Estimation of Heavy Metals in Selected Medicinal Herbs

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ABSTRACT
Worldwide nearly 80% of peoples are using the medicinal plants for basic illness due to the presence of primary metabolites, secondary metabolites, minerals and inorganic salts. Some Inorganic minerals available in plants are useful for health and have therapeutic value and some heavy metals are dangerous for health and may cause damage to internal organs. In view of that, the present study designed to quantitatively evaluate the presence of inorganic minerals like Sodium, Potassium, Calcium, Magnesium, Zinc and Copper and heavy metal like Mercury, Arsenic, Lead, Cadmium, Selenium, Cobalt and Chromium in selected medicinal herbs using Flame photometric method and Atomic absorption method. There is a limitation for the presence of heavy metals in herbal drugs, The distributions of these metals are not uniform all areas, so before using for formulation development all herbs should be taken for the estimation of heavy metals. In this study an attempt to estimate the inorganic elements and heavy metals present in some of the important medicinal plants like Moringa oleifera Lam, Achyranthes aspera Linn, Scoparia dulcis Linn, Achyranthes aspera Linn. Castus pictus Linn, are mostly used in traditional medicines. Based on the analysis, the result shows the minerals like Sodium, Potassium, Calcium, Magnesium, Zinc and Copper were present in considerable quantity and heavy metals like Mercury, Arsenic, Lead, Cadmium, Selenium, Cobalt and Chromium were within the limit as per WHO standard values. The plants are containing heavy metals were within the limit as per WHO standard. Further these medicinal plants can use for new herbal formulations.

INTRODUCTION
The selected plants Moringa oleifera Lam.(MO), Achyranthes aspera Linn.(AA), Scoparia dulcis Linn.(SD), Saropus androgynus Linn. (SA), (Gokhale et al., 2002) and Castus pictus Linn.(CP) are more useful in the traditional system of medicine for many diseases (Ezeamuzie et al., 1996). Herbal medicines when used for internal administration they must be standardized before formulation for the quantitative estimation of Physical parameters like heavy metals, Phytochemicals estimations. Heavy metals are the substances can be toxic when consumed...
by humans especially like Lead, Cadmium, Mercury, Chromium and Arsenic (Gasser et al., 2009). They are naturally present in the earth in different places in different concentration and it may absorbed and occur in plants (Karayil et al., 2014). Some of the metals are necessary and do some beneficial effect on human like iron and some do toxic effect selenium, arsenic (Graeme and Pollack, 1998a). Heavy metals can, in certain level, causes illness (Karadi et al., 2008), as well as carcinogenic, have adverse reproductive effects, (unfavorably impact nutrition), and displace more geographically valuable metals such as magnesium and zinc (Duffus, 2003). This document is focused on the estimation of heavy metals like lead, mercury, arsenic, cadmium, selenium, chromium, cobalt and nickel. The guidelines developed to determine with accompanying explanations by the American Herbal Products Association (AHPA) and given the quantitative limits for it. As well as it debates relevant regulations about the prevalence of these chemicals in products sold in the United States. The heavy metals are wide spreader environmental contaminant and potential toxin that may adversely affect human health (Johri et al., 2010). When exposure to cadmium towards the respiratory or gastrointestinal tract (Rao et al., 2006), important non-industrial source of exposure are cigarette smoke and food (from contaminated soil and water). The kidney is the main organ affected by chronic cadmium exposure and toxicity (Rai et al., 2001). Environmental cadmium exposure is also the significant contributory factor may cause the development of chronic kidney diseases, (Wang et al., 2007). And in the presence of other metals also especially for diabetes, hypertension, therefore the source and environmental impact of and efforts to limit Cd exposure, justify more attention (Graeme and Pollack, 1998a). Metals may produce toxic and sometimes produce effect as same as that of the essential elements in body and interact with metabolic process and produce problems in body (Pasquale et al., 1993).

Environmental Cd exposure may be a significant contributory factor to the development of chronic kidney disease, especially in the presence of other co-morbidities such as diabetes or hypertension; therefore, the sources and environmental impact of Cd, and efforts to limit Cd exposure, justify more attention (Graeme and Pollack, 1998b). Metals may produce toxic and sometime produce effect as same as that of the essential elements in body and interact with metabolic process and produce problems in body. This present study focused towards the importance of the quantitative evaluation of heavy metals in herbal products.

### MATERIALS AND METHODS

#### Collection and Authentication of Plant

The plants *Moringa oleifera* Lam. And *Achyranthes aspera* Linn., *Saropus androgynus* Linn., *Castus pictus* Linn, available locally and they were collected from in and around Coimbatore. The plant *Scoparia dulcis* Linn. was collected from Palakad district in Kerala. The botanical identity has been authenticated by the Director, Botanical Survey of India, Coimbatore (No:BSI/SRC/5/23/2011-12/Tech/376;BSI/SRC/5/23/2012-13/Tech/496). The voucher specimens had been submitted and preserved in herbarium for future reference.

#### Preparation of Drug Sample

For the estimation of heavy metals the ash from the plant leaves were prepared and the ash dissolved in dilute hydrochloric acid, filtered. The clear liquid was taken for the quantitative estimation of inorganic metals by flame photometer and Atomic Absorption Spectroscopic method (Becketta and Stenlakej, 1987).

#### Quantitative Estimation

##### Estimation of Inorganic Minerals by Flame photometer Method

The alkalies and alkaline earth metals such as Na, K, and Ca can be determined by flame photometry. Flame emission photometry is also successfully used for estimation of certain transition elements such as copper, iron and manganese as the energy obtained by the flame is quite sufficient for the excitation of these elements. It is used by the flame to provide the energy of excitation to atoms introduced into the flame. The quantitative results can be obtained by plotting a calibration curve between the standard and sample concentration of the element and flame induction.

Liquid sample containing element, is aspirated into a flame to the formation of the liquid droplets. Evaporation of liquid droplets, resulting formation of salt residue. Then decomposition of residue to formation of free neutral atoms or radicals. These neutral atoms are excited by the thermal energy of the flame. The excited atoms which are unstable, quickly emit photons(radiation) and return to the lower energy state. The measurement of emitted radiation in terms of wavelength or intensity is basic of flame photometry. If $E_2$ and $E_1$ represent the energy of the higher and lower energy state. The radiation emitted during the changing energy level may be defined...
Table 1: Estimation of mineral salts in selected medicinal plants

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>AA (ppm)</th>
<th>MO (ppm)</th>
<th>SD (ppm)</th>
<th>CP (ppm)</th>
<th>SA (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sodium</td>
<td>56</td>
<td>28</td>
<td>39</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>Potassium</td>
<td>57</td>
<td>36</td>
<td>36</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>Calcium</td>
<td>144</td>
<td>528</td>
<td>304</td>
<td>126</td>
<td>203</td>
</tr>
<tr>
<td>4</td>
<td>Manganese</td>
<td>0.58</td>
<td>0.02</td>
<td>0.61</td>
<td>0.87</td>
<td>0.76</td>
</tr>
<tr>
<td>5</td>
<td>Iron</td>
<td>16.67</td>
<td>5.23</td>
<td>11</td>
<td>09.31</td>
<td>09.75</td>
</tr>
<tr>
<td>6</td>
<td>Magnesium</td>
<td>96</td>
<td>67</td>
<td>77</td>
<td>46</td>
<td>53</td>
</tr>
<tr>
<td>7</td>
<td>Zinc</td>
<td>0.61</td>
<td>0.3</td>
<td>0.52</td>
<td>0.12</td>
<td>0.24</td>
</tr>
<tr>
<td>8</td>
<td>Copper</td>
<td>0.3308</td>
<td>0.1077</td>
<td>0.2385</td>
<td>0.213</td>
<td>0.132</td>
</tr>
</tbody>
</table>

*Results are mean of three sample preparations. Where Moringa oleifera Lam (MO), Achyranthes aspera Linn (AA), Saropus androgynus Linn. (SA), Castus pictus Linn (CP) and Scoparidulcis Linn (SD).

Table 2: Estimation of heavy metals in selected medicinal plants

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Metals</th>
<th>AA (ppm)</th>
<th>MO (ppm)</th>
<th>SD (ppm)</th>
<th>CP (ppm)</th>
<th>SA (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mercury</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.004</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>2</td>
<td>Arsenic</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>&lt;0.001</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>3</td>
<td>Lead</td>
<td>&lt;0.004</td>
<td>&lt;0.004</td>
<td>&lt;0.004</td>
<td>&lt;0.002</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>4</td>
<td>Cadmium</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.00</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>5</td>
<td>Selenium</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.006</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>6</td>
<td>Cobalt</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>7</td>
<td>Nickel</td>
<td>&lt;0.001</td>
<td>&lt;0.095</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>8</td>
<td>Chromium</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.076</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

*Results are mean of three sample preparations.

by equation as,

\[ E_2 - E_1 = hv \]

Where \( h \) = planks constant, \( v \) = the frequency of emitted light.

Which is defined as,

\[ \gamma = c/\lambda \]

We get

\[ E_2 - E_1 = hc/\lambda \text{ or } \lambda = hc/E_2 - E_1 \]

From the above equation calculate the wave length of the emitted radiation which is characteristic of the particular element.

Weigh accurately 100 mg of sodium chloride and potassium chloride separately dissolve in 100 ml of distilled water. Prepare a series of standard solutions of sodium chloride, potassium chloride 10, 20, 30, 40, 50 \( \mu \)g/ml concentrations. Switch the flame photometer and select the sodium filter. Set the gas in flame and air pressure at 0.4 to 0.5 kg/cm\(^2\). Automize the flame intensity to 0% using distilled water with the knob. Automize the flame intensity to 100% using 50 \( \mu \)g/ml standard solution (highest concentrations). Measure the flame intensity of all the standard solutions with different concentrations like 10, 20, 30, 40, 50 \( \mu \)g/ml, and unknown sample solution. Plot the graph between concentration and percent flame intensity. From the graph, make the percentage flame intensity of the known sample and by extrapolating, determine corresponded concentration (Becketta and Stenlakej, 1987).

Atomic Absorption method

This technique is closely related to flame emission spectrometry. They use a flame as the atomizer. The sample solution is aspirated into the flame same as flame emission spectrometry, here the element is converted to atomic vapor, it contains the atoms of the elements. Some are remain in the ground state. These ground state atoms can absorb radiation of a particular wavelength that is produced by a special source made from that element. The wavelengths of radiation given by the atoms in the flame is as identical in principle to absorption spectrometry (Abou-Arab et al., 1999). The absorbance is directly proportional to the path length in the flame and the concentration of atomic vapor in the flame. Both of the variables are difficult to determine, but the path length in the flame can be held constant and the concentration of atomic
vapour is directly proportional to the concentration of the analyte in the solution being aspirated. The calibration curve is prepared with concentration in the solution versus absorbance. The inorganic elements like magnesium, Zinc, Iron, Manganese, and copper like elements are estimated by Atomic absorption method. The heavy metals like mercury, Lead, Arsenic, Cadmium, Selenium, Chromium, Nickel, Cobalt were also estimated by same Atomic Absorption method (Honary et al., 2007). The estimations were carried out in triplicate and calculated statistically.

RESULTS AND DISCUSSION

Estimation of Inorganic Elements

The quantity of inorganic elements like Potassium, Manganese, Sodium, Calcium, Magnesium, Zinc, Iron and Copper were estimated by flame photometry and atomic absorption methods and the quantity present in the selected plant leaves were expressed in the Table 1.

Estimation of mineral salts in selected medicinal plants

Figure 1: Quantity of minerals present in the selected herbs

Estimation of Heavy Metals

The selected plants leaves powder were estimated for the presence of Heavy metals by atomic absorption spectra method. The quantities of all heavy metals present in these plants were tabulated below. Where Moringa oleifera Lam (MO), Achyranthes aspera Linn.(AA), Saropus androgynus Linn.(SA), Castus pictus Linn. (CP) and Scoparia dulcis Linn.(SD).

The herbal drugs usage has been increased in recent years. The inorganic minerals which are necessary for human health were quantified and tabulated and also the quantity of heavy metals present in the selected plants leaves were estimated and tabulated. According to the WHO (2005) the standard quantity or limits of heavy metals in the medicinal plants were listed as Arsenic 3ppm, Lead 10ppm, Cadmium 0.3ppm, Chromium 1ppm and Mercury 0.03ppm. The results shows the quantity of these heavy metals present in the selected plants were within the limit and the results were tabulated in the Table 2, (Commission Regulation, 2006). The results of this study reveals that the concentration some of the inorganic minerals like Ca, Na, K, Cu, Mn, Mg and Zn present in the samples are in good quantity expressed in figure 1, because all these minerals are necessary for human life, where as the heavy metals are within or remarkably much lower when comparing with the limits recommended by WHO. Different region of the world and countries set on maximum values for toxic metals in various quantities and now the world has recently been published by the WHO (WHO, 2007). In this context, the WHO propose some limit for Lead 10mg/kg and for Cadmium 0.3mg/kg in dried herbs (WHO, 2007).

These heavy metals determination was performed by using atomic absorption spectrometry (AAS). Here the concentration of lead was found to be much lesser quantity when compared with the standard limit 10mg/g of Lead (WHO, 1999). Because the more quantity of lead in herbal medicines will produce hazardous effect like deleterious effect on central nervous system (Khan et al., 2001), side effects in kidney and bones etc (Abou-Arab et al., 1999). Minerals like Copper and Zinc are important but there should be a limits, (de Souza et al., 2008) they were also determined and documented in the table no.1, that also found within the limit as per WHO guideline for copper 40mg/kg and Zinc 60mg/kg (FDA, 1993). Zinc is essential for plant growth and also for human body normal growth, brain development and bone formation. Copper is also playing important role in human body like oxidative defence system where as the concentration increases it will leads to toxic (Jones et al., 1977). Copper and lead are required for metabolic functions. The development of new blood vessels, for wound healing property is promoted by copper. Zinc enhances the human immune system, work as cofactor for some enzymes involved in metabolic pathways (Baker and Brooks, 1989). If the level increases it also leads to toxicity and interfere with copper metabolism (Maqsood and Khan, 2005). The arsenic and Cadmium content were determined and found that are within the permissible limit (WHO, 2003). Exposure in high dose they may produce nausea, vomiting, abnormal heart beat, damages blood vessels (Zhao et al., 2009). Arsenic poisoning
leads to convulsion, confusion, vomiting, diarrhea,
and in severe condition it may lead to coma and
death.

CONCLUSIONS

On basis of this experimental study, result shows
the quantity of all the heavy metals estimated are
below the permissible limit of WHO guideline. The
result shows that all these selected plants are having
the inorganic minerals in the considerable quantity
and may helpful for health requirements. The result
shows *Moringa oleifera* Lam. *Achyranthes aspera*
Linn. *Saropus androgynus* Linn. and *Scoparia dulcis* Linn. are safe for internal admin-
istration for their therapeutic values and will not
produce any harmful effect by heavy metal toxicity.
These plants are advisable for the development of
new herbal formulations.

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Conflict of Interest

There are no conflicts of interest.

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