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SPECTROSCOPIC AND HPTLC STUDY FOR QUALITATIVE AND QUANTITATIVE STUDY OF PHYTOCOMPOUND IN EXTRACT OF *PUERARIA TUBEROSA*

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ABSTRACT

The present study investigates the qualitative and quantitative phytochemical composition of the ethanolic extract of *Pueraria tuberosa*. The extraction yielded 7.2% (w/w) of the dried extract. Comprehensive phytochemical screening revealed the presence of flavonoids, phenols, proteins, saponins, and diterpenes, while alkaloids, glycosides, carbohydrates, and tannins were absent. Quantitative analysis indicated significant amounts of total phenols (0.622 mg/100 mg) and flavonoids (0.835 mg/100 mg) in the extract. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) confirmed the presence of quercetin, a potent antioxidant, with a concentration of 0.75%. These findings underscore the potential therapeutic value of *Pueraria tuberosa*, warranting further investigation into its pharmacological applications.

Key words: *Pueraria tuberose*, Phytochemical screening, Flavonoids, High-Performance Thin Layer Chromatography (HPTLC), Quercetin.

INTRODUCTION

Pueraria tuberosa, commonly known as Indian Kudzu, is a perennial climbing plant found in various regions of India and Southeast Asia. Traditionally, it has been used in Ayurvedic medicine for its potential therapeutic properties, including anti-inflammatory, antipyretic, and antidiabetic effects. Recent scientific investigations have focused on its phytochemical composition, revealing a rich array of bioactive compounds such as isoflavonoids, tuberosin, puerarin, and other phenolic constituents (Chatterjee *et al.,* 2001; Tripathi *et al.,* 2006).

Isoflavonoids, particularly puerarin, have garnered significant interest due to their wide-ranging pharmacological activities, including antioxidant, anti-inflammatory, and estrogenic effects (Chatterjee *et al.,* 2001). These compounds are believed to contribute to the therapeutic potential of *Pueraria tuberosa*, making it a promising candidate for further pharmacological and phytochemical studies. High-Performance Thin-Layer Chromatography (HPTLC) and spectroscopic techniques such as UV-Vis, IR, and NMR are pivotal in the qualitative and quantitative analysis of phytocompounds. HPTLC is a versatile, rapid, and cost-effective analytical technique widely used for the identification, quantification, and quality control of herbal medicines and their active constituents (Patel *et al.,* 2010). It provides high-resolution separation and allows simultaneous analysis of multiple samples, making it ideal for the phytochemical evaluation of complex plant matrices. The use of spectroscopic methods complements HPTLC by providing detailed structural information about the isolated compounds. UV-Vis spectroscopy is useful

for detecting conjugated systems and aromatic compounds, while IR spectroscopy provides insights into functional groups present in the molecules. NMR spectroscopy offers detailed information about the molecular structure and dynamics of the phytocompounds (Sarker and Nahar; 2006).

This study aims to employ HPTLC and various spectroscopic techniques to qualitatively and quantitatively analyze the phytocompounds present in the extract of *Pueraria tuberosa*. By doing so, it seeks to establish a comprehensive profile of its bioactive constituents and validate its traditional uses through modern scientific methods.

Material and Methods

Material

The study utilized a variety of chemicals sourced from reputable suppliers. Potassium mercuric iodide, iodine, and picric acid were procured from Thomas Baker in Mumbai. Loba Chemie Pvt. Ltd., also based in Mumbai, supplied several key reagents including potassium iodide, sodium nitroprusside, sodium hydroxide, lead acetate, and the Folin-Ciocalteu reagent. Potassium bismuth iodide, pyridine, gelatin, nitric acid, copper acetate, and sodium chloride were obtained from S. D. Fine Chem. Ltd., Mumbai. Ferric chloride was sourced from Thomas Baker, while Fehling's solution was acquired from Central Drug House Ltd., New Delhi. For solvent needs, methanol and ethanol were provided by Qualigens Fine Chemicals, Mumbai. These high-quality reagents ensured the reliability and accuracy of the experimental procedures conducted in this study.

Instruments

The study utilized several key instruments to ensure precise and accurate results. A UV-Visible Spectrophotometer (Labindia 3000+) was employed for spectroscopic analysis. Sample preparation was facilitated by a Micro Centrifuge from REMI Laboratory, Mumbai, while a pH Meter from Accumax India, New Delhi, was used for measuring pH levels. An Electronic Balance provided accurate weight measurements.

Collection of Plant material

In the month of November 2023, leaves of *Pueraria tuberosa* have been obtained from a local area of Bhopal (M.P.).

Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs (Kokate, 1994, Mukherjee, 2007):

Defatting of plant material

Soxhlet extraction is also known as the hot continuous extraction process the main advantage of this method is complete extraction in minimum amount of solvent. The selection of the solvent for soxhlet extraction is based on the phyto constituent isolation process. The solvent should be easy to remove and inert. Normally the solvent selection is based on the increasing polarity order. 62 gram of dried *Pueraria tuberosa leaves* were coarsely pulverised and extracted with petroleum ether using the soxhlation technique. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhlation technique

Defatted dried powdered of *Pueraria tuberosa* has been extracted with ethanolic solvent using soxhlation method for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} x100$$

Phytochemical screening

Phytochemical screening refers to the systematic process of identifying and analyzing the chemical constituents present in plant extracts. Various methods and assays are employed to detect the presence of different classes of phytochemicals such as alkaloids, flavonoids, phenols, terpenoids, tannins, saponins, glycosides, and steroids. Phytochemical screening is a fundamental technique in botanical research aimed at elucidating the chemical composition of plant extracts and uncovering their potential pharmacological activities. This method involves a series of qualitative tests designed to detect the presence of specific classes of phytochemicals within the plant material. All of the extracts were subjected to phytochemical analysis using conventional procedures ((Audu *et al.,* 2007; Mishra *et al.,* 2017).

Quantitative studies of phytoconstituents

Total phenol content estimation

Principle: The modified folin-ciocalteu method was used to find out the total phenol concentration of the extract [52].

Preparation of Standard: In methanol, 10 mg Gallic acid was dissolved in 10 ml methanol, and various aliquots of 10- 50g/ml were produced.

Preparation of Extract: Ten milligram of dried extract were dissolved in ten millilitres of methanol and filtered. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 1 ml folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml extract and each standard. For colour development, the mixture was vortexed for 15 seconds before being set aside for 10 minutes. A spectrophotometer was used to measure the absorbance at 765 nm.

Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.,* 2017).

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to remain at room temperature for 15 minutes before measuring absorbance at 420 nm.

Estimation of Quercetin using HPTLC method

A CAMAG HPTLC system (Switzerland) comprising CAMAG Linomat 5 applicator, CAMAG TLC scanner 3, CAMAG Wincats software, version 1.44, Hamilton syringe (100µl), CAMAG Reprostar 3, CAMAG TLC plate heater, CAMAG UV Cabinet were used for the study.

Preparation of the Standard

1mg/ml of the standard, quercertin was prepared with methanol. From this 100µl was diluted with 950µlof methanol and hence the concentration of the standard was 100 µg/ml.

Preparation of the extract Sample

1mg/ml of the all extract was prepared with methanol seperately. From this 100µl was diluted with 950µlof methanol and hence the concentration of the extract was 100 µg/ml.

Preparation of the plates

The plates used for HPTLC was silica gel 60 F 254 (E.MERCK KGaA). 100 µg/ml of the Standard was applied in the form of bands using LINOMAT IV applicator. The volumes applied were 2, 4, 6, 8, and 10µl.

The concentration of the sample was 1.0mg/ml, and the different amounts were 2µl. The mobile step used was toluene: formic acid (5:4:1) ethyl acetate. Built the chromatograph For 15 minutes, dried at room temperature and scanned at 254 nm. The normal maximum peak area was measured. Average peak area of the standard was calculated. The calibration curve of the standard drug concentration (X-axis) over the average peak height / area (Y-axis) was prepared to get a regression equation by Win Cats software.

Estimation of quercetin in herbal extracts

Estimation of quercetin in Ethanolic *leaves extract of Pueraria tuberosa.* The mean peak height/area of the sample was calculated and the content of quercetin was quantified using the regression equation obtained from the standard curve.

Results and Discussion

The study focused on the extraction and analysis of phytocompounds from Pueraria tuberosa, highlighting both qualitative and quantitative assessments. The ethanolic extract yielded 7.2% (w/w), demonstrating a considerable extraction efficiency (Table 1).

Phytochemical Screening (Table 2) revealed diverse constituents in the ethanolic extract of *Pueraria tuberosa*. The presence of flavonoids was confirmed through positive results in both Lead Acetate and Alkaline tests, indicated by yellow color formation. Phenols were identified via the Ferric Chloride test, turning the solution black. Proteins and diterpenes were also detected, with positive results in the Xanthoproteic and Copper Acetate tests respectively. Saponins were confirmed by the formation of a foam layer in the Foam test. However, tests for alkaloids, glycosides, carbohydrates, and tannins returned negative, indicating their absence in the extract.

Quantitative Analysis (Table 3) showed significant amounts of phenolic and flavonoid compounds, with total phenol content at 0.622 mg/100 mg of dried extract and total flavonoid content at 0.835 mg/100 mg of dried extract. These results underscore the rich phenolic and flavonoid composition of the extract, contributing to its potential therapeutic effects. High performance thin layer chromatography (HPTLC) provided visual separation of the extract components. Figure 1 and Figure 2 displayed the chromatograms in normal light and short UV light, respectively, revealing distinct spots corresponding to different phytocompounds. Figure 3 presented the chromatogram of standard quercetin for comparison. Figure 4 showed the HPTLC chromatogram of the hydroalcoholic extract, validating the presence of quercetin in the sample.The HPTLC Estimation (Table 4) quantified the percentage of the identified compound, finding 0.75% quercetin in the ethanolic extract. This finding is crucial as quercetin is known for its antioxidant and anti-inflammatory properties, which can contribute to the therapeutic efficacy of the extract.

Table 1: % Yield of *Pueraria tuberosa*

S. No.	Extracts	% Yield (w/w)
1.	Ethanolic	7.2%

Table 2: Phytochemical screening of extract *of Pueraria tuberosa*

S. No.	Constituents	Ethanolic extract	Observation
1.	**Alkaloids**		
	Dragendroff's test	-ve	Green coloured
	Hager's test	-ve	Not yellow coloured
2.	**Glycosides**		
	Legal's test	-ve	Green coloured
3.	**Flavonoids**		
	Lead acetate	+ve	Yellow colour precipitate
	Alkaline test	+ve	Yellow colour
4.	**Phenol**		
	Ferric chloride test	+ve	Black coloured
5.	**Proteins**		
	Xanthoproteic test	+ve	Yellow coloured
6.	**Carbohydrates**		
	Fehling's test	-ve	Sky coloured
7.	**Saponins**		
	Foam test	+ve	Layer of foam
8.	**Diterpenes**		
	Copper acetate test	+ve	Green coloured
9.	**Tannins**		
	Gelatin Test	-ve	White colour

Table 3: Estimation of total phenolic and flavonoids content of *Pueraria tuberosa* extract

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.622	0.835

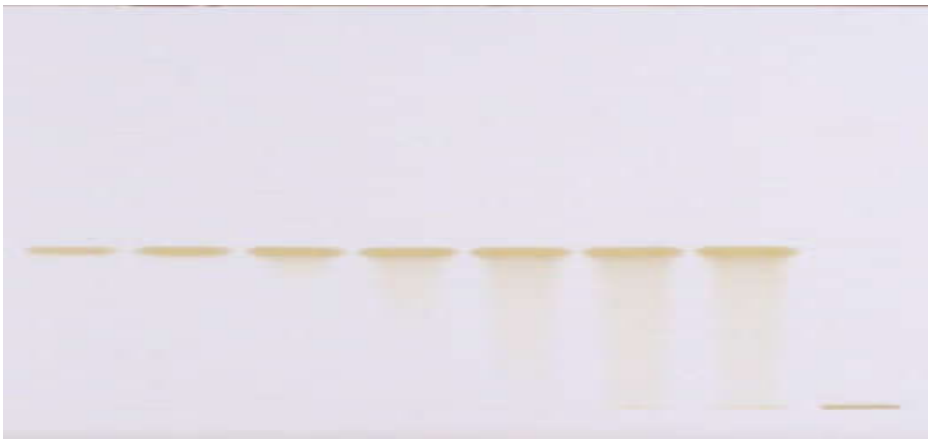

Figure 1: Thin layer chromatography in Normal light

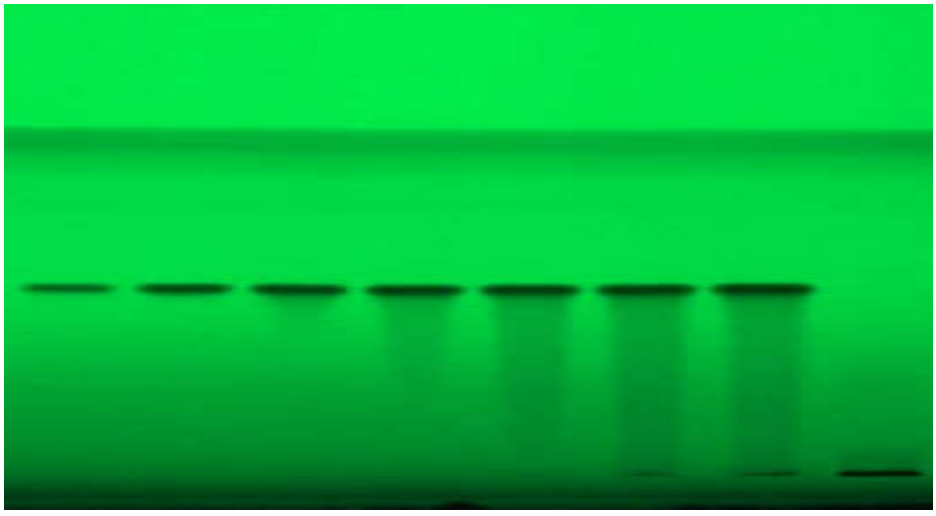

Figure 2: Thin layer chromatography in Short U.V

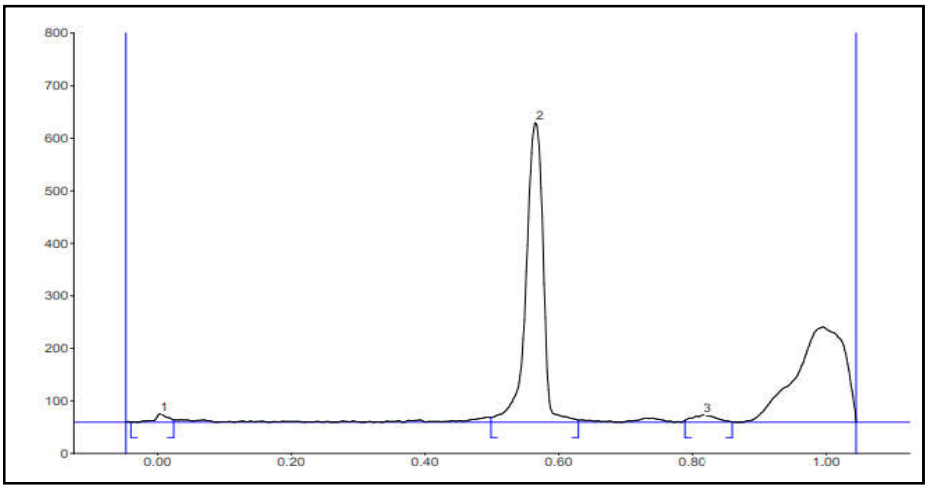

Figure 3: Chromatogram of standard quercetin

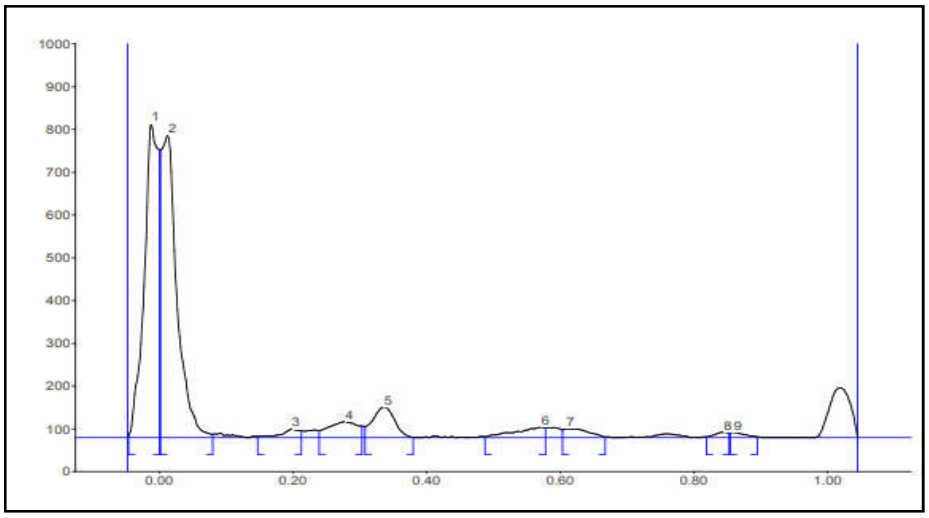

Figure 4: HPTLC chromatogram of Hydroalcoholic extract

Table 4: Results of HPTLC estimation

Estimation	Ethanolic extract
Percentage Found	0.75

Conclusion

In conclusion, the study successfully demonstrated the presence of important phytocompounds in the ethanolic extract of *Pueraria tuberosa* through comprehensive phytochemical screening, quantitative analysis, and chromatographic techniques. These findings pave the way for further exploration of *Pueraria tuberosa* as a potential source of natural bioactive compounds with significant pharmacological properties.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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