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Research Article

### IMPORTANCE OF QUALITATIVE ANALYSIS IN ENSURING QUALITY OF HERBAL MEDICINES W.R.T. INDIAN KUDZU (PUERARIA TUBEROSA DC.)

Mahesh TS<sup>1\*</sup>, Shreevidya M<sup>2</sup>, Ravishankar AG<sup>3</sup>

<sup>1</sup>Assistant Professor, Department of Post Graduate Studies in Dravyaguna Vijnana, Alva's Ayurveda Medical College Moodbidri, Karnataka, India.

<sup>2</sup>Lecturer, Department of Post Graduate Studies in Rasashastra, Alva's Ayurveda Medical College Moodbidri, Karnataka, India.

<sup>3</sup>Professor, Department of PG studies in Shalya tantra, Alvas Ayurveda Medical College Moodbidri, Karnataka, India

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\*Corresponding Author: **Mahesh T S**

Assistant Professor, Dept Of Dravyaguna, Alva's Ayurveda Medical College, Vidyagiri, Moodbidri, - 574227 D.K. District, Karnataka, India

Email: dtsmv2002@gmail.com

#### ABSTRACT

*Pueraria tuberosa* DC. known as Indian Kudzu is one of the most important drugs that have been used in the system of Ayurveda from time immemorial. The references in the Sanskrit texts depict its use as a vegetable in the past which is also seen even today. Today the tender root tubers are being sold as vegetables in some part of India. Other than nutritional effect, the plant tuber is known to produce the aphrodisiac effect for which it is mainly regarded in the system of Ayurvedic medicine. Thus it is very necessary to have the qualitative analysis to ensure better effect either in the form of nutrition through food or medicine.

**Keywords:** Ayurveda, *Pueraria tuberosa* Dc, Nutritional, Indian Kudzu, Phytochemicals and Herbal medicina

#### INTRODUCTION

Plants have been the indispensable materials that have been used by man right from the point of creation in different forms food, clothing, shelter, medicine, etc. Its use especially in the field of healing has been an immense sense of relief for all living organisms. The systems like Ayurveda have used the plants for its majority of preparations for the purpose of treatment.

Plants that are identified to be of the same species also tend to produce different effects. The main reason may be the variation in the environmental factors in which it is grown or collected. Thus to ensure quality the analysis of the phytochemicals is necessary. The plant Indian Kudzu is referred to as 'Kandashaaka' which denotes its use as a vegetable in the past. In Ayurveda it is indicated as Balya (Strength promoting), Brimhana (Nutritive) and Vaajikarna (Aphrodisiac) by Charaka<sup>1</sup>. Sushruta enumerated it under Vallipanchamoola<sup>2, 3</sup>. The Ayurvedic Pharmacopoeia of India (API) has laid down certain values to ensure the purity, strength and the Identity of medicinal plants used in Ayurveda.

The values for *Pueraria tuberosa* DC. mentioned are Foreign matter (not more than 2%), Total Ash (not more than 17%), Acid insoluble Ash (not more than 4.5%), Alcohol soluble extractive (not less than 4%) and Water soluble extractive (not less than 24%)<sup>4</sup>.

#### MATERIALS AND METHODS

##### Collection of sample:

The sample of the drug namely *Pueraria tuberosa* Dc. tubers were obtained from Alva Pharmacy, an Ayurvedic GMP certified drug manufacturing company.

##### Place of Work:

The Phytochemical study of the drug was carried out in the Department of PG Studies in Dravyaguna Vijnana Laboratory of Alva's Ayurveda Medical College, Moodbidri D.K.

##### Procedure:

The present study about the drug *Pueraria tuberosa* Dc. comprises of five parts namely: Analysis of Physical standards, Extractive values in various solvents, Preliminary Phytochemical studies, Ash analysis and Qualitative analysis.

The analysis consists of tests namely Determination of moisture content, Total Ash percentage, ash value, acid insoluble ash, water insoluble ash and pH value. For this, the air dried samples of *Pueraria tuberosa* Dc tubers that are finely powdered are taken. For each test 5 gm of the drug is taken and the procedures as mentioned in Ayurvedic Pharmacopoeia of India (API) are followed. The obtained values are tabulated in the table No. 1 below. These values are useful in knowing the Strength and Identity of the given drug in gross manner.

The extractive values in various solvents provide the information regarding the percentage of the content that gets soluble in different media like water, Alcohol, etc. It also

provides sufficient amount of data for the purpose of understanding the purity and Identification of the drug too. It helps to understand the best media for extracting and obtaining the maximum benefit from the substance. In the study soluble extractive values of Water, Ethyl alcohol, Methanol, Chloroform, Petroleum ether and Acetone are analyzed and tabulated in the table No. 2 below.

The preliminary phytochemical studies are essential to know the basic constituents present in drug. It helps to get an idea about the rich varieties of organic substances present in the drug that may or may not be instrumental in bringing out a drug action. These constituents can be a mixture or a single chemical substance. Single chemical substances are alkaloids, enzymes, proteins, Sugars and Vitamins. Fixed oils, fat, volatile oils, oleo-resins, oleo-gum, resins are the mixed chemicals. In the present study the samples of the trial drug is subjected to preliminary screening for the detection of various plant constituents.

The extracts obtained from various solvent extractions like Methanol, Ethanol, Chloroform, Water and Petroleum ether. These extracts evaporated on a waterbath and after cooling each of them were collected in clean containers which are subjected into various tests. The obtained results are noted and tabulated in the Table No. 3.

The air dried powdered drug was taken in a crucible and heated in an electric Bunsen to make the ash. Then it was diluted with distilled water, boiled and filtered. The filtrate is used for the testing the various contents of the ash. The obtained results are noted and tabulated in the Table No. 4.

The qualitative analysis consisted of Chromatographic fingerprinting of alcoholic extract of *Pueraria tuberosa* Dc. was performed by HPTLC. 1 g of the vidari sample 1 and 2 was extracted with 10 ml ethanol 2 times by cold percolation for 48 hrs. 10 and 20  $\mu$ l of the alcohol extracts were applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in toluene – ethyl acetate – formic acid (1:0.7:0.1) and the developed plates were visualized and scanned under UV 254, 366 and after derivatisation in vanillin-sulphuric acid spray reagent at 620 nm and densitometric scan were recorded.

## RESULTS AND OBSERVATION

### Analysis of Physical Standards:

On observing the values that are tabulated in Table No. 1 it is observed that the tuber of the plant contains very low value of ash. The pH value obtained suggests that it tends to produce an acidic solution with water. On comparison with the standard values mentioned in the API, the values obtained appeared to be of much lower.

### Extractive values in various solvents:

From the extractive values obtained (Table No. 2.), it can be observed that the tuber gives maximum extract with water as solvent while it gives the least extract with Ethanol and Acetone as solvent. On comparison with the extractive values mentioned in API the pattern of the values are similar but the value obtained appeared to be of much lower.

### Preliminary Phytochemical Studies:

The results thus obtained (Table No. 3) show that the tuber consists of Reducing sugars, saponins, non-reducing sugars, phenols and steroids.

### Ash Analysis:

Analysis of Ash gives an idea of the inorganic content of the plant. In the present study the analysis of Ash of the tuber showed the presence of Sodium content only (Table No. 4).

### Qualitative analysis:

The knowledge of essential chemical constituents in a medicinal plant and detection of their presence in a preparation can be readily ascertained by chromatography, particularly thin layer chromatography. It is really a competent tool to standardize a drug and even a semi quantitative assessment to the chemical constituents of the preparation is also possible by this technique. High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements. The Densitometric scan at 254 nm and 366 nm of *Pueraria tuberosa* DC sample's alcohol extract showed 3 (plate no. 1) and 17 peaks (plate no. 2) respectively as mentioned in Table No. 5.

## DISCUSSION

On analysis of the physical standards we observe that the tuber of the plant is having an acidic pH and that it has a very low percentage of Ash (Table No.1). This suggests that the inorganic contents in the tuber are very less. This is also supported by the observations with respect to ash analysis (Table No. 4) too which suggests only the presence of Sodium in the ash. Moreover the comparison in the table also suggests that the sample is quite inferior in reference to the standards.

Among the various extractive values obtained (Table No.2), the water extract contained the highest value compared to others. This shows that the preparations like decoctions where water is the base will be having an utmost effect and medicinal value. The pattern is undoubtedly the similar to that of the standard values but the values are suggestive of its inferior quality when compared.

According to the results that are noted in the table No. 3 the sample of the drug *Pueraria tuberosa* DC consists of Reducing sugar, Non-reducing sugar, Saponin, Phenol, Steroids and Starch. This suggests that the drug possesses all those chemicals that can result in the increase of capacity to perform physical activity. This is because sugar or carbohydrates are the best energy providing constituents in the substance which help in supplementing the energy required for the physical activity.

As per the tests conducted the  $R_f$  value 0.54 is the value corresponding to the compound Puerarin which is observed in the sample. Puerarin is usually considered as the marker compound or the major chemical constituent of the plant.

From the above aspect it can be considered that the sample which is obtained for the study is undoubtedly a genuine sample of *Pueraria tuberosa* DC. but the quality is of an inferior one. Thus its expected effect in the form of medicine

or nutrition can surely be noticed but not to the extent of a superior quality sample.

### CONCLUSION

The obtained sample is found to be genuine. It can also be concluded that the plant is suitable to enhance the capacity to perform physical activity as indicated in the texts of Ayurveda for it contains the necessary chemicals and nutrients which can perform the same. But due to its inferior quality observed on comparison with the standard values, it is concluded that the sample will have a lesser effect than expected. Thus the analysis will surely give an idea of the nature, strength and purity of the plants such that it can result in proper utilization. The analysis methods also help one to substantiate the uses of the plant as a nutrient and a potent medicinal substance.

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**Table 1: Comparison of the documented and obtained values of Physical standards of Vidaarikanda.**

Sl. No.	Physical Standard	% Obtained Value	Values in AFI
1.	Moisture Content	2.2	5.21
2.	Percentage of Total Ash	5.38	Less than 17
3.	Percentage of Acid insoluble Ash	0.36	Less than 4.5
4.	Percentage of Water Soluble Ash	3.88	Not available
5.	pH value	5.5	Not available

**Table 2: Comparison of the documented and obtained values of Extractive values in various solvents of Vidaarikanda.**

Sl. No.	Solvent for Extract	% Obtained Value	Values in AFI
1.	Water	8.8	Less than 24
2.	Ethanol	0.4	Less than 4
3.	Methanol	1.4	Not available
4.	Chloroform	0.6	Not available
5.	Petroleum ether	1.0	Not available
6.	Acetone	0.4	Not available

**Table 3: The Results of the Chemical Analysis**

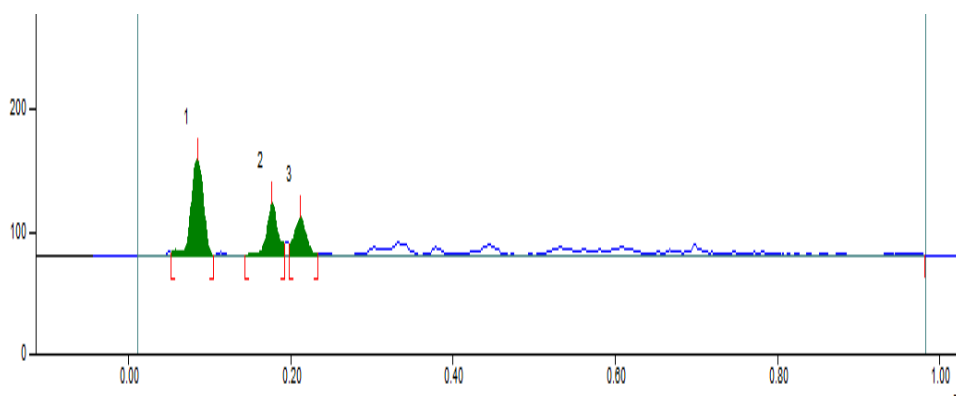
Sl. No	Name of the Tests	Observation	Results
1	Biuret test for Proteins	No colour change	Absent
2	Carbohydrate test		
	Benedict's test (Reducing sugar)	Green Precipitate	Present
	Fehling's test (Non-reducing sugar)	Red Precipitate	Present
3	Tannins	Brown colour of FeCl <sub>3</sub>	Absent
4	Saponin (Foam Test)	Persisting Foam Present	Present
5	Test for Flavanoids	No Colour Change	Absent
6	Test for Phenols	Dark Brown colour seen	Present
7	Steroids		
	Liebermann-Buchard's test	Green colour precipitate	Present
	Salkowski's test	Red colour precipitate	Present
8	Alkaloids (Mayer's test)	No precipitate found	Absent
9	Triterpenoides	No colour change	Absent
10	Starch	Solution turns blue	Present

**Table 4: On the basis of the observations the results of Ash analysis**

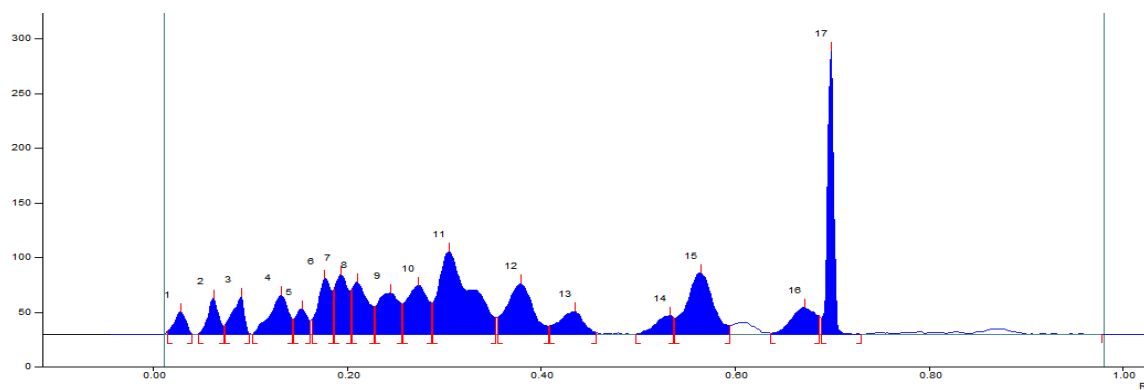
Components	Pueraria Tuberosa
Carbonates	Negative
Flourides	Negative
Chlorides	Negative
Sulphate	Negative
Chromate	Negative
Phosphate	Negative
Potassium	Negative
<b>Sodium</b>	<b>Positive</b>
Aluminium	Negative
Calcium	Negative

**Table 5: The R<sub>f</sub> values of Pueraria tuberosa DC. obtained on Densitometric scan at 254nm and 366nm.**

Sl. No.	254 nm	366 nm
1.	-	0.02
2.	0.05	0.05
3.	-	0.07
4.	-	0.10
5.	0.14	-
6.	-	0.15
7.	-	0.16
8.	-	0.19
9.	0.20	-
10.	-	0.21
11.	-	0.23
12.	-	0.26
13.	-	0.29
14.	-	0.36
15.	-	0.41
16.	-	0.50
17.	-	0.54
18.	-	0.64
19.	-	0.69



**Plate No. 1: HPTLC Densitometric scan of Alcohol extract of *Vidaarikanda* 20 µl at UV 254 nm**



**Plate No. 2: HPTLC Densitometric scan of Alcohol extract of *Vidaarikanda* 20 µl at UV 366 nm**

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