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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND HPTLC FINGER PRINTING ANALYSIS OF TRADITIONAL MEDICINAL PLANT *PUERARIA TUBEROSA* (ROXB. EX WILLD.)DC

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ABSTRACT

The medicinal value of a plant lies in the phytoconstituents present in it. These phytochemical compounds form the base of modern drugs. The aim of the present study is to identify the phytochemical constituents present in the traditional medicinal plant *Pueraria tuberosa* and to develop HPTLC fingerprint profile of acetone extract. Preliminary phytochemical screening was done to identify the phytoconstituents and HPTLC studies were carried out. CAMAG make HPTLC system equipped with Linomat 5applicator, TLC scanner 3, Reprostar 3 and WINCATS-1.4.3 software were used. The present study revealed the presence of carbohydrates, proteins alkaloids, flavonoids, saponins, phenols and tannins in various extracts. The HPTLC fingerprint analysis of acetone extract of *Pueraria tuberosa* showed 10 peaks at 254nm. The components with Rf values 0.05, 0.21 and 0.72 were predominant with the percentage area of 34.52, 16.16 and 10.10 respectively. The preliminary phytochemical analysis revealed the presence of various phytochemicals, which were confirmed by the HPTLC fingerprint profile.

Keywords: *Pueraria tuberosa*, phytochemical analysis, HPTLC.

1. INTRODUCTION

Plants are endowed with various phytochemicals and secondary metabolites which include alkaloids, tannins, saponins, coumarins, glycosides etc. Products of primary metabolism like amino acids, carbohydrates and proteins are vital for the maintenance of life processes, while secondary metabolites like alkaloids, phenolics, steroids, and terpenoids are of toxicological, pharmacological and ecological importance (1). About three quarters of the world's population relies on plant products for good health care. People living in rural areas largely depend up on herbal remedies for the treatment of different types of diseases. *Pueraria tuberosa* is one such plant used in traditional medicine as fertility control agent, aphrodisiac, cardiotonic, diuretic, antihyperglycemic, anti hyperlipidemic etc. (2,3,4)

Pueraria tuberosa (Roxb.ex Willd.) DC belongs to Fabaceae family. It is an important plant used in Indian medicine, commonly called as Vidarikand or Indian Kudzu. The plant is described as rasayana and tonic in Ayurvedic Pharmacopoeia of India. The herb acts as rasayana and slows down the ageing process. It strengthens body and boosts its immunity. Kudzu is used for the treatment of dysuria, cough, rheumatism, erysipelas and malarial fever. The roots are used as a demulcent and refrigerant in fevers (5). The study by Nagendra Singh Chauhan *et al.* (6) provides evidence for the role of phytoestrogenic compounds from *Pueraria* function and testosterone production in male rats and thus adds to the evidence for its ethnopharmacological utilization as an Ayurvedic herb for improvement of sexual potency.

2. MATERIALS AND METHODS

For the present investigation the medicinal plant Vidari (*Pueraria tuberosa* (Roxb. ex Willd.) DC. (Fabaceae) was selected. The root tubers of *Pueraria tuberosa* were collected from Nelliampathy region of south Western Ghats during the month of April and identified. The collected samples were washed thoroughly in running tap water and shade dried under room temperature.

2.1. Preparation of extracts

The collected tubers were cut into small pieces, shade dried, powdered and extracted with organic solvents like petroleum ether, chloroform, acetone, methanol and hot water in the increasing order of polarity using a soxhlet apparatus for 8-10 hours. After each solvent extraction, the material was dried in hot air oven at 40°C. And all the extracts were dried under vacuum in a rotary evaporator at 40°C to pursue further analysis. The dried extracts were obtained and the percentage yield was expressed in terms of air dried weight of plant material. For further studies the evaporated extracts were dissolved in respective solvents at the concentration of 1mg/mL.

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2.2. Preliminary phytochemical analysis

The phytochemical test of these extracts was performed using the method adopted by Harborne (7) and Sofowora (8).

2.2.1. Test for Carbohydrates (Molisch's test)

To 2ml of plant extracts, 1ml of Molisch's reagent and a few drops of conc. sulphuric acid were added. Presence of purple or reddish colour indicates the presence of carbohydrates.

2.2.2. Test for Proteins (Ninhydrin test)

To the extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins

2.2.3. Test for Alkaloids (Mayer's test)

To 2ml of plant extract, 2ml of conc. hydrochloric acid was added. Then add few drops of Mayer's reagent presence of green colour or white precipitate indicates the presence of alkaloids.

2.2.4. Test for Flavonoids (Shinoda test)

To 2ml of plant extract, 1ml of 2N of sodium hydroxide was added. Presence of yellow colour indicates the presence of flavonoids.

2.2.5. Test for Saponins (Froth test)

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes length wise. Formation of a 1cm layer of foam indicates the presence of saponins.

2.2.6. Test for Tannins and Phenols (Ferric chloride test)

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.3. High Performance Thin Layer Chromatography - HPTLC Studies

HPTLC technique was carried out using the method of Harbone (9). HPTLC is an important tool in drug analysis. It has the ability to analyze several samples simultaneously with small quantity of mobile phase with precession and shorter time.

2.3.1. Sample preparation

The acetone tuber extract was dissolved in 1ml of acetone and centrifuged for about 5 minutes at 3000rpm, and this solution was utilized as test solution for HPTLC analysis

2.3.2. Developing solvent system

Mobile phase used was ethyl acetate - 80%, water-10%, acetic acid- 5% and formic acid- 5%.

2.3.3. Sample application

1µl of test solution was spotted on the form of band of 8mm length using Hamilton syringe on silica gel $60F_{254}$ (precoated on aluminium plate 10x10 cm) with the help of CAMAG LINOMAT 5 applicator which was programmed through WINCATS software.

2.3.4. Development of chromatogram

The chromatogram was developed in ascending order with CAMAG twin trough glass chamber (20x10cm) which was pre saturated with mobile phase for 15min.The length of each run is cm. The TLC run was performed under laboratory conditions of temperature 25±2 and humidity 60±5°C. The plates were air dried by hot air to evaporate solvents.

2.3.5. Photo documentation

The Reprostar 3 (CAMAG, Switzerland) was used for documenting and evaluating the planar chromatograms at UV 366 nm, UV 254 nm and in white light.

2.3.6. Scanning

After derivatization the plate was fixed in CAMAG TLC scanner 3 and scanning was done at UV254nm.The peak numbers with their height and area, peak display and peak densitogram and Rf values were programmed through WINCATS software 1.3.4 version.

3. RESULTS AND DISCUSSION

Phytochemicals are chemical compounds synthesized during various metabolic processes. These may possess a variety of pharmacological activities, some are found to have antimicrobial activity and serve as plant defense mechanisms against pathogenic organisms. The present study revealed the presence of carbohydrates, proteins alkaloids, flavonoids, saponins, phenols and tannins in various extracts (Table 1). In the root tubers of *Pueraria tuberosa* alkaloids were detected in all the fractions in *Pueraria tuberosa* root extracts carbohydrates, saponins and proteins were found only in methanol and hot water extracts. It was detected that methanol extract had higher number of secondary metabolites.

High performance Thin Layer chromatography (HPTLC) is most simple and reliable separation technique which gives better precision and accuracy at various steps. The acetone

root tuber extract of *Pueraria tuberosa* was subjected to high performance thin layer chromatography to analyse the fingerprint profile of secondary metabolites. The acetone extract of *Pueraria tuberosa* showed 10 peaks at 254nm in 2µl of the sample (Fig.1). Rf values ranged from 0.05 to 0.85. It was clear from Table 2 (Fig. 2 & 3), the components with Rf values 0.05, 0.21 and 0.72 were predominant as the percentage area is more with 34.52, 16.16 and 10.10 respectively. The photo documentation of acetone extract of *P. tuberosa*, observed at 254 nm and 366 nm is given (Fig. 2). The 3D densitogram of acetone extract at 254nm was given (Fig. 3).

Table 1. Phytochemical screening of various fractions of *Pueraria tuberosa* root tuber extracts

Fractions	C	P	A	F	S	T&P	
Petroleum ether	-	-	+	-	-	+	
Chloroform	-	-	+	+	-	+	
Acetone	-	-	+	+	-	+	
Methanol	+	+	+	+	+	+	
Hot Water	+	+	-	+	+	+	

C- Carbohydrates; P – Proteins; A – Alkaloids; F – Flavonoids; S-Saponins; T&P-Tannins & Phenols.
(+) – Presence (-) -Absence

Table 2. HPTLC –Flavonoid profile of acetone extract of root tuber of *Pueraria tuberosa*

Peak	Retention factor (Rf)	Height AU	Peak area AU	Area %
1	0.05	751.5	49956.4	34.52
2	0.14	283.2	10768.8	7.44
3	0.21	403.6	23388.5	16.16
4	0.37	109.0	3854	2.66
5	0.43	112.5	5039	3.48
6	0.51	229.5	7922.6	5.47
7	0.59	407.1	12381.3	8.56
8	0.66	364.1	9034.8	6.24
9	0.72	417.4	14615.8	10.10
10	0.85	226.2	7753.0	5.36

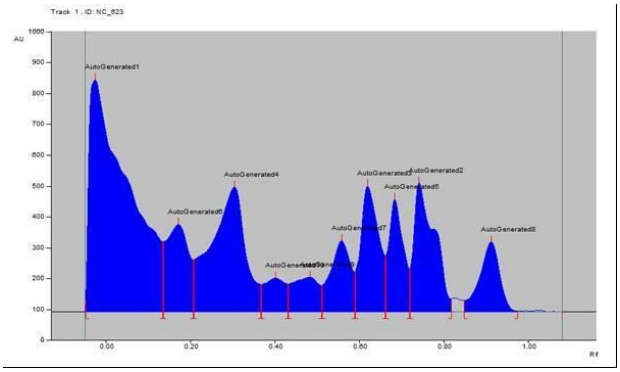

Fig. 1. Chromatogram for acetone solvent extract of *Pueraria tuberosa* at 254nm

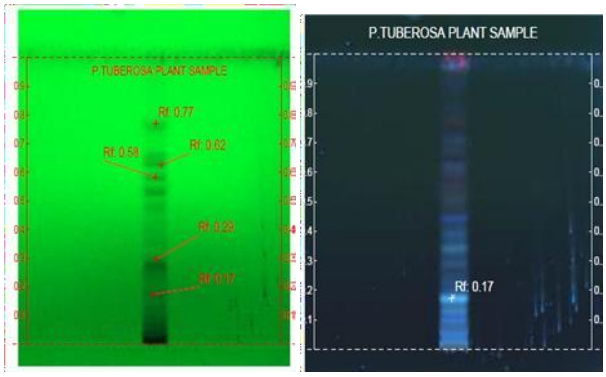

Fig. 3. 3D diagram of HPTLC densitogram for acetone solvent extract of *Pueraria tuberosa* at 254nm

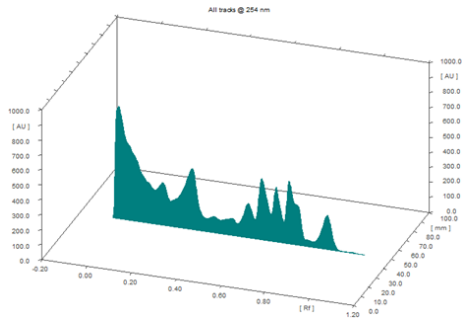

Fig. 3. 3D diagram of HPTLC densitogram for acetone solvent extract of *Pueraria tuberosa* at 254nm

Authentication of medicinal plants at chemical and genetic level is an imporant step for both research purposes and commercial preparations. A thorough understanding of the chemical composition is essential for conducting saftey assesment. HPTLC technique is an invaluable quality assesment tool for the analysis of broad number of compounds costeffectively and efficiently Prema *et al*. (10) has done the HPTLC fingerprint analysis of bark of *Stereospermum colais* which could be used as a diagnostic tool for the correct identification of the plant and also as a phytochemical marker and a good estimator of genetic variability in plant populations.

4. CONCLUSION

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. It can serve as a tool for identification, authentication, and quality control of herbal drug. In the present study

preliminary phytochemical screening showed presence of alkaloid, flavonoids, tannin, and phenolic compounds. HPTLC chromatogram of acetone extract results showed that there are many compounds in *Pueraria tuberosa.* So it is established that the pharmacological activity shown by the study species is due to the cumulative effect of all the compounds in composite.

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