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## PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON TUBER OF *PUERARIA TUBEROSA*

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and pharmacological studies

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**ABSTRACT:** Medicinal herbs have an important role in illness prevention, and their promotion and usage are compatible with all current disease preventive techniques. *Pueraria tuberosa*, widely known as kudzu, is a climber with tuberculated woody stems. It has exhibited Cardio-tonic, Aphrodisiac, Abthypoglycemic Galactogogic, and anti-lipidemic characteristics Daidzin, Puerarin, Puerarone, Genistein, Puetuberosanol, Tuberosin, Tuberosin and Puerarin 4',6' diacetate are all found in high amounts in the tubers of *Pueraria tuberosa*. Numerous bioactive phytochemicals, mostly Isoflavonoids such as Puerarin, Genistein, Daidzein and Tuberosin, were soon identified in the tuber. In the present study, three types of studies, Pharmacognostical Studies, Phytochemical Studies, and Pharmacological Studies, have been done using methanolic extracts of tuber powder; Pharmacognostical Studies include organoleptic evaluation, microscopical evaluation, and physical evaluation. In pharmacological studies, analgesic activity was recorded using the Hot plate Method. A suitably designed transverse section of the tuber was studied and different tissue organizations were viewed at 10x and 40x. Foreign organic matter, ash values, extractive values, moisture content, swelling index, and foaming index were all measured, analyzed and recorded. In accordance of increasing polarity, successive Soxhlet extractions were done with different solvents, e.g., the % yields of petroleum ether, chloroform, ethanol and distilled water were computed. Alkaloids, carbohydrates, phenolic compounds, flavonoids, fixed oils and other substances were identified throughout the testing.

**INTRODUCTION:** Medicinal plants have been used by mankind as a source of medicine since immemorial time.

Medicinal plants are commonly referred to as "Chemical Goldmines" because they contain a wide range of natural compounds that are safe for human and animal systems <sup>1</sup>.

*Pueraria tuberosa*, Fabaceae whose tuber is widely used in ayurvedic and local medicine to cure & treat a variety of diseases & disorders. *Pueraria tuberosa*, often known as kudzu, is a climber woody tuberculated stem included in the Indian Ayurvedic Pharmacopoeia as Vidari.

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It is mature in the autumn with 20°C temperature, high moisture soil and full sunlight, showing sensitivity against mechanical disturbances. With particularly large tuberous roots, it is a climbing, coiling, and trailing vine. The tubers are globose or pot-shaped, measuring about 25 cm across, with white, starchy and faintly sweet inside.

The hairless leaflets (18 ×16 cm) are egg-shaped, with a circular base and uneven sides and the leaves are trifoliate and alternating. Blue or purplish blue bisexual Flowers are around 1.5 cm in diameter. The fruit pods are linear, measuring 2 to 5 cm in length and densely packed between the seeds<sup>2</sup>. Their reddish brown hair is silky and bristly. The number of seeds varies from three to six.

- It strengthens the body, improves immunity, and enhances muscle mass.
- Dysuria, cough, rheumatism, erysipelas, and malarial fever are all treated by kudzu. It is commonly used in the treatment of skin illnesses that produce discoloration, and it helps to slow down the aging process.
- They're beneficial for easing constipation.
- Many Ayurvedic medicine use kudzu as a component like Planetary herbals(Kudzu), Vidhari Kand, Kudzu Root extract capsule, GE GEN TANK PIAN, Nesh mukti, Kudzu Root Extract Powder and Ayushkar Vidarikand powder<sup>3</sup>.

Traditional medicine is estimated to be utilised by about 80% of the world's population for basic health care, according to the WHO<sup>4-5</sup>. Organoleptic, macroscopic, and microscopic examination is the initial stage toward establishing the identification and purity of pharmaceuticals and is required before undertaking subsequent tests<sup>6</sup>. Pharmaceutical companies can use phytochemical research of medicinal plants to help them design new drugs. HPLC is a dependable, safe and sensitive approach for quality control of medicinal components. It's a great resource for ayurvedic formulation standardization. HPLC was used to standardize ayurvedic medicines such as dhanyapanchak kwathaurna, guduchyadiyana kwathaurna and stanyajanana kashayaurna<sup>7-8</sup>.

## MATERIAL AND METHOD:

**Collection of Plant Material:** The plant material of kudzu; *Pueraria tuberosa* collected from Herbal & Medicinal Garden Haridwar, Uttarakhand. The tuber of *Pueraria tuberosa* linn. (family-Fabaceae) authenticated by Dr. Devendra Kumar Panday; assistant professor, Domain of Botany, Department of Biotechnology & Dr. Udai Chand Agrahari, assistant professor, Domain of Pharmacognosy, Department of Pharmaceutical Science, Lovely Professional University (LPU) Jalandhar- Delhi G.T. Road (NH-1), Phagwara, Punjab, India-144411 at 16 December 2015.

**Analgesic Activity:** Analgesic evaluation was performed by using the Hot-Plate method at S.D. College of Pharmacy and Vocational Studies, Muzaffarnagar under the CPCSEA, Registration No. SDCOP & VS/C PCSEA/ IAEC/016/M. PHARM/2016 following the below-mentioned procedures.

**Animals:** The impact of the test medication on hot plate-generated seizures was studied in mice weighing 20-30 g. Because the estrus cycle affects the seizure threshold, female mice were removed from the study. The animals were taken from the S. D. College of Pharmacy and Vocational Studies' animal house in Muzaffarnagar, Uttar Pradesh, India. The Institutional Animal Ethical Committee gave their consent for the use of 30 animals for scientific purposes. Animals were housed in polypropylene cages (3 per cage) with dust-free rice husk as a bedding material in a laboratory setting with a temperature of 25±2°C, the humidity of 60%–10%, and a 12-hour light/dark cycle, as per CPCSEA recommendations. They were given balanced food and water and permitted a week to acclimate to the laboratory surroundings before being subjected to experiments. Each group consists of six animals that are employed in the research activities mentioned. The chemical was now given at a fixed dose of 50 mg/kg orally (Tween80) every day at a set time for 10 days. Anticonvulsant and neurotoxic effects were evaluated at 30 minutes and 2 hours after delivery.

## Pharmacognostical Studies:

**Macroscopic and Microscopic Analysis:** The authenticated plant *Pueraria tuberosa* was coarsely powdered with the help of a mechanical grinder.

The coarse powder was stored in an airtight container for Pharmacognostical studies and successive extraction. It was carried out using sense organs to assess colour, scent, size, form, taste and specific properties such as touch, fracture, texture, etc. It was carried out by using the fresh *Pueraria tuberosa* tuber parts for section cutting were soaked in chloral hydrate for few minutes in order to make them soft, and then the cross sections were prepared by taking free hand section.

The thin section of the stem, fruit and flower were selected and stained with chemical dye (colorant), safranin, iodine solution, Sudan red III, and mounted with the help of glycerin. The finally prepared slides were then captured through a compound microscope and labeled.

**Powder Microscopy:** It was done by using the fine powder of the fruit parts of *Pueraria tuberosa*. The powder was treated with chloral hydrate for a few minutes. Further, this powder drug was transferred on a microscopy slide with a drop of glycerin to prevent drying, a cover-slip was then placed on the powdered drug, and finally, the slide was observed under a microscope. Different cells, tissues, and their arrangement were observed and identified from the microscopic slide.

**Extraction of Plant Drug:** The grinded, fine powder of tuber was subjected to extraction by using the Soxhlet apparatus. In that order, tuber powder was extracted using petroleum ether, chloroform, ethanol and acetic acid. The extract obtained in each solvent was separately concentrated, solidified, and used for preliminary phytochemical analysis.

**Physico-chemical Investigation:** Foreign organic matter, ash value (total ash, acid insoluble ash and water-soluble ash), extractive value, moisture content (loss on drying), swelling index, and foaming index were all investigated.

**Phytochemical Evaluation:** To identify the presence of diverse phytoconstituents such as alkaloids, carbohydrates and glycosides, steroids, saponins, proteins and amino acids, phenolic compound, tannins and flavonoids and other phytoconstituents, each tuber extract was subjected to several qualitative chemical analyses.

**Physicochemical Evaluation:** The total ash value, acid insoluble ash, water soluble ash, extractive value and moisture content were all used in the physicochemical examination of the powder.

**Fluorescence Evaluation:** Fluorescence analysis was carried off all extracts and powder using different solvents and observed in visible and UV rays (for both short & long wavelength).

#### **Pharmacological Evaluation:**

**Evaluation of Analgesic Activity:** An analgesic, usually known as a painkiller, is any of a class of medications intended to provide analgesia or pain reduction. Analgesic widespread affects the peripheral and central nervous systems in various ways. Various pharmacological actions have been attributed to many Indian medicinal plants. Because it has a wide variety of phytochemicals. Because of their adverse effects and poor potency, existing analgesic-inducing medicines such as opiates and non-steroidal anti-inflammatory drugs are considered ineffective in all circumstances. *Pueraria tuberosa* analgesic efficacy was investigated utilizing Eddy's hot plate, acetic acid-produced writhing, and yeast-induced hyperthermia procedures at different doses (100, 150 and 200 mg/kg). Methanolic extract of *Pueraria tuberosa* exhibited impressive analgesic and antipyretic effects in all mice investigated at 200 mg/kg doses.

**Hot Plate Method:** Woolfe and MacDonald's approach was the first to be devised (1944). Mice and rats' paws are extremely sensitive to heat at temperatures that do not harm the skin. Jumping, withdrawing the paws, or licking the paws are all examples of responses<sup>9-10</sup>.

The animals were placed on Eddy's hot plate, which was held at 550.5°C. To avoid paw damage, a 15-second cutoff time was observed. A stopwatch was used to record the reaction time and type of response. The vehicle was given to control rats (12 percent Tween 80, 1 ml/kg). Ethanolic extracts (100, 300, and 500 mg/kg, IP) of *Pueraria tuberosa* were employed as a positive control (paracetamol, 10 mg/kg). The latency was measured before and after intraperitoneal injection of 100, 300 and 500 mg/kg of each extract to different groups of six mice for 15, 30, 60, and 120 minutes<sup>11-12</sup>. The percentage variance was computed by multiplying



the average reaction times by the following ratio:  
115

$$\text{Percentage protection} = \frac{\text{Drug latency} - \text{Baseline latency}}{\text{Baseline latency}} \times 100$$

**Organoleptic Evaluation:** The investigation on the organoleptic study of the tuber part of the plant indicated the characters like colour, odour and taste **Fig. 1** and **Table 1**.



**FIG. 1: TUBER OF PUERARIA TUBEROSA**

**TABLE 1: ORGANOLEPTIC CHARACTERS OF PUERARIA TUBEROSA (TUBER PART) POWDER**

Tuber Part	
Characters	Observation
Colour	Brown
Texture	Coarse
Taste	Sweet taste
Odour	Characteristic pleasant
Shape & size	Pot-like, 60 cm. long & 30 cm. width

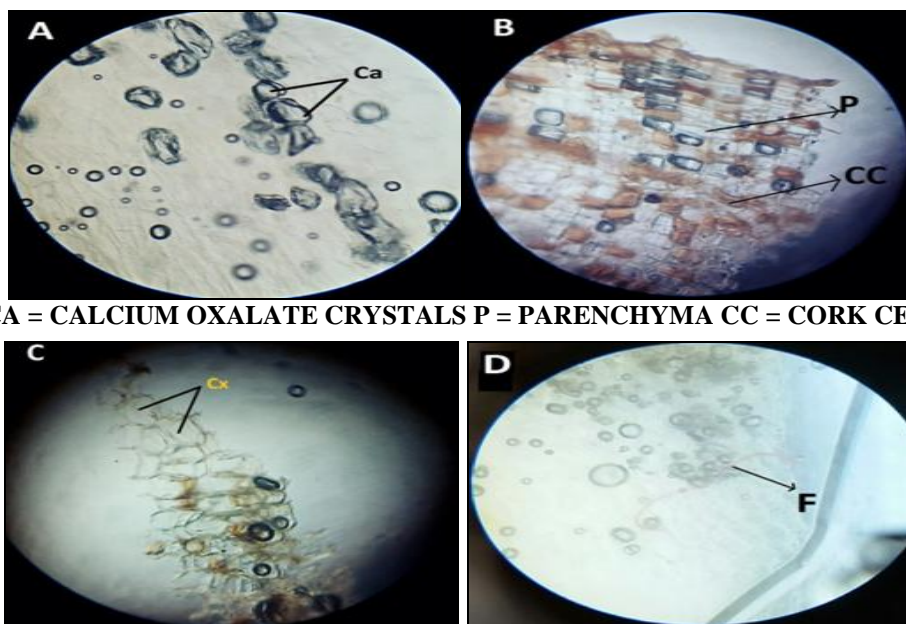
trifoliolate and alternating. They're 18cm long, 16cm wide, and hairless on top. Flowers are bisexual, measuring around 1.5 cm in diameter, and are blue or purplish-blue.

The fruit pods are linear in shape, measuring 2 to 5 cm long and tightly packed between the seeds. They have silky, bristly reddish-brown hair with three to six seeds.

Kudzu, or *Pueraria tuberosa*, is a climber with a woody tuberculated stem. With huge tuberous roots, it is a climbing, coiling, and trailing vine. The tubers are globose or pot-like, measuring about 25cm across, with white, starchy and faintly sweet interiors. The leaflets are egg-shaped, with a circular base and uneven sides, and the leaves are

**Microscopic Evaluation of Pueraria tuberosa:**  
**Transverse Section (Tuber) Examination:** A suitably designed transverse section of fruit was studied, and different tissue organizations were viewed at 10x and 40x. Labeled characters showed in **Fig. 2** and mentioned in **Table 2**.

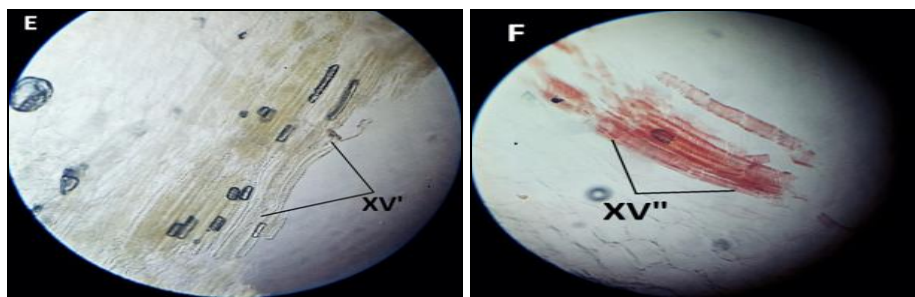
**Transverse Section of Tuber:**



**CA = CALCIUM OXALATE CRYSTALS P = PARENCHYMA CC = CORK CELLS**

**CX = CORTEX**

**F = FIBER**

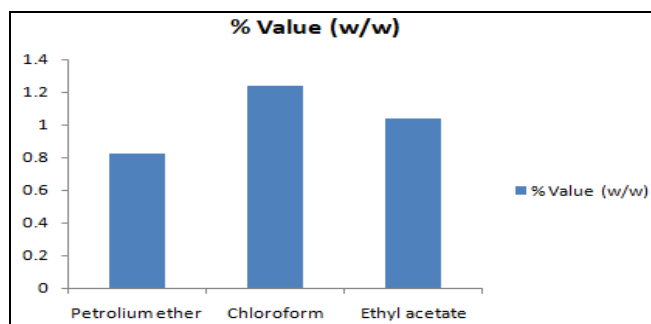


XV' = XYLUM VESSELS (FLUROGLYCEROL) XV'' = XYLUM VESSELS (SUDAN RED)  
**FIG. 2: T.S. OF TUBER OF PUERARIA TUBEROSA**

**TABLE 2: FOREIGN ORGANIC MATTER OF PLANT PUERARIA TUBEROSA**

Parameter	% yield (w/w)
Foreign Organic matter	None

**Physico-chemical Investigation:** Foreign organic matter, ash values, extractive values, moisture content, swelling index, and foaming index were performed, evaluated, and shown in **Fig. 3** and noted in **Table 3**.



**FIG. 3: ASH VALUE OF TUBER PART OF THE PLANT PUERARIA TUBEROSA**

**TABLE 3: ASH VALUE OF TUBER PART OF THE PLANT PUERARIA TUBEROSA**

S. no.	Test	Percentage (%w/w)
1	Total Ash	25%
2	Acid Insoluble Ash	20%
3	Water Soluble Ash	15%

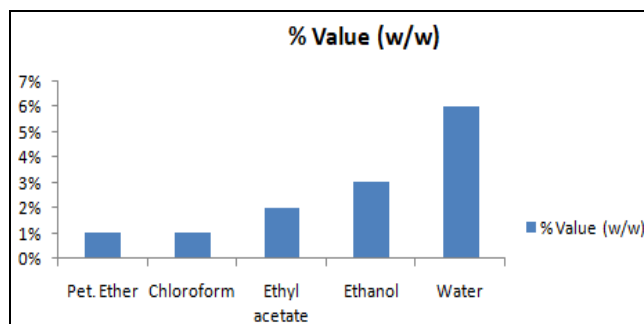
The foreign organic matter value of the plant drug is absent. The plant's various ash values determined that the acid insoluble ash value was the greatest (20%), while the water-soluble ash value was the lowest (15 percent). The moisture content was determined to be 0.25 percent using the loss on drying method. The swelling and foaming index measurements were 1.2 cm and less than 100, respectively.

**Phytochemical Investigation:**

**Extractive Values using Successive Extraction:**

The % yields were calculated after successive Soxhlet extractions using different solvents in

increasing order of polarities, such as petroleum ether, chloroform, ethanol, and distilled water. The percentage yield of the aqueous extract was the highest compared to petroleum ether, chloroform, and ethanolic extracts. The obtained percentage of extractive values were found to be 0.82%, 1.24%, 1.04%, 3.07%, 5.37%, 33.11% as shown in **Fig. 4** and in **Table 4**.



**FIG. 4: EXTRACTIVE VALUE (COLD EXTRACTION) OF PUERARIA TUBEROSA IN DIFFERENT SOLVENTS**

**TABLE 4: EXTRACTIVE VALUE (COLD EXTRACTION) OF PUERARIA TUBEROSA IN DIFFERENT SOLVENTS**

S. no.	Solvent	% Value (w/w)
1	Petroleum Ether	1%
2	Chloroform	1%
3	Ethyl Acetate	2%
4	Ethanol	3%
5	Water	6%

**Preliminary Phytochemical Investigation:** It was investigated using several extracts, such as petroleum ether, chloroform, ethanol, and water, which were subjected to preliminary phytochemical screening using qualitative chemical assays, as shown in **Table 5**.

**TABLE 5: MOISTURE CONTENT OF PUERARIA TUBEROSA**

Parameter	% Value (w/w)
Moisture content	0.25%

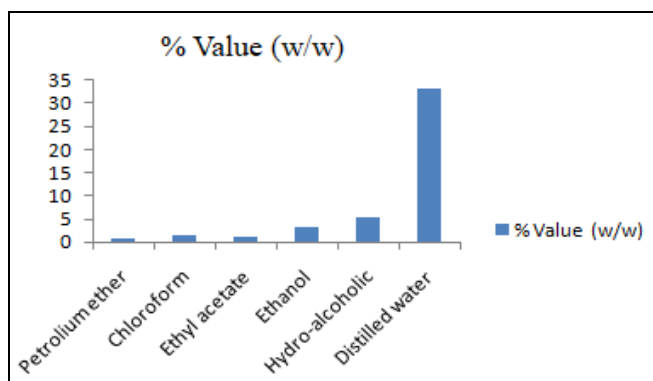


FIG. 5: EXTRACTIVE VALUE (SUCCESSIVE EXTRACTION) OF *PUERARIA TUBEROSA* WITH DIFFERENT SOLVENTS

**Detection of Organic Constituents:** Preliminary qualitative analysis of *Pueraria tuberosa* extracts for the presence of various functional groups is shown in Table 6.

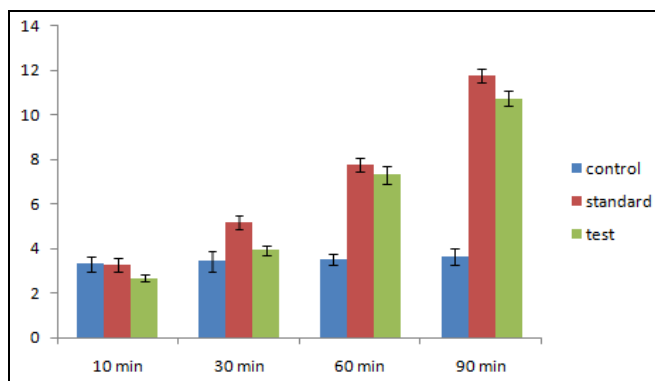


FIG. 6: EFFECT OF ETHANOLIC EXTRACT OF *PUERARIA TUBEROSA* BY EDDY'S HOT PLATE METHOD IN RATS

TABLE 6: SWELLING INDEX OF *PUERARIA TUBEROSA*

Parameter	Value (c.m)
Swelling index	1.2

**Fluorescence Analysis:** The sample of different extracts were treated with various reagents separately *i.e.*, methanol, water, 1N HCl, 1N HNO<sub>3</sub>, 1N H<sub>2</sub>SO<sub>4</sub>, *etc.*, and subjected to fluorescence studies, including the crude extract specimens also, as shown in Table 7.

TABLE 7: FOAMING INDEX OF *PUERARIA TUBEROSA*

Parameter	Value
Foaming index	Less than 100

**Pharmacological Studies:**

**Analgesic Activity of the Compound:** Effect of Ethanolic Extract of *Pueraria tuberosa* by Eddy's Hot Plate Method in rats as shown in Fig. 2 and in Table 7.

TABLE 8: EXTRACTIVE VALUE (SUCCESSIVE EXTRACTION) OF *PUERARIA TUBEROSA* WITH DIFFERENT SOLVENTS

S. no.	Solvent	% Value(w/w)	Consistency
1	Petroleum ether	0.82%	Brown
2	Chloroform	1.24%	Brown
3	Ethyl Alcohol	1.04%	Brown
4	Ethanol	3.07%	Brown
5	Hydro-Alcoholic	5.37%	Brown
6	Distilled water	33.11%	Brown

TABLE 9: PRELIMINARY QUALITATIVE ANALYSIS OF *PUERARIA TUBEROSA* EXTRACTS FOR THE PRESENCE OF VARIOUS FUNCTIONAL GROUP

Category	Test	Pet. Ether	Chloroform	Ethyl acetate	Ethanol	Hydro alcoholic	Aqueous
Alkaloids	Mayer's Test	+	+	-	-	-	-
	Hager's Test	+	+	-	-	-	-
	Wagner's Test	-	+	-	-	-	-
	Dragondroff	-	+	+	-	-	+
	Tannic acid	-	+	+	-	-	+
Carbohydrates	Molish test	+	+	-	-	-	-
	Fehling test	+	+	-	+	+	+
	Barford's test	-	-	-	-	+	-
	Benedict test	-	+	-	+	+	+
Glycosides	Legal's test	+	+	-	-	-	-
	Killar killani test	-	-	-	-	-	-
	Borntrager test	-	-	-	-	-	-
Saponin	Foam test	-	+	-	-	-	-
Tannins	FeCl <sub>3</sub>	-	-	-	-	-	-
	Lead acetate solution	+	-	-	+	-	+
	Acetic acid solution	-	-	-	-	-	-

Flavonoids	Dil. Iodine solution	-	+	+	-	-
	Dil. HNO <sub>3</sub>	-	-	-	-	-
	H <sub>2</sub> SO <sub>4</sub> solution	+	+	-	+	+
	Lead acetate solution	-	-	-	-	-
Proteins	NaOH solution	-	-	-	-	-
	Biurete's test	-	-	-	-	-
	Millon's test	-	-	-	-	-

“+” = Present “-” = Absent

As can be seen in **Table 9**, the tests revealed the presence of alkaloids, carbohydrates, phenolic compounds, flavonoids, fixed oils, and fats.

**TABLE 10: EFFECT OF ETHANOLIC EXTRACT OF PUERARIA TUBEROSA BY EDDY'S HOT PLATE METHOD IN RATS**

Group	Mean latency time (in sec)			
	10min	30min	60min	90min
Control	3.318±0.35	3.462±0.47	3.476±0.25	3.652±0.38
Standard	3.268±0.30	5.17±0.31	7.77±0.30	11.78±0.31
Test	2.66	3.925	7.31	10.74

**CONCLUSION:** The tuber part of *Pueraria tuberosa*; Fabaceae, was selected to evaluate the pharmacognostic, phytochemical & Pharmacological activity. With the use of Pharmacognostical characteristics, we discovered that *Pueraria tuberosa* is a climber with a woody tuberculated stem and massive tuberous roots in the current study. It was a vine that climbed, coiled, and trailed. The tuber was globose or pot-shaped, about 25cm broad, and had white, starchy and faintly sweet contents. Microscopical examinations revealed Calcium Oxalate Crystals, Parenchyma, Cork Cells, Cortex, and Xylem Fibers.

In phytochemical evaluation, we determined Foreign Organic matter (0%), Total ash (25%), Acid-insoluble ash (20%), Water soluble ash (15%), Moisture content (0.25%), Swelling index (1.2 cm.), Foaming index-less than 100. Extractive values with cold extraction method (Pet. ether-1%, Chloroform-1%, Ethyl Acetate-2%, Ethanol-3%, Water-6%), and the extractive values with Successive extraction method were found to be Pet. ether, Chloroform, Ethyl alcohol, Ethanol, Hydroalcoholic, Distilled water (0.82%, 1.24%, 1.04%, 3.07%, 5.37%, 33.11%. respectively) were detected in Phytochemical evaluation also. Alkaloids, glycosides, saponins, tannins, flavonoids, and carbohydrates were identified in the ethanolic extract of vidarikand during preliminary phytochemical screening. Using Eddy's hot-plate method, we discovered that ethanolic extract of kudzu has strong analgesic efficacy.

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**CONFLICTS OF INTEREST:** There is no conflict of interest regarding the publication of this paper.

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