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Pharmacognostic, Phytochemical and Physicochemical Investigation of *Pueraria tuberosa* (Roxb. ex Willd.) DC. Tuber

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Abstract: ***The present investigation was carried to determine the pharmacognostic characters and the presence of different phytochemical compounds of Pueraria tuberosa tuber. Phytochemical analysis of tuber was carried out by using series of solvents such as petroleum ether, chloroform, ethanol and acetic acid by soxhlet extractor. Qualitative phytochemical analysis showed the presence of carbohydrates, glycosides, tannins and phenolic compound, flavonoids, steroid, triterpenoids, alkaloid, proteins, amino acids, gums and mucilage, etc. Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, extractive value and moisture content were determined. Qualitative phytochemical and physicochemical analysis is essential for standardization and in determination of medicinal value of crude drugs.***

Keywords: *Pueraria tuberosa*, Bhuikohla, Botha forest, Indian tribes.

1. Introduction

Medicinal plants are being used by mankind as a source of medicine since immemorial time. Medicinal plants are generally known as "Chemical Goldmines" as it contain a variety of natural chemicals, which are acceptable to human being and animal systems [(1)]. A medicinal plant possesses curative properties due to the existence of various complex chemical substances of different composition known as secondary metabolites [(2)].According to World Health Organization more than 80% of the World's population depends on traditional medicine for their primary healthcare requirements [(3)]. Approximately 75% of the medicinally useful plant species produce in wild condition [(4)]. *Pueraria* tuber is sweet in taste and used in indigenous system of Indian medicine as antirheumatic, aphrodisiac, tonic for strength, diuretic and galactogogue [(5)]. Tubers are consumed as supplementary food and for birth control by assured Indian tribes [(6)].

Pueraria tuberosa belongs to family Fabaceae, large herbaceous perennial twiner with tuberous root. Leaflets are 10-16 X 8-14 cm, terminal one is broadly ovate, apex acuminate, base cuneate, laterals ovate-oblong, inequilateral, base truncate. Flowers are many, blue or purplish blue in lax, axillary racemes. Pods are linear, flat, constricted between the seeds, clothed with long, silky, brown hairs. Seeds are 3-10, reddish-brown, ellipsoid-oblong.

2. Materials and Methods

a) **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. Fresh tuber were collected, thoroughly washed with water to remove foreign matter; shade dried and then grinds into fine powdered by using mechanical grinder.

b) **Pharmacognostic studies:** During pharmacognostic study, a free hand thin section of tuber was taken. After staining and mounting, permanent slide was observed under microscope. Microphotograph of the section was made using digital camera [(7)].

c) **Extraction of plant drug:** The grinded, fine powder of tuber was subjected to extraction by using soxhlet apparatus. Tuber powder was successively extracted with petroleum ether, chloroform, ethanol and acetic acid. Each time before extracting with next solvent, the powder residue was dried properly. The percentage yield of different extract and their colour were reported (table.1). Extract obtained in each solvent was separately concentrated, solidified and used for preliminary phytochemical analysis [(8, 9, 10, 11 and 12)].

d) **Phytochemical evaluation:** For preliminary phytochemical evaluation each extract of tuber was subjected to various qualitative chemical tests and determine the presence of different phytoconstituents like alkaloids, carbohydrates and glycosides, steroids, saponins, proteins and amino acid, phenolic compound, tannins and flavonoids etc.(table. 2).

e) **Physicochemical analysis:** [(13, 14 and 15)] Physicochemical analysis of powder was carried out by using different parameters such as total ash value, acid insoluble ash, water soluble ash, extractive value and moisture content. (Fig.3)

f) **Fluorescence analysis:** [(16 and 17)] Fluorescence analysis was carried off all extracts as well as powder by using different solvents and observed in visible rays and UV rays (for both short & long wave length). (Table.3)

3. Result and Discussion

Pharmacognostic Study

Figure 1: T. S. *Pueraria tuberosa* tuber

Where, A: Cork, B: Cortex, C: phloem, D: Cambium, E: Secondary xylem, F: Medullary rays, G: Primary xylem. The transverse section of *Pueraria tuberosa* tuber is circular in outline. The outer cork region containing 18-24 layers of radially arranged, rectangular, elongated and thin walled cells of dark reddish brown colored. Next to cork is cortex containing 8-14 layers of tangentially elongated, thin walled parenchyma cells. Endodermis is indistinct with secondary growth. Pericycle forms moderately thick walled patches of stone cells. Calcium oxalate crystals and spherical or oval shape starch grains are scattered throughout the parenchymatous cells of cortex.

In vascular tissue, secondary phloem form large zone, as it comprises sieve tubes, companion cells, phloem parenchyma and number of tangential phloem fiber bands. Parenchymatous cells form multilayer medullary rays and get divided into number of patches. Cambium is followed by secondary xylem, it consist of xylem vessels, xylem parenchyma dispersed amongst the conjunctive tissue. Numbers of primary xylem bundles are present towards centre. Pith is absent in centre.

Table 1: Successive solvent extract shows colour and yield percent of *Pueraria tuberosa* tuber

Sr. No.	Solvent extract	Colour	Yield percent
1	Petroleum ether	Light yellowish	6.20%
2	Chloroform	Light brown	5.40%
3	Ethanol	Light brown	17.2%
4	Acetic Acid	Yellow	16.6%

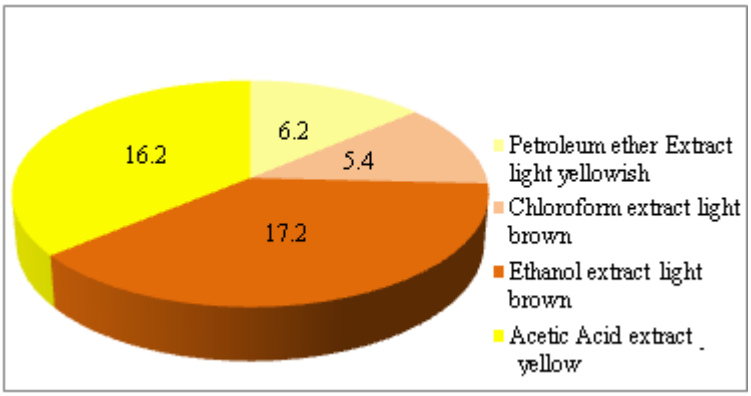

Figure 2: Successive extractive value of *Pueraria tuberosa* tuber

Table 2: phytochemical analysis of *Pueraria tuberosa* tuber extracts

Sr. No.	Test For Phytochemical	Test	Pet. ether extract	Chloroform extract	Ethanol extract	Acetic acid extract
I	Alkaloids					
1		Dragandorff's Test	+	-	+	+
2		Hager's Test	+	+	+	-
3		Mayer's Test	-	+	-	-
4		Wagner's Test	+	+	+	+
II	Carbohydrates					
1		Fehling's Test	+	-	+	-
2		Molisch's Test	-	+	+	-
3		Benedicts Test	+	-	+	+
III	Glycosides					
1		Borntrager's Test	+	+	+	-
2		Legal's Test	+	+	+	+
IV	Saponin					
1		Foam Test	-	+	+	-
V	Tannin and phenolic compound					
1		Ferric chloride Test	+	-	+	-

2		Lead acetate Test	+	-	+	+
VI	Proteins					
1		Millon's Test	+	-	+	-
2		Biuret's Test	+	-	+	-
VII	Amino Acid					
1		Ninhydrin Test	+	-	-	+
VIII	Phytosteroids					
1		Lieberman- Burchards Test	+	+	+	+
2		Salkowski Test	+	+	+	+
IX	Flavonoids					
1		Alkaline reagent test	+	+	+	+
X	Gums and Mucilage					
1		Alcohol test	+	-	+	-
XI	Fixed oil and Fats					
1		Soap test	-	-	-	-

Where, + = present and - = absent.

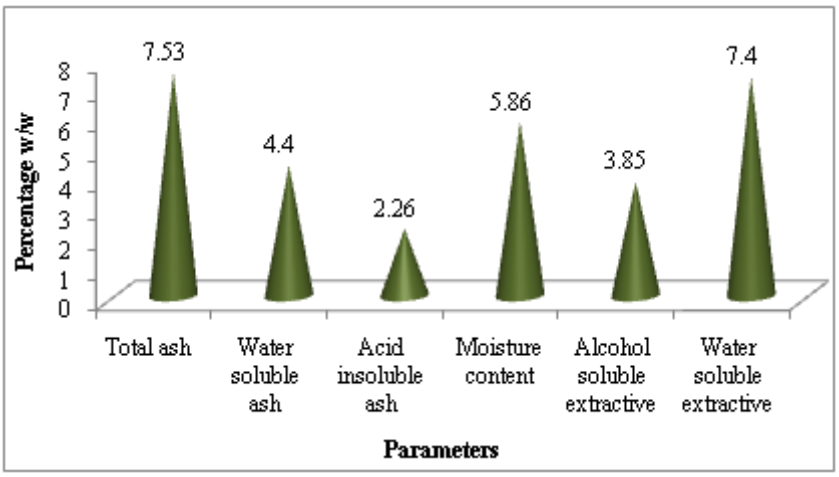

Figure 3: Physicochemical parameters of *Pueraria tuberosa* tuber.

Table 3: Fluorescence analysis of different extracts and powder of *Pueraria tuberosa* tuber

Sr. No.	Extract/ Powder	Visible light	U.V. light	
			Short wave (254nm)	Long wave(366nm)
1	Pet. ether extract	Light yellowish	Light green	Light brown
2	Chloroform extract	Light brown	Green	Blackish brown
3	Ethanol extract	Light brown	Light green	Light brown
4	Acetic acid extract	Yellow	Light brown	Brown
5	Powder as such	Light brown	Brown	Light blackish brown
6	Powder + D. water	Light brown	Dark brown	Brown
7	Powder + Conc.HCL	Reddish brown	Dark brown	Light brown
8	Powder+ Conc.H_2SO_4	Reddish	Reddish	Reddish black
9	Powder + Methanol	Light brown	brown	Brownish black
10	Powder + $Fecl_3$	Blackish green	Light brown	Blackish
11	Powder + Picric acid	Yellowish black	Greenish	Black brown
12	Powder + NaOH	Light green	Light green	Brown

During phytochemical analysis ethanol showed higher percentage yield (17.2%) than acetic acid (16.6%), (4.3%) petroleum ether (6.20) and chloroform (5.40%). Phytochemical analysis revealed the presence of carbohydrates, glycosides, tannins and phenolic compound, flavonoids, steroid, triterpenoids, alkaloid, proteins, amino acids, gums and mucilage, etc. Different physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, moisture content, alcohol soluble extractive value and water soluble extractive value were determined as 7.53%, 4.4.%%, 2.26%, 5.86%, 3.85% and 7.40% respectively. Fluorescence analysis of powder and its various extracts was carried out under visible and UV light (at 254 nm and 366 nm) revealed the presence of various constituents in the tuber.

4. Conclusion

Presence of different phytochemicals confirmed it's used as antirheumatic, aphrodisiac, tonic for strength, diuretic and galactogogue properties and as a supplementary food for Indian tribes. Physicochemical parameters help in determining pharmacopoeial standard and identification of impurities in crude drugs. Fluorescence analysis helps to find out adulteration of crude drugs.

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