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Heavy Metal Analysis from Traditionally used Herb *Ceropegia juncea* (Roxb.)

Sudha Karayil ,Bhavani & Vivek. Ch
Department of Biotechnology
Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

ABSTRACT: *Natural products had been indispensably used by many cultures and traditions from thousands of years. Plants synthesis enormous varieties of substances called secondary metabolities (Phytocompounds) and accumulated in it. The potential bioactive phytocompounds like alkaloids, flavanoids, and phenolic compounds, steroids and coumarins etc are potential source for drug discovery. There has been considerable increase in the usage of herbal products and drugs from medicinal plants in recent years. Because of this, it is essential that the quality of plant-based drugs must be assured safe prior to their use. A study was conducted to analyse the presence of traces of essential and non essential heavy metals in the selected medicinal plant. AAS is used to investigate the presence of heavy metals in the selected medicinal plant collected from hill ranges of Palakad district, Kerala, India. (Western ghats). Results obtained in the present study showed that the medicinal herb analysed contain heavy metals chromium, copper, zinc, manganese and nickel that are considered essential elements and lead, cadmium and mercury which are nonessential. The concentration (ppm) of heavy metals in the plant extracts was found to be as follows: chromium (0.036 µg/g), manganese (0.017µg/g), copper (1.637 µg/g), zinc (0.247 µg/g), cadmium (0.053 µg/g), mercury (0.0) and lead (0.002 µg/g), arsenic (0.60 µg/g). From the comparison of the results with the defined permissible limits, it was concluded that the levels of heavy metals present in the herb fall in the permissible limits for consumed medicinal herbs.*

KEY WORDS: *Heavy metals, Ceropegia, Soma plant, Traditional medicinal plant*

I. INTRODUCTION:

In India, the use of plants for medicinal treatment dates back to Vedic era. About 500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in indigenous system of medicine (Perumal Samy and Gopala Krishna kone, 2007). Vast ethnobotanical and ethnopharmacological knowledge exists in India from ancient times. According to world health organization report about 80% of the world population depend on plant based medicines and traditionally used herbs as their primary health care (WHO). The herbal drugs are well established for their therapeutical benefits. Nutritionally important mineral elements accumulate in the plants which are used as herbs and food supplements. Elements like Lead, cobalt, Chromium, Cadmium etc which do not use the plants directly but accumulate in the plants and are detrimental to human health when consumed(Baker and Brook, 1989; Lasisi *et al*.,2005).The most common heavy metals which implicated toxicity in humans include lead, mercury, arsenic, and cadmium, although aluminum and cobalt may also cause toxicity. Therefore, the world health organization recommends that medicinal plants, which form the raw materials for most herbal remedies, should be checked for the presence of heavy metals.

Plants are the source of natural products which act as models for new pharmacologically active compounds. *Ceropegia juncea* (Roxb.) is the plant belongs to the family *Asclepiadoideae* having wide medicinal properties and is being used in different traditional medical systems and by tribal people for curing different ailments. The present test plant has vast ethnobotanical and ethnomedicinal properties (Meve, 2002b, Jadaja, 2004). The plant *Ceropegia juncea* (Roxb.) was also claimed as one of the *Soma* plant (Alam Muzaffer *et al.*, 1982 BMER). There has been considerable increase in the usage of herbal products and drugs in recent years. Because of this, it is essential that the quality of plant-based drugs must be assured safe prior to their use. Several works have been reported on the phytochemical and biological activities of medicinal plants, although there is few reports in regard to the heavy metal concentrations in the medicinal plants and herbal drugs used. The medicinal herbs can cause health risks due to the presence of toxic metals such as Nickel, Lead, Cadmium, Manganese and Mercury, which are hazardous to humans. Pharmacological evaluation of the medicinal plants was recommended for purity and quality of the drugs coming from the botanicals (Peter and Smet, 2002). Heavy metals are ubiquitous in trace concentrations in soils and the plants grown in these soils face the heavy metal stress, and causes changes in production of secondary metabolites. High levels of heavy metal contamination in medicinal or other plants may suppress secondary metabolite production. It is essential to maintain safety, quality and efficacy of the plants and their products to avoid serious health problems. Hence in the present study an attempt has been also made to analyze heavy metals (Cadmium, Lead, Copper, Znic, Arsenic, Mercury, Manganese and Chromium) by AAS.

II. MATERIALS AND METHODS:

The traces of heavy metals were detected through atomic absorption spectrometry analysis. The selected medicinal plant was collected from the hill places (Western Ghats) of Palakkad, Kerala, India.

Reagents: Analytical grade nitric acid, hydrochloric acid (Sigma&Merc grade) was used.

Plant material for analysis: The whole plant (without flowers and seeds) was collected and washed thoroughly. The plant is shade dried and finely powdered.

Analysis: Heavy metal analysis of the selected plant material was done on Atomic Absorption Spectrometry (AAS) with slight modified optimized conditions. The whole plant (except flowers and seeds) *Ceropegia juncea* (Roxb.) was taken for heavy metal analysis. The shade dried sample was converted into a finely powdered form and was subjected to microwave digestion method. 5.0 g of crude sample was placed in an Erlenmeyer flask and 20 ml of the extracting solution of 3M HNO_3 was added to it and it was placed in a magnetic stirrer and the mixture was stirred for 20 minutes. It was allowed to stand overnight and the solution was heated carefully in a water bath until red nitrous oxide fumes ceased and allowed to cool. The resulting solution was filtered through a Whatman filter paper No. 42 and transferred into a 50 ml polypropylene vial and diluted to 50 ml with the extracting solution. The final residue was dissolved in HNO_3 solution and made up to 50ml. Standard solutions were prepared by diluting the stock solution with 0.1 M nitric acid for checking the linearity.

The analytical reagent blanks were prepared. Wavelength is fixed between 185 to 900 nm. Atomic Absorption Spectrophotometer was equipped with high intensity hollow cathode. Compressed air and Acetylene gas was used to analyze the selected metals in the sample and the Air Flow is adjusted to 17.0 (L/min), Acetylene flow 1.5 (L/min) and the Lamp current is mA 15. Burner Head is 10 cm. The test samples were analyzed against the standard for measuring the concentration of the desired data.All measurements were run in triplicate for the samples and standard solutions. Standard operating parameters for working elements were set. All the metals were extracted into the HNO_3 in the form of metal nitrites. Plotted the response (absorbance or peak) versus concentration of each standard solution. Heavy Metals selected for this study in the selected medicinal plant was found Arsenic, Cadmium, Lead, Mercury, Zinc, Copper, Chromium, Manganese were analyzed and the results were shown.

Instrument details:

A.A.S (Atomic Absorbance Spectrometer) - n o v AA 350
Lamps are Hallow – Cathode and Deuterium
AA280FS Atomic Absorption Spectrometer
Agilent Technologies.

AAS is the analytical technique mostly employed for heavy metal analysis because of its low interference level and reasonable sensitivity (Caldas and Machado, 2004). Determination of heavy metals in the selected plant material was carried out by using Atomic Absorption Spectrometry (AAS) reported by Hussain *et al.*, (2005,2006) and modified appropriately with optimized conditions. Arsenic, Cadmium, Lead, Mercury, Zinc, and Copper, Manganese, Chromium were traced in the plant material. Absorbance was measured through atomic absorption spectrometer and the concentration of different trace and heavy metals in the plant samples were calculated.

III. RESULTS:

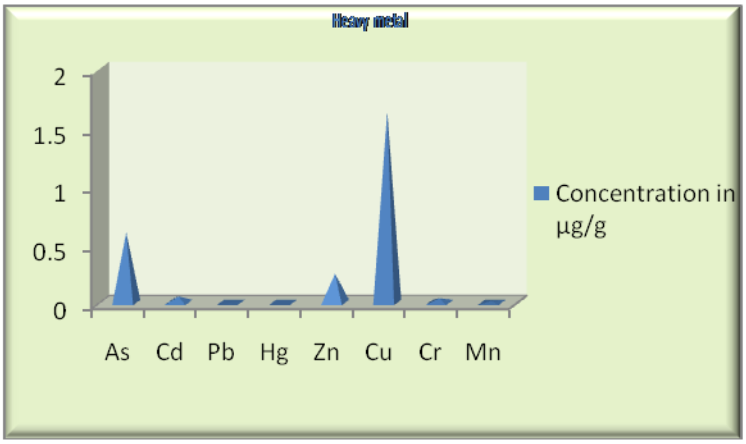
The metals traced in the present study were Arsenic (Ar), Cadmium (Cd), Lead (Pb), Mercury (Hg), Copper (Cu) and Zinc (Zn), Chromium (Cr), Manganese (Mn). The presence of these heavy metals were analyzed in the sample and the results were tabulated and represented in graph. Arsenic was estimated at 0.60 μg/g in the test plant. The concentration of Cadmium, Lead, Zinc, Copper, Chromium and Manganese were 0.053 μg/g, 0.002 μg/g, 0.247μg/g, and 1.637μg/g, 0.036 μg/g, and 0.017 μg/g, respectively were determined in the test plant. No traces of Mercury were identified in the plant. The tests were carried out in triplicate and analyzed statistically. The concentrations of all the heavy metals analyzed were found to be within the permissible limits (WHO/FDA). The permissible limits of the heavy metals in herbal ingredients as per WHO (World Health Organization) and FDA (Federal Drug Administration) were shown below (Hussain, 2006).

Maximum permissible limits of heavy metals in herbs:
Arsenic (Ar) 10^{3} ng/g
Cadmium (Cd) 0.3 µg/g
Lead (Pb) 10 µg/g
Mercury (Hg) 1 µg/g
Copper (Cu) 40mg/kg
Zinc (Zn) 60mg/kg

Estimation of Heavy Metals in *Ceropegia juncea.* Roxb.

Table.1. Concentration of heavy metals in *Ceropegia juncea* (Roxb.)

S.NO	Metals	Concentration in µg/g
1	As	0.60±0.13
2	Cd	0.053±0.12
3	Pb	0.002±0.001
4	Hg	0.00±0.00
5	Zn	0.247±0.121
6	Cu	1.637±0.142
7	Cr	0.036±0.016
8	Mn	0.017±0.004



The contents of heavy metals were found to be within the prescribed limit. The test medicinal plants contain trace metals such as cadmium (Cd), copper (Cu), chromium (Cr), manganese (Mn),, zinc (zn) as well as non essential heavy metals such as arsenic (As), lead (Pb) and mercury (Hg), which were present within the permissible limit.

IV. DISCUSSION:

There has been increased use of herbal drugs in recent years. Heavy metals are ubiquitous in trace concentrations in nature. Plants grown on heavy metal rich soils and waters undergo stress and shows changes in production of secondary metabolites. High levels of heavy metal contamination in medicinal or other plants may suppress secondary metabolite production. WHO (1998) recommended that medicinal plants which form the raw material for the finished product must be checked for the presence of heavy metal and pesticide residues etc. The traditional medicines cater about 85% of the world population for their health needs. It is essential to maintain safety, quality and efficacy of the plant and their products to avoid serious health problems.

Determination of toxic heavy metals in plants with potentially medicinal properties has not drawn the same research effort and importance as that of isolation and standardization of phytochemicals in plants (Branter and Males, 1999). Radhika Singh, (2008) reported heavy metal contamination in Ayurvedic drugs. Research reports about the levels of the above said heavy metals in the test plant sample (*Ceropegia juncea* Roxb.) is rather scanty. From the present results, it was found that no traces of mercury in the plant. The presence of heavy metals in different medicinal plants were reported by several authors. Zero levels of mercury residues were reported by Sudha *et al.* (2011) in *Sarcostemma acidum*. The present results were in accordance with the reports showed in the plant *Sarcostemma acidum.* Khan *et al*. (2001) reported the least concentration of mercury in ginseng. Mercury exposure for the general population occurs mainly from consumption of fish, as methyl mercury (Baht and Moy, 1997; Barbosa, 1997) and possibly from dental amalgam fillings (WHO, 2003) and it is unlikely that the exposure through medicinal herbs will affect human health. From the present report, the Cadmium was found in trace concentration 0.053µg/g in the plant sample and it is remarkably lower than permissible limits recommended by WHO as 0.3mg/kg (WHO, 1999). The concentrations of cadmium found in the present study were in accordance with the earlier reports. Various herbal medicines of 79 samples had shown that the concentrations ranging from 0.01 to 0.75 mg/g, with higher levels reported in cinchona extracts by De Pasquale et al. (1993). Khan et al. (2001) reported that the levels of cadmium in ginseng products varied from 0.008 to 0.12 mg/g. Generally it is accepted that the normal limits of Cadmium content in plants were between 0.2-0.8mg/kg and toxic content of Cadmium were defined as 5-30mg/kg.

In the present analysis, the concentration of lead was found to be 0.002 µg/g and was lower than the permissible limits. Maximum Recommended limit of 10 mg Pb/g (WHO, 1999). Much lower concentrations (0.001 to 2.6 mg Pb/g) were reported in medicinal plants (Aboub-Arab *et al.,* 1999; De Pasquale *et al.,* 1993; Khan *et al.,* 2001). Lead is one of the toxic elements that cause hazardous effect on health. Lead shows deleterious effect on central nervous system, kidneys, bones etc (Marina *et al.,* 2009) WHO (1998) prescribed limit for Pb contents in herbal medicine is 10 ppm while the dietary intake limit for Pb is 3 mg/week. Copper and Zinc were determined in the test plant and the concentration of the copper in the plant is 1.637µg/g and was within the WHO limit (40mg/kg). The concentration of Zinc was 0.247µg/g and below the recommended limit by WHO (60mg/kg). Zinc is an essential trace element for plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of perception and also cause loss of sense of touch and smell. The dietary limit of Zn is 100 ppm. (Jones, 1987).Copper and Zinc acts as essential micronutrients up to certain concentration. Copper plays an important role in the oxidative defense system but the high levels of copper leads to toxic conditions. WHO (1996) has recommended the lower limit of the acceptable range of Cu as 20 µg/mg body weight per day (FDA, 1993 & Waston 1993). Copper deficiency results in anemia and congenital inability to excrete copper resulting in Wilson's disease (Gupta, 1975).Zinc is co-factor of over 200 enzymes and involved in metabolic pathways. If the concentration exceeds the limits it becomes toxic to the body and interferes with copper metabolism. The present findings showed similarities with previous studies (Fatima *et al.,* 2005). These two elements are the essential trace elements and were required for major metabolic functions. Adequate amounts of copper are required for the growth of new blood vessels, and in wound healing property. Korban, (2006) Zinc enhances immune system.

The Arsenic content was recorded as 0.60µg/g in the present findings. The concentration of the metal was found to be within the permissible limits (WHO). Arsenic and Cadmium are potential threats for human health (Zhao *et al.,* 2009). Exposure to higher doses of arsenic can cause nausea and vomiting, decreased production of red and white blood cells, abnormal heart beat, damage to blood vessels, and a sensation of pinching by pins and needles in hands and feet. Symptoms of arsenic poisoning include headache, confusion, convulsion, diarrhoea, vomiting, and in severe cases coma and death. The present values showed the arsenic and cadmium was within the permissible limits and showed similarities with the previous findings. The observed concentrations of Chromium and Manganese in the selected plant were found well under prescribed values. The present study revealed that the concentration of Hg, Cd, Pb, Cu, Zn, Ar,Cr, Mn metals are within or remarkably much lower in limits recommended by WHO. The test plant *Ceropegia juncea* showed no traces of mercury. Copper and Zinc were found higher in concentrations compared to other metals. Presence of Cu, and Zn might be a useful supplement because these two elements are essential elements in trace concentrations for body metabolism. The other heavy metals like arsenic, cadmium, lead, mercury which are non essential elements were found in low levels when compared to other findings.Based on these results, all the metals are below the WHO permissible limits. Hence, the plant can be used for the development of drug and herbal products. Thus, on the basis of experimental outcome, it can be concluded that the plant ceropegia juncea which has vast ethnomedicinal values collected are safe and may not produce any harmful effect of metal toxicity during their therapeutic application and could also develop new drug entities from the plant.

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