Heavy Metal Analysis of Selected Phytotherapeutic Plants in Sanamavu Forest, Krishnagiri District of Tamil Nadu, India

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ABSTRACT

Phytotherapeutic plants such as *Aristolochia indica* L. *Bidens pilosa* L. *Clematis zeylanica* L. (Pori.) Syn: *Naravelia zylanica* L. *Corallocarpus epigaeus* (Rottl.) Ex Willd. and *Plectranthus caninus* L. are used as important source of medicine for village people near the Sanamavu Forest area, Krishnagiri District. The concentration of five heavy metals Cadmium (Cd), Lead (Pb), Arsenic (As), Mercury (Hg) and Thalium (TI) are investigated using Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Heavy metal analysis shows that the high amount of lead (Pb) 2.36 mg/kg is identified in *Plactranthus caninus* L. and 1.80 mg/kg is in *Clematis zeylanica* L. (Pori.) The high amount of Cadmium (Cd) 0.143 mg /kg is identified in *Plactranthus caninus* L. and 0.097 mg/ kg in *Corallocarpus epigaeus* (Rottl.) Ex Willd. and the high amount of Arsenic (Ar) 0.11 mg/kg found in *Plactranthus caninus* L. and 0.039 mg/kg in *Biden pilosa* L. The high amount of Copper (Cu) 19.01mg/kg found in *Clematis zeylanica* L. (Pori.) Mercury (Hg) and Thalium(TI) are not detected in the five selected medicinal plants. This research goal is to identify and report heavy metals in the selected medicinal plants.

Keywords: Heavy metals (Pb, Cd, Ar, Cu, Hg, TI), ICP-MS, Phytotherapeutic plants, Sanamavu Forest.

1. INTRODUCTION

Traditional medicine are safety and effectiveness have been proven over a long period of time by the usage of many of its practices, including knowledge passed down from generation to generation (WHO, 2000). Since ancient times, phytotherapeutic plants, also known as medicinal herbs, have been identified and used as traditional medicine, for a variety of purposes, including defence and protection against insects, fungi, illnesses and herbivorous mammals, because plants synthesise hundreds of different chemical compounds (Jonathan and Chhana, 2022).

Ayurveda, Siddha, and Unani are the three main medical systems used in India. These systems make use of medicines made from plants, animals, and minerals that are derived naturally. Pharmaceutical, health food, and natural cosmetic businesses have all begun to place an increasingly significant emphasis on medicinal plants (Kim et al., 2016). Medicinal herbs are popular because they are inexpensive and easy to obtain without a prescription. The use of medicinal plants and phytonutrients or nutraceuticals has increased rapidly in recent years all over the world (WHO, 2004).

However, these medicinal plants contain heavy metals in varying amounts of concentration. Because of the presence of hazardous substances such as heavy metals in many medicinal herbs utilized in the formulation of these medicines can pose a health risk. Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Molybdenum (Mo) and Nickel (Ni) are among the micronutrients that plants essentially need in modest amounts for normal growth. (Emamverdian et al., 2015, Nagalakshmi et al., 2022). Metal levels that are too high, on the other hand, may be hazardous to the organism (Singh et al., 2011). But the elements with metallic properties and a relatively large density are known as heavy metals or contaminant, which are not essential for plant normal growth. It include lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), selenium (Se), nickel (Ni), silver (Ag), aluminium (Al), caesium (Cs), cobalt (Co), strontium (Sr), and uranium (U) (Mcintyre, 2003). If heavy metal uptake by plants and subsequent accumulation along the food chain poses a risk to animal and human health (Jiwan and Kalamdhad, 2011). Pb and Cd are not necessary elements in the human body or in plants. They induce different biomolecular detrimental functional consequences at low levels (Maiga et al., 2005) and also they may also react directly with DNA, resulting in a variety of DNA lesions such as DNA strand breakage and DNA protein cross-linkage (Harris et al., 2011).

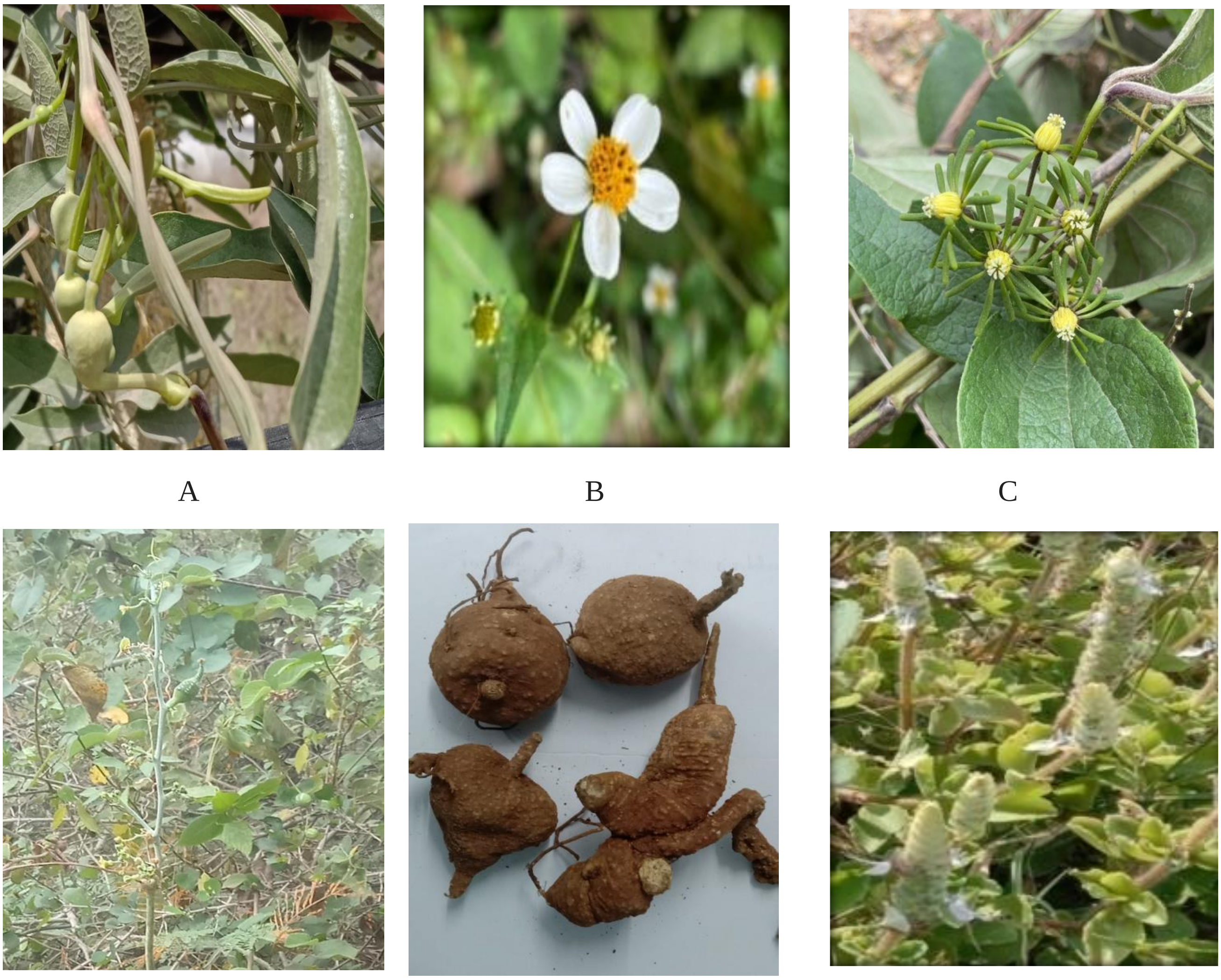
Arsenic (As) and Mercury (Hg) have the potential to harm the pulmonary, neurological, renal and respiratory systems, as well as cause skin pathology (Jarup, 2003; Mahurpawar, 2015). And also some herbal treatments had significant levels of heavy metals; this was an issue more frequently found in conventional medical therapy and was thought to be the root cause of a number of health conditions (Brookes et al., 2019). Humans are globally at risk from heavy metal contamination in herbal remedies, especially at doses exceeding recognized threshold values. Pb, Cd, As, and Hg have higher than acceptable risk in 25 different types of herbs for exposure evaluation (Lu Luo et al., 2021). So, the element levels of As, Cd, and Pb must be determined due to their toxicity (Jan et al., 2015).

The current study is concerned with determining the heavy metal content of various medicinally significant herbs and plants. The purpose of this study was to assess the amounts of several poisonous or heavy metal ions in medicinal plants like *Aristolochia indica* L. *Bidens pilosa* L. *Clematis zeylanica* L. (Pori.) Syn: *Naravelia zylanica*, *Corallocarpus epigaeus* (Rottl.) Ex Willd. and *Plectranthus caninus* L. were collected from the Sanamavu forest in the Krishnagiri District of Tamil Nadu, India. Only six heavy metals (Cd, Pb, As, Cu, Hg, and TI) were chosen for heavy metal analysis.

2. MATERIALS AND METHODS:

2.1. Collection of plant sample

Phytotherapeutic plant samples were collected from the Sanamavu Forest of Tamil Nadu, Krishnagiri District. It is located 8 kilometres southeast of Samanapalli village and 9 kilometres southwest of Kelamangalam village in Krishnagiri District.



A B C

D E F

Fig: 1. Habitat of selected medicinal plants for heavy metal analysis

A. *Aristolohia indica* L. B. *Biden pilosa* L. C. *Clematis zeylanica* L. (Pori.)

D & E. *Corallocarpus epigaeus* (Rottl. & Willd.) Hook.f. E. *Plectranthus caninus* L.

2.2 Sample preparation

Collected leaves and tubers were washed using clean running tap water and distilled water, dried in the room temperature under the shadow, powdered, sieved and stored in the closed vessel in the refrigerator for further heavy metal analysis.

2.3 Microwave Digestion Procedure

Collected, powdered, sieved and stored 0.5g of plant samples were taken in each plant separately in a microwave digestion closed (MDC) vessel. After addition of 4.0 millilitres of milli-Q water, 5 millilitres of (Trace metal-grade) concentrated nitric acid (HNO_3, 65%) and 1 millilitre of (Trace metal grade) hydrogen peroxide (H_2O_2, 30%) in to the tightly closed MDC vessel and kept at room temperature for 5 minutes. After 5 minutes the vessels kept in the rotor inside the microwave digester for digestion and rehydrate the vessel at room temperature.

Table: 1. Microwave Digestion Heating Program

S.No	Ramping stage	Hold Time (Min)	Temperature (^{0}C)	Power (Watt)
1	**01**	**20**	**180**	**1200**
2	**Cool Down**	**10**	**-**	**-**

The digested solution was filtered using Watman No. 42 filter paper, and the volume was increased up to 25 mL using milli-Q water (Shaole *et. al.*, 1997).

2.4. Method

The heavy metals of Cadmium (Cd), Lead (Pb), Arsenic (As), Mercury (Hg) and Thalium (TI) were investigated by using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Agilent -7850. ICP-MS is capable of detecting metals at very low concentration, as low as 1ppq (One part per quadrillion). So, it is used in the investigation of heavy metals analysis and working conditions of ICP-MS given in the table-2.

Table: 2. working conditions of ICP-MS

Plasma condition	**a. Plasma Flow-Argon (15L/min)** **b. Nebulizer pump uptake speed (0.5rps)** **c. RF power 1550 watts**
S/C Temperature	2^{0}C
Uptake Time	40 Sec
Delay Time	40 Sec
Stabilization Time	40 Sec
Probe Rinse	Rinse 1-2.0% Nitric acid (20 Sec) Rinse 2-Milli-Q water (20 Sec)
Nebulizer Flow	0.98 to 1.2 L/min (Optimal 1.05 L/min)
Reaction Cell	ORS and KED with Helium Flow 4.1 to 4.5 ml/min (Optimal 4.3ml/min)
Number of Replicates	3.0
Energy Discrimination	3.0 V
Mode	He mode for both Selenium and Zinc
TMP Revolution	100%
Oxide ratio (He and NO gas mode)	156 / 140
Doubly charged (He and NO gas mode)	70 /140
Stabilization time	40 Sec
Integration time	0.1 Sec
Sensitivity (He mode)	Mass 59-minimum 2400 counts)
Auto sampler conditions	Working mode – continuous wash – between Runs
P/A Factor	P/A factor ON 'Automatic Mode'.

	Low Concentration – Pulse Mode High Concentration – Analogue.

2.5 Calibration Techniques

The ICP-MS detector measures the signal in 'counts per second' (CPS). This is the number of ions that strike the detector every second. Calibration standards having known concentrations of elements can be used to generate a calibration curve in order to translate the data to a concentration value.

3. RESULTS AND DISSCUSION:

3.1 Results

The concentration of five heavy metals Cadmium (Cd), Lead (Pb), Arsenic (As), Copper (Cu), Mercury (Hg) and Thalium (TI) were investigated in the selected medicinal plants using Inductively Coupled Plasma Mass Spectrometer (ICP-MS). In *Aristolochia indica* L. 0.8mg/kg of Lead (Pb), 0.035mg/kg of Cadmium (Cd), 0.017mg/kg of Arsenic (As) and 9.39mg/kg Copper (Cu) were reported. In *Biden pilosa* L. 0.81mg/kg of Lead (Pb), 0.025kg/mg of Cadmium (Cd), 0.039kg/mg of Arsenic (As) and 15.55mg/kg were reported. In *Clematis zeylanica* L. (Pori.) 1.80mg/kg of Lead (Pb), 0.038kg/mg of Cadmium (Cd), 0.032kg/mg of Arsenic (As) and 19.01mg/kg Copper (Cu) were reported. In *Corallocarpus epigaeus* (Rottl. & Willd.) Hook.f 0.38mg/kg of Lead (Pb), 0.097kg/mg of Cadmium (Cd), 0.025kg/mg of Arsenic (As) and 6.26mg/kg were reported. In *Plectranthus caninus* L. 2.36mg/kg of Lead (Pb), 0.143kg/mg of Cadmium (Cd), 0.11kg/mg of Arsenic (As) and 13.44mg/kg Copper (Cu) were reported.

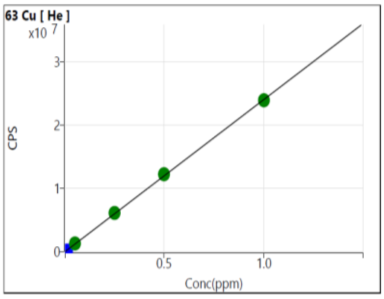
Table: 3. Heavy metal analysis of selected phototherapeutic plants

Samples	Lead (Pb) (mg/kg)	Cadmium (Cd) (mg/kg)	Arsenic (As) (mg/kg)	Copper (Cu) (mg/kg)	Mercury (Hg) (mg/kg)	Thallium (TI) (mg/kg)
***Aristolohia indica* L.**	**0.87**	**0.035**	**0.017**	**9.39**	**<LOD**	**<LOD**
***Biden pilosa* L.**	**0.81**	**0.025**	**0.039**	**15.55**	**<LOD**	**<LOD**
***Clematis zeylanica* L. (Pori.)**	**1.80**	**0.038**	**0.032**	**19.01**	**<LOD**	**<LOD**
***Corallocarpus epigaeus* (Rottl. & Willd.) Hook.f**	**0.38**	**0.097**	**0.025**	**6.26**	**<LOD**	**<LOD**
***Plectranthus caninus* L.**	**2.36**	**0.143**	**0.11**	**13.44**	**<LOD**	**<LOD**

*** <LOD – Blow the limits of detection.**

3.2 Calibration curve:

Aristolohia indica L



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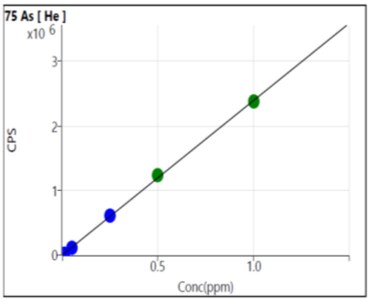

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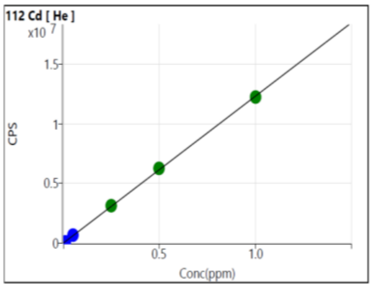

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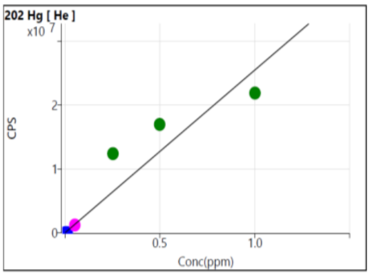

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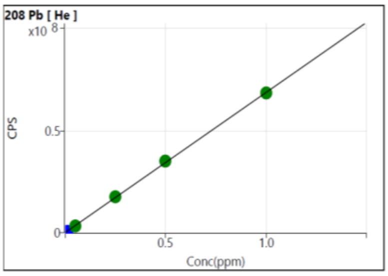

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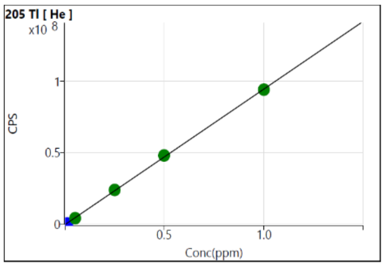

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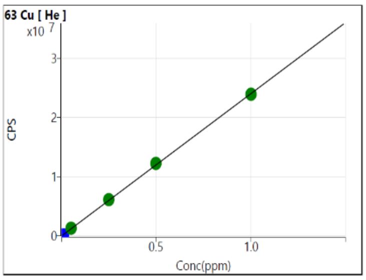
Biden pilosa L



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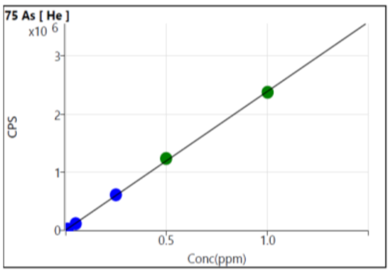

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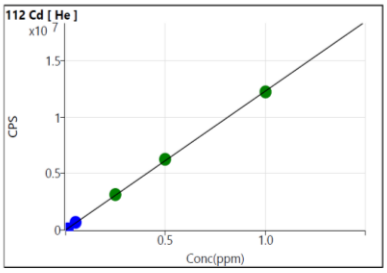

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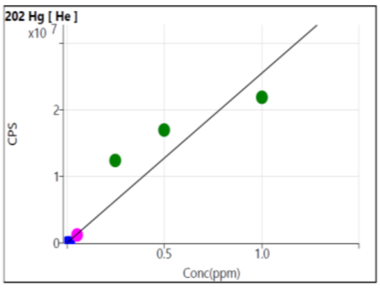

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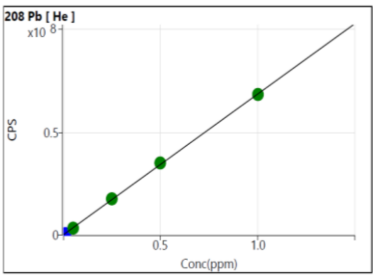

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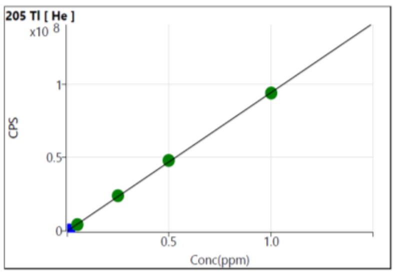
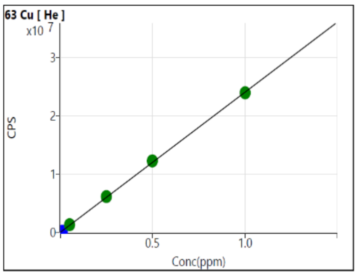


y=94256562.5361*x+66.7400

Fig. 2. Calibration curves for Pb, Cd, As Cu, Hg and TI in phytotherapeutic plants *Aristolohia indica* L. and *Biden pilosa* L.

Clematis zeylanica L. (Pori.)



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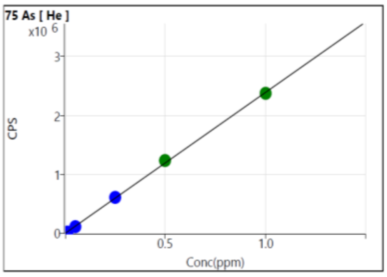

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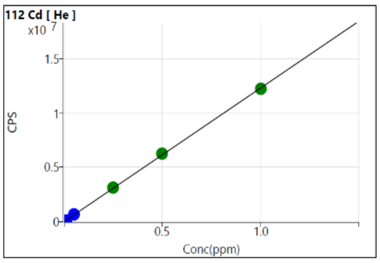

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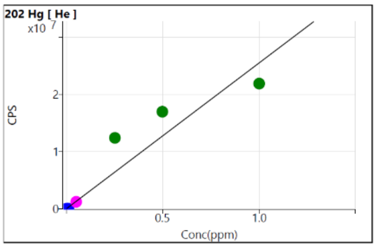

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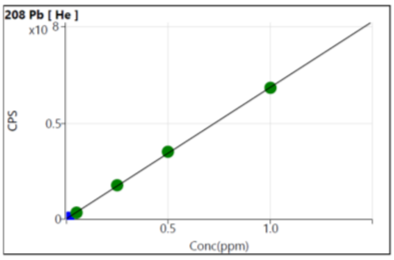

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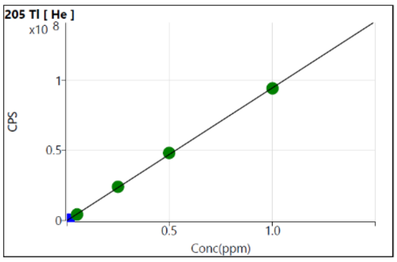

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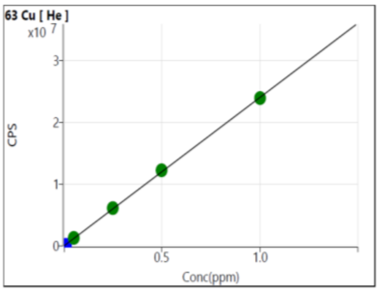
Corallocarpus epigaeus (Rottl. & Willd.) Hook.f



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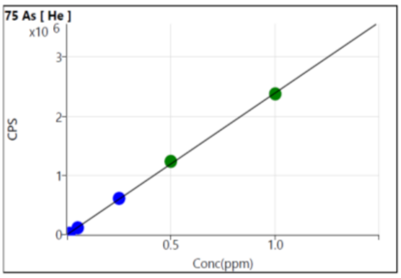

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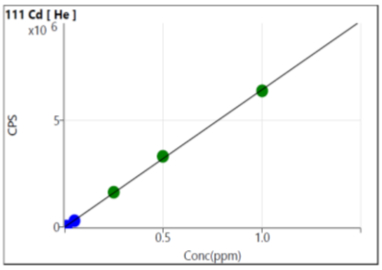

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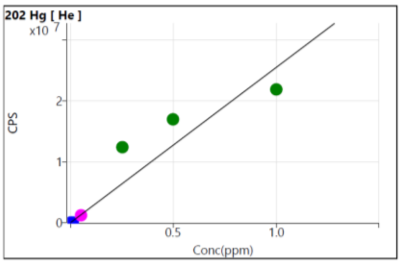

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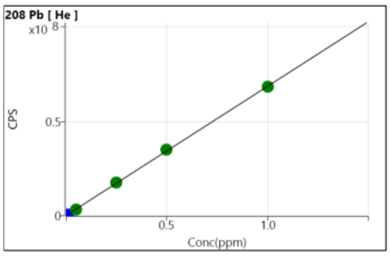

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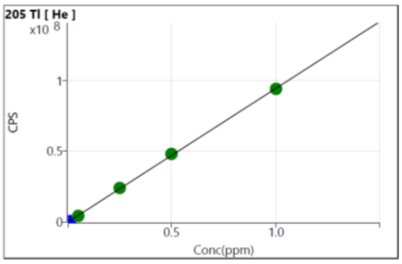

y=94256562.5361*x+66.7400

Fig. 3. Calibration curves for Pb, Cd, As Cu, Hg and TI in phytotherapeutic plants *Clematis zeylanica* L. (Pori.) and *Corallocarpus epigaeus* (Rottl. & Willd.) Hook.f

Plectranthus caninus L.

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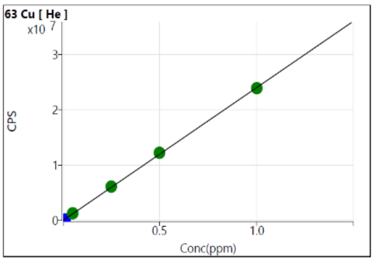

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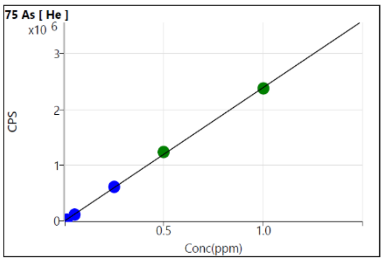

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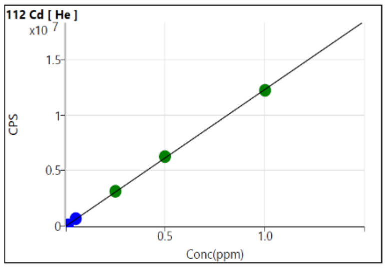

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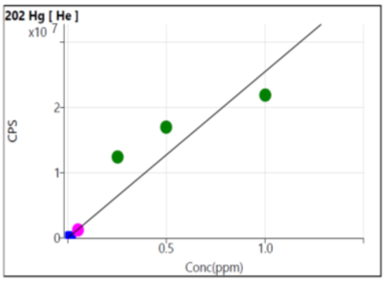

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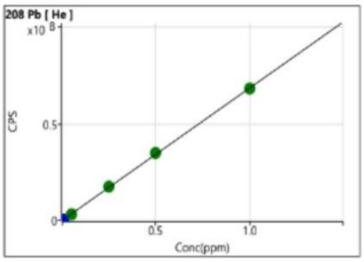

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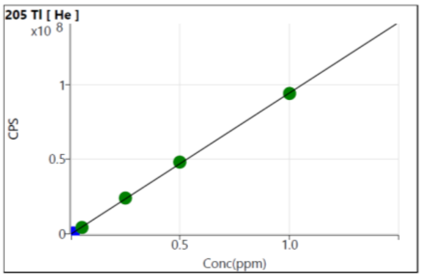

y=94256562.5361*x+66.7400

Fig. 4. Calibration curves for Pb, Cd, As Cu, Hg and TI in phytotherapeutic plant *Plectranthus caninus* L.

3.3 Discussion:

The World Health Organization (WHO) and Food and Drug Administration (FDA) states that the maximum permissible values are 0.3 mg/kg dry weight (d.w.) for Cadmium (Cd) 1 mg/kg d.w. for arsenic (As), and 10 mg/kg d.w. for Lead (Pb) in medicinal herbs (Stanojkovic-sebic *et al.*, 2015, Asgari Lajauer ,2017, Maria Gavrilescu, 2020).

Heavy metal analysis shows the high amount of Lead (Pb) 2.36 mg/kg is identified in *Plactranthus caninus* L. 1.80 mg/kg is in *Clematis zeylanica* L. (Pori.) 0.87 mg/kg is in *Aristolochia indica* L. 0.87 is in *Biden pilosa* L. and 0.38 mg/kg is in *Corallocarpus epigaeus* (Rottl. and Willd.) Hook.f. According to the WHO, the maximum permissible concentration of Lead (Pb) is in herbal medicine and product is 10 mg/Kg dry weight (WHO, 2007). Thus, all the samples are found to have Lead (Pb) concentrations below the acceptable limit.

Heavy metal analysis shows the high amount of Cadmium (Cd) 0.143 mg/kg is identified in *Plactranthus caninus* L., 0.097 mg/kg is in *Corallocarpus epigaeus* (Rottl. and Willd.) Hook.f. 0.038 mg/Kg is in *Clematis zylanica* L. (Pori.) 0.035 mg/kg is in *Aristolochia indica* L. and 0.025 mg/kg is in *Biden pilosa* L. According to the WHO, the maximum permissible concentration of Cadmium (Cd) in plants is 0.3 mg/Kg dry weight (WHO, 2007). Thus, all the samples are found to have Cadmium (Cd) concentrations below the acceptable limit.

The high amount of Arsenic (As) 0.11 mg/kg is found in *Plactranthus caninus* L. 0.039 mg/kg is in *Biden pilosa* L. 0.032 mg/kg is in *Clematis zylanica* L. (Pori.) and 0.025 mg/kg is in *Crollacorpus epigaeus (*Rottl. and Willd) Hook.f. According to the WHO, the maximum permissible concentration of

Arsenic (As) in fresh plant product in Malaysia is 5 mg/Kg and in China 2 ppm. Thus all the samples were found to have Arsenic (As) concentration below the acceptable limit.

The high amount of Copper (Cu) 19.01 mg/kg is found in *Clematis zylanica* L. (Pori.). 15.55 mg/kg is in *Biden pilosa* L. 13.44 mg/kg is in *Plactranthus caninus* L. 9.39mg/kg is in *Aristolochia indica* L. and 6.26 mg/kg is in *Crollacorpus epigaeus (*Rottl. and Willd) Hook.f. According to the WHO, the maximum permissible concentration of Copper (Cu) in finished herbal products at Singapore is 150 mg/Kg. All the samples are found to have Copper (Cu) concentration below the acceptable limit. Mercury (Hg) and Thalium (TI) were below the limit of detection from five selected medicinal plants.

4. CONCLUSION

In all the five selected medicinal plants have heavy metals such as Cadmium (Cd), Lead (Pb), Copper (Cu), Arsenic (As), but the concentration of heavy metal is lower than the accepted limit according to the WHO guidelines. Heavy metal like Mercury (Hg) and Thalium (Tl) concentrations were below the limit of detection.

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