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SCIENTIFIC VALIDATION OF LEAD IN 'LEAD CONTAINING PLANTS' IN SIDDHA BY ICP-MS METHOD

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ABSTRACT

Siddha system is one of the oldest medicinal systems of India. In Siddha medicine the use of metals and minerals are more predominant in comparison to other Indian traditional medicinal systems. A major portion of the Siddha medicines uses herbs and green leaved medicines. Ancient Siddha literature specifies medicinal plants containing various metal constituents. In the Classical Text of Siddha system of medicine Gunapadam Thathu Jeeva Vaguppu, a verse narrates 11 plants as 'Lead containing'. These plants are Seethai, Muthirukanchevi, Vellaisaarvelai, Pathukai, Vaeliparuthi, Musthaiyum, Surai, Seenthil, Vizhuthi, Sirupeelai and Vellarugu. This paper deals with the scientific validation of this verse by estimating Lead in the different anatomical parts of the mentioned plants using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The anatomical parts were selected as per the usage of the plants mentioned in the Classical texts of Siddha system of medicine. Three accessions of the plant materials were studied and the results of the analysis were compared and found that all the plant materials studied contain Lead in detectable range. The Lead level of every accession of each plant material varied from one another. The Lead content was varied from 0.36 ppm to 6.65 ppm in all the three accessions of plants studied. As per the guidelines of WHO and FDA, the permissible limit for Lead is <10 ppm. In this study Lead content was observed within the aforesaid permissible limit and thereby proving the level of safety for the consumption. By this study, the ancient claim of the presence of Lead in these medicinal plants is proved scientifically.

KEYWORDS: Lead content, Medicinal plants, Classical text, Siddha system, ICP-MS.

INTRODUCTION

Research on herbal drugs has invited global attention and the research for 'miracle cures' from the plant kingdom continues all over the world. Recent years have witnessed resurgence of interest in traditional medical systems and more and more people are turning to use herbal drugs and medicinal plant products. Siddha system is one of the oldest medicinal systems of India. In Siddha medicine, the use of metals and minerals are more predominant in comparison to other Indian traditional medicinal systems. A major portion of the Siddha medicines uses herbs and green leaved medicines. Ancient Siddha literature specifies medicinal plants containing various metal constituents. This paper deals with the scientific validation of a verse, which indicates and reflects some medicinal plants containing Lead, mentioned in the Classical Text of Siddha System of Medicine Gunapadam Thathu Jeeva Vaguppu (Thiyagarajan, 1981). The verse is as follows

"Seethai Muthirukanchevi Vellaisaarvelai
Pathukai Vaeliparuthi Musthaiyum – Kothilsurai
Seenthil Vizhuthi Sirupeelai Vellarugum
Enthizhaiyereyamooli"

The verse narrated in Tamil reflects the following plants.

1. *Alternanthera sessilis* (L.) R. Br., ex DC. (Seethai)

It is a small prostrate perennial herb belongs to the family Amaranthaceae. The species occurs throughout tropical and subtropical regions of the world. Its leaves and young shoots are eaten as vegetable and also used as traditional medicine. Leaves possess high antioxidant properties in general and hence it can be recommended to be included in our daily diet, as it will protect us from commonly occurring chronic diseases. (The Wealth of India, 1985). The vernacular names of the plant are Tamil – Seethai; Malayalam –Ponnankanni; Bengali – Sanchesak; Gujarati – Jalajambo; Hindi – Gudari; Kannada -Honagonne, Soppu; Marathi - Kanchari; Oriya - Matsagandha, SalinchaSaaga; Sanskrit -Matsyaksi, Matsyagandha; Telugu – Ponnaganti (SPI, 2008). The leaves are used in eye diseases, in cuts and wounds, antidote to snake bite and scorpion sting, in skin diseases and diabetes (Gupta, 2004). The plant contains hydrocarbons, ester, and sterols, such as stigmasterol, campesterol, β -sitosterol, α- & β-spinasterol, α-stigmasteanol and palmitates of sterol, 24-methylenecycloartanol, cycloeucalenol and saponins. 5α-stigmast-7-en-3-ol, lupeol, oeucalenol,oleanolic acid glycosides and robinetin-7-O-s-glucopyranoside (Sharma, 2002).

2. *Merremia emarginata* (Burm. f) Hall. f. (Muthirukanchevi)

Merremia emarginata (Burm. f) Hall.f. (Fam: Convolvulaceae) is a perennial, much branched creeper. The species frequently found on sandy banks of river and in fields with fertile soil. It mainly grows in rainy and winter season. It is found widely distributed all over India, especially in damp places in upper gangetic plain, Gujarat, Bihar, West Bengal, ascending up to 900 m in the hills, Goa and Karnataka in India, Ceylon and Tropical Africa (Kirtikar, 2005; Ghosh, 1984). The vernacular names of the plant are Tamil - Muthirukanchevi, Elikathukeerai; Malayalam - Elichevi; Marathi – Undirkani; Hindi – Musakani; Sanskrit - Mushakarni; Bengali - Bhuikamri, Indurkani; Telugu - Elika-jemudu (Wealth of India, 1988). The plant contains terpenes, steroids, polyphenols, glycosides, flavanoids, carbohydrates and proteins. It contains luteolin, caffeic acid, stigmasterol, betulin, (E)-3-(4-hydroxy-3-methoxyphenyl) acrylic acid, hentriacontane, tritriacontane and vanillin acid (Trease & Evans, 1989; Ross, 1980). The plant is useful for cough, headache, neuralgia, rheumatism, inflammation, troubles of nose, and fever due to enlargement of liver and also for treating cancer. Powder of leaves is used as a snuff during epileptic seizures, juice acts as purgative and the root is having diuretic and laxative activity. It is used in the disease of the eyes and gums. Anticancer activity of the plant against human cervical and breast carcinoma was reported (Prabhu, 2014; Eluomalai, 2011).

3. *Trianthema portulacastrum* Linn. (Vellaisaarvellai)

Trianthema portulacastrum L. belongs to the family Aizoaceae. It is an annual herb found in tropical and sub-tropical region and almost throughout India as a weed. Its infestation is very common in various agricultural and vegetable crops, such as mustard, maize, moong bean, potato, onion, cotton, pearl millet, and sugarcane, especially during the rainy seasons. It is rapidly growing, much branched, succulent, prostrate and annual terrestrial weed. Two forms of this plant is reported, a red coloured form in which the stem, leaf margin and flowers are red; and a green coloured form which has a green stem and white flowers. In India it is used as green leaf vegetable and is considered to be useful for the purpose of medicinal value (Kirtikar, 2005). The vernacular names of the plant are Tamil - Vellaisaarvelai, Saranai, Mukuruttai; Malayalam –Thazhuthama; Bengali – Saburi; English - Horse purselane; Marath - Sweta, Punarnava; Punjabi – Sanaya; Sanskrit – Sothaghni; Hindi - Pathar; Telungu – Galijeru; Kannada-SihiPunarnava (API, 2004). The plant contains trianthemine, punarnavin, ecdysterone, tetraterpenoid, β-cynin, saponins, trianthenol, 3-acetylaleuritolic acid, 5,2'-dihydroxy-7-methoxy-6,8-dimethylflavone, leptorumol, 3,4-dimethoxy cinnamic acid, 5-hydroxy-2-methoxybenzaldehyde, p-methoxybenzoic acid, and β-cyanin (Banerji, 1971; Poddar et al. 2020). The plant parts such as leaves, fruits and roots have various medicinal properties and are used as analgesic, antipyretic, anti-inflammatory and antibacterial. Pharmacological activities like hypoglycemic, hypolipidemic, analgesic, hepatoprotective, anthelmintic,

anticancer, diuretic, antioxidant activities and mosquito larvicidal activities have been reported for different extracts of the plant and served as alterative cure for bronchitis, heart disease, blood anaemia, inflammation, piles and ascites. The plant is used against throat troubles and as an anti-fungal agent. The root paste applied to the eye cures corneal ulcers, itching, dimness of sight and night blindness. In the Indian traditional medical systems, the plant is considered as a diuretic. The leaves possess diuretic properties. The fleshy nature of leaves makes them suitable for use as a wound-dressing. It is also used as vegetable in various parts of the world due to its high nutritional value (Ibn Baitar, 2000; Kirtikar, 2005; Prajapati, 2003).

4. *Coldenia procumbens* Linn. (Pathukai)

Coldenia procumbens L. (Fam: Boraginaceae) is an annual herb and common weed in India. It is found widely in south India on waste lands, common in dry rice grounds. The genus is having 24 species of prostate. *Coldenia procumbens* is only species of its genus has a place both in the Hortus Bengalensis and Moon's Catalogue of Ceylon plant. This plant is widely used in traditional medicines in India, Africa and Malaysia. (Krishnarao, 1955; The Wealth of India, 1950). The vernacular names of the plant (SPI, 2011) are Tamil-Patukai; English - Trailing coldenia; Gujarat – Khasadiya Okharad; Hindi - Tripunkhi; Kannada - Tripakshi; Malayalam - Cherupadi; Marathi - Tripunkhi; Odia - Gondrilota; Telugu - Hamsapadu, Chepputhatteku. The chemical constituents present in the plant are glycosides, phytosterols, proteins, amino acids, fixed oils, flavonoids, gums and mucilage as chief constituents. Saponins and fixed oils & fats are present. Wedelolactone is identified in the plant (Senthamari, 2002; Beena, 2011). In villages, the fresh leaves are ground and applied to rheumatic swelling. The whole plant used in external application of causing suppuration of boils. The leaves are also used to cure fever, piles and scorpion sting (Pullaiah, 2006; Krishnarao, 1955; Chopra, 1986).

5. *Pergularia daemia* (Forssk.) Chiov. (Vaeliparuthi)

Pergularia daemia Forsk. Chiov. (Fam: Asclepiadaceae) is a perennial twinning herb grows widely along the roadsides of India and also in the tropical and subtropical regions. It is a slender, hispid, fetid smelling laticiferous twiner found in the plains throughout the hot parts of India. The whole plant possess high medicinal value and traditionally used in treating various ailments for human beings (Cook, 1906). The vernacular names of the plant (API, 2008) are Tamil – Vaeliparuthi; Malayalam – Veliparuthi; Bengali – Chhagalbete; Gujarathi - Nagaladudhi, Amaradudheli; Marathi - Mendhadhdhi, Utarana; Odia – Utrali; Panjabi - Karial, Siali; Telungu - Dustuputige. The leaves of the plant contain flavonoids, alkaloids, terpenoids, tannins, steroids, cardenolides, triterpenes, saponins, steroidal compounds and carbohydrates. The seeds contain uzarigenin, coroglaucigenin, calactin, calotropin and a bitter resin (Sathish, 1998; Anjaneyulu, 1998). Extract of this plant is taken orally for gastric ulcers, uterine and menstrual complaints. The stem bark of this plant is a good remedy for cold, it is also used to treat malaria and the twig is used as an antipyretic and appetizer. Dried roots are used as an abortifacient, emetic, bronchitis and used for cough, asthma and constipation, while the fresh roots used to treat gonorrhea. The leaf latex is used as pain killer and as a relief for toothache (Hebbar, 2010). The leaves are useful in leprosy and haemorrhoids. The fresh, pulped leaves are applied as a poultice to relieve carbuncles. Leaf juice is used as an amenorrhea, catarrhal infections and dysmenorrheal, infantile diarrhea and also used to reduce body pain. In addition dried leaves are used as an antirheumatic, asthma, amenorrhea, dysmenorrheal, bronchitis, whooping cough, heals cuts and wounds and finally to facilitate parturition (Dutta 1947).

6. *Cyperus rotundus* Linn. (Musthaiyum)

Cyperus rotundus L. (Fam: Cyperaceae), is a colonial, perennial herb considered to have originated in India 2000 years ago. It is also known as purple nut sedge or nut grass or java grass, belongs to the sedge family Cyperaceae. It is a colonial, perennial herb, 7–40 cm tall with fibrous roots and reproduces largely by rhizome and tubers. Rhizomes may grow in any direction in the soil. Those growing upward produces shoot and roots. The rhizomes that are growing downwards or horizontally form individual tubers or chains of tubers. The upright culms or stems support a much-branched inflorescence with bisexual flowers with three stamens. (Govaerts, 2007). The vernacular names of the plants (API, 2001) are Tamil - Musthaiyum, Korai; Malayalam – Muthanga; Sanskrit - Mustaka, Virida; Assam - Mutha, SomadKoophee; Bengali - Mutha, Must; English - Nut Grass; Gujarati - Moth, Nagarmoth; Hindi - Motha, Nagarmotha; Kannada – KonnariGadde; Marathi - Moth, Nagarmoth, Motha, Bimbal; Punjabi –Mutha. The plant material contains alkaloids, flavonaids, tannins, starch, glycosides, furochromones, monoterpenes, sesquiterpens, α-sitosterol, glycerol, linolenic, myristic and stearic acid and essential oils. The major compounds isolated from essential oil are α-cyperone, cyperene, cyperotundone, cyperol, β-selinene, β-caryophyllene, valerenal, sugeonylacetate, α-copaene, patchoulene, transpinocarveol, patchoulenenone, aristro l9-en-3-one, selina-4,11-diene, aristrol-9-en-8-one, kobusone, sugetriol, isokobusone, isocyperol, and sugeonol (Meena, 2010). The plant material is used in the treatment of nausea and vomiting, dyspepsia, colic, flatulence, diarrhoea, dysentery, intestinal parasites, fever, malaria, cough, bronchitis, renal and vesical calculi, urinary tenesmus, skin diseases, wounds, amenorrhea, dysmenorrhea, deficient lactation, loss of memory, insect bites, food poisoning, indigestion, nausea, dysuria, bronchitis, infertility, cervical cancer and menstrual disorders (Yeung, 1985; Duke, 1985; Chopra,1986).

7. *Lagenaria siceraria* (Molina) Standl. (Surai)

Lagenaria siceraria (Molina) Standl. (Fam: Cucurbitaceae) is an annual herbaceous climbing plant with a long history of traditional medicinal uses in many countries, especially in tropical and subtropical regions. Geographically it occurs throughout India and is now cultivated worldwide. It is generally accepted that this plant was indigenous to Africa and that it reached temperate and tropical areas in Asia and the Americas about 10,000 years ago. The vernacular names of the plant are Tamil – Surai; Malayalam – Pechura; Sanskrit - Tiktalabu; Hindi – Lauki; English - Bottle Gourd; Gujarathi – Tumada; Manipuri – Khongdrum; Kannada - Isugumbala, Tumbi; Marathi – Phopla; Telungu – Sorakkaya; Urdu - Lauki (API, 2001). Alkaloids, phenols, tannins, triterpenoids, flavonoids, amino acid and steroidal compounds (fucosterol and campesterol) are known in the genus. Edible portion of the fruit is a good source of glucose and fructose. The fruit is a good source of vitamins B and a fair source of ascorbic acid. Bitter fruits yield 0.013% of solid foam containing cucurbitacins B, D, G and H, mainly cucurbitacin B. Leaves contain cucurbitacin B and roots contain cucurbitacins B, D and E (The Wealth of India, 1988). Oleanolic acid, β-sitosterol, campesterol, isoquercitrin, saponin and fatty oils are also reported (Shirwaikar, 1996; Gangwal, 2010). The fruits, leaves, oil, and seeds are edible and used by local people as folk medicines in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases. The fruit pulp is used as an emetic, sedative, purgative, cooling, diuretic, anti-bilious and pectoral. The flowers are an antidote to poison. The stem bark and rind of the fruit are diuretic. The seed is vermifuge. Extracts of the plant have shown antibiotic activity. Leaf juice is widely used for baldness (Kirtikar, 2005; Duke, 1985; Rahman, 2003).

8. *Tinospora cordifolia* (Willd.) Miers. (Seenthil)

Tinospora cordifolia (Willd.) Miers. belongs to the family Menispermaceae. It is a large, deciduous, climbing shrub found throughout India, especially in the tropical parts ascending to an altitude of 300 m and also in certain parts of China (The Wealth of India, 1989). The vernacular names of the plant (SPI, 2008) are Tamil - Seenthil; Malayalam - Chittamrut; Sanskrit – Guruchi; Bengali – Gulancha; Gujarati – Galac; Marathi – Gulvel; Punjabi – Gilo; Odia – Guluchi; Assamese - Siddhilata, Amariat; Telugu – Thippateega; Kannada – Amruthaballi. The plant contains alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, essential oils, a mixture of fatty acids, and polysaccharides. Leaves are rich in protein, calcium and phosphorus. The stem contains clerodanefuronoditerpene glucoside (amritoside A, B, C, and D) (Singh, 2003). The plant is useful in the treatment of helminthiasis, heart diseases, leprosy, rheumatoid arthritis, support the immune system, the body's resistance to infections, supports standard white blood cell structure, function, and levels. It is used for diabetes, high cholesterol, allergic rhinitis, upset stomach, gout, lymphoma and other cancers, rheumatoid arthritis, hepatitis, peptic ulcer disease, fever, gonorrhea and syphilis. It also helps in digestive ailments such as hyperacidity, colitis, worm infestations, loss of appetite, abdominal pain, excessive thirst, and vomiting, and even liver disorders like hepatitis. Antioxidant, Antimicrobial, Anti-toxic, Antidiabetic, Antistress, Hypolipidemic, Anticancer and Anti-osteoporotic activities of the plant have been reported (Parthipan, 2011).

9. *Cadaba fruticosa* (L.) Druce. (Vizhuthi)

Cadaba fruticosa (L.) Druce. is a climbing shrub belonging to the Capparaceae family. In Tamil Nadu, *Cadaba fruticosa* is known as "'Vizhuthi" and is used in Siddha medicine for more than 2000 years. The shrub is widely distributed in the Indian subcontinent, commonly seen in scrub Jungles and Rocky areas (Prajapati, 2003). The vernacular names of the plant (http) are Tamil -Vizhuthi, Adamorinika; Malayalam – Kattakatti; Hindi - Kodhab, Dabi, kadhab; Marathi - Hapal, Vaelivee; Telugu - Chekonadi, chemudu; Gujarati - Kalokat-tiyokhordu. The leaves contain terpenoids, flavanoids, steroids, proteins, alkaloids, furans, gums, sugars, saponins. non-tannin phenolics, kaempferol, new spermidine alkaloids cadabicine, capparisine L-stachydrine and 3- hydroxystachydrine, an aromatic acid, ά, β-dihydroferulic acid, novel sesquiterpenoidcadabicine methyl ether and cadabicine diacetate, besides a sesquiterpene and cadibicilone. It also contains 3(4-formylphenoxy) -4- methoxybenzaldehyde, methyl cinnamate, methyl ferulate ether, ether of p-cinnamic acid-m- ferulic acid, thiazolidine compound. It also shows the presence of quercetin, isoorientin, hydroxybenzoic acid, syringic acid, vanillic acid and 2-hydroxy- 4-methoxy benzoic acid (Arokiyaraj, 2008).

The fruits are used to treat worm infestation, swellings, eczema and constipation**.** The leaves are used as a poultice to promote healing of sores. The juice of the leaves is especially used to cure gonorrhea and syphilis. Leaves are used to relieve rheumatic pain and used in the treatment of boils. It is also used in the treatment of cough, fever, dysentery and as antidote against poisoning (Arokiyaraj, 2008).

10. *Aerva lanata* (L.) Juss. (Sirupeelai)

Aerva lanata (L.) Juss. is an erect or prostrate under shrub (Fam: Amaranthaceae) with a long tap-root and many wolly-tomentose branches. It is commonly known as polpala. It is an important gregarious shrub growing throughout Bangladesh, particularly along waste places in India. It is one of the plants included in Dasapushpam, the sacred flowers of Kerala (Manoj, 2011; Varghese et al. 2010). The vernacular names **(**SPI, 2008) of the plant is Tamil – Sirupeelai, Malayalam – Cherula; English – Polpala; Marathi – Kapuri; Punjabi - Bui-kaltan; Rajasthani – Bhui; Kannada – Bilesuli; Sanskrit – Astmabayda; Hindi – Gorakhganja; Bengali – Chaya. Plant contains alkaloids, flavanoids, steroids and their

glycosides, β- sitosterol, β-sitosterol palmitate, α-amyrin, terpenoids, tannic acid, sugar, saponins and minerals, (Chandra & Sastry, 1990). The plant is mainly used for urinary disorder. The roots are used in the treatment of headache. It cures pneumonia, typhoid and other prolonged fevers. It has many pharmacological uses identified like urolithiasis, diuretic activity, anti-microbial activity, anti-fertility and antiulcer activity (Singh, 1998).

11. *Enicostemma axillare* (Poir.ex Lam.) A. Raynal. (Vellarugu)

Enicostemma axillare (Fam: Gentianaceae) is glabrous perennial herb found throughout the greater part of India up to an altitude of 1500 ft. It is also common in coastal area (Struwe and Albert, 2002). The vernacular names (SPI, 2011) of the plant are Tamil – Vellarugu; Malayalam – Vallari; Sanskrit - Nahi; Gujarathi – Maamijvaa; Kannada – Karibandit; Marathi –Kadvinaai; Punjabi – Bahuguni; Urdu – Naay; Odia – Sweta. The chemical constituents of the plant are genkwanin, apigenin, swertisin, saponarin, swertiamarin, betulin, enicoflavine, gentiocrucine, gentianine, erythrocentaurine, ephelicacid glycoside, sylswertisioside, isoswertisin-5-O-glucoside, sylswertisin - 5- O- glucoside (Ghosal et al., 1974). The fresh juice of leaves has been used as a bitter tonic and cooling agent. It is used to control arthritis and in typical fever. It is used as stomachic and laxative, blood purifier in dropsy, rheumatism, abdominal ulcers, hernia, swellings, itches and insect poisoning. The plant paste is applied on boils. The leaves are fed to cattle to increase appetite (Raghu Bir, 2006). It also acts as ethno medicine for snakebite. The plant possesses stimulant, astringent, diuretic and anthelmintic properties (Garg, 2000).

Lead content in plants

According to Siddha literature, the minerals and metallic particles with good medicinal value is present in the medicinal plants. There is a list of medicinal plants containing Lead (Plumbum). Lead is a metal having the anti-septic, astringent and diuretic properties and is useful in the treatment of venereal diseases, leprosy, tuberculosis, ulcers and neurological ailments. The Siddha formulations – *Vanga parpam, Vanga chendhooram* prepared by the calcination process of Lead are beneficial in the treatment of Vatha diseases and *Mega rogangal*. Lead is also an ingredient of many external medications like *Vanga virana kalimbu, Viranatthukku vanga pugai, Viranatthukku vanga seelai*, etc which are useful for healing ulcers. The metal thread made of Lead - *Kareeyakkol* is useful for the prevention of eye diseases. As per Siddha concept, the treatment shall be initiated with herbal drugs and if not cured therewith it will be followed with metallic or mineral preparations. Most of the medicinal plants have phytochemicals that can act efficiently in the prevention and management of diseases. The Lead containing plants are commonly used for vatha diseases and urinary disorders. In chronic stage of illness, Siddha formulations prepared with Lead (Pb) is indicated and beneficial (Thiyagarajan, 1981).

In the study, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was employed for the detection of Lead present in the plants. Good detection limits, a large linear dynamic range, relative freedom from chemical inter-element interferences, simultaneous multi-element capability make ICP-MS a powerful analytical tool for many applications over other techniques (Hiçsönmez et al. 2009). ICP-MS is a type of mass spectrometry which is capable of detecting metals and several non-metals at concentrations as low as parts per billion on non-interfered low-background isotopes. An ICP-MS instrument consists of the components - Sample introduction system, ICP torch and RF coil, Interface, Vacuum system, Collision/reaction cell, Ion optics and Detector. The ICP-MS allows determination of elements with atomic mass ranges 7 to 250 (Li to U) (Nageswaran et al. 2017). In the study, ICP-MS was employed for the detection of Lead present in the selected plants.

MATERIALS AND METHODS

1. Site selection and study area

Three accessions of the selected plants were taken for the study. First accession of the plants were collected from Siddha Medicinal Plants Garden, Mettur Dam, and third accession from Thirunelveli, Tamilnadu. The second accession was taken up for the study from different places in Kerala as follows. Sirupeelai and Vellaisaarvelai – Kozhikode; Vaeliparuthi, Seethai, Surai and Pathukai – Wayanad; Seenthil, Musthaiyum and Vellarugu- Thiruvananthapuram; Muthirukkanchevi - Malappuram and Vizhuthai - Idukki.

2. Collection of plant materials

Three accessions of the plant materials were collected and authenticated by Botany experts. Herbarium specimens of these plants were prepared following standard botanical procedure. The collected samples were shade dried and stored in airtight containers. These dried samples were used for the quantitative analysis of Lead. Each collection is labeled with botanical name, family, vernacular name, place of collection and date of collection.

3. Estimation of Lead

According to the Classical Text of Siddha system of medicine, the selected plants contain Lead. The Lead content in these plant materials were estimated using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) (Make : Thermo Electron Corporation, Model :iCAP RQ) by out sourcing at 'Sophisticated Instrumentation and Computation Centre (SICC)', University Campus, University of Kerala, Karyavattom, Thiruvananthapuram and 'Sophisticated Tests and Instrumentation Centre (STIC), Cochin University of Science and Technology, Cochin.

Procedure

ICP- MS working standard: Working standards prepared from ICP single/ multi elements 1000 ppm certified standard solutions are used for instrument calibration. Minimum five working standards were prepared in the range between 1 ppb to 250 ppb according to the concentration of the elements and matrix of the sample.

Sample analysis: Solid samples are typically digested in strong and hot acids (either open digestion or closed vessel digestion in a microwave digester). The acids themselves range from simple nitric acid (for relatively simple matrices) to hydrofluoric acid (for samples containing high silicon dioxide content). Hydrogen peroxide may be added to the samples containing organic matter during the digestion step because H_2O_2 breaks down organic matter efficiently.

Modes of operation: Standard mode - the instrument performs like a standard ICP-MS. High sensitivity is achieved for all elements. This mode is typically used for uninterfered elements such as Be, Hg, Pb.

RESULTS AND DISCUSSION

Estimation of Lead

Estimation of Lead in each plant material of the three accessions was carried out using ICP-MS and the results are tabulated in the Table 2.

Table 2: Lead content in plant materials as estimated by ICP-MS.

Sl. No.	Name of plant material		Part used	Lead content in plant materials (ppm)		
	Botanical name	Tamil name		Accession 1	Accession 2	Accession 3
1	*Alternanthera sessilis* (L.) R.Br., ex DC.	Seethai	Leaf	1.83	4.82	1.24
2	*Merremia emarginata* (Burm. f) Hall.f.	Muthirukanchevi	Leaf	2.93	3.04	2.65
3	*Trianthema portulacastrum* Linn.	Vellaisaarvelai	Leaf	3.04	1.02	0.64
			Root	2.91	6.14	1.02
4	*Coldenia procumbens* Linn.	Pathukai	Whole plant	0.89	6.65	1.35
5	*Pergularia daemia* (Forssk.) Chiov.	Vaeliparuthi	Whole plant	1.56	2.29	0.85
6	*Cyperus rotundus* Linn.	Musthaiyum	Root tuber	0.60	2.99	0.92
7	*Lagenaria siceraria* (Molina) Standl.	Surai	Whole plant	0.88	1.70	2.48
8	*Tinospora cordifolia* (Willd.) Miers.	Seenthil	Whole plant	0.96	6.64	0.46
9	*Cadaba fruticosa* (L.) Druce	Vizhuthi	Leaf	1.59	1.53	0.73
			Fruit	1.17	1.15	0.36
10	*Aerva lanata* (L.) Juss.	Sirupeelai	Whole plant	3.32	3.13	0.93
11	*Enicostemma axillare* (Poir.ex Lam.) A. Raynal.	Vellarugu	Whole plant	2.68	1.59	1.53

The results of the analysis of three accessions of the plant materials were compared and found that all the plant materials studied contain Lead in detectable range. The Lead level of every accession of each plant material varied from one another. The Lead content was varied from 0.36 ppm to 6.65 ppm in all the three accessions of plants studied. There is no regularity or order in the quantity of Lead present among the accessions of the plants. The high Lead level was observed in the second accessions of *Coldenia procumbens* (whole plant) - 6.65 ppm, *Tinospora cordifolia* (whole plant) - 6.64 ppm, *Trianthema portulacastrum* (root) - 6.14 ppm and *Alternanthera sessilis* (leaf) – 4.82 ppm and the low Lead level was observed in the third accession of *Tinospora cordifolia* (whole plant) – 0.46 ppm and *Cadaba fruticosa* (fruit) – 0.36 ppm. Out of 13 plant materials studied, the second accessions of 7 plant materials (collected from Kerala) have more quantity of Lead ranging from 2.29 ppm to 6.65 ppm compared to other accessions.

There are many previous reports proved that Lead is a non-essential element which is toxic when present beyond the permissible limit (Peralta et al. 2009). In the current study, the Lead content in all the three accessions of plants was found to be within the permissible limit (<10 ppm) which is in agreement with WHO and FDA guidelines. Mineral and trace elements present in considerable amount is directly or indirectly useful in the management of different diseases (Shashikanth et al., 2014). Previous studies suggested that *T. portulacastrum*, *C. rotundus* and *A. sessilis* are efficient phytoaccumulators of Lead and other heavy metals. Messou et al. 2013 and Chinmayee et al. 2012 reported that two species of *Alternanthera* (*A. sessilis* and *A. tenella*) have the tolerance ability against Lead induced

stress and suggested as a suitable candidate for phytoremediation of Lead.

CONCLUSION

ICP-MS was employed for the estimation of Lead present in the plants and thereby scientifically validated the presence of Lead in detectable amount. The Lead level was found to vary from accession to accession as well as plant to plant. In this study Lead content was observed within the permissible limit of WHO and FDA and thereby proving the level of safety for the consumption at nutritional as well as medicinal level. Therefore, plants selected for the present study can be exploited as suitable candidates for phytoremediation of Lead in the soil.

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