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RESEARCH ARTICLE

Atomic Spectroscopy Analysis of Heavy Metals in Plants

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ABSTRACT

This study depicts a profile of existence of heavy metals (Cu, Cr, Cd, Hg, As, and Pb) in some important medicinal plants such as *Hibiscus rosa-sinensis, Eclipta alba*, and *Solanum nigrum* using atomic absorption spectroscopy. *H. rosa-sinensis, E. alba*, and *S. nigrum* these are indigenous plants having tremendous medicinal properties. Extracts of these plants are already reported to have many pharmacological activities. The purpose of this study is to determine heavy metal contents in these medicinal plants. The findings of this study were compared with prescribed limits of these metals in the WHO guidelines, and the content of all these heavy metals was found to be within safe limits. These findings indicate that the extract of *H. rosa-sinensis, E. alba,* and *S. nigrum* is safe from the point of view of heavy metal toxicity.

Keywords: Atomic absorption spectroscopy, *Eclipta alba*, heavy metals, *Hibiscus rosa-sinensis, Solanum nigrum.*

INTRODUCTION

In the environment, heavy metal pollutants are released from many different anthropogenic sources.[1] Heavy metals are released in the form of atmospheric particulates in different sizes which may be found as very minute solid, liquid, and gaseous particles. Depending on the geographical sources, heavy metals are trace elements may differ which may lead to severe toxicity. These particulate matters are produced during the incomplete combustion of diesel fuel. It has been shown that diesel engines, which power most of the nation's transportation (buses, trains, ships, cars, and trucks), produce tonnes of air pollutants, and account for more than two-thirds of all particulate matter from transportation sources.[2] Lead and cadmium are among the most abundant heavy metals and are particularly toxic.[3] Excessive content of these metals in food is associated with a number of diseases, especially of the cardiovascular, renal, nervous, and skeletal systems.[4-6] These heavy metals are also implicated in carcinogenesis, mutagenesis, and teratogenesis. Other metals, such as copper and zinc, are essential for important biochemical and physiological functions and necessary for maintaining health throughout life.

Heavy metals are reported to accumulate in plants in various concentrations. Its excessive consumption may cause intake of toxic heavy metals, which may result in serious complications such as accumulative poisoning, nervous disorder, and cancer and leads to mortality.[7] Heavy metal contamination with cadmium, copper, lead, nickel, mercury, andarsenic when accumulated in plants that are above the standard permissible limits causes environmental pollution and can cause major health complications. It is mandatory to test the presence of highly toxic heavy metals such as arsenic, mercury, lead, and cadmium in the plant extract for food safety and quality control.

Hibiscus rosa-sinensis, Eclipta alba, and *Solanum nigrum* these plants have various kinds of pharmacological actions:

E. alba is a famous hair tonic for maintaining dark hair and reversing baldness. It is often translated as "king of the hair." It is decocted in coconut oil, and as this is a "cooling" oil, it is used externally for "hot" and inflammatory head problems such as headaches, sinusitis, and ear infections. The herb also benefits heat problems.

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E. alba is also used as medicine alterative, anti-inflammatory, hemostatic, antipyretic, vulnerary, tonic, cholagogue, and hepatoprotective.[8]

The aqueous ethanolic extract of aerial parts of *H. rosa-sinens* was reported for its use in constipation and diarrhea. The alcoholic extract of flowers of *H. rosa-sinensis* has been proved to possess anticonvulsant property. In traditional medicine, the leaves of the plant are used in fatigue and skin disease. Fresh root juice of the plant is given for gonorrhea and powder root for menorrhagia. Flowers of the plant are used in epilepsy, leprosy, bronchial catarrh, and diabetes.

The berries and leaves of *S. nigrum* are mainly used for medicinal purposes, besides the other parts of the whole plant.[9] The leaves are used as poultice for rheumatic and gouty joints (disease causing the joints to swell and become painful) and skin diseases, used in the treatment of antituberculosis, and are said to produce phoresis. Leaves are also used in dropsy, nausea, and nervous disorders.[10-12] The berries and flowers is useful in cough and erysipelas as specific, acute, cutaneous inflammatory disease caused by a hemolytic streptococcus and are characterized by red hot. These are a remedy for pulmonary tuberculosis, bronchitis, and diuretic. The juice of the berries is used as an antidiarrheal, ophthalmopathy, and hydrophobia.[13-15]

The objective of the study is to evaluate the presence of four toxic heavy metals in *H. rosa-sinensis, E. alba,* and *S. nigrum.*

MATERIALS AND METHODS

Instrumentation of atomic absorption spectra (AAS)

As early as 1860, Kirchhoff described the basic principle of AAS. It was not until 1955, the analytical background for its analytical applications was demonstrated by Walsh, Alkemade, and Milatz. The simplicity of this technique marks it an attractive tool for the analysis of many elements. In AAS, the elements are transformed into the atomic vapor form by drawing an aerosol of the sample solution into an open flame. Most of the freed atoms are then excited by exposure to a suitable source of radiation. The radiation absorbed by the unexcited atomizes related to the sample concentration [Table 1]. In this sense, atomic absorption spectrometry then could been visage as the inverse of emission spectrometry where the radiation emitted by the thermally excited atoms is related to concentration. It should be emphasized that usually the fraction of atoms excited by heat (through flame or an electric arc) is relatively small for most elements.

Anatomic absorption spectrometer consists of the following elements.

Source[16]

Single-element or multi-element hollow cathode tubes generally are employed as source in atomic absorption. Less frequently, the bright continuum of a xenon arc has been used as a source. Collision of these atoms with an inert gas such as argon induces excitation of the metal atoms and subsequent emission of characteristic radiation.

Burner[17]

The quality of the burner, the type of fuel, and the ratio of fuel to oxidize are the important factors which affect the result of analysis by an atomic absorption instrument. The burner can be compared to a sampling cell in a spectrometer.

Monochromator

The monochromator should be able to pass the resonance line and filter out other.

Phototube and amplifier[18]

Following factors are affecting the atomic absorption spectrometer. In general, an organic solvent enhances the absorption signal, and therefore, it may alter the absorption intensity. These can bond strongly with metal sand tend to reduce the signal intensity. EDTA could eliminate such effect.

Chemicals and reagents

Nitric acid (HNO3), perchloric acid (HClO4), hydrochloric acid (HCl), and sulfuric acid (H2SO4) were used and all were of analytical grade.[19] The concentration of stock solution was 1000 ppm.

Sample preparation

For the analysis, samples were ground to a fine powder and dried at 55–70°C for 6–8 h in a

Table 1: Standard permissible limits of heavy metals as per the WHO/FDA

Heavy metals	Permissible limit (PPM)
Arsenic	Not more than 3
Cadmium	Not more than 1
Mercury	Not more than 5
Lead	Not more than 1

controlled environment, to remove moisture. Immediately after drying, accurately weighed 1.0 g was placed in a flask and treated with 12 ml of concentrated HNO3 for 24 h. A mixture of H_2SO_4 and HNO_3 (3:1) was added 5 ml in each of the conical flask. The mixture was heated at 120–130°C for 5–6 h, until fumes stop, and the resulting solution was become clear. It was cooled at room temperature and filtered using Whatman filter Paper No. 42. The entire filtrate was mixed and made the volume upto 50 ml with Milli-Qwater. A blank was also prepared for every sample in the same way. Each sample was aspirated twice and the experiment was repeated for 5 times.

The AAS system (AA6300, Shimadzu, Japan) used was equipped with flame and graphite furnace having wavelength range of 185–900 nm and detector photomultiplier of 185.0–600.0 nm. The solvents used in the study were of analytical grade. The concentration of the stock solution used in AAS such as copper, chromium, cadmium, lead, arsenic, and mercury was 1000 ppm. From the afore-mentioned stock solution, the concentration of working ranges as mentioned in Table 2 was prepared. Instrumental condition for trace and heavy metal analysis is also given in Table 2.

RESULTS AND DISCUSSION

Heavy metals are found everywhere in the environment and enter through human activities, mining, power generation, and leaded gasoline. Humans risk to exposure from environmental concentrations that occur naturally or human activities. People who are not occupationally exposed may also carry certain metals in their body as a result of exposure from other sources, such as food, beverages, or air.[20] It is, however, possible to reduce metal toxicity risk through lifestyle changes that diminish the probability of harmful heavy metal uptake, such as dietary measures that may promote the safe metabolism or excretion of heavy metal consumption.

Mercury, cadmium, and lead can effectively inhibit cellular glutathione peroxidase, reducing the effectiveness of this antioxidant defense system for detoxification.[20] Mercury gets deposited invitrogen's such as brain, nervous system, heart, liver, kidneys, and bone marrow[21] and known to cause dementia, peripheral neuropathy, Parkinson's disease, and cancer.[22]

The result analysis of the levels of heavy metal present in the selected herbs is discussed in this section and the concentration of As, Cd, Hg, and Pb in the herbs is presented in Table 3. The heavy metals analyzed in the herbal extract are less than the permissible limits.[16] The levels of heavy metals present in the extract were expressed as mean of heavy metal concentration (ppm) ± SD of three replicates. Calibration functions[23-25] for each element were determined. Concentrations of each heavy metal in the medicinal herb were calculated from the calibration functions. Statistical analysis is done in the herbal extract, and there is no significant heavy metal present in it.

Heavy metal analysis is calculated using this formula:

Actual concentration = Concentration × VF × DF × [CF/WF].

Where
CF=Correction factor,
DF=Dilution factor,
VF=Volume factor,
WF=Weight factor.

Atomic absorption spectroscopy detection was carried out on positive ionization mode. It was optimized using a standard line calibration curve for various concentrations. The calibration curves were constructed by plotting the response against the concentration. A linear relationship was obtained for each compound. The heavy metals (cadmium, lead, arsenic, and mercury) were analyzed at their particular wavelength, and the ion with the upper intensity was selected as the basic ion. The study revealed that there were no resultant spectral peaks of Cd, Pb, As, and Hg in *Hibiscus rosa-sinensis, E. alba,* and *S. nigrum.*

It is concluded from the present study that heavy metal analysis of *H. rosa-sinensis, E. alba,* and *S. nigrum* extract obtained below standard prescribed limits. The concentration of trace metals and heavy metals of three plants was determined by AAS.[26] The quantitative determinations were carried out using

Table 2: Instrumental condition for trace and heavy metal analysis by atomic absorption spectroscopy

ASS specification	Elements					
	Copper	Chromium	Cadmium	Arsenic	Lead	Mercury
Wavelength	324.8	357.9	232.0	193.7	217.0	253.7
Current (mA)	5.0	5.0	9.0	12.0	9.0	3.0
Flame	AA	AA	AA	AA	AA	AA
Fuel (L/min)	3.05	2.90	2.94	240	2.90	7.66

Table 3: Results of heavy metal analysis by atomic absorption spectroscopy in plants

Plantsused	Copper (ppm ± SEM)	Chromium (ppm ± SEM)	Cadmium (ppm ± SEM)	Lead (ppm ± SEM)	Arsenic (ppm ± SEM)	Mercury (ppm ± SEM)
Ecliptaalba	1.151 ± 0.031	0.308 ± 0.012	0.021 ± 0.035	0.860 ± 0.009	0.081 ± 0.007	0.036 ± 0.010
H. rosa-sinensis	2.605 ± 0.045	0.450 ± 0.025	0.018 ± 0.050	0.630 ± 0.025	0.041 ± 0.014	0.023 ± 0.006
S. nigrum	1.549 ± 0.087	0.304 ± 0.055	0.012 ± 0.150	0.945 ± 0.005	0.034 ± 0.011	0.040 ± 0.015

H. rosa-sinensis: Hibiscus rosa-sinensis, S. nigrum: Solanum nigrum, SEM: Scanning electron microscopy

standard calibration curve obtained by the standard solution of metals having optimal detectable concentration ranges.[27-30] The concentration of the metal obtained in plant material was expressed in terms of parts per million. Form the results obtained, it was concluded that levels of heavy metals fall within the permissible range and can be preferred to consume by humankind for various medicinal purposes.[31-36]

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