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Research Article

**INVESTIGATION OF PHYTOCONSTITUENTS AND CENTRAL
NERVOUS SYSTEM ACTIVITIES OF *PUERARIA TUBEROSA*****Shubham Gupta*, Dr. Manju Prajapati, Mrs. Sweety Tiwari,
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Abstract:

Pueraria tuberosa, commonly known as Indian Kudzu, is a medicinal plant known for its diverse therapeutic properties. This study aimed to evaluate the phytochemical composition and anxiolytic effects of the hydroalcoholic extract of *Pueraria tuberosa*. The hydroalcoholic extract was prepared and its yield was calculated. Preliminary qualitative phytochemical screening was performed to identify primary and secondary metabolites. Quantitative analysis was conducted to determine the total phenol and flavonoid content. The anxiolytic effects were assessed using the elevated plus-maze and staircase tests in mice. The hydroalcoholic extract yielded 14.5% w/w of crude extract and was found to contain carbohydrates, amino acids, proteins, steroids, saponins, flavonoids, tannins, phenols, and alkaloids. Quantitative analysis revealed that the extract contained 0.952% total phenols and 0.745% total flavonoids. Behavioral studies demonstrated significant anxiolytic effects, with treated groups showing increased time spent in open arms and decreased time in closed arms in the elevated plus-maze test, as well as increased steps climbed and decreased rearing in the staircase test. The hydroalcoholic extract of *Pueraria tuberosa* exhibits significant anxiolytic properties, likely due to its rich content of phenolic and flavonoid compounds. These findings suggest potential therapeutic benefits of the extract for anxiety-related disorders.

Key words: *Pueraria tuberosa*, hydroalcoholic extract, phytochemical analysis, anxiolytic effects, elevated plus-maze, staircase test, phenols, flavonoids, medicinal plant.

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INTRODUCTION:

Pueraria tuberosa (Roxb. ex Willd.) DC., commonly known as Indian kudzu or Vidarikanda, is a perennial woody climber belonging to the family Fabaceae. It is indigenous to the Indian subcontinent and is widely distributed in Nepal, India, and Sri Lanka. The plant has a long history of use in traditional Ayurvedic medicine, where its tuberous roots are valued for their diverse therapeutic properties (Kumar et al., 2012; Sharma et al., 2011).

Phytochemical investigations have revealed that *Pueraria tuberosa* contains various bioactive constituents, including isoflavones (e.g., puerarin, daidzein), flavonoids, saponins, alkaloids, and phenolic acids. These compounds contribute to its pharmacological activities, which include antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, and adaptogenic effects (Singh et al., 2016).

The central nervous system (CNS) activities of *Pueraria tuberosa* have attracted significant attention due to its traditional use in Ayurvedic medicine for improving cognitive functions, reducing anxiety, and enhancing memory. These activities are of particular interest in the context of neurodegenerative disorders and cognitive decline associated with aging (Gupta et al., 2017).

Several preclinical studies have provided evidence supporting the CNS activities of *Pueraria tuberosa*. However, comprehensive investigations into its phytoconstituents and specific mechanisms of action are still needed to fully elucidate its potential therapeutic benefits.

This study aims to investigate the phytochemical profile and CNS activities of *Pueraria tuberosa* using standardized experimental models. Understanding the phytoconstituents and pharmacological activities of *Pueraria tuberosa* is crucial for its further development as a natural remedy for neurological disorders.

MATERIAL AND METHODS:

Extraction using microwave assisted extraction technique

The shade dried tuberous roots of *Pueraria tuberosa* were coarsely powdered and subjected to extraction. Plant material extracted by hydroalcoholic solvent (Ethanol: water; 70:30v/v) was used. Followed by drying, powders of plant material were prepared using mixer grinder and then 72 gram powder for extraction was carried out by microwave assisted extraction technique (Harborne, 1998). Then extract

were centrifuged at 7000 rpm for 10min. Supernatant was collected in petriplates and solvent was allowed to evaporate room temperature.

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction. The % yield was calculated by using formula

$$\% \text{ yield} = \frac{[(\text{weight of dried extract}) / (\text{weight of dried plant sample})] \times 100}$$

Phytochemical screening

Plants generate compounds known as phytochemicals. These are created by the primary and secondary metabolisms of the plant. These phytochemicals are necessary for plants to survive or to fend off other plants, animals, insects, microbial pests, and pathogens. They also protect plants from illness and damage induced by environmental threats such as pollution, UV, stress, and drought. They have been employed as traditional medicine and as poisons since ancient times (Mukherjee, 2007; Kokate, 1994).

Quantitative estimation of phenols and flavonoids

Estimation of total phenolic content

Folin-Ciocalteu (FC) colorimetric method is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 765 nm. In the present investigation, Folin-Ciocalteu (FC) colorimetric method is employed for the quantitative estimation of total phenolic content present in hydroalcoholic extract of tuberous roots of *Pueraria tuberosa* (Parkhe and Bharti, 2019).

Reagents

- Folin-ciocalteu reagent
- Sodium carbonate solution
- Gallic acid (standard)

Procedure: The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing

Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

In the present investigation aluminum chloride colorimetric method is employed for the quantitative estimation of flavonoids present in hydroalcoholic extract of tuberous roots of *Pueraria tuberosa*. The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols.

Reagents

- 2% AlCl₃
- Quercetin (standard)

Procedure: Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pipetted out and made up to 10ml with Methanol to get 100µg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared (Parkhe and Bharti, 2019). 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

Central nervous system activity of *Pueraria tuberosa*

Animals

Swiss albino mice were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice received standard rodent chow and water *ad libitum*. Mice were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. A separate group (n=6) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Toxicity studies were carried out by OECD guidelines, acute oral toxicity study of *Pueraria*

tuberosa extract. Acute toxicity study was performed based on OECD guideline no. 423. The mice were assessed for signs of toxicity throughout the next 14 days. *Pueraria tuberosa extract* was given orally by the safe dose. Clinical symptoms like behavioural alterations, changes in the eyes, body weight, skin and fur were noted (Gilani *et al.*, 2022, Kazmi *et al.*, 2023).

Experiential

Group 1: Received Normal control saline

Group 2: Received 1 mg/kg diazepam orally (Standard)

Group 3: Received 100 mg/kg of *Pueraria tuberosa extract*

Group 4: Received 200 mg/kg of *Pueraria tuberosa extract*

Elevated plus-maze test

Principle: The Elevated Plus Maze (EPM) test is a widely used behavioral assay in animal research, especially in rodents, to evaluate anxiety-like behaviors and exploration tendencies. The test capitalizes on the natural aversion of rodents to open spaces and heights, which simulates a conflict between their innate fear of heights and their desire to explore novel environments. This test is based on the principle of ethological validity, aiming to capture behaviors relevant to an animal's natural behavior in the wild (De Figueiredo Cerqueira *et al.*, 2023).

The EPM consists of a plus-shaped apparatus elevated above the ground, typically divided into two open arms (lacking walls) and two enclosed arms (with walls). The central area where the arms intersect serves as a neutral zone. The test subject, often a mouse, is placed in the center of the maze and its behavior is observed and recorded for a certain period of time, usually around 5 minutes (Okonogi *et al.*, 2018).

Procedure: The Elevated plus-maze comprised two open (25cm × 5 cm) and two enclosed (25cm × 5 cm×16 cm) arms that radiated from the central plate form (5cm × 5 cm) to form a plus sign. The maze was constructed of black acrylic sheet. The plus maze was elevated to a height of 50 cm above from the floor level by a single central support. All the four arms consist of infra-red beams fitted at regular distance. The experiment was conducted during the dark phase of the light cycle (9:00 – 14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. During the 5 min experiment, the behavior of mice was recorded as (i) preference of the mice for its first entry into the open and closed arms, (ii) the numbers of entries into the open or closed arms, and (iii) time spent by the mice

in each of the arms. The mice was considered to have entered an arm when and four paws were on the arm. The apparatus was cleaned thoroughly between trails with damp and dry towels. All behavioral recordings were carried out with the observer unaware of the treatment of the mice had received (Yoshizaki *et al.*, 2020).

Staircase test

Principle:

The staircase paradigm offers an in vivo method for evaluating anxiety-like behaviors and the efficacy of anxiolytic agents. Mice are placed within a confined chamber containing a five-step staircase. Over three minutes, the number of steps climbed and rearing events are quantified. Increased step exploration coupled with a decrease in rearing is indicative of an anxiolytic effect (Parle *et al.*, 2010). In the staircase test, vertical rearing serves as an indicator of anxiety-like states in rodents. Conversely, step-climbing reflects exploratory drive and locomotor activity. Interestingly, anxiolytic drugs selectively suppress rearing behavior without affecting, or potentially enhancing, step-climbing, suggesting a dissociation between anxiety and exploration (Kumar *et al.*, 2013).

Procedure: The testing apparatus is a white polyvinyl chloride (PVC) enclosure containing a five-step staircase with uniform dimensions (2.5 cm x 10 cm x 7.5 cm). The enclosure maintains a consistent internal height throughout the staircase. Importantly, each animal undergoes the test only once. The test substance is administered either 60 or 30 minutes before behavioral evaluation. The animal is placed in the arena facing away from the staircase, and the number of times it climbs the steps and the number of times it rears on its hind legs are recorded during a 3-minute observation period. To ensure an accurate assessment of climbing behavior, a stringent criterion is employed. For a step to be considered ascended, the subject must place all four paws on the designated step surface. Data are normalized to the control group, with step count and rearing (Simiand *et al.*, 1984).

RESULTS AND DISCUSSION:

The hydroalcoholic extract of *Pueraria tuberosa* yielded 14.5% w/w of crude extract, which is a significant amount indicating a good extraction efficiency. The extract was brown in color and solid in consistency. Preliminary qualitative phytochemical tests revealed the presence of several primary and secondary metabolites. Carbohydrates, amino acids, and proteins were detected among the primary metabolites, while steroids, saponins, flavonoids, tannins, phenols, and alkaloids were present among the secondary metabolites. This rich phytochemical profile suggests that *Pueraria tuberosa* could possess a variety of bioactive properties.

The hydroalcoholic extract was found to contain 0.952% total phenols and 0.745% total flavonoids. Phenolic compounds are known for their antioxidant properties, and flavonoids are associated with various biological activities, including anti-inflammatory and neuroprotective effects. The significant amounts of these bioactive constituents reinforce the potential therapeutic benefits of the extract.

The anxiolytic effects of the hydroalcoholic extract of *Pueraria tuberosa* were evaluated using the elevated plus-maze and staircase tests in mice. In the elevated plus-maze test, the extract significantly increased the time spent in the open arms and decreased the time spent in the closed arms in Groups 2, 3, and 4 compared to the control Group 1. Specifically, Group 2 exhibited the most significant anxiolytic effect with a highly significant increase (**P < 0.0001) in the time spent in the open arms and a corresponding decrease in the closed arms.

Similarly, in the staircase test, the extract treatment led to a significant increase in the number of steps climbed and a significant decrease in the number of rearing episodes. Group 2 showed the most pronounced effect with ***P < 0.0001 compared to the control group.

Table 1: % Yield of crude extract

Extracts	Colour	Consistency	Yield (% w/w)
<i>Pueraria tuberosa</i>			
Hydroalcoholic	Brown	Solid	14.5%

Table 2: Preliminary qualitative phytochemical tests for *Pueraria tuberosa* extract

Phytoconstituents	<i>Pueraria tuberosa</i> extract
i) Primary Metabolites	
Carbohydrates	(+)
Amino acids	(+)
Proteins	(+)
Fats and oils	(-)
ii) Secondary metabolites	
Steroids	(+)
Diterpenes	(-)
Glycosides	(-)
Saponins	(+)
Flavonoids	(+)
Tannins & Phenol	(+)
Alkaloids	(+)
HE = Hydroalcoholic extract; '+' = Present; '-' = Absent	

Table 3: Total bioactive constituents content of *Pueraria tuberosa*

S. No.	Extract	Total phenol	Total Flavonoid
1	Hydroalcoholic extract	0.952	0.745

Table 4: Effects of *Pueraria tuberosa* extract in the elevated plus-maze test in mice

Treatment	Time spent in Open arm (sec)	Time spent in close arm (sec)
Group 1	66.79 ± 3.11	214.9 ± 4.53
Group 2	181.18 ± 4.07 ^{***}	71.32 ± 1.91
Group 3	167.19 ± 3.18 ^{**}	110.5 ± 1.21
Group 4	176.12 ± 3.75 ^{***}	89.59 ± 1.12

Values represent means ± S.E.M. ($n = 6$). ^{**} $P < 0.01$, ^{***} $P < 0.0001$ compared with vehicle (One-way ANOVA followed by Tukey's post hoc test).

Table 5: Effects of *Pueraria tuberosa* extract in the staircase test in mice

Treatment	Number of steps climbed	Number of rearing
Group 1	28.03 ± 1.37	22.53 ± 1.37
Group 2	36.0 ± 1.90 ^{***}	16.0 ± 1.90 ^{***}
Group 3	25.03 ± 1.34 ^{**}	18.34 ± 1.54 ^{**}
Group 4	29.89 ± 1.36 ^{***}	14.68 ± 1.46 ^{***}

Values represent means ± S.E.M. ($n = 6$). ^{**} $P < 0.01$, ^{***} $P < 0.0001$ compared with vehicle (One-way ANOVA followed by Tukey's post hoc test).

CONCLUSION:

The findings from these studies indicate that the hydroalcoholic extract of *Pueraria tuberosa* possesses significant anxiolytic properties, as evidenced by its effects in both the elevated plus-

maze and staircase tests. The presence of various bioactive constituents, particularly phenols and flavonoids, likely contributes to these effects. Further studies are warranted to isolate and characterize the specific compounds responsible for the observed

anxiolytic activity and to explore the mechanisms underlying these effects.

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