

ESTIMATION OF VARIATION IN THE ELEMENTAL CONTENTS OF METHANOLIC SOXHLET LEAF EXTRACT OF *AMPELOCISSUS LATIFOLIA* (ROXB.) PLANCH. BY ICP-AES TECHNIQUE

PARAG A. PEDNEKAR¹, BHANU RAMAN¹

¹Department of Chemistry, K.J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai 400077.
Email: paragpednekar81@gmail.com, professor.bhanuraman@gmail.com

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ABSTRACT

Objective: To investigate the elemental contents of methanolic soxhlet leaf extract of *Ampelocissus latifolia* (Roxb.) Planch. by ICP-AES technique. The quantitative estimation of various concentrations of elements in medicinal plants is necessary for determining their effectiveness in treating various diseases and for understanding their pharmacological action.

Methods: Elemental concentrations of methanolic soxhlet leaf extracts of *Ampelocissus latifolia* was measured by the ICP-AES technique. 41 elements Na, Mg, Si, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Co, Cd, Se, Al, S, Pb, Ba, Hg, As, B, P, Sr, Br, Ti, Bi, Ge, In, La, Li, Mo, Pd, Sb, Sc, Sn, Te, V, W, I, Th were screened.

Results: Depending on geo-environmental factors and local soil characteristics, 11 elements were found to be present in the above leaf extract in different concentrations. 41 elements and their role in treating various diseases are discussed in this research paper.

Conclusion: Data on elemental concentration in the above medicinal plant will be useful to set new standards for prescribing the dosage and duration of administration of these herbal medicines.

Keywords: Pharmacological action, *Ampelocissus latifolia* (Roxb.) Planch., ICP-AES, Herbal medicines.

INTRODUCTION

Ampelocissus latifolia belongs to family Vitaceae (Grape family). It is a large herbaceous climber with tuberous root stock. Stem and branches are hollow and smooth. Leaves are circular or broadly heart-shaped with lobes acute and toothed. The roots and barks of this plant are used in the treatment of bone fracture, dysentery, fever, menstrual complaints [1]. The roots are also employed in the treatment of pain in stomach, snake bites, sores and wounds [2]. The sandals of Bihar used this plant for muscular pains, sores and fractured bones [3]. The stem ash of this plant is applied abdominally for easy delivery in pregnancy [4]. The antimicrobial activity of the methanolic leaf extract of *Ampelocissus latifolia* was evaluated against medicinally important bacteria *Staphylococcus epidermidis*, *Micrococcus luteus*, Methicillin-resistant *Staphylococcus aureus*, *Propionibacterium acnes* and yeast, *Malaassezia furfur* using the MIC and MBC/MFC analysis. The same methanolic leaf extracts were also tested for antioxidant activity by DPPH free radical scavenging method and Nitric oxide radical scavenging method. The functional groups present in the crude powder and methanolic extract of *Ampelocissus latifolia* leaves were identified through Fourier Transform Infrared (FT-IR) spectrometry [5].

In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems. The therapeutic effect of these plants for the treatment of various diseases is based on the chemical constituents present in them [6,7]. Plant extracts are used to produce therapeutic tinctures, syrups, tablets, snuff, saucers or oral sprays [8,9]. It is well known that medicinal herbs are not completely safe. Sometimes allergic and toxic reactions occur, as well as drug interactions. Some herbs could be hepatotoxic [10]. Therefore, the objective of the present study is to determine the levels of Na, Mg, Si, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Co, Cd, Se, Al, S, Pb, Ba, Hg, As, B, P, Sr, Br, Ti, Bi, Ge, In, La, Li, Mo, Pd, Sb, Sc, Sn, Te, V, W, I, Th in this medicinal plant by nitric acid digestion procedure with ICP-AES technique [11]. The data obtained will provide information on whether this plant contains some metal elements in amounts that could be toxic at the normal doses if consumed as medicine.

MATERIALS AND METHODS

Collection and Authentication of the plant

The leaves of *Ampelocissus latifolia* were collected from an open field around Mumbai, Maharashtra. The identification of the plant was done

at the Blatter Herbarium, St. Xavier's College, Mumbai. The *Ampelocissus latifolia* (Roxb.) Planch specimen matches with the Blatter Herbarium specimen no. Shah-I of G. L. Shah. The leaves were thoroughly washed with distilled water, dried in an oven at 40°C and subsequently ground into fine powder by using a mechanical grinder.

Chemicals

A standard solution of each element was prepared by dilutions of a 1000 mg/L stock solution (Merck, Darmstadt, Germany) prior to use. Milli-Q deionized water (Millipore, Milford, MA) was used throughout this experiment. Solvents such as nitric acid and methanol were of analytical reagent grade (Merck). All glassware were soaked with 10% nitric acid overnight and then rinsed with deionized water prior to use.

Soxhlet Extraction of Plant Material

The leaf powder of *Ampelocissus latifolia* (20 gm) was extracted with 250 ml methanol by soxhlet extraction for 8 hours. The extract was concentrated on water bath at 60°C. The dark greenish brown thick liquid obtained was stored in a glass vial in refrigerator.

Digestion Procedure of Soxhlet Leaf Extracts

1 gm of sample by weight was taken in a beaker and 5 ml of 60% concentrated nitric acid was added and kept on hot plate at 85°C for 15 mins. Subsequently it was cooled and then made to 50 ml with the help of distilled water. Blank samples were also processed and analyzed simultaneously. The solutions were then analyzed with an inductively coupled plasma atomic emission spectrometer.

Elemental Analysis of Plant Leaf Extracts

All spectrometric measurements were performed with ICP spectrometer (Arcos from M/S. Spectro, Germany). The software used was Smart Analyzer Vision 5.01.0921. The detector was charge coupled device (CCD). The instrumental parameters and operating conditions are given in Table 1. All the samples were analyzed in triplicate and mean values of concentrations for each element are given along with standard deviation in Table 2.

RESULTS

Various elements of biological importance in man's metabolism were found to be present in varying concentrations in the present

studied medicinal plant and toxic heavy metals were also analyzed for their presence. Table 2 shows the concentrations of various elements present in the medicinal plant under study. Elemental analysis has revealed the presence in order of $K > Na > Si > Mg > B > Ca > S > Cu > Mn > Ni, Zn$. The results show higher concentration of potassium. Elements such as Cl, Cr, Fe, Co, Cd, Se, Al, Pb, Ba, Hg, As, P, Sr, Br, Ti, Bi, Ge, In, La, Mo, Pd, Sb, Sc, Sn, Te, V, W, Th, I, Li were not detected. The differences in the concentration of these elements within the plant is attributed to factors such as the preferential absorbability of a particular plant for the corresponding elements, the age of the plant, the mineral composition of the soil in which the plant grows as well as its ambient climatological conditions [12].

DISCUSSION

It is well known that the level of elements in plants depends on environmental conditions, such as type of soil, rainfall, vicinity of industry and extensive agricultural activity [13]. Essential elements perform various complimentary vital functions in the body to keep the organism healthy. Sodium and potassium are reported to be important elements for the maintenance of acid-base equilibrium and of osmotic pressure of body fluids. It has also been reported that potassium, if taken simultaneously with sodium, prevents increase in blood pressure [14]. The concentration of potassium was highest than the other reported elements present in methanolic leaf extract of *Ampelocissus latifolia*, 225.45 ± 1.779 ppm. The concentration of sodium was found to be 44.633 ± 1.109 ppm in this plant extract. Calcium is known to enhance the qualities of bones and teeth and also of neuromuscular systemic and cardiac functions. The concentration of calcium was found to be 4.4 ± 0.132 ppm. Magnesium is an important electrolyte and is also responsible for proper nerve and muscle function. It also works as co-factor in more than 300 metabolic reactions [15]. Magnesium acts in the cells of all the soft tissues, where it is part of the protein-making machinery and is necessary for the release of energy [16]. The concentration of magnesium was found to be 12.71 ± 0.305 ppm. Iron regulates the function of T lymphocytes and many studies (in vivo and in vitro) reported that a deficiency in iron results in impaired cell-mediated immunity [17-21]. Iron was not detected in this plant extract. Zinc is an important element responsible for many enzymatic processes and is involved in working of genetic materials, proteins, immune reactions, wound healing, development of the foetus and sperm production. It has been suggested that normal levels of zinc can prevent diarrhoea [22]. The concentration of zinc was found to be 0.05 ± 0.0 ppm in this plant extract.

Manganese is an antioxidant nutrient and is important in the breakdown of fats and cholesterol and also helps in the nourishment of the nerves and the brain [23]. Manganese is essentially required for the metabolism of Vitamin B₁, C and E and for the activation of various enzymes which are important for proper digestion, utilization of foods and hence in regulating immune response of the body [23]. The concentration of manganese was found to be 0.1 ± 0.0 ppm in this plant extract. Copper is a factor necessary for the absorption and use of iron in the formation of haemoglobin, part of many enzymes, and helps in the formation of protective covering around nerves [22]. The concentration of copper was found to be 0.55 ± 0.0 ppm in this plant extract. Phosphorous is essential for growth and renewal of tissues. In metabolism of energy nutrients, phosphorus compounds carry, store and release energy and they assist many enzymes and vitamins in extracting the energy from nutrients. Phosphorous is essential for growth and renewal of tissues [16]. Phosphorous was not detected in this plant extract. Alfrey *et al.* reported that orally administered aluminium compounds reduce the absorption of a number of other elements and compounds including strontium, iron, fluoride, phosphorus and to a lesser extent calcium [24]. Aluminium was not detected in this plant extract. The biochemical role of **boron** is not well understood, but it is known that it affects brain function, psychomotor performance, the metabolism of macrominerals, energy, nitrogen and reactive oxygen [25]. The concentration of boron was found to be 5.63 ± 0.513 ppm in this plant extract.

Chloride is the chief anion of extracellular fluid which is responsible for muscular irritability [26]. Chlorine was not detected in this plant

extract. Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration [27,28]. Lead was not detected in this plant extract. Acute or chronic exposure of cadmium causes respiratory distress, lung and breast cancers, haemorrhagic injuries, anemia and cardiovascular disorders [29-31]. Cadmium was not detected in this plant extract. Arsenic is reported to cause hypertension, peripheral arteriosclerosis, skin diseases and neurotoxicity whereas mercury causes neurological disorders, paralysis, digestive tract inflammation, uremia, acrodynia and immunotoxicity [32-36]. Both arsenic and mercury were not detected in this plant extract. Nickel has been reported to cause contact dermatitis, nasal, sinus and lung cancers, kidney disorders, chronic bronchitis, acute respiratory distress syndrome and pulmonary fibrosis [37]. The concentration of nickel was found to be 0.05 ± 0.0 ppm in this plant extract. Chromium is known to cause nephrotoxicity, nasal and lung ulcers, skin ulcers, hypersensitivity reactions and "chrome holes" of the skin [38,39]. Chromium was not detected in this plant extract. Cobalt is an essential trace element and forms part of the active site of Vitamin B₁₂. The amount of cobalt required in the human body is very small and it contains only about 1 mg [40,41]. Cobalt was not detected in this plant extract. Sulphur is an important element that is used in small amounts to construct all parts of human body. It is essential as a component of fats, body fluids and bones. Sulfur is essential to all living things and there is a sulfur cycle in nature. The average human contains 140 gm and takes in about 1 gm of sulphur a day [40,41]. The concentration of sulfur was found to be 0.766 ± 0.583 ppm in this plant extract.

Silicon is common mineral required by our body along with calcium to grow and maintain strong bones, hair, skin and fingernails. It is essential to plant and animal life. In humans, it is found in connective tissue and skin [40,41]. The concentration of silicon was found to be 24.5 ± 13.519 ppm in this plant extract. Titanium is a suspected carcinogen [40,41]. Titanium was not detected in this plant extract. Strontium is the fifteenth most abundant element on earth. Its occurrence in plants is a due to the type and chemical composition of soil, rainfall, agricultural practice and kind of plant [40,41]. Strontium was not detected in this plant extract. The best known use of barium is in the form of barium sulfate, which can be drunk as a medical cocktail to outline the stomach and intestines for medical examination. Barium and all its compounds that are water or acid soluble are toxic [40,41]. Barium was not detected in this plant extract. Selenium is an essential trace element but is toxic in excess. It is carcinogenic and teratogenic. Hydrogen selenide and other selenium compounds are extremely toxic [40,41]. Selenium was not detected in this plant extract. Bromine has no known biological role. It has an irritating effect on the eyes and throat, and produces painful sores when in contact with the skin [40,41]. Bromine was not detected in this plant extract. Bismuth (III) chloride oxide (BiClO) is used in cosmetics to give a pearly effect. Bismuth has no known biological role and is non-toxic [40,41]. Bismuth was not detected in this plant extract. Germanium has no known biological role. It is non-toxic. Certain germanium compounds have low mammalian toxicity but marked activity against some bacteria, which has stimulated interest in their use in pharmaceutical products [40,41]. Germanium was not detected in this plant extract.

Indium has no known biological role but has been shown to cause birth defects in unborn children [40,41]. Indium was not detected in this plant extract. The ion La³⁺ is used as a biological tracer for Ca²⁺, and radioactive lanthanum has been tested for use in treating cancer. Lanthanum has no known biological role, but both the element and its compounds are moderately toxic [40,41]. Lanthanum was not detected in this plant extract. Lithium has no known natural biological role. It is non-toxic, teratogenic, stimulatory and an anti-depressant. Lithium carbonate therapy has become standard treatment for manic depression, although its action on the brain is still not fully understood [40,41]. Lithium was not detected in this plant extract. Molybdenum is an essential element for animals and plants. If soil lacks this element the land is barren. Leguminous plants use the nitrogen-fixing enzyme nitrogenase, which contains molybdenum [40,41]. Molybdenum was not detected in this plant extract. Palladium has no known biological role and is

non-toxic [40,41]. It was not detected in this plant extract. Antimony and many of its compounds are toxic [40,41]. It was not detected in this plant extract. Scandium has no known biological role, but is a suspected carcinogen [40,41]. It was not detected in this plant extract. Tin is non-toxic [40,41]. It was not detected in this plant extract. Tellurium has no known biological role [40,41]. It is very toxic and teratogenic. It was not detected

in this plant extract. Vanadium is essential to some species, including humans, although we require very little, less than the 0.04 mg, which we take in each day [40,41]. It was not detected in this plant extract. Tungsten has no known biological role and has low toxicity [40,41]. It was not detected in this plant extract. Thorium has no known biological role. It is toxic due to its radioactivity [40,41]. It was not detected in this plant extract.

Table 1: The instrumental parameters and operating conditions for ICP-AES.

ICP-AES parameter	Value
R.F. Generator	1.6 KW, 27.12 MHz
Plasma Power	1400 W
Pump Speed	30 rpm
Coolant Flow	12.00 l/min
Auxiliary Flow	1.00 l/min
Nebulizer Flow	0.80 l/ml

Table 2: Elemental concentrations in methanolic soxhlet leaf extracts of *Ampelocissus latifolia* by the ICP-AES technique.

Elements	Wavelength Selected in nm	Concentrations in ppm Mean \pm SD
Na	589.592	44.633 \pm 1.109
Mg	279.079	12.71 \pm 0.305
Si	251.612	24.5 \pm 13.519
Cl	134.724	ND
K	766.491	225.45 \pm 1.779
Ca	422.673	4.4 \pm 0.132
Cr	267.716	ND
Mn	257.611	0.1 \pm 0.0
Fe	259.941	ND
Ni	231.604	0.05 \pm 0.0
Cu	324.754	0.55 \pm 0.0
Zn	213.856	0.05 \pm 0.0
Co	230.786	ND
Cd	214.438	ND
Se	196.09	ND
Al	176.641	ND
S	180.731	0.766 \pm 0.583
Pb	220.353	ND
Ba	455.404	ND
Hg	184.95	ND
As	189.042	ND
B	208.959	5.63 \pm 0.513
P	177.495	ND
Sr	460.733	ND
Br	154.065	ND
Ti	132.171	ND
Bi	223.061	ND
Ge	164.919	ND
In	230.606	ND
La	408.672	ND
Mo	202.095	ND
Pd	340.458	ND
Sb	206.833	ND
Sc	361.384	ND
Sn	189.991	ND
Te	170	ND
V	292.464	ND
W	239.709	ND
Th	401.913	ND
I	178.276	ND
Li	670.780	ND

Mean concentration \pm standard deviation (n=3); ND= Not Detected means less than 0.01 ppm.

CONCLUSIONS

Review of the literature on bioelements content of *Ampelocissus latifolia* showed that there was no data available on the elemental composition of this plant. The studied plant contains essential elements which can play an important role in the various

biochemical and physiological processes in humans and hence can be considered as a potential source for providing trace elements other than diet. ICP-AES technique was employed for the determination of the elemental compositions of 41 elements and their concentrations were estimated. 11 elements were found to be present in the above leaf extract in different concentrations. The

nonessential toxic elements like Al, As, Cd, Pb and Hg were not detected in the present plant extract. A fair amount of information on these elements and their roles in various physiological processes, their ways of functioning and necessity would be of paramount importance to understand the progression of various diseases and their remedies. The data obtained in this work could serve as an important resource for further studies on the above medicinal plant.

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