International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Quantitative Estimation of Some Phytochemical and Determination of Metalic Elements from *Pueraria tuberosa* (Roxb. ex Willd.) DC. Tuber

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Abstract: ***In present study quantitative estimation of some phytochemicals and metalic elements were carried out. Quantitative estimation of P. tuberosa tuber was performed to identify the constituents as total alkaloids, total flavonoids, total saponin and total terpenoid by standard method. Quantitative estimation was shown that P. tuberosa tuber powder contained alkaloid: 9.84%, flavonoids: 4.23%, terpenoids: 1.32% and saponin: 4.00%. The ICP-AES analysis of ethanolic soxhlet extracted powdered of P. tuberosa tuber was showed the presence of total 22 elements such as Al, B, Ba, Be, Ca, Co, Cr, Cu, Fe, Hf, K, Mg, Mn, Na, Ni, P, S, Sc, V, Y, Yb and Zr. Quantitative analysis of six major elements was revealed their concentration as Fe= 0.37ppm, Mg= 0.4ppm, Al= 1.56ppm, K= 1.1ppm, Ca= 1.52 ppm and Cr= 0.0071ppm. The elements obtained during XRD analysis were also showed similarity with ICP-AES result. The result was showed that P. tuberosa tuber contain various medicinally important phytoconstituents and metalic elements. Hence this study revealed that the further studies are necessary for identification, isolation and purification of biologically active compounds and various elements.***

Keywords: Phytochemical, *Pueraria tuberosa*, ICP-AES spectrometry, X-Ray Diffraction

1. Introduction

Medicinal plants can serve as a source of novel therapeutic agent due to the presence of diverse bioactive compounds like alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides etc in plants (Hasan *et al*., 2009). These phytocompounds are synthesized by primary or rather secondary metabolism of living organisms. They are widely used in the human therapy, veterinary, agriculture, scientific research, etc (Vasu *et al*., 2009).

The utilization of several medicinal plants as medicine lies in the fact that they contain various phytoconstituents of therapeutic value (Ncube *et al*., 2008). Extraction and characterization of various active phytoconstituents from these green factories have given birth to some highly active profile drug (Mandal *et al*., 2007).

Most of the plants are found to be rich in one or more elements, thus providing a probable relation to the therapeutic action of the medicine (Ray *et al*., 2004; Singh and Garg, 1997). The deficiency & excess of essential micronutrients and trace toxic metals can cause serious effects on health (Reilly, 1980; Underwood, 1977). Plants alter the elemental distribution and metal accumulation to a large extent without toxic effects in response to environmental stress (Toapsern *et al*., 2000). World Health Organization (WHO) recommended that the analytical control of metal elements in medicinal plants is part of quality control, which should establish their purity, safety and efficacy in a number of resolutions (WHO, 1992).

Usually mineral elements form a small portion of total combination of most plant material and of total body weight; however it was of immense physiological significance mostly in body metabolism (Hameed *et al*., 2008). Inductively coupled plasma atomic emission spectrometry (ICP-AES) is normally used to determine trace elements in medicinal herbs. The study investigates on the quantitative phytochemical screening and determination of metalic elements of *P. tuberosa* tuber.

2. Material and Methods

Collection of Plant Material

The plant material of *Pueraria tuberosa* was collected during October-November 2013 from Botha forest area of Buldana District, Maharashtra, India. Plant was identified by using various floras. Herbarium specimen of the plant was deposited at Department of Botany, Shri Shivaji Science and Arts College Chikhli for further references. Fresh tubers were collected, thoroughly washed with water to remove foreign matter; shade dried and then grinds into fine powdered by using mechanical grinder.

Extraction of Plant Sample

The grinded, fine powder of tuber was subjected to extraction by using soxhlet apparatus. About 20 gm of tuber powder was successively extracted with ethanol for 8 hrs. Ethanol extract was filtered through Whatman No. 1 filter paper and the filtrate was collected (crude extracts). Ethanol extract was concentrated, solidified and used for further studies.

Quantitative phytochemical analysis

During previous study, qualitative phytochemical analysis of *P. tuberosa* tuber was revealed the presence of alkaloid, carbohydrates, glycosides, saponin, tannin and phenolic compound, proteins, amino acids, phytosteroids, flavonoids, gums and mucilage, etc. Hence based on qualitative analysis, quantitative tests were carried out of *P. tuberosa*

tuber to identify the constituents as total alkaloids, total flavonoids, total saponin and total terpenoid.

a) Determination of Alkaloid:
About 5 gm *P. tuberosa* tuber powdered 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 a one quarter of the original volume. Conc. ammonium hydroxide was added dropwise to the extract up to the precipitation was complete. This solution was allowed to settle. The precipitated was collected, washed with dilute ammonium hydroxide and filtered. The remaining residue was alkaloid, completely dried and finally weighed (Harborne, 1973).

b) Determination of Flavonoid:
Weighed 10 gm of *P. tuberosa* tuber powdered and repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. This solution was filtered through whatman filter paper No 42. The filtrate obtained was transferred into the crucible and evaporated till dryness over water bath, weighed up to a constant weight obtained (Boham and Kocipai- Abyazan, 1974).

c) Determination of Terpenoids: About 10 gm of *P. tuberosa* tuber powdered was taken and soaked in alcohol for 24 hours. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids (Ferguson, 1956).

d) Determination of Saponin:
About 10 gm of *P. tuberosa* tuber powdered was put into a conical flask and then 50 ml of 20% aqueous ethanol was added. The sample was heated with continuous stirring at 55^0c 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 90^0c. The concentrate obtained was transferred into a 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 the saponin percentage was calculated (Obadoni and Ochuko, 2001).

Inductive Coupled Plasma – Atomic Emission Spectrometry (ICP-AES):
Spectrometric analysis of ethanol extracted powdered of *P. tuberosa* tuber 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 (30rpm), Coolant Flow (12.00 l/min), Auxiliary Flow (1.00 l/min), Nebulizer Flow (0.80 l/ml). The software used was Smart Analyzer Vision 5.01.0921 and the detector was used as charge coupled device (CCD). Powdered sample was analyzed in triplicate and mean values of concentrations for each element determined.

XRD (X-Ray Diffraction) study by powder method:
Dried raw powder of *P. tuberosa* tuber 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: 25^0c, angle range: 5^0-120^0, voltage: 40 KV, Electron probes current range: 40mA. Leptos and EVA software was used for measurement and analysis.

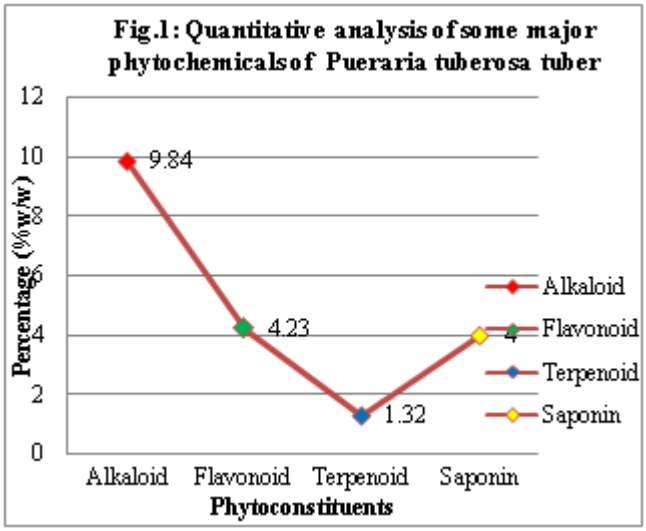
3. Results and Discussion

Quantitative estimation of some major phytochemicals:
On the basis of preliminary phytochemical test result obtained, quantitative estimation of *P. tuberosa* tuber powdered was carried out by standard methods for some major phytochemicals such as alkaloid, flavonoid, saponin and terpenoids. Comparative quantitative estimation was shown that *P. tuberosa* tuber powdered was contained alkaloid: 9.84%, flavonoids: 4.23%, terpenoids: 1.32% and saponin: 4.00% which were shown in table 1.

Table 1: Quantitative estimation of some major phytochemicals of *Pueraria tuberosa* tuber

Sr.No.	*Plant sample*	*Alkaloid Extraction: Each 5gm*		*Flavonoids Extraction: Each 10gm*		*Terpenoids Extraction: Each 10gm*		*Saponin Extraction: Each 10gm*	
		(gm)	(%)	(gm)	(%)	(gm)	(%)	(gm)	(%)
1	*Pueraria tuberosa* tuber	0.49	9.84	0.423	4.23	0.132	1.32	0.400	4.00

The percentage of alkaloid, flavonoids, terpenoids and saponin obtained during quantitative estimation of *P. tuberosa* tuber powdered were represented in figure 1.



Qualitative and quantitative estimation of elements of *P. tuberosa* tuber by ICP-AES technique:

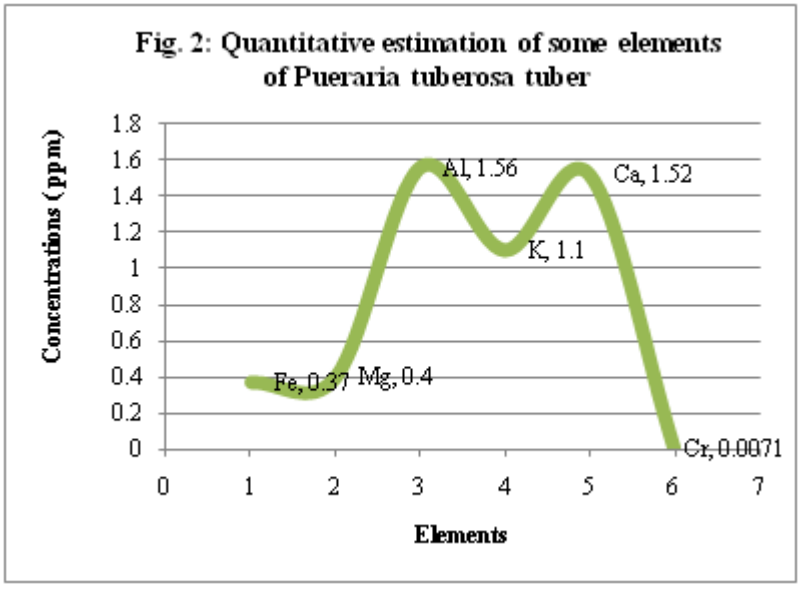
The ICP-AES technique was used for qualitative estimation and the determination of concentration of some major elements from ethanolic soxhlet extracted powdered of *P. tuberosa* tuber. Ethanolic soxhlet extracted powdered of *P. tuberosa* tuber was revealed the presence of total 22 elements such as Al, B, Ba, Be, Ca, Co, Cr, Cu, Fe, Hf, K, Mg, Mn, Na, Ni, P, S, Sc, V, Y, Yb and Zr.

Among these elements, the quantitative analysis was carried out of six major elements such as Fe, Mg, Al, K, Ca and Cr. The concentration of these six major elements was calculated as Fe= 0.37%, Mg= 0.4%, Al= 1.56%, K= 1.1%, Ca= 1.52 % and Cr= 0.0071% 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	*Pueraria tuberosa* tuber	Al, B, Ba, Be, Ca, Co, Cr, Cu, Fe, Hf, K, Mg, Mn, Na, Ni, P, S, Sc, V, Y, Yb, Zr	0.37	0.4	1.56	1.1	1.52	0.0071

The concentration of six major elements such as Fe, Mg, Al, K, Ca and Cr calculated during quantitative estimation of *P. tuberosa* tuber by ICP-AES technique were represented in figure 2.



XRD (X-Ray Diffraction) analysis by powdered method:
The XRD technique was used to authenticate the presence of various nanoparticles in *P. tuberosa* tuber. The XRD pattern for *P. tuberosa* tuber was depicted in figure 3. It was consisted of broad hump but of less marginal intensity height. It was directly reflected the less degree of crystallization. It was comprised prominent diffraction peaks at 2θ-positions such as 11.22, 15.21, 22.06 and 24.64° results in d-spacing value 7.88, 5.86, 4.02 and 3.6 Å; which confirms the presence of Al, K, Ni and Ca elements respectively. The elements obtained during such analysis were showed similarity with Inductively Coupled Plasma Atomic Emission Spectroscopy result.

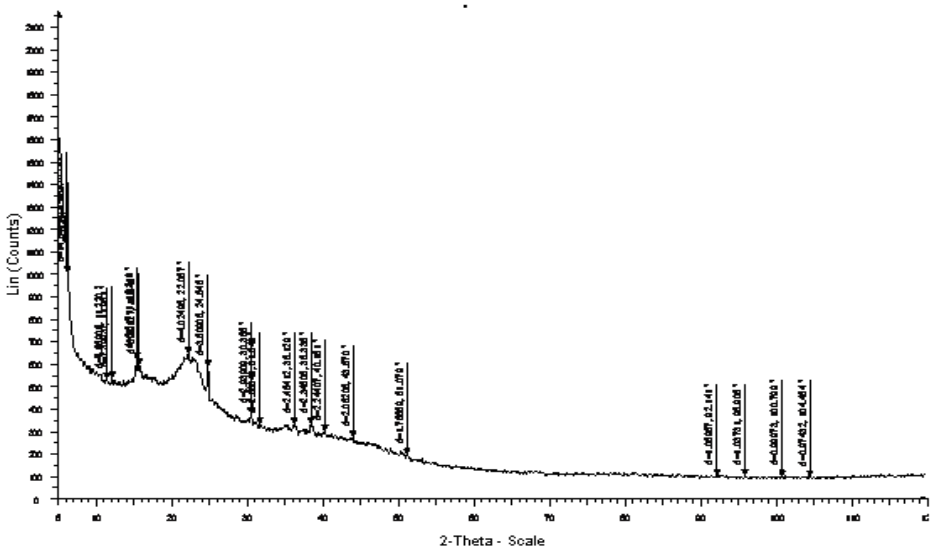


Figure 3: XRD spectrum of *Pueraria tuberosa* tuber powdered

4. Conclusion

The present study on *P. tuberosa* tuber quantitatively revealed the presence of alkaloid, flavonoids, terpenoids and saponin which contribute to medicinal value of plant. The ICP-AES technique was showed the presence of different elements in *P. tuberosa* tuber that used to cure various diseases. The element obtained during XRD analysis also showed similarity with ICP-AES result.

Hence this study revealed the identification, isolation and purification of biologically active compounds are necessary for their further studies.

5. Acknowledgments

We are grateful to SAIF, IIT, Bombay, Powai, Mumbai for providing the ICP-AES facility for elemental analysis. Authors are also thankful to Institute Instrumentation Centre, IIT, Roorkee, Uttarakhand, India for providing the X-Ray Diffraction analysis facility.

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