

INDUCTIVELY COUPLED PLASMA OPTICAL- EMISSION SPECTROSCOPY of GLORY LILY (*GLORIOSA SUPERBA.L*)

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ABSTRACT

The *Glory Lily plant* parts (Leaf, Tuber and Seed) and its relationship with element (Cr, Cu, Fe, Mg, and Zn) status were analysed through Inductively Coupled Plasma Optical-Emission Spectroscopy (ICP-OES), Atomic Absorption Spectrometer method. The quantitative of elements these parts of plants showed a different distribution in each part. This information could be used to identify which parts of plants are more effective for therapeutic use i.e. the required availability of such element to the particular disease may be chosen from particular part of the plant material. Based on the results obtained in the present work ICP-OES, AAS, technique is suitable for the determination of elemental concentration in plant. The simplicity and versatility of the procedure makes it attractive for its use in the pharmaceutical quality control of medicinal plants. The results are discussed.

Key Words: ICP-OES, AAS, medicinal plant, glory lily, trace elements and linear regression.

1. Introduction

Glory lily (*Gloriosa Superba* Linn.) belonging to the family Liliaceae., is one of the endangered species among the medicinal plant [1-7]. It is an ancient medicinal plant in India. Indian was the first to use it as a Ayurveda medicine, later as a source of pharmaceutically important alkaloids such as colchicines and colchicoside in Glory Lily [8-12]. This species is used as an

anticancer, antimicrobial, antifungal, anticoagulant, antilipoxygenase agent and antidote in snake bite. This seed germination, susceptibility towards many pests and excessive collections of tubers in type of environment in which an organism for medicinal purposes have pushed this taxon towards endangered category [13-18]. Poor seed sprouting, susceptibility toward many pests, and excessive collection in habitats for medicinal purposes have pushed this taxon to endanger. In order to provide enough plant material for commercial exploitation, the cultivation of the plant in a large scale is taken up. The seeds of this taxon are highly priced in the world market as they are the main source of colchicines and colchicoside [19-24]. Due to its over-exploitation in wild as well as problems faced during field cultivation, it was on the verge of extinction [5] and was one of the endangered species among the most valued medicinal plants [6]. Both its tuber and seeds have similar medicinal properties Pharmacies and drug manufacturers often fulfill up to 75% of their raw material demand from wild. Presently it is been cultivated in Tamil Nadu and other parts of South India[25-30]. Various Botanical Research Institutes and Nurseries are also propagating this important plant. The fondness for flower beauty has also placed Kalihari as a pot plant in gardens. Leaves are blanced, alternate, stalkless, (of a leaf shape) shaped like a lance head; narrow and tapering to a pointed apex, and spear shaped with curved end, which helps them to climb and creep[7,8].The alkaloids from plants (Colchicine and Gloriosine) are used in treatment of gout [9] and medicine that prevents or retards the clotting of blood [10]. Colchicine is used in the treatment of cancer [31-37].

2. Materials and Methods

2.1. Plant Material

The plant samples were collected from Jayamkondam cultivated plot area. The plant samples (leaves, tubers, seeds) were collected for the analysis of constituents. The samples were ground well into a fine powder by using an agate mortar. The samples were powdered well and

oven dried to remove the moisture content. The leaves were given code such as L₁, L₂, L₃, L₄ and L₅. The tubers were given code such as T₁, T₂, T₃, T₄ and T₅ and seeds were given code such as S₁, S₂, S₃, S₄ and S₅.

2.2. Sample preparation (Tri-acid method)

The different part of the plant were washed with distilled water and dried and the constant weight is obtained, the dried plant material was then ground to powder[38-45]. One gram of oven dried sample is transferred to a Teflon beaker and 10 ml of concentrated nitric acid and 2.5 ml of concentrated perchloric acid are added. The sample is then brought very slowly to boiling on a hot plate and heated to dryness. In sample of blackening occur during the fuming stages, nitric acid is added drop by drop. The sample is then cooled, redissolved in 10 ml of distilled water and 1 ml of concentrated hydrochloric acid and brought to volume of 25 ml in a volumetric flask. The solution is then analysed against calibration curves established. The prepared plant samples (leaf-5, tuber-5, and seed-5) were subjected to ICP-OES, AAS and Flame photometry analysis [46-50].

2.3. ICP-OES in Elemental Analysis

Emission spectroscopy using inductively coupled plasma has been developed in the mid of 1960's as a rapid sensitive and convenient method for the determination of elements in water, wastewater and other materials. The existence of a high performance ICP analysis method is a landmark for the development of basic and applied research, as well as for a number of industrial applications, especially in the field of assaying elements as trace or present in very high concentrations. ICP analysis involves introducing the elements to be analyzed into argon plasma induced by a high frequency (27 or 40 MHz). At temperature of 6500 K, the elements are atomized or ionized. The temperature is high enough to excite atoms and ions electronically, so

that they emit light. The resulting spectrum consisting of many emission lines is split up by Echelon optics. The intensity of the emission lines is directly proportional to the concentration of the analyses.

2.4. AAS in Elemental Analysis

AAS is well established and generally, the most convenient analytical techniques available for the determination of a metal ion in solution, if analyze concentration is above the milligram per litter range [13]. At concentrations is the milligram per litter level are below, the more sensitive techniques of electro thermal AAS is performed over the flame method. An important advantage of furnaces atomic absorption spectrometer is the small sample size requirement while direct analysis of solid samples is also possible. The main problem in atomic absorption spectrometer is that of interference from chemical and biological material in the samples, which is not present in the standard solutions used for calibration [14]. The sensitivity of AAS may vary element to element and under the normal instrumental conditions, AAS has no promising potential for multi-element determination [15]. Here, three methods of sample preparation were discussed and also compared. The formances of ICP-OES, AAS were compared through the determination Cr, Cu, Fe, Zn and Mg.

3. Results

The fifteen samples (Leafs-5, Tubers-5 and Seeds-5) have been subjected to ICP-OES, AAS, using the standard procedure already described. The plant samples analyses are found to show quantitative amount of the following elements viz., Cr, Cu, Fe, Zn and Mg.

From the quantitative analysis of the plant, it has been found that the elements like Cr, Cu, Fe, Mg and Zn are found to be present in major quantities where as element like Cr are found to be present in minor quantities (Table 1 and 2) in all the fifteen samples of the plant.

Chromium

Chromium is one of the known environmental toxic pollutants the world. Although it is an essential element for plants to serves as a cofactor and activator for several enzymes involving the synthesis of fatty acids, DNA and RNA, and cholesterol from acetate [16]. Chromium toxicity in plans has been related to the interaction between uptake and translocation of mineral nutrients [17]. The interaction may be distributed their concentration in plants and manifested by physiological disorders and a retarded growth [18].

3.2. Copper

Copper is one of the essential micro nutrients for normal plant growth. It is an essential compound of the plant enzymes diamine oxidase, ascorbate oxides, o-diphenol oxidase, cytochrome coxidase, superoxide dismutase, and plastocyanin oxidase and quinol oxides. In the absence o Cu these enzymes are inactivated [19]. Copper is an essential enzymatic element for normal plant growth. In humans, copper deficiency can cause anaemia and congenital inability to excrete copper resulting in Wilson's disease [20].

3.3. Iron

Iron is very essential for human beings and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and Fat to control body weight [21]. The

synthesis of chlorophyll, when iron becomes limiting, the chlorophyll synthesis slow down and chlorophyll is diluted due to continuous leaf expansion [22]. Iron is one the most common deficiency in men as well as women. Iron combines with other nutrients to produce vital blood proteins and is involved in food metabolism, digestion, elimination, circulation, and helps to regulate blood pressure. Iron needs the help of Vitamin C to improve iron absorption.

3.4. Zinc

Zinc is an essential trace element for plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioral response, bone formation and wound healing [23]. Zinc plays an important role in production, storage, and regulation of insulin [24]. First recognized the relationship between zinc and insulin.

3.5. Magnesium

Magnesium is the fourth most abundant mineral in the body and is essential to good health. Approximately 50% of total body magnesium is found in bone. The other half is found predominantly inside cells of body tissues and organs. Only 1% of magnesium is found in blood, but the body works very hard to keep blood levels of magnesium constant [25]. Magnesium is an essential element for macronutrients, the quantity of magnesium accumulated will differ for various plant organs, with a tendency toward greater allocation of magnesium in transpiring organs such as leaves and flowers, rather than the roots [26].

4. Statistical analysis

ICP-OES and AAS data obtained for the *Gloriosa Superba .L* plants part were statistically analyzed by one way ANOVA method. The statistical data were shown in the table 1 and 2. The statistical analysis of ICP-OES and AAS data in these different parts plant clearly shows, the probability and their actual quantitative value can be estimated. In the case of Cr, Cu, Fe, Mg and Zn the probability value is less than 0.001 ($P < 0.001$) i.e. table value; there is significant relationship among the elements.

5. Discussion

5.1. ICP-OES method

Table (1) shows that they are having high amount of Mg, moderated amount of Fe and lesser amount of Cu and Zn. Cr is very less concentration compared to other elements. Figure (1) shows that concentration elements in the relative distribution of trace elements in the Glory lily plant by ICP-OES. The Tubers are having high amount of Mg and Fe and medium concentration of Cu and Zn and less concentration of Cr compared to other elements. The seeds are having high amount of Mg and Fe and moderated amount of Cu and Zn and less amount of Cr compared to other elements the amount of Mg and Fe are in high level and moderate amount of Cu and Zn is compared with Cr for all the three parts of the plant.

Table 1

Elements	Glory lily						F Values	p Value
	Leaf		Tuber		Seed			
	Mean	SD	Mean	SD	Mean	SD		
Chromium	9.120	1.214	10.630	3.424	23.694	7.881	12.791	0.001 ^S
Copper	110.210	23.929	118.950	29.040	161.610	20.869	6.128	0.001 ^S
Iron	349.988	17.820	415.740	52.827	413.324	64.658	2.861	0.001 ^S
Magnesium	3626.224	189.433	4498.540	491.135	4391.754	480.971	6.679	0.001 ^S
Zinc	34.036	10.821	41.812	9.529	40.552	7.683	0.979	0.001 ^S

5.2. AAS method

Table (2) shows that they are having high amount of Fe and moderated amount of Cu and Zn and less amount of Cr compared to other elements. The Tubers are having high amount of Fe and medium amount of Cu and Zn and less amount of Cr compared to other elements. Figure.2 shows that concentration elements in the relative distribution of trace elements in the Glory lily plant by AAS. The seeds are having high amount of Fe and moderated amount of Cu and Zn. Cr is very less in amount. Overall the three parts of the plant. In which the amount of Fe is in high level and moderate level of Cu and Zn. Cr is very less when compared to other elements.

Table 2

Elements	Glory lily						F Values	p Value
	Leaf		Tuber		Seed			
	Mean	SD	Mean	SD	Mean	SD		
Chromium	9.120	1.214	10.630	3.424	23.694	7.881	12.791	0.001 ^S
Copper	110.210	23.929	118.950	29.040	161.610	20.869	6.128	0.001 ^S
Iron	349.988	17.820	415.740	52.827	413.324	64.658	2.861	0.001 ^S
Magnesium	3626.224	189.433	4498.540	491.135	4391.754	480.971	6.679	0.001 ^S
Zinc	34.036	10.821	41.812	9.529	40.552	7.683	0.979	0.001 ^S

5.3. Comparison with Absorption atomic spectroscopy

The procedure described above we analysed on Cr, Cu, Fe and Zn in plant samples. We compared the results obtained with an AAS method. From the Regression analysis the data gave the following linear regression equations. Table (4) shows that y-axis AAS and X-axis ICP-OES techniques. The good concordance among data obtained by both methods is assessed by r-values

mentioned above in Table (3). The figure (3, 4) and (5) shows comparison with linear regression curve of the three parts of Glory Lily plants [27].

In linear regression for AAS and ICP-OES the five elements of leaf, tuber and seed samples is studied. From the Table 3 we observe that the 'r' value for all the elements is greater than 0.90, which indicates there is high relationship among the AAS and ICP-OES in leaf, tuber and seed samples of Glory Lily plant. Except for the element Zn in seed, it is observed that there is less significant relationship. Since, the 'r' value is 0.60. Plants have been the major source of medicine through out human life. Even today this trend continues. More over, Scienfic query and cultual histry indicate, that the modern therapeutic Sciences envolved from the folk remedies. Therefore proper scientific investigations are required to expllore the extract medicinal potential of plants. Oneway to add economic value to such product is by physical, chemical and biological standardization. *Glory Lily* is highly useful for the Anticancer activity and From the systematic analysis of the literature survey reveals only few spectroscopic works and limited investigations has been studied in medicinal plant *Glory Lily* so far. To fill up the lacunae the present investigation has been undertaken.

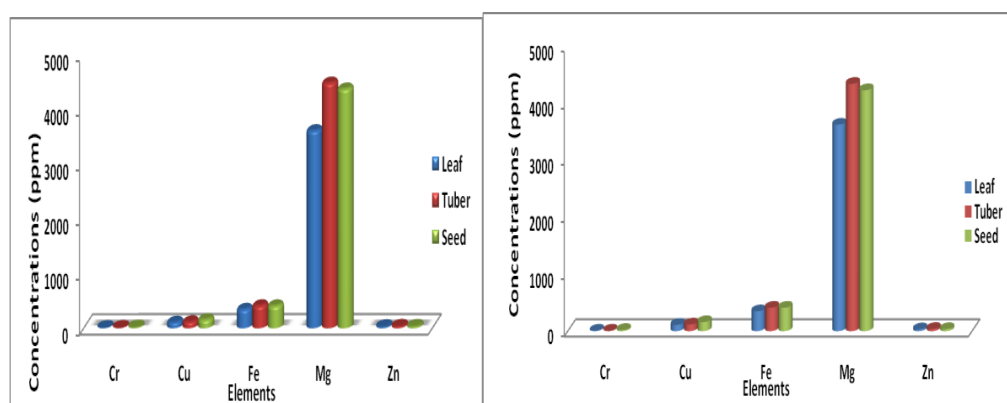


Figure 1

The present work on *Glory Lily plant* parts (Leafs, tubers and seeds) and its relationship with element (Cr, Cu, Fe, Mg, and Zn) status were analysed through Inductively Coupled Plasma Optical- Emission Spectroscopy (ICP-OES), Atomic Absorption Spectrometer, method. The quantitative of elements these parts of plants showed a different distribution in each part. This information could be used to identify which parts of plants are more effective for therapeutic use i.e. the required availability of such element to the particular disease may be chosen from particular part of the plant material. Based on the results obtained in the present work ICP-OES, AAS, technique is suitable for the determination of elemental concentration in plant. The simplicity and versatility of the procedure makes it attractive for its use in the pharmaceutical quality control of medicinal plants.

Elements	Analytical method				r ² values	
	Pairs	ICP-OES		ASS		
		Mean	SD	Mean		SD
Chromium	Leaf	9.120	1.214	9.100	1.306	0.991
	Tuber	10.630	3.424	9.586	3.425	0.998
	Seed	23.694	7.881	22.012	7.528	0.990
Copper	Leaf	110.210	23.929	110.462	23.892	0.999
	Tuber	118.950	29.040	117.646	28.597	0.996
	Seed	161.610	20.869	160.106	20.549	0.996
Iron	Leaf	349.988	17.820	349.794	18.169	0.990
	Tuber	415.740	52.827	412.582	49.662	0.997
	Seed	413.324	64.658	412.402	64.423	0.999
Magnesium	Leaf	3626.22	189.433	3635.034	211.380	0.982
	Tuber	4498.540	491.135	4353.812	544.977	0.987
	Seed	4391.754	480.971	4242.924	526.891	0.986
Zinc	Leaf	34.036	10.821	32.950	11.376	0.992
	Tuber	41.812	9.529	39.632	9.446	0.988
	Seed	40.552	7.683	37.448	9.523	0.997

Conclusion:

The quantitative of elements these parts of plants showed a different distribution in each part. Based on the results obtained in the present work ICP-OES, AAS, technique is suitable for the determination of elemental concentration in plant. The simplicity and versatility of the procedure makes it attractive for its use in the pharmaceutical quality control of medicinal plants.

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