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METAL DETERMINATION FROM MEDICINAL HERBAL PRODUCT OF SARPGANDHA BY AAS (ATOMIC ABSORPTION SPECTROPHOTOMETRY)

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ABSTRACT

Sarpagandha is one of the most important medicinal plant in Ayurvedic medicine. Over 30 alkaloids[1] have been isolated. A synthetic derivative Reserpine, was marked for many years as an effective medicine for blood pressure (Hyper tension) the roots of this plant are used in Ayurvedic medicines for the treatment of Hypertension, Insomnia, and Insanity, acute stomach ache and painful delivery. It is used in snake-bite, insect stings, and mental disorders. Having multiple influences on environment, biodiversity, rural economy, health and culture. Apart from traditional use in health care and culture, it has been increasingly used in pharmaceutical industries in the country as well as abroad.[2] Therefore this plant received international attention worldwide. Due the use of this plant in the treatment of Insanity this is also known as Pagle ki booti [1]. Sarpagandha contain many Toxic, Essential, Trace metals. Minerals, Alkaloids, Steroids and organic compounds, enzymes, proteins. For the present study Sarpagandha tablet was scanned and detection of the heavy metals like Cd (Cadmium), Cr (chromium), Cu(Copper), Fe(Iron), Mn (manganese) etc. is carried out, these metals are evaluated quantitatively by using AAS (Atomic Absorption Spectrometry), which should be incorporated in routine quality control parameters.

KEYWORDS: Sarpgandha, pagali ki booti, AAS, Alkaloids, herbal tablets, elemental analysis, validation, Herbal quality controlled.

INTRODUCTION

Ayurveda, Siddha, Unani and tribal medicines are the major ancient systems of medicines. Ayurveda is based on three essential elements called Tridosh like Vata, Pitta and Kapha. Theses includes seven basic entities (dhatu), which are plasma (rasa), blood (rakta), muscles (māmsa), fat (meda), bone (asthi), marrow (majja), and semen (shukra).[3,4] These are subject to wear and tear so that mala (excretory material) is formed from them. When tridosha, saptadhatu and mala are in balance with each other, it is called a healthy condition, while imbalance results in diseases. Plant-based treatments in Ayurveda derived from roots, leaves, flowers, fruits, bark, stems, and seeds. The origins of Ayurveda have been traced back to around 5,000 BC, found in Atharvaveda.[5] Nature has provided a complete store house of remedies to cure all ailment of mankind. In the past almost all the medicines used were from the plants. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. Quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with Ayurvedic and herbal products, which is neglected till this date. The traditional approach towards standardization and validation is insufficient for current herbal products and hence there is need for more advanced techniques for standardization and validation, using sensitive instrumental analysis. The World Health Organization (WHO) in 1999 and 2011 has given a detail guideline for the standardization of herbal products (WHO 2011).[7]

Ayurveda, an ancient science of life the well-known treatises in Ayurveda are Chakra Samhita and Sushrutha Samhita.

Sarpagandhaa mitigates kapha and vaata. It induces sleep (niddrapradaa) slows heart (hardvasaadinee), suppresses libido (kaamaavasaadinee), is beneficial in colic pain (chaiv), fever (jvara) and parasitic infestation (krimi).[1] Sushruta Uttara, 60:47. Sarpagandha is a species of flowering plant in the family Apocynaceae. It is nativeto the Indian subcontinent and East Asia. Root, bark is the richer in alkaloids .The alkaloids content varies depending on the local, season, and plant age at the time of harvesting of roots.

Different names of Sarpagandha

Common English names: Devil-pepper, Snake-root,

Sanskrit: Sarpagandhaa (Ayurveda).

Scientific Name: Rauvolfia serpentina (L.) Benth. Ex Kurz.

Common Name: Serpentine, Serpentine root, Rauwolfia.

Trade name: Rauwolfia, Sarpagandha.

Bengali: Sarpagandha.

Hindi: Sarpagandha.

English: Serpentina.

Indian Trade Name: Sarpagandha.

Family: Apocynaceae.

Genus: Rauvolfia.

Species : R. serpentine.

Binomial name: Rauvolfia serpentin.

Urdu: Asrel.

Indonesia: Pule Pandak[5]

Medicinal qualities of Sarpagandha

Rasa (Taste) – Tikta (Bitter)

Guna (qualities) – Rooksha – dryness

Vipaka- Katu – Undergoes pungent taste conversion after digestion

Veerya- Ushna – Hot potency

Effect on Tridosha – Balances Vata and Kapha Doshas.

Prabhava – Nidrajanaka – induces sleep.

Part used- Roots with bark, roots and leaves

Sarpagandha chemical constitution

Ajmalicidine, Ajmalicine, Rouhimbine, Indobinine, Reserpiline, Reserpine, Sarpagine, Serpentine, Serpentinine, Yohimbine, Ajmalimine, Ajmaline, Rauwolfinine (Perakenine), Sandwicolidine, Serpinine etc.

Table: 1 Tablet name with the company name and plants as per label.

Sr.No	Brand and Company Name	Medicines Name	Plants as per label *
1	Baidyanath (Mfg. Lic .No- ND/AYU/4)	Sarpagandha	Sarpagandha
2	Unjha (Mfg. Lic .No- GA/435A)	Sarpagandha	Sarpagandha, Pimpri mul, Khurasi ajmo, Jatamasi
3	Zandu (Mfg. Lic .No- GA/80)	Sarpagandha	Sarpagandha powder
4	Local powder	Sarpagandha	Sarpagandha powder
5	Local powder	Sarpagandha	Sarpagandha powder

Figure: 1 Sarpagandha root

Figure: 2 Sarpagandha plant

MATERIALS AND METHOD

Chemicals

1) Double Deionised water used for all dilution.

2) Hydrogen peroxide (H_2O_2) AR (100 Volume, SDFSL.M.W. 34.04).

3) Concentrated Sulphuric Acid (H_2SO_4) (98%).

4) Acetone.

5) Paraffin.

Sampling

In the present study, the marketed herbal tablets of Sarpagandha from different brands are selected for the analysis. The brand names of products, license number and content as per company's label are included in table 1.

Experimental design

Code numbers namely Sp1, Sp2, Sp3, Sp4, Sp5 was assigned for Sarpagandha Tablet of five different brands. By taking the weight of each tablet Sarpagandha on digital balance, each tablet sample was gently ground to fine powder using mortar and pestle and packed in butter paper until the analysis. To determine the concentration and validation of heavy metals, a wet digestion of the powder sample was done according to the new method developed. Table number 2 shows the weight of Sarpagandha samples taken for analysis. The each sample was placed separately in 100 mL round bottom flask and 3 mL concentrated sulphuric acid was

added. The mixture was allowed to stand for 30 minutes at room temperature. After 30 minutes about 4 mL of 30% hydrogen peroxide was added to the round bottom flask and allowed to cool at room temperature. The sample was then refluxed at 190^{o}C for 40 minutes. The sample was cooled down to room temperature. 2 mL of 30% hydrogen peroxide was added and the solution heated once again until the digest was clear upon cooling. it was filtered through Whatman No. 42 filter paper and transferred quantitatively to a 25 mL volumetric flask by adding distilled water. The concentration of Cu, Cr, Mn, Fe, and Cd in the final solution were determined by using Atomic Absorption Spectrophotometer (AAS). Same method was applied for other Sarpagandha samples Sp2, Sp3, Sp4, and Sp5, by Model AA7000F ROM version 1.012. The General analytical conditions are given in table number 5.

Table 2- Sample weight and dilution

Sr. No	Sample	Weight (g)	Dilution
1	Sarpagandha [Sp1]	0.375	25 mL in conc H_2SO_4
2	Sarpagandha [Sp2]	0.256	25 mL in conc H_2SO_4
3	Sarpagandha [Sp3]	0.392	25 mL in conc H_2SO_4
4	Sarpagandha [Sp4]	0.336	25 mL in conc H_2SO_4
5	Sarpagandha [Sp5]	0.346	25 mL in conc H_2SO_4

Table 3- Accuracy of elements in ppm and percentage by weight

Sr. No	Elements	Concentration	Sarpgandha Samples				
			Sp1	Sp2	Sp3	Sp4	Sp5
1	Copper	Ppm	0.0433	0.1216	0.0361	0.0381	0.0399
		% by Weight	0.00000433	0.00001216	0.00000361	0.00000381	0.00000399
2	Iron	Ppm	1.963	1.553	0.5074	1.7588	1.8111
		% by Weight	0.0001963	0.0001553	0.00005074	0.00017588	0.00018111

Table 4-Accuracy of Elements in ppb

Sr. No	Elements	Concentration	Samples				
			Sp1	Sp2	Sp3	Sp4	Sp5
1	Cadmium	ppb	45.0	44.7	44.3	40.32	41.52
		% by Weight	0.0000045	0.00000447	0.00000443	0.000004032	0.000004152
2	Chromium	ppb	381.5	405.3	410.0	341.82	351.99
		% by Weight	0.00003815	0.00004053	0.000041	0.000034182	0.000035199
3	Manganese	ppb	113.9	142.3	159.5	102.5	105.09
		% by Weight	0.00001139	0.00001423	0.00001595	0.00001025	0.000010509

Table 5- Optical parameters / General analytical conditions

Sr. No	Parameters	Elements				
		Cu	Cd	Cr	Fe	Mn
1	Socket #	6	3	5	4	2
2	Lamp Current Low(Peak)(mA)	8	8	10	12	10
3	Wavelength(nm)	324.8	228.8	357.9	248.3	279.5
4	Slit Width(nm)	0.7	0.7	0.7	0.2	0.2
5	Lamp Mode	BGC-D2	BGC-D2	BGC-D2	BGC-D2	BGC-D2
6	Fuel Gas Flow Rate(L/min)	1.8	1.8	2.8	2.2	2.0
7	Support Gas Flow Rate (L/min)	15.0	15.0	15	15.0	15.0
8	Flame Type	Air-C2H2	Air-C2H2	Air-C2H2	Air-C2H2	Air-C2H2

Table 6- LD 50 of the elements (The Merck Index, 1989)[6]

Sr. No	Elements	Compounds	LD50
1	Cadmium	Cadmium chloride	88 mg/kg orally in rat
2	Chromium	Chromium carbonyl	100 mg/kg iv in mice
3	Copper	Cupric acetate	0.71 g/kg orally in rat
4	Iron	Iron pentacarbonyl	02.19 mg/l in mice 0.91 mg/l in rat
5	Manganese	Manganese dioxide Manganese chloride	45 mg/kg in rabbit 180-250 mg/kg in mice

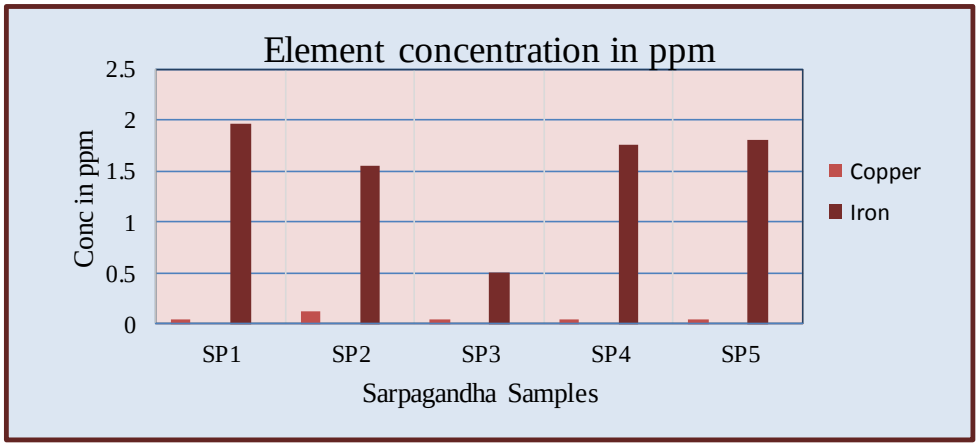

Fig: 3. Graphical representation of Elements of each sample in ppm

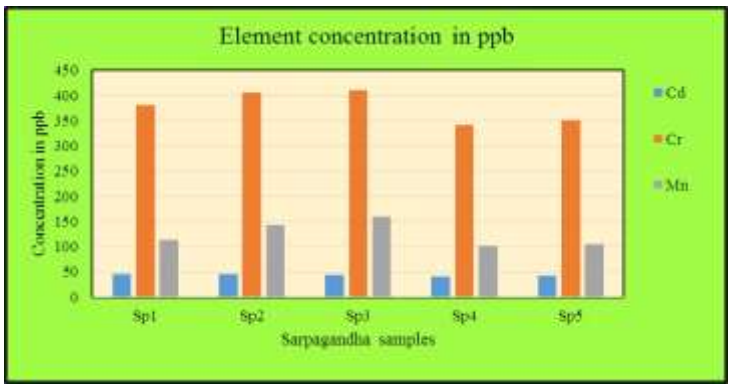

Fig: 4. Graphical representation of Elements concentration of each sample in ppb

RESULT AND DISCUSSION

The detected accuracy of Iron (Fe), copper (Cu), Cadmium (Cd), Chromium(Cr), and Manganese(Mn) elemental concentration in selected sample by AAS is given in Table 3 and Table number 4. Recently published FDA regulation hold supplement manufacturers or distributers responsible for the content of the dietary supplements which should only contain what they are labelled and not any harmful or undesirable substances, including pesticides and heavy metals.

Figure-3. indicate the graphical representation of each elements per sample X- axis indicate the sample code Sp1 to Sp5 and Y-axis indicate the detected concentration of copper and Iron .As shown in figure sample Sp1, Sp2, Sp3, Sp4 and Sp5 detected the highest concentration value of Iron (Fe) 1.9630 ppm, 1.5530 ppm, 0.5074 ppm, 1.7588 ppm and 1.8111 ppm respectively. And lowest concentration of copper was found in all samples.

Sp1 Sample: In Sp1 Sample of Sarpagandha most abundant element was iron (Fe) 1.9630 ppm, Whereas Copper (Cu) was found in lowest concentration 0.0433 ppm.

Sp2 Sample: In Sp2 Sample of Sarpagandha most abundant element was iron (Fe) 1.5530 ppm, Whereas Copper (Cu) was found in lowest concentration 0.1216 ppm.

Sp3 Sample: In Sp3 Sample of Sarpagandha most abundant element was iron (Fe) 0.5074 ppm, Whereas Copper (Cu) was found in lowest concentration 0.0361 ppm.

Sp4 Sample: In Sp4 Sample of Sarpagandha most abundant element was iron (Fe) 1.7588 ppm, Whereas Copper (Cu) was found in lowest concentration 0.0381 ppm.

Sp5 Sample: In Sp5 Sample of Sarpagandha most abundant element was iron (Fe) 1.8111 ppm, Whereas Copper (Cu) was found in lowest concentration 0.0399 ppm.

Figure-4 indicate the graphical representations of each element per sample in ppb X axis indicate the sample code Sp1 to Sp5 and Y-axis indicate the detected concentration of Cadmium, Chromium and Manganese. As shown in figure sample Sp1, Sp2, Sp3, Sp4 and Sp5 detected the highest concentration value of Chromium (Cr) 381.5 ppb, 405.3 ppb, 410.0 ppb, 341.82 ppb, 351.99 ppb respectively and Cadmium was found in lowest concentration.

Sp1 Sample: In Sp1 Sample of Sarpagandha most abundant element was Chromium (Cr) 381.5 ppb, Whereas Cadmium (Cd) was found in lowest concentration 45.0 ppb.
Sp2 Sample: In Sp1 Sample of Sarpagandha most abundant element was Chromium (Cr) 405.3 ppb, Whereas Cadmium (Cd) was found in lowest concentration 44.7 ppb.

Sp3 Sample: In Sp1 Sample of Sarpagandha most abundant element was Chromium (Cr) 410.0 ppb, Whereas Cadmium (Cd) was found in lowest concentration 44.3 ppb.

Sp4 Sample: In Sp1 Sample of Sarpagandha most abundant element was Chromium (Cr) 341.82 ppb, Whereas Cadmium (Cd) was found in lowest concentration 40.82 ppb.

Sp5 Sample: In Sp1 Sample of Sarpagandha most abundant element was Chromium (Cr) 351.99 ppb, Whereas Cadmium (Cd) was found in lowest concentration 41.52 ppb.

Table 6 shows the LD 50 of the elements (The Merck index)

HABITAT

Global

India, Srilanka, Malaysia, Burma, Bangaladesh, Andaman Islands, Indonesia (Tropical region of Asia), America, Africa, and Java.

National

Moist tropical hills of different states of India. Regional: Sub himalayna tract from Sirhind eastwards from Dehradun to Aasam- till 4000 feet ht abundant in darjeeling and Sikkim Tarai.

Biological role

Iron (Fe)-Iron plays an important role in living organism forming complex with molecular oxygen in haemoglobin and myoglobin and in varies biological processes.

Toxicity

High blood level of free ferrous iron react with peroxide to produced free radicals which are highly reactive and can damage DNA, Proteins, Lipids and other cellular compounds. Iron can damage cell in the heart, liver and other organs which can cause adverse effect includes coma, metabolic acidosis, shock, liver failure, long term organs damage and even death.

Toxicology

Most of the adverse effect of reserpine are due to its effect on the C.N.S. sedation, etc., inability to concentrate or perform complex tasks are the most common adverse effects, more serious in the occasional psychotic depression that can lead to suicide. Other side effects include nasal stuffiness and exacerbations of peptic ulcer disease which is uncommon with oral doses in small quantity.

Sarpagandha side effects

1It is contra indicated in – Bronchial asthma, duodenal ulcer, hyper acidity, renal disorder.

2It is not suitable to be used in case of depression.

3It is not ideal to use this in children and after delivery.

4It is not ideal to take this medicine along with Digoxin, Levodopa.

5Special care is needed when it is administered along with other medicines for high BP. The combined effect may lower the BP to a great extent.

6Its sleep enhancing effect may further increase if taken by an alcoholic. Hence, precaution is needed.[5]

Levels in humans (Fe)

Human abundance by weight is 60000 ppb by weight and 6700 atoms by atoms.

CONCLUSION

The above study shows that the amount of the elements present in Sarpagandha preparation is very much safe and below toxicity limit for human consumption, however Sarpagandha have few side effects. This shows that effective Ayurvedic ingredients are in micro quantities which actually in pictograms/ fento gram levels per kg body weight. Therefore Ayurvedic medicines are nontoxic.

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