 1.02	752.1 ± 3.29
	AE	277**±0.15	213**±0.20	401.2±0.22	342.2±0.02
	ME	311±0.06	333±0.20	1002.4±0.20	1390**±0.82
Na	CP	130.7 ± 1.28	147.1 ± 0.46	126.9 ± 1.05	163.8 ± 0.37
	AE	69.9±0.06	86.1±0.04	58.2±0.03	54.7±0.02
	ME	159.9±0.06	163.4±0.05	165.2±0.07	110.9±0.20
K	CP	1194**± 0.42	285.5 ± 0.49	123.1± 0.82	137.9± 1.30
	AE	390.4±1.41	147.4±1.44	74.9±0.52	69.4±0.06
	ME	1525**±0.25	407.4**±0.92	149.1±0.85	247.4±0.94
Mg	CP	261.7 ± 0.13	319.2 ± 0.05	310± 1.37	328.9 ± 1.71
	AE	113.30±0.01	80.3±0.38	97.3±0.52	102.7±0.82
	ME	144.8±0.05	130.3±0.97	291.3±1.23	256.7±0.02
Fe	CP	477 ± 0.68	227.4 ± 0.05	182.0 ± 0.16	101.2 ± 2.11
	AE	116.5±0.09	79.0±0.02	85.1±0.09	25.4±0.12
	ME	1041.9±2.45	135.5±0.08	196.9±0.20	38.0±0.18
Mn	CP	285.0±0.67	79.2±0.46	94.5 ± 0.78	47.1±0.42
	AE	42.2±0.35	68.2±0.45	73.10±2.47	53.2±0.08
	ME	307.1±0.13	118.3±0.05	322.8±0.51	95.4±0.58
Zn	CP	82.5±0.91	75.4 ± 0.53	91.7 ± 0.79	96.3 ± 0.78
	AE	47.40±0.42	54.0±1.28	108.4±2.50	128.2±2.71
	ME	53.40±0.81	69.4±0.85	115.5±0.64	133.5±1.09
Co	CP	0.22 ± 0.06	BDL	BDL	4.02±0.15
	AE	BDL	BDL	BDL	0.08 ± 0.07
	ME	0.12±0.01	0.18±0.008	0.49±0.02	6.02±0.20
Cr	CP	3.52±0.53	BDL	11.53 ± 0.05	19.39± 0.31
	AE	0.40 ± 0.00	2.71±0.09	3.10±0.04	3.70±0.05
	ME	2.33 ± 0.08	2.86± 0.07	7.20 ± 0.12	9.29 ± 0.38
Cu	CP	1.02 ± 0.05	BDL	BDL	0.84 ± 0.01
	AE	2.16±0.02	2.66±0.03	1.76±0.04	13.0±0.40
	ME	7.16±0.01	4.66±0.02	2.46±0.09	22.1±0.28
Cd	CP	2.39 ± 0.02	3.75 ± 0.03	2.80 ± 0.00	4.73 ± 0.02
	AE	0.06±0.01	0.10±0.02	0.61±0.02	0.82
	ME	0.60±0.02	0.99±0.01	1.20±0.01	1.49±0.00
Ni	CP	4.73 ± 0.02	5.80 ± 0.00	4.24 ± 0.03	8.99 ± 0.03
	AE	BDL	BDL	BDL	1.80±0.05
	ME	0.50±0.00	0.61±0.01	1.71±0.03	2.26±0.02
Pb	CP	BDL	BDL	BDL	BDL
	AE	BDL	BDL	BDL	BDL
	ME	BDL	BDL	BDL	BDL

BDL = below detection limit; Values are showed as mean ± SD (n=3); CP= crude powder; AE= aqueous extract; ME= methanolic extract; MOG=*Moringa oleifera* gum; SCR= *Smilax china* rhizome; VAS; *Vernonia anthelmintica* seed; CAC= *Colchicum aitchisonii* corm; **P≤=0.01 were considered statistically significance damaged to liver, brain and DNA lead to mutations (Jadoon and Malik, 2017). Mn require for various vitamins metabolism and in proper digestion by activation of enzymes (Devi *et al*., 2008). Zn was high in all plants with ranged from 75.4-96.3 mg/Kg. In both extract VAS contain highest values of zinc 128.2-133 mg/Kg, whereas lowest 47.4-53.4 mg/Kg was recorded in SCR. Zinc plays a crucial role in insulin storage and secretion (Li, 2014), served as antioxidant and anti-inflammatory and in cell signaling. High dose of zinc can induce nausea and vomiting (Ibrar *et al*., 2013). Only powder analysis of SCR (0.22 mg/Kg) and VAS (4.02 mg/Kg) showed the presence of cobalt. However, in aqueous extracts, Co was detected only in VAS (0.08 mg/Kg) and in methanolic extracts all plants showed Co in the range of 0.18-6.02 mg/Kg with lowest in SCR and highest in VAS. A known biologically vital function of trace metal cobalt in the body is as a component of vitamin B12 (Mekassa and Chandravanshi, 2015). Excluding CAC, all plants accumulate Cr within the range of 3.52-19.39 mg/Kg in plant and 0.40-9.29 mg/Kg in extracts. Cd showed array 2.39-4.73 mg/Kg in plants, and in extracts 0.06-1.49 mg/Kg. Non essential metal cadmium induced hepatotoxicity and nephron toxicity when combine with cysteine protein and form complex metalothione (Jadoon and Malik, 2017) Highest concentrations of Ni was present in the order of VAS>CAC>SCR>MOG, except VAS, Ni was not detected in an aqueous extracts. Nickel has been known as serious toxicant to human health on ground to causes high incidence of carcinoma to different organs (Khuda *et al*., 2012). However, due of very low absorption by human body toxicity related with nickel is not very common (Onianwa *et al*., 2000). Pb was not spotted in any extracts. According to WHO (Table 1), plants accumulate exceeds level of Fe, Mn, Zn, Cr, Ni and Cd, except Cd in aqueous extracts of SCR and CAC. Previous reported work of VA seeds shows high Zn and Mn while, in accordance with Cr, Fe and Co amount (Fatima *et al*., 2013). However, there are no previously published data available for metals contents of CAC, SCR and MOG. Variations in metal contents than that of publishing verdict reflect deviation in soil geochemical composition where the plants have been belong (Glavac *et al*., 2017; Randelovic *et al*., 2013).

In comparison with the crude powder, acquired metals values of both extracts were decreased or increased. Water extracted minimal amounts of metals then methanol. Literatures support that in comparison with water, other organic solvent exhibited stronger extraction power of influence on dissociations of organic complexes (Adie and Adekunle, 2017; Kostic *et al*., 2011). Furthermore, the extraction efficiency of metals also depends on the plant species (Randelovic *et al*., 2013). Taken together, the results suggest that for the extraction of medicinal plants, water served as safe and optimal solvent than any category of alcohol.

Conclusion

Most of the metals were found higher than the permissible limits in the per milligram selected plants. Besides appreciably higher values of essential metals accountable for anti-inflammatory properties Cr, Cd, Ni, and Co we are recovered in the exceeded amount. The utilization of these medicinal plants can cause persistent toxic consequences. Therefore, the present work suggests the use of these remedies with caution and directed the regular checking of commonly consumed medicinal plants that have been found or utilized in different localities.

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