Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2014, 5(2), 223-233

Analysis of the Mercury in commonly used Medicinal Plants

Research Article

Meenakshi N[1*], Sarath Babu B[2], Pavan Kumar S[3]

1. M.Tech Scholar, 2. Asst. Professor, Department of Chemical Engineering,
Sri Venkateshwara University College of Engineering, Tirupati.
3. Lecturer, Department of Dravyaguna, SV Ayurvedic College, Tirupati.

Abstract

Medicinal plants are used in various herbal products as food supplements and food additive. The requirement of medicinal plants is tremendously increasing in the global market. The presence of variousl heavy metals such as Arsenic, Mercury, Lead, Cadmium, Chromium, Nickle,silver, Atimony,Copper etc in herbal formulations result in several adverse effects. The present study was done to determine the presence of Mercury in some of the selected medicinal plants namely *Hemidesmus indicus* (L.) R.Br. *(Sariba), Cyperus rotundus* L. *(Musta), Glycyrrhiza glabra* L. *(Yashtimadhu), Rubia cordifolia* L. *(Manjishta), Eclipta alba* Hassk *(Bhringaraj), Hedychium spicatum* Ham.ex Smith *(Karchura), Emblica officinalis* Gaertn. *(Amalaki) and Acacia concinna* (Willd.) DC. *(Shikakai),* which were procured from local market of Chennai, Tirupati and Hyderabad. The samples were digested by Wet digestion method and analysed by UV-Vis Spectrophotometer. The results were compared with permissible limits recommended by WHO. Mean levels were evaluated with respect to their procurement. It was found that the analyzed plant species contained safe levels of the heavy metals concentration excepting Sariba Tirupati sample, Yastimadhu Chennai sample and Manjishta Hyderabad sample. There was a considerable variation of heavy metal concentration for the examined medicinal plant species. This may be due to the difference in physiological properties of plant uptake.

Key words: Mercury, herbal drugs, UV Spectrophotometer, heavy metal concentration

Introduction

According to the world health organisation(WHO), traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercises, applied singularly or in combination to treat, diagnose, and prevent illnesses or to maintain well-being. If the material being used is of plant origin, then it is called traditional herbal medicine. Plant derived drugs were classified for the treatment and evaluation based on their therapeutic action from the ancient time itself.

These Medicinal plants have different chemical compositions due to influence of climatic conditions, nature and properties of soil, fertilizer, pesticide, geographical distribution, age of the plant, source of collection, altitude, period of harvesting, manufacturing practices etc(1).

Medicinal plants may be easily contaminated by absorbing heavy metals from soil, water and air. Usually soil is subjected to contamination through atmospheric deposition of heavy metals from point sources including metalliferous

*Corresponding Author:
Meenakshi N
M.Tech Scholar,
Dept of Chemical Engineering,
SV University College of Engineering
Tirupati
E-mail: meena1114@gmail.com

mining, smelting and different industrial activities. Some other sources of soil contamination involve use of fertilizers, pesticides, sewage sludge and organic manures (Singh *et al*., 1997). (3,4,5,6). Additional sources of these elements for plants are rainfall, atmospheric dusts and plant protection agents, which could be absorbed through leaf blades (7).

The term heavy metal refers to any metallic chemical element that has a relatively high density(>5g/cm^3) or molecular weight (>60g/mol) and is toxic or poisonous even at very low concentrations. Some of the heavy metals are essential in very low concentrations for the survival of all forms of life. Heavy metals such as iron, chromium, copper, zinc, cobalt, manganese and nickel are called essential metals, because they play a significant role in biological systems; whereas mercury, lead, arsenic and cadmium are called nonessential metals, as they are toxic even at very low concentration. Various cases of human disease, disorders, malfunction and malformation (deformity) of organs due to heavy metal toxicity have been reported in the past few decades. Along with human beings, animals and plants are also affected by toxic levels of heavy metals (8, 17, 18).

Mercury is the only common metal which is liquid at ordinary temperatures. Mercury is also known as quicksilver. It is a heavy (Atomic weight = 80g/mol), silvery-white (d-block element) liquid metal. It is a poor conductor of heat when compared with other metals. However, it is a good conductor of electricity. It forms amalgams with many metals, such as gold, silver, and tin.

Mercury metal has many uses. Since it has high density, it is used in barometers and manometers (to calculate pressure). It is extensively used in thermometers because it has high coefficient of expansion. It can easily amalgamate with gold and hence it is used in the recovery of gold from its ores.

It has a number of unwanted effects on humans like Disruption of the nervous system, damage to brain functions, DNA damage and chromosomal damage, Allergic reactions resulting in skin rashes, tiredness and headaches, Negative reproductive effects, such as sperm damage, birth defects and miscarriages, tremors, vision changes, deafness, muscle in coordination and memory loss (9).

In this present work, UV-VIS spectrophotometer is used because most of the phenolic compounds, such as flavonoids, anthroquinones, coumarins, anthocyanins, and other compounds containing conjugated double bond (s) with chromophore (s) in herbs have strong UV-Vis absorption. The use of UV-VIS spectrophotometer in determination of heavy metals in medicinal samples is becoming popularin many laboratories because it provides for easy, economical, efficient, robust simple and rapid determination in low and high concentration at cheap cost (16).

Aims and Objectives

The present study is concerned with the assessment of Mercury [Hg] content in some of the selected medicinally plants namely

- ***Hemidesmus indicus (Sariba),***
- ***Cyperus rotundus (Musta),***
- ***Glycyrrhiza, glabra (Yashtimadhu),***
- ***Rubia cordifolia (Manjishta),***
- ***Eclipta alba Hassk (Bhringaraj),***
- ***Hedychium spicatum Ham.ex Smith (Karchura),***
- ***Emblica officinalis (Amalaki) and***
- ***Acacia concinna (Shikakai)***

were procured from local market of **Chennai, Tirupati and Hyderabad** respectively.

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2014, 5(2), 223-233

Materials and Methods

Chemicals:

Sulphuric acid, hydrogen peroxide, nitric acid, deionised water, Mercury metal.

Apparatus:

- 1000 ml standard flask,
- 100 ml standard flask,
- 50 ml standard flask,
- Tissue papers,
- Whatman filter papers,
- Beakers,
- Hot plate,
- Electronic weighing machine,
- Pipette,
- Measuring jar

Preparation of Stock Solution

Mercury stock solution

Dissolve 1.0g of mercury metal in 20ml of conc. nitric acid by constantly stirring the volumetric flask. Dilute to 1 litre in a volumetric flask with deionised water.

$$Hg + 4HNO_3\,(Conc.) \rightarrow Hg(NO_3)_2 + 2NO_2 + 2H_2O$$

Mercury does not react with non-oxidizing acids but does react with concentrated nitric acid, HNO_3, or concentrated sulphuric acid, H_2SO_4, to form mercury (II) compounds together with nitrogen or sulphur oxides.

Mercury dissolves slowly in dilute nitric acid to form mercury(I) nitrate, mercurous nitrate, $Hg_2(NO_3)_2$.

Sample preparation

Sample preparation for analysis of Heavy metals in medicinal plants was done according Wet digestion method (AOAC 1995) for non volatile heavy metals. Wet digestion involves the destruction of organic matter through the use of both heat and acid.

Procedure

- Weigh accurately 1.0 g of dried sample and place in a beaker or digestion tube.
- Add 16 ml concentrated H_2SO_4 and place the beaker on hot plate and then temperature was gradually increased to 125^0C at which the sample was boiled for 1hour.
- Remove beaker and allow cooling.
- Add 4 ml H_2O_2 (30%) and digest at the same temperature. As the reaction finished another 4 ml H_2O_2 (30%) was added. The mixture was heated till the digestion is complete.
- After cooling, the content was filtered into 100 ml volumetric flask using Whatman filter paper No.41 and the solution was completed to the mark using deionized water.

Concentrated Sulphuric Acid is been used in this procedure. Hydrogen peroxide is also used to enhance reaction speed and complete digestion. Hot plates or digestion blocks are utilized to maintain temperatures of 80 to 125^0C. After digestion is complete and the sample is cooled and filtered into standard flask which is filled to volume and dilutions are made to meet analytical requirements.

Critical factors in wet digestion procedures include selection of the digestion vessel, temperature and its control, time, the digestion mixture, and final volume. Selection of a digestion vessel is dependent on the elements of interest and the heat source. Time and temperature are interrelated and are dependent on the digestion mixture.

Wet digestion procedures generally require greater analyst supervision and intervention than dry procedures.

The addition of H_2SO_4 is used to raise digestion temperature and H_2O_2, 30% are used to increase speed of reaction

and ensure complete digestion (Jones and Case, 1990).

Wet digestion is recommended for plant materials.

Instrumentation: Agilent Cary 60 UV-Vis spectrophotometer

The Agilent Cary 60 UV-Vis spectrophotometer is efficient, accurate and flexible, and is designed to meet both current and future measurement needs. The proven, robust design of the Cary 60 comprises a double beam, Czerny-Turner monochromator, 190–1100 nm wavelength range, 1.5 nm fixed spectral bandwidth, full spectrum Xenon pulse lamp single source with exceptionally long life, dual silicon diode detectors, quartz overcoated optics, scan rates up to 24, 000 nm/min, 80 data points/sec maximum measurement rate, non- measurement phase stepping wavelength drive, room light immunity, central control by PC with Microsoft® Windows® operating system. Supported by GLP software, optional 21 CFR Part 11 capable software, and dedicated instrument validation software which includes pharmacopeia test suites (10-15)

Agilent Cary 60 UV-Vis spectrophotometers are manufactured according to a quality management system certified to ISO 9001. The guaranteed specifications are listed in this document and are based on the 4 sigma statistical confidence level of the final acceptance tests performed at the factory.

Working principle UV-Vis spectrophotometer

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (A) versus wavelength.

The concentration of an analyte in solution can be determined by measuring the absorbance at a given wavelength and applying the Beer-Lambert Law, as follows:

$$A = \varepsilon bc$$

where ε is a constant of proportionality, called the molar absorbtivity. Absorbance is therefore directly proportional to the path length, b (cm), and the concentration, c (mol/L), of the absorbing species.

Observations and results

Sample is prepared using Wet Digestion method. Mercury was analysed at a maximum wavelength of 252 nm and at different conc. (0, 0.02, 0.04, 0.06, 0.08 and 0.1) and the corresponding absorbance was obtained. A graph is plotted between concentration and absorbance is called Calibration Curve. Based on this graph, the concentration of Mercury in various samples was identified.

S.No	Concentration	Absorbance
1	0	0
2	0.02	0.1754
3	0.04	0.444
4	0.06	0.459
5	0.08	0.5865
6	0.1	0.7089

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2014, 5(2), 223-233

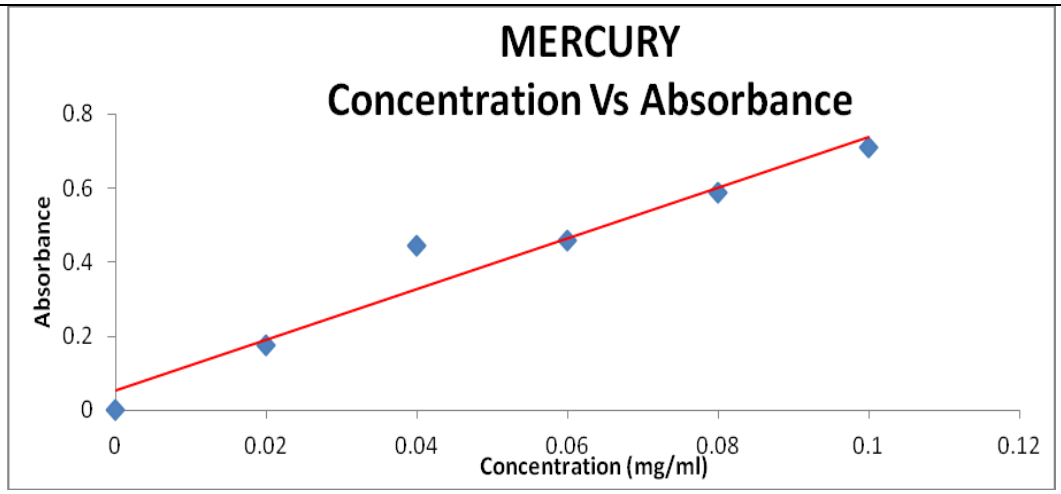

Fig 24: Calibration curve of Mercury

Maximum Wavelength	252 nm
Calibration equation	Abs = 6.80938 * Conc. +0.05604
Correlation Coefficient	0.94958

MERCURY					
Name of the sample	Sample taken from	Absorbance	Concentration (mg/ml)	Concentration (ppm)	Concentration (mg/kg)
SARIBA	Chennai	0.056	0.002	2	1.3783
	Tirupati	0.0385	0	0	0
	Hyderabad	0.0407	0	0	0
MUSTA	Chennai	0.0386	0	0	0
	Tirupati	0.0385	0	0	0
	Hyderabad	0.0383	0.001	1	0.6678
YASTIMADHU	Chennai	0.0488	0.002	2	1.3412
	Tirupati	0.0446	0.001	1	0.7371
	Hyderabad	0.0373	0	0	0
KARCHURA	Chennai	0.0365	0	0	0
	Tirupati	0.0409	0	0	0
	Hyderabad	0.0471	0.001	1	0.5892
MANJISHTA	Chennai	0.0346	0	0	0
	Tirupati	0.0396	0	0	0
	Hyderabad	0.0522	0.002	2	1.2232
BHRINGARAJ	Chennai	0.035	0	0	0
	Tirupati	0.0473	0.001	1	0.9938
	Hyderabad	0.0407	0	0	0
AMLA	Chennai	0.0127	0	0	0.0000
	Tirupati	0.0401	0	0	0.0000
	Hyderabad	0.032	0	0	0.0000
SHIKAKAI	Chennai	0.0524	0.001	1	0.6173
	Tirupati	0.0315	0	0	0.0000
	Hyderabad	0.0313	0	0	0.0000

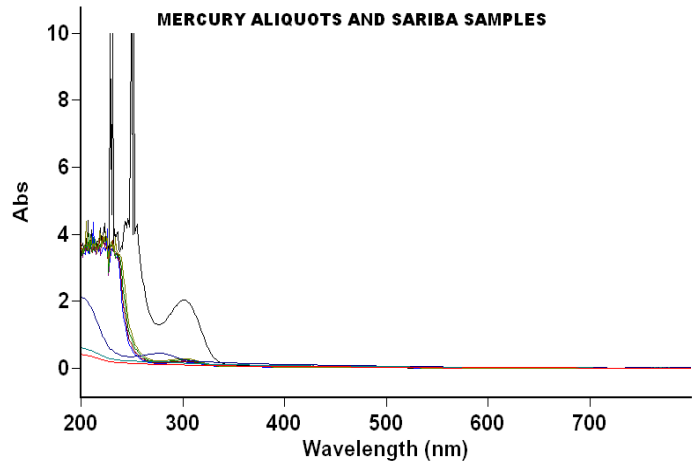

Fig 25: Spectrophotometric analysis of Mercury and Sariba samples

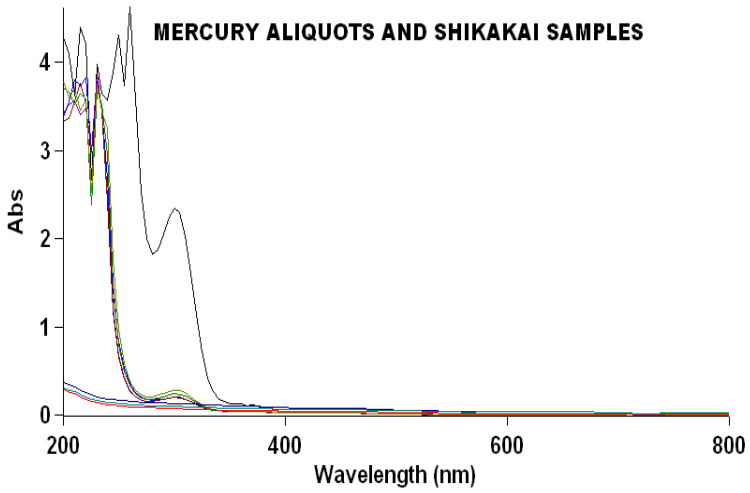

Fig 26: Spectrophotometric analysis of Mercury and Shikakai samples

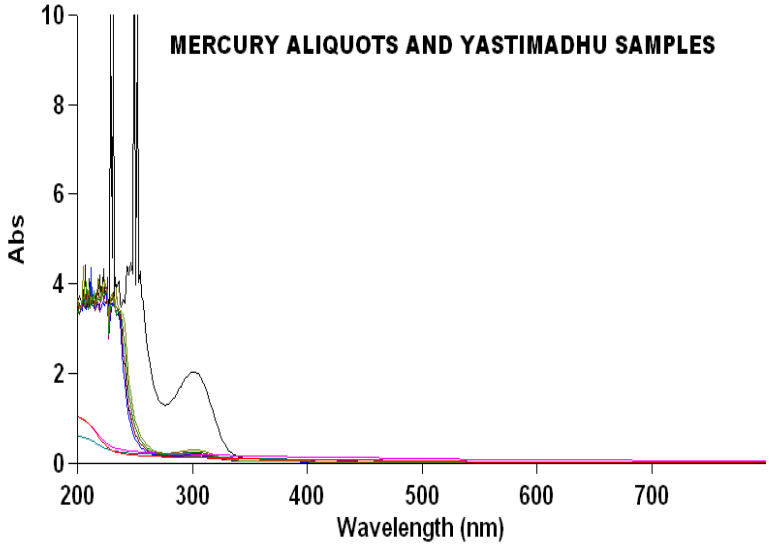

Fig 27: Spectrophotometric analysis of Mercury and Yastimadhu samples

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2014, 5(2), 223-233

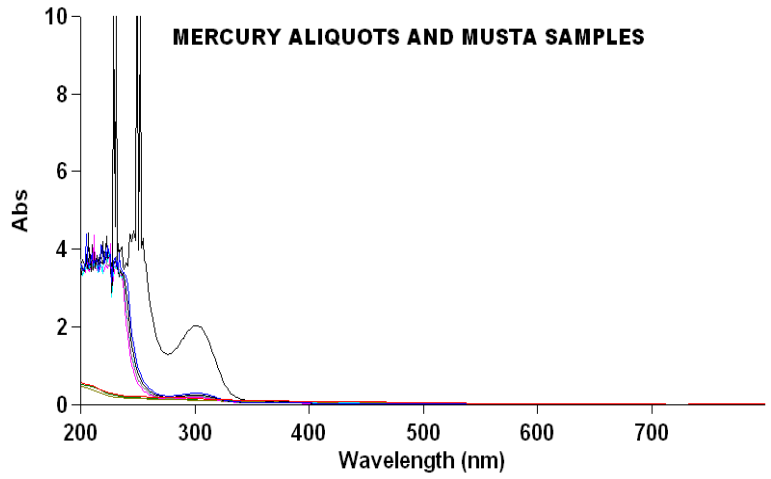

Fig 28: Spectrophotometric analysis of Mercury and Musta samples

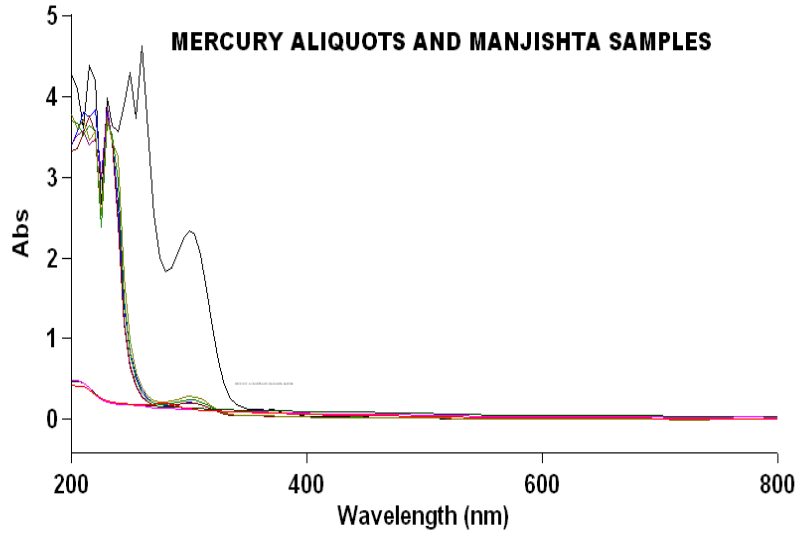

Fig 29: Spectrophotometric analysis of Mercury and Manjishta samples

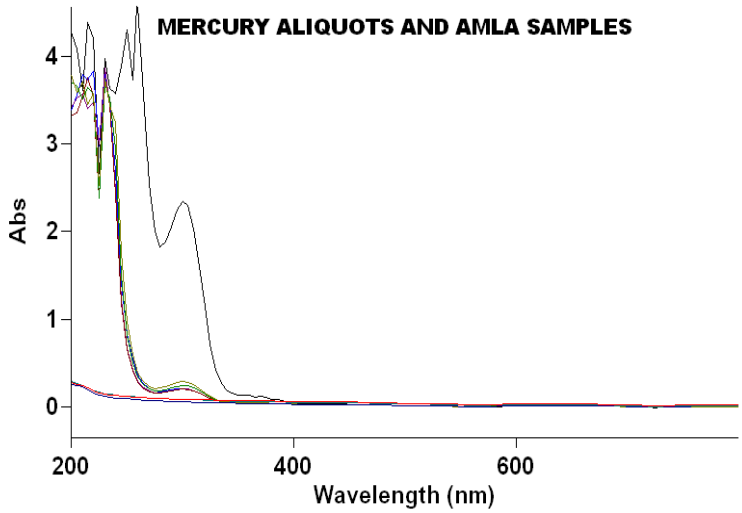

Fig 30: Spectrophotometric analysis of Mercury and Amalaki samples

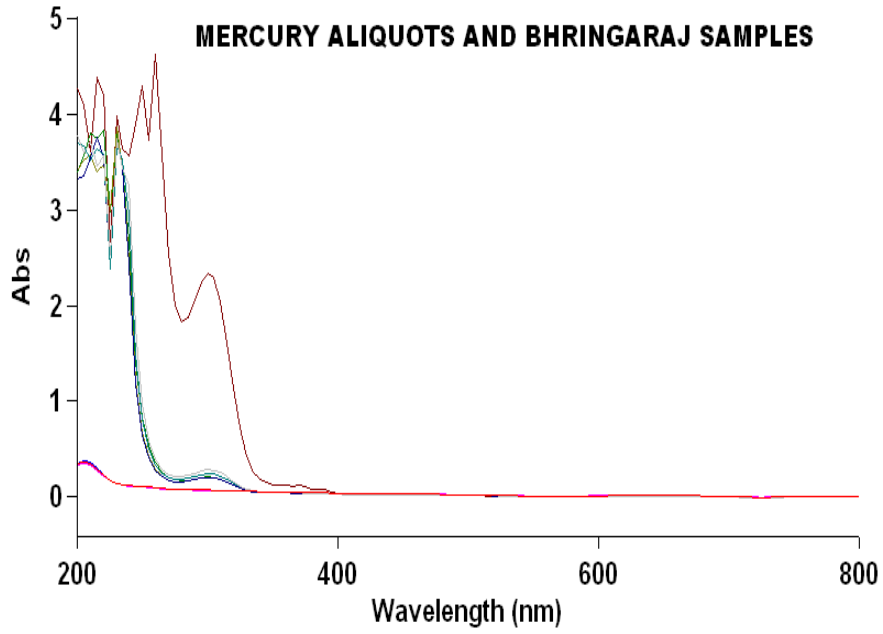

Fig 31: Spectrophotometric analysis of Mercury and Bhringaraj samples

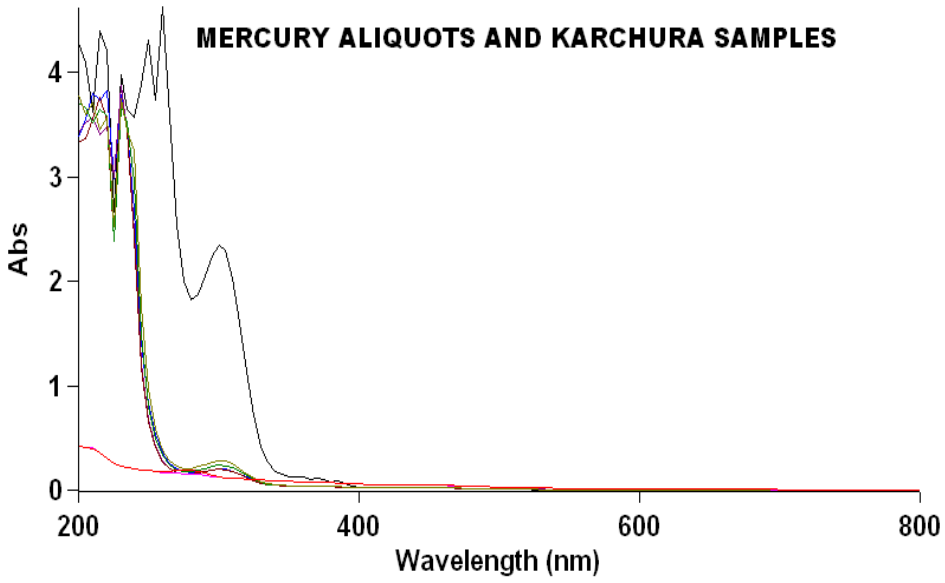

Fig 32: Spectrophotometric analysis of Mercury and Karchura samples

Metal	Mean	Standard deviation (SD)	Standard error of mean (SEM)	T value	P value	Significance
Mercury	0.46	0.72	0.15	3.6802	0.0012	Significant
Number of sample (N) = 24; Degree of Freedom (df= N-1) = 23 Hypothetical mean = 1						

Results and Discussion

From the Observation it was found that, the conc. of mercury in the Sariba Chennai sample is 2 ppm (1.3783 mg/Kg) whereas Sariba Tirupati sample and Hyderabad sample did not show the trace of mercury.

In the Musta Chennai sample and Tirupati sample, mercury was not found but in Musta Hyderabad sample the conc. of mercury was found to be 1 ppm (0.6678 mg/Kg).

In the Yastimadhu samples, Chennai sample has 2 ppm (1.3412 mg/Kg) conc. of mercury, Tirupati sample has 1 ppm (0.7371 mg/Kg) conc. of mercury and Hyderabad sample has no trace of mercury.

Out of the three samples of Karchura, Chennai sample and Tirupati sample did not contain traces of mercury whereas the Hyderabad sample showed 1 ppm (0.5892 mg/Kg) conc. of mercury.

In the Manjishta samples, Tirupati sample and Hyderabad sample did not show any trace of mercury. However, Hyderabad sample showed 2 ppm (1.2232 mg/Kg) conc. of mercury.

Tirupati sample and Hyderabad sample of Bhringaraj did not show any trace of mercury, whereas Tirupati sample contained 1 ppm (0.9938 mg/kg) conc. of mercury.

Amalaki samples did not show any traces of Hg. In the Shikakai samples, samples 2 and 3 did not show the traces of Hg whereas Chennai sample contained 1 ppm (0.6173 mg/kg) of Hg.

The results of the present analysis showed that the levels of Mercury in all samples were 0-2 ppm (0-1.3783 mg/Kg) with a mean of 0.46 ppm, which is much lower than the acceptable limit (1 ppm) recommended by World Health Organization (WHO). It was observed that most of the samples have not shown any traces of the mercury. Only three samples i.e., Sariba Tirupati sample, Yastimadhu Chennai sample and Manjishta Hyderabad sample contain mercury in the conc. of 2 ppm. Musta Hyderabad sample, Yastimadhu Tirupati sample, Karchura Hyderabad sample, Bhringaraj Tirupati sample and Shikakai Chennai sample has the 1 ppm conc. of mercury.

Results reveal that the contents of Mercury in some samples like Sariba Tirupati sample, Yastimadhu Chennai sample and Manjishta Hyderabad sample are slightly higher than the acceptable safe limit for the body. The elevated level of Hg may lead to the mercury toxicity and potential health hazards for the consumers. No samples of Musta, Karchura, Bhringaraj, Amla and Shikakai contain mercury above allowable limit recommended by WHO.

Conclusion

From the above study it can be concluded that the analyzed plant species contained safe levels of the heavy metals concentration excepting Sariba Tirupati sample, Yastimadhu Chennai sample and Manjishta Hyderabad. There was a considerable variation of heavy metal concentration for the examined medicinal plant species collected from three local markets of Chennai, Tirupati and Hyderabad. This may be due to the difference in physiological properties of plant uptake.

It is therefore suggested that awareness of this phenomenon should be disseminated to prevent collecting

medicinal herbs from non- cultivated, polluted areas and other sources, which are prone to heavy metal pollution. The analysis of heavy metals is highly essential for raw drugs used for the preparation of compound formulations.

The periodic assessment is essential for quality assurance and safer use of herbal drugs.

References

1. Willow J.H.LIU. Traditional Herbal Medicines Research Methods: Identification, Analysis, Bioassay and pharmaceutical and clinical studies.
2. Singh, RP. Tripathi, RD. Sinha, S.K. Maheshwari, R.and. Srivastava, H.S. 1997. Response of higher plants to lead contaminated environment. Chemosphere. 34:2467-2493.AOAC (1995). *Official methods of analysis of AOAC International (*16th ed.).
3. Jones, J.B., Case, V.W., 1990. Sampling, handling and analyzing plant tissue samples. In: Westerman, R.L. (Ed.), Soil Testing and Plant Analysis. Third ed., Soil Science Society of America, Book Series No. 3, Madison, Wisconsin, pp. 389–427.
4. A.Sathiavelu et al; "Evaluation of heavy metals in medicinal plants growing in Vellore District", European Journal of Experimental Biology, 2, 5, 2012, 1457-1461
5. Bempah et.al, "Heavy Metals Contamination In Herbal Plants From Some Ghanaian Markets", Journal of Microbiology, Biotechnology and Food Sciences, 2012/13 : 2 3 886-896
6. Divrikli U, Horzum N, Soylak M and Elci L, *Trace heavy metal contents of some spices, Int. J. Ayur. Pharma Research, 2014; 2(1): 77-83* ISSN: 2322 – 09103 *and herbal plants from western Anatolia, Turkey*. International Journal of Food Science & Technology, 2006. 41(6): 712-716.
7. Khan, I. A., Allgood, J., Walker, L. A., Abourashed, E. A., Schelenk, D., & Benson, W. H. (2001). Determination of heavy metals and pesticides in ginseng products. *Journal of AOAC International, 84*, 936–939.
8. Kirmani et al; "Determination of some toxic and essential trace metals in some medicinal and edible plants of karachi city", journal of basic and applied sciences vol. 7, no. 2, 2011, 89-95
9. Lim et al; "Total Silica Analysis Using a Double Beam Atomic Absorption Spectrophotometer", 24-29, April 2005
10. Okoye et al; "Simultaneous ultraviolet-visible UV–VIS Spectrophotometric quantitative determination of Pb, Hg, Cd, As and Ni ions in aqueous solutions using cyaniding as a chromogenic reagent", International Journal of Physical Sciences
11. Bukhari et. al; Determination of trace heavy metals in different varieties of vegetables and fruits available in local market of Shorkot Pakistan", International Journal of Current Pharmaceutical Research, Vol 5, Issue 2, 2013, Vol. 83, pp. 98-102, 23 January, 2013
12. Ranjan et al; "Comparative analysis for metal binding capacity of cysteine by using UV-VIS spectrophotometer", International journal of applied biology and pharmaceutical technology, Volume-3, Issue-2, April-June 2012.
13. Rao et.al. "Detection of toxic heavy

metals and pesticide residue in herbal plants which are commonly used in the herbal formulations", environ monit assess, doi 10.1007/s10661-010-1828-2, 2011 181: 267–271

14. Subramanian R, Gayathri S, Rathnavel C and Raj V, *Analysis of mineral and heavy metals in some medicinal plants collected from local market.* Asian Pacific Journal of Tropical Biomedicine, 2012, 2(1), 74-78.
15. Tatjana et al; "Concentration of Heavy Metals in Medicinal Plants in Serbia - Potential Health Risk".
16. Soomro MT, et al, Quantitative assessment of metals in local brands of tea in Pakistan, Pak J Biol Sci. 2008 Jan 15; 11(2):285-9.
17. Jitender K Malik *et al;* "Heavy Metals In Herbal Preparations - A Review", *International Journal of Drug Research and Technology* 2012, Vol. 2 (6), 430-439
18. Moses et al; "Profile of Heavy Metals in Selected Medicinal Plants Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya", Global Journal of Pharmacology 6 (3): 245-251, 2012

*****