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Characterization of Heavy Metals in Extracts of *Hypericum* Medicinal Plant by Flame Atomic Absorption Spectrometry

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Heavy metals ion contents (Fe, Ni, Cu, Cr, Pb, Cd and Co) in methanolic extracts of five species of Hypercum plant were studied. Two Pakistani species, *Hypericum perforatum* and *Hypericum oblangifolium* and three UK species, *Hypericum olympicum*, *Hypericum moserarum* and *Hypericum androsaemum* were analyzed for metal content by flame atomic absorption spectrometry. Level of Pb and Ni was high in Pakistani species as compared to UK species. The highest concentration of Cr was found in *Hypericum perforatum* (Pak) while minimum concentration was found in *Hypericum moserarum* (UK). The highest mean value of copper was found in *Hypericum olympicum* (UK) followed by *Hypericum perforatum* (Pak). There was a little variation in case of Cd and Co while highest mean value of Fe content was found in *Hypericum androsaemum* (UK). However overall level of heavy metal content in the extracts of all the species under study was well below the critical limit.

Key Words: *Hypericum*, Extracts, Heavy metals.

INTRODUCTION

Herbal medicine is a global phenomenon, a multibillion dollar industry and its raw materials phytochemicals are widely used as the precursors for regulated pharmaceutical products. The contributions of medicinal plants in the traditional system of medicine for curing diseases have been documented. Nowadays increased scientific interest and consumer demand have promoted the development of herbal products as dietary supplements. In view of renewed interest, oriental herbal medicines have a prominent role to play in the pharmaceutical and health markets of the 21st century[1].

The pharmacological properties of the medicinal plants have been attributed to the presence of active constituents which are responsible for important physiological function in living organisms. It has been reported that trace elements play an important role in the reactions which will lead to the formation of these active constituents[2]. However, a correlation between elemental composition of medicinal plants and their curative properties have not been established yet. Besides, element concentrations present in medicinal plants are of great importance to understand their pharmacological actions[3].

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WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals. Further it regulates maximum permissible limits of toxic metals like arsenic, cadmium and lead, which amount to 1.0, 0.3 and 10 ppm, respectively[4]. Medicinal herbs are easily contaminated during growth, development and processing. After collection and transformation into dosage form the heavy metals confined in plants finally enter the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancers[5].

The objective of the present study is to investigate the level of some heavy metals lead, cadmium, cobalt, iron, copper and chromium in crude extract of five species of *Hypericum*. The genus *Hypericum* consists of more than 370 species. This genus is widely used in folk medicine since ancient time. Several pharmacological uses of *Hypericum* are known worldwide.

The most popular plant of this genus is the *Hypericum perfotatum* L. (Saint John's Wort) mainly due to its use as a healing agent and by its antidepressive and antiviral applications[6].

In present study, two species of *Hypericum* collected from Pakistan (*Hypericum perforatum* and *Hypericum oblangifolium*) and three species of *Hypericum* collected from England (*Hypericum olympicum*, *Hypericum moserarum* and *Hypericum androsaemum*) were studied. Comparison of heavy metal content in crude extract of different species was made.

EXPERIMENTAL

Sample collection and processing: *Hypericum perforatum* and *Hypericum oblangifolium* were collected from Murree Hills, Pakistan while other species were collected from green house of university of Portsmouth, UK. The samples were dried at room temperature under shade for seven days and powdered. Powered samples were macerated of methanol at room temperature for three days. The extract was dried by rotary evaporator until 95 % of solvent was dried.

Dried extract sample (0.5 g) was digested with 20 mL of 2:1 $HNO_3/HClO_4$ (Analytical grade) and heated until evolution of white fumes. Where necessary more acid mixture was added and the sample digested until evolution of white fumes marking the end of the digestion process. The digests were filtered into standard 50 mL volumetric flask and made up to mark with distilled water. This was subsequently analyzed for Pb, Cd, Cu, Cr, Co and Fe by air-acetylene flame atomic absorption spectrometry (Hitachi Z-5000) by the standard calibration technique. Calibration standards were prepared by dilution of the high purity commercial metal standards (Applichem) for atomic absorption analysis. Adequate quality assurance measures were carried out to ensure reliability of results. Glassware was properly cleaned and reagents (HNO_3, $HClO_4$ and distilled water) were of analytical grade. Spikes and blanks were also introduced. Results reported are average of duplicates.

RESULTS AND DISCUSSION

The arithmetic mean values of the heavy metals concentrations obtained in five different *Hypericum* species are given in Table-1. Copper and iron were the mot abundant elements present in *Hypericum* samples. Cadmium presented the lowest concentration.

Nickel: The concentration of Ni in different plants was in the order of; *Hypericum oblangifolium* 0.1000 mgg^{-1} > *Hypericum perforatum* 0.0685 mgg^{-1} > *Hypericum olympicum* 0.0570 mgg^{-1} > *Hypericum androsaemum* 0.0487 mgg^{-1} > *Hypericum moserarum* 0.0447 mgg^{-1} (Table-1, Fig. 1). The higher concentrations of Ni in plants may be due to anthropogenic activities. The most common ailment arising from Ni is an allergic dermatitis known as nickel itch, which usually occurs when skin is moist, further more Ni has been identified as a suspected carcinogen and adversely affects lungs and nasal cavities. Although Ni is required in minute quantity for body as it is mostly present in the pancreas and hence plays an important role in the production of insulin. Its deficiency results in the disorder of liver. The recommended daily intake of Ni (EPA) should be less than 1 $mgkg^{-1}day^{-1}$ beyond which it is toxic[5,7,8].

TABLE-1
MEAN VALUES OF HEAVY METALS IN METHANOL EXTRACT OF FIVE DIFFERENT SPECIES OF *Hypericum* PLANT

	H. oblangifolium	*H. perforatum*	*H. androsaemum*	*H. olympicum*	*H. moserarum*
Ni	0.1000 ± 0.009	0.0685 ± 0.007	0.0487 ± 0.009	0.0570 ± 0.000	0.0440 ± 0.000
Cr	0.0344 ± 0.009	0.0538 ± 0.004	0.0535 ± 0.007	0.0354 ± 0.000	0.0288 ± 0.002
Cu	0.1240 ± 0.008	0.2104 ± 0.003	0.2924 ± 0.009	0.0354 ± 0.003	0.1976 ± 0.008
Pb	0.0819 ± 0.000	0.0460 ± 0.000	0.0164 ± 0.003	0.0177 ± 0.009	0.0082 ± 0.000
Cd	0.0053 ± 0.000	0.0053 ± 0l.001	0.0082 ± 0.002	0.0089 ± 0.001	0.0133 ± 0.005
Co	0.0135 ± 0.004	0.0538 ± 0.009	0.0494 ± 0.009	0.0443 ± 0.008	0.0575 ± 0.004
Fe	0.0600 ± 0.000	0.3181 ± 0.009	0.4077 ± 0.000	0.0220 ± 0.009	0.0930 ± 0.005

Chromium: The concentration of chromium ranged from 0.0288-0.0538 mg g^{-1}. The minimum concentration was found in *Hypericum moserarum* (UK) while highest concentration was found in *Hypericum perforatum* (Pak). Chromium particularly Cr(III) plays an important role in the body function in trace amount but it is toxic in excess amount. It is thought to regulate carbohydrate, nucleic acid and lipoprotein metabolism and it also potentiates insulin action[9].

Copper: As revealed by analytical results (Table-1, Fig. 1) copper content of *Hypericum* samples ranged between 0.0354-0.204 mg g^{-1}. The highest mean level of copper was found in *Hypericum olympicum* sample and lowest mean value was found in *Hypericum perforatum*.

Although copper is an essential element in trace amount but can be toxic at excess level. Copper build can result in a tendency for hyperactivity in autistic children. An excess of copper can cause oily skin loss of skin tone (due to ability to block vitamin C) and cause a dark pigmentation of skin specially, around face. It can attribute to hair loss specially, in women[10].

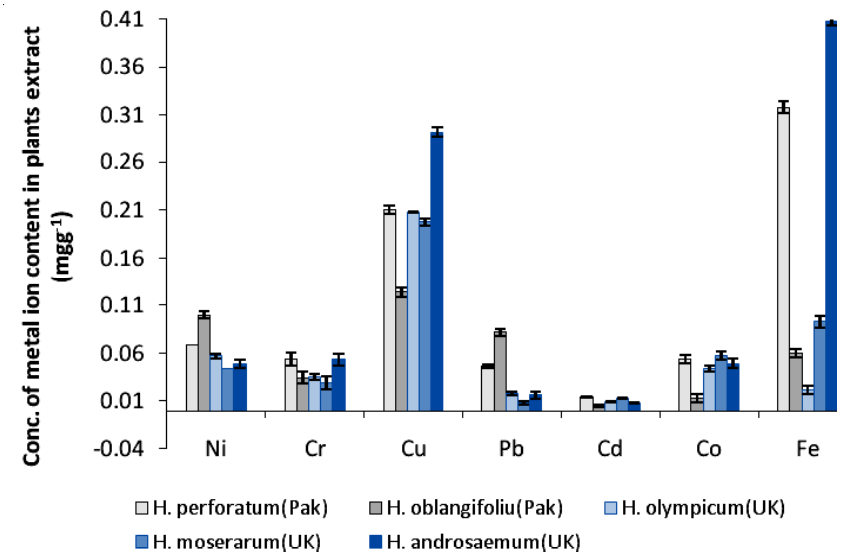

Fig. 1. Comparison of heavy metals in methanol extract of Pakistani and UK species of *Hypericum* plant

Lead: As revealed by the analytical data high concentration of Pb was found in sample of *Hypericum oblongifolium* extract. In other samples concentration of Pb ranged between 0.0164-0.0460 mg g^{-1}. In all the samples concentration of Pb was less than maximum acceptable limits (Table-1, Fig. 1).

Lead is a heavy metal poison which forms complexes with oxo-groups in enzymes to affect virtually all steps in the processes of heamoglobin synthesis and porphyrin metabolism[11]. Toxic levels of Pb in man have been associated with encephalopathy seizures and mental retardation[12]. WHO prescribed limit for Pb contents in herbal medicine is 10 ppm while the dietary intake limit for Pb is 3 mg/week[13].

Cadmium: There was a little variation in case of cadmium. The concentration ranged between 0.0053-0.0133 mg g^{-1}. The highest concentration was found in *Hypericum moserarum* extract sample. In other samples concentration of Cd ranged between 0.0053-0.0089 mg g^{-1} (Table-1, Fig. 1).

Cadmium is non-essential trace elements having functions neither in human body nor in plants. It induces various toxic effects in humans at low doses. Cadmium accumulates in human body and damages mainly the kidneys and liver The lowest level of Cd which can cause yield reduction is 5-30 ppm, while the maximum acceptable concentration for food stuff[14] is around 1 ppm. The concentration of Cd was less than maximum acceptable limit in all samples of *Hypericum* extracts.

Cobalt: Cobalt is an important component of vitamin B12 which participates as a coenzyme in important enzymatic reactions[15]. The highest mean level of this element was found in sample of *Hypericum moserarum* extract. Lowest mean value was found in sample of *Hypericum oblangifolium* extract.

Iron: Iron was the most abundant element present in *Hypericum* samples. As revealed by analytical results (Table-1, Fig. 1) iron content of *Hypericum* extract samples ranged between 0.022-0.4077 mg g^{-1}. The highest mean level of Fe was found in *Hypericum androsaemum* sample. On the other hand, lowest mean value was found in sample *Hypericum olympicum*.

Iron is an essential element and constituent of active site of various reproductive hydrogenases, most frequently associated with sulfur containing ligands. Iron together with heamoglobin and ferrodoxin plays a central role of metabolism. Iron facilitates the oxidation of carbohydrates, proteins and fat to control body weight which is an important factor in some diseases (diabetes)[16].

Conclusion

In this study, concentrations of seven elements were determined in methanol extract samples of five species of *Hypericum* using FAAS. The results obtained showed differences among the elemental concentrations determined for five species of *Hypericum* plant. The overall conc. Of heavy metals was more in Pakistani species than UK species. However the concentrations of heavy metals determined in extracts of all the selected species of *Hypericum* plant were well below the critical limit. Iron and copper contents were found in relatively large amount followed by Ni, Cr and Pb. Cobalt and cadmium were found in small amounts. Lead and cadmium were below the detection limits. The implication of findings may be taken into consideration while using the medicinal plants for human consumption.

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