



Quantitative Analysis of Some Major Phytochemicals and Determination of Elements from *Ampelocissus Latifolia* (Roxb.) Planch

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ABSTRACT

Quantitative analysis of *Ampelocissus latifolia* tuberous root was carried out to identify phytoconstituents such as total alkaloid, flavonoids, saponin and terpenoids by standard method. Quantitative estimation was shown that *Ampelocissus latifolia* tuberous root powder contained alkaloid: 9.6%, flavonoids: 8.14%, terpenoids: 5.23% and saponin: 13.58%.

Elements analysis of ethanolic soxhlet extracted powder of *Ampelocissus latifolia* tuberous root was carried out by using ICP-AES technique, showed the presence of total 24 elements such as Al, B, Ba, Be, Ca, Cr, Cu, Fe, Hf, K, Li, Mg, Mn, Mo, Na, Ni, P, Sc, Sr, Ti, V, Y, Yb and Zr. Quantitative analysis of six elements was showing their concentration as Fe = 0.051 ppm, Mg = 0.045 ppm, Al = 2.06 ppm, K = 0.49 ppm, Ca = 0.09 ppm and Cr = 0.00009 ppm. Elements obtained during XRD analysis was also supported the ICP-AES result. Element content is depending on various factors such as the climate, location of plant specimen and soil composition in which plant grows. Presence of various phytochemicals and elements in tuberous root indicates the promising potential of this plant in medicine.

Keywords: ICP-AES spectrometry, X-Ray Diffraction, *Ampelocissus latifolia*, soxhlet extraction.

I. INTRODUCTION

Medicinal plants are used in various drugs preparation either singly or in combinations ⁽¹⁾. Some major secondary metabolites found in plants as alkaloid, steroid, tannin and phenol compounds that are produced and deposited in particular part or in almost all parts of the plant ⁽²⁾. Various active constituents of medicinal plants and mineral elements play an important role in metabolism ⁽³⁾.

Determination of mineral elements in plant is very important as the quality of many medicines depends upon the content and type of minerals ⁽⁴⁾. Presences of inorganic elements in medicinal plants are of great importance due to their pharmacological actions ⁽⁵⁾. It

has been found that trace elements play a vital role in the formation of active constituents in medicinal plants ⁽⁶⁾.

Plant was selected for quantitative analysis due to its availability and used against various ailments. *Ampelocissus latifolia* (Family: Vitaceae) used to treat various ailments in ethno medicine practices such as antidote, dyspepsia, gout, tuberculosis and as a health tonic ⁽⁷⁾. Few root pieces of *Ampelocissus latifolia* chewed or half glass decoction was taken orally for complicated deliverance ⁽⁸⁾. Fresh tuber of *Ampelocissus latifolia* with *Ricinus communis* oil was used to treat gout, tuber extract used to treat fracture bones of animal, dyspepsia, indigestion and tuberculosis ⁽⁹⁾.

With reference to this knowledge, present study aimed to determine the concentration of some major

phytochemicals and elements in tuberous roots of selected medicinal plant as an extensively used in curing various diseases by tribal peoples.

II. MATERIALS AND METHODS

Collection of plant materials:

Whole plant of *Ampelocissus latifolia* was collected from Wasali forest area of Buldana District, Maharashtra, India. Plant was identified by using various floras^(10, 11) and also from experts of this region. Tuberous roots were collected, thoroughly washed with distilled water; shade dried and then grinded into fine powder by using mechanical grinder.

Successive solvent extraction:

The grinded fine tuberous root powder of *Ampelocissus latifolia* was subjected to successive solvent extraction by using ethanol as solvent by soxhlet apparatus. About 20 gm of tuberous root powder was successively extracted with 250 ml of ethanol for 8 hrs. Ethanol extract was filtered through Whatman No. 1 filter paper and the filtrate was collected (crude extract). Ethanol extract was concentrated, solidified and used for further studies.

Quantitative estimation of some major phytochemicals:

a) Determination of Alkaloid:

About 5 gm tuberous root powder was taken into 250 ml beaker; 200 ml of 10% acetic acid was added into beaker, covered it and allowed to stand for 4 hours. Content in beaker was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Conc. ammonium hydroxide was added drop wise to extract up to the precipitation was complete. This solution was allowed to settle. The precipitate was collected, washed with dilute ammonium hydroxide and filtered. The remaining residue was alkaloid, completely dried and finally weighed⁽¹²⁾.

b) Determination of Flavonoid:

Weighed 10 gm of tuberous root powder and repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. This solution was filtered through Whatman filter paper No. 42. Filtrate obtained was transferred into the crucible and evaporated till dryness over water bath, weighed to a constant weight obtained⁽¹³⁾.

c) Determination of Terpenoids:

Weighed 10 gm of tuberous root powder and soaked in alcohol for 24 hrs. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids⁽¹⁴⁾.

d) Determination of Saponin:

Weighed 10 gm of tuberous root powder, put into a conical flask and then 50 ml of 20% aqueous ethanol was added. The sample was heated with continuous stirring at 55°C over a hot water bath for 4 hours. This mixture was filtered and the remaining residue re-extracted with another 100 ml 20% ethanol. Both the extract combined and reduced up to 40 ml over water bath at 90°C. The concentrate obtained was transferred into 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. In separating funnel, two separate layers were observed out of which aqueous layer was recovered and the ether layer was discarded.

The process of purification was repeated. To the aqueous extract 30 ml of n-butanol was added. A combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample obtained were dried in oven to the constant weight and saponins percentage was calculated⁽¹⁵⁾.

Inductive Coupled Plasma-Atomic Emission Spectrometry (ICP-AES):

Spectrometric analysis of ethanolic soxhlet extracted powdered of tuberous root was done with the help of ICP spectrometer (Arcos from M/S. Spectro, Germany). The instrument was operated by adjusting various parameters such as R.F. Generator (1.6 KW, 28 MHz), Plasma Power (1400 W), Pump speed (30 rpm), Coolant Flow (12.00 l/min), Auxiliary Flow (1.00 l/min) and Nebulizer Flow (0.80 l/min). The software used was Smart Analyzer Vision 5.01.0921 and the detector was used as charge coupled device (CCD). Each sample was analyzed in triplicate and mean values of concentrations for each element determined.

X-Ray Diffraction (XRD) Study by powder method:

Dried raw powder of *Ampelocissus latifolia* tuberous root was used for XRD analysis. D8 ADVANCE (BRUKER) computer controlled diffractometer was used for automatic operation. The instrument was facilitated to conduct experiment by adjusting various

parameters such as temperature: 25⁰C, angle range: 5⁰-120⁰, voltage: 40 KV, Electron probes current range: 40mA. The results obtained were plotted against diffraction angle (2-θ) versus intensity (Counts). Leptos and EVA software was used for measurement and analysis.

III. RESULTS AND DISCUSSION

Quantitative estimation of some major phytochemicals:

Quantitative estimation of *Ampelocissus latifolia* tuberous root powdered was carried out by standard methods for some major phytochemicals such as alkaloid, flavonoids, saponin and terpenoids.

Quantitative estimation was showed that the *Ampelocissus latifolia* tuberous root powdered contained alkaloid: 9.6%, flavonoids: 8.14%, terpenoids: 5.23% and saponin: 13.58% as shown in table 1.

Table 1: Quantitative estimation of some major phytochemicals of *Ampelocissus latifolia* tuberous root

Sr. No.	Plant parts	Alkaloid Extraction: Each 5gm		Flavonoid Extraction: Each 10gm		Terpenoid Extraction: Each 10gm		Saponin Extraction: Each 10gm	
		(gm)	(%)	(gm)	(%)	(gm)	(%)	(gm)	(%)
1	<i>Ampelocissus latifolia</i> - tuberous root	0.48	9.6	0.814	8.14	0.523	5.23	1.358	13.58

The percentage of alkaloid, flavonoids, terpenoids and saponin obtained during quantitative estimation of *Ampelocissus latifolia* tuberous root powdered were represented in figure 1.

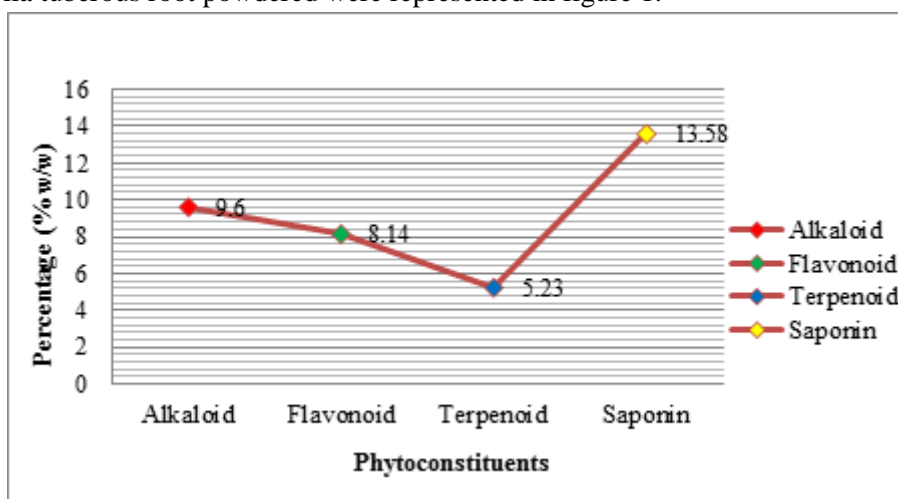


Figure 1. Quantitative analysis of some major phytochemicals of *Ampelocissus latifolia* tuberous root

Qualitative and quantitative estimation of some major elements of *Ampelocissus latifolia* tuberous root by ICP-AES technique:

Inductively Coupled Plasma- Atomic Emission Spectrometry technique was used for qualitative estimation and the determination of concentration of some major elements from ethanolic soxhlet extracted powder of *Ampelocissus latifolia* tuberous root. Ethanolic soxhlet extracted powder of *Ampelocissus latifolia* tuberous root was revealed the presence of total 24 elements such as Aluminum (Al), Boron (B), Barium (Ba), Beryllium (Be), Calcium (Ca), Chromium (Cr), Copper (Cu), Iron (Fe), Hafnium (Hf), Potassium (K), Lithium (Li), Magnesium (Mg), Manganese (Mn),

Molybdenum (Mo), Sodium (Na), Nickel (Ni), Phosphorus (P), Scandium (Sc), Strontium (Sr), Titanium (Ti), Vanadium (V), Yttrium (Y), Ytterbium (Yb) and Zirconium (Zr). Raman and Pednekar, (2013) was also reported forty one elements during estimation of variation in the elemental contents of methanolic soxhlet leaf extract of *Ampelocissus latifolia* (Roxb.) by ICP-AES technique⁽¹⁶⁾. The content of elements in plant is depends on environmental conditions such as rainfall, type of soil, industrial area and large scale activities in agriculture⁽¹⁷⁾. Such inorganic elements in plant carried out crucial role in accumulation of secondary metabolites such as alkaloids, glycosides, terpenoids and phenolic compounds etc.⁽¹⁸⁾.

Among these elements, quantitative analysis was carried out of six major elements such as Iron (Fe), Magnesium (Mg), Aluminum (Al), Potassium (K), Calcium (Ca) and Chromium (Cr). The concentration of these six major elements was calculated as Iron (Fe) = 0.051 ppm,

Magnesium (Mg) = 0.045 ppm, Aluminum (Al) = 2.06 ppm, Potassium (K) = 0.49 ppm, Calcium (Ca) = 0.09 ppm and Chromium (Cr) = 0.00009 ppm as shown in table 2.

Table 2. Qualitative and Quantitative estimation of elements by ICP-AES technique:

Sr. No.	Plant sample	Qualitative analysis of elements	Quantitative analysis of some major elements (ppm)					
			Fe	Mg	Al	K	Ca	Cr
1	<i>Ampelocissus latifolia</i> tuberous root	Al, B, Ba, Be, Ca, Cr, Cu, Fe, Hf, K, Li, Mg, Mn, Mo, Na, Ni, P, Sc, Sr, Ti, V, Y, Yb and Zr.	0.051	0.045	2.06	0.49	0.09	0.00009

The concentration of six major elements such as Iron (Fe), Magnesium (Mg), Aluminum (Al), Potassium (K), Calcium (Ca) and Chromium (Cr) calculated during quantitative estimation of *Ampelocissus latifolia* tuberous root by ICP-AES technique represented in figure 2.

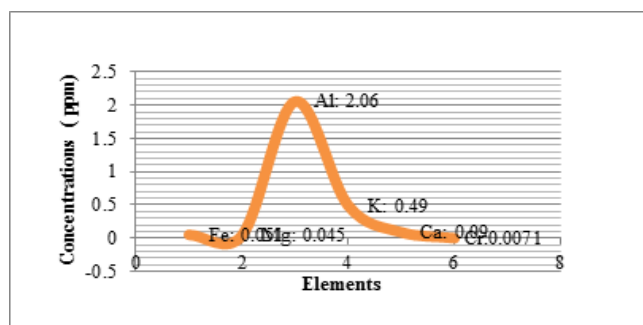


Figure 2. Quantitative estimation of elements of *Ampelocissus latifolia* tuberous root

X-Ray Diffraction (XRD) analysis by powdered method: XRD technique was used to authenticate the presence of various elements in *Ampelocissus latifolia* tuberous root powdered. XRD pattern for *Ampelocissus latifolia* tuberous root powdered was depicted in figure 3. The XRD pattern was shown that it comprised prominent diffraction peaks at 2θ -positions 12.55, 15.09, 24.57, 30.30, 38.36 and 46.04.

Diffraction peaks at 2θ -positions was resulted in d-values at 7.04, 5.86, 3.6, 2.9, 2.3 and 1.96 Å which revealed the presence of Aluminum (Al), Potassium (K), Calcium (Ca), Iron (Fe), Chromium (Cr) and Titanium (Ti) respectively. Besides this sharp diffraction peaks, pattern was consisted of some noisy peaks, which reflected the less crystallinity of *Ampelocissus latifolia*

tuberous root powdered. The above obtained results were found in good agreements with Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

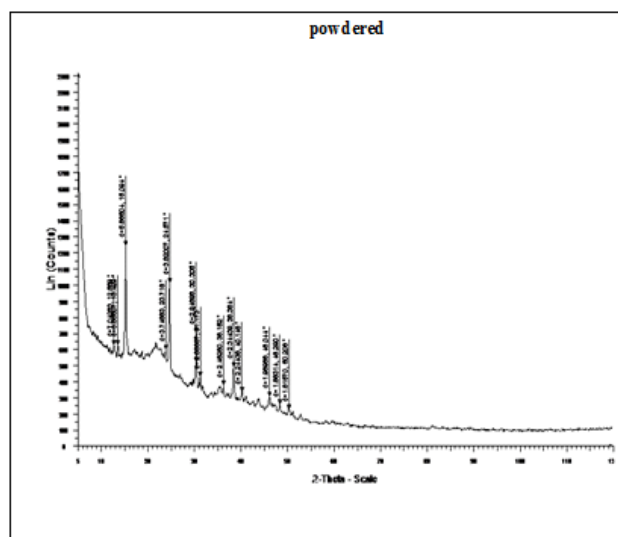


Figure 3. XRD spectrum of *Ampelocissus latifolia* tuberous root

IV. CONCLUSION

Present study on *Ampelocissus latifolia* tuberous root was quantitatively shown presence of alkaloid, flavonoids, terpenoids and saponin in variable concentration which contributes to medicinal values of plant. An ICP-AES result clearly indicated the presence of various elements in *Ampelocissus latifolia* tuberous root. Data obtained on elemental concentration of this medicinal plant became useful for prescribing the quantity and period of administration of these herbal

medicines as a new standard. The XRD analysis results were also showed similarity with ICP-AES result.

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