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Chemical fingerprinting and quantification of bioactive metabolites in *Pueraria tuberosa* (Willd.) DC. and its marketed samples: Implications for quality control in herbal medicine

Adarsh Tiwari[a], Mridul Kant Chaudhary[a], Ankita Misra[a], Vairavan Ramesh[a], Rupali Sharma[a], Sanjeev Kumar Lale[b], Nagayya Shiddamallayya[c], Rabinarayan Acharya[c] & Sharad Srivastava[a,*]

[a]Pharmacognosy Division, CSIR-National Botanical Research Institute, Lucknow 226 001, UP, India
[b]Central Ayurveda Research Institute, Jhansi 284 003, UP, India
[c]Central Council for Research in Ayurvedic Sciences, New Delhi 110 058, India
[*]E-mail: sharad_ks2003@gmail.com

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The present study aims to distinguish *Pueraria tuberosa* (Willd.) DC. from its common adulterants, sold in Indian market as the Ayurvedic drug "*Vidarikand"*. Authentic samples of *Vidarikand* were collected from three natural locations, and marketed samples were procured from twelve major herbal markets in India. Pharmacognostic parameters of collected samples were standardized as per API guidelines, and known bioactive metabolites *viz*., gallic acid (1), p-coumaric acid (2), puerarin (3), biochanin A (4), daidzein (5), and genistein (6) were quantified using RP-HPLC. The separation was achieved using a mobile phase containing 0.1% orthophosphoric acid in water (A) and acetonitrile (B). Elution was carried out in an isocratic manner (65:35% v/v; A: B), with a flow rate of 0.7 mL min^{-1}. HPLC data revealed significant variation in the quantification of bioactive metabolites between natural populations and marketed samples. Analysis of HPLC fingerprints showed that authentic samples contained a higher number of peaks (both known and unknown), whereas marketed samples exhibited fewer peaks, except for PT-8. The sample procured from the Trivandrum market (PT-8) displayed a chemical profile somewhat similar to authentic *P. tuberosa*, suggesting that it could be genuine. However, based on its morphology and the absence of puerarin, daidzein, and genistein, it was identified as *Ipomoea* sp. This study will aid in the quality control of *Vidarikand* by distinguishing it from visually similar adulterants in various herbal markets in India, helping to ensure the safety and efficacy.

Keywords: Adulteration, *Pueraria tuberosa*, Puerarin, RP-HPLC, *Vidarikand*

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Pueraria tuberosa (Willd.) DC. (Fabaceae), also known as *Vidarikand* in Ayurveda, *Bankumara* in Hindi, and *Indian kudzu* in English, is a large woody climber with tuberous roots[1]. Its distribution ranges from the Western Himalayas to Sikkim, Madhya Pradesh, Odisha and the southern parts of India, up to an elevation of 4000 ft. The species also covers tropical and subtropical regions in South Asia, including Nepal, China and Pakistan[2]. The tubers of *Pueraria* hold an important place in Ayurveda and other traditional systems of medicine due to its various therapeutic benefits, such as aphrodisiac, galactagogue, rejuvenating, emetic, cardiotonic, refrigerant, expectorant, laxative, diuretic, and emollient. *Vidarikand* is one of the key ingredients of "Chyawanprash"[3,4] and is classified as a "Rasayana" drugs in Ayurveda[4]. The tuber is a rich source of various classes of bioactive compounds, mainly phenolics and isoflavones *i.e*., gallic acid, puerarin, genistein, daidzein, puerarone, diadzin, tuberosin, biochanin A and B, irisolidon, 4-methoxypuerarin, genistin, etc[4,5]. The pharmacological potential of *Vidarikand* is attributed to these compounds, which exhibit antidiabetic, antioxidant, anti-inflammatory, anticancer, hepatoprotective, immunomodulatory, cardioprotective, hypolipidemic, neuroprotective, wound healing, and nootropic effects[4-7].

Herbal drug industries around the globe often struggle to procure quality raw materials of commercially viable medicinal plants, largely due to common practices of adulteration and substitution[8,9]. Similarly, various species are sold under the name *Vidarikand* in India because it is required in bulk by industries, is expensive, and is difficult to collect. The

[*]Corresponding author

authentic source of *Vidarikand* is *Pueraria tuberosa* (Willd.) DC., while common adulterants are *Ipomoea* sp., and recently an endangered Gymnosperm *Cycas circinalis* L., which is also sold as *Vidarikand* in South India[10].

Previous studies on *Pueraria tuberosa* have reported validated RP-HPLC and HPTLC methods for the quantification of key bioactive metabolites, including puerarin, genistein, and daidzein, in tuber extracts[11-13]. However, these investigations have largely been restricted to a limited number of metabolites. Despite these advances, there is still a lack of consistent quality control and standardization of *Vidarikand* in herbal markets, where adulteration and substitution continue to affect the safety, effectiveness, and reliability of these herbal products.

Considering these facts, the present study was aimed to evaluate the authenticity of *Vidarikand* sold in various Indian markets. The variability in bioactive metabolites *viz*., gallic acid, p-coumaric acid, puerarin, biochanin A, daidzein, genistein profile were established through RP-HPLC. Further, the pharmacognostical standards were also established as per Ayurvedic Pharmacopeia of India (API). This study highlights the need to regulate the quality of raw material in herbal medicine and to restrict adulteration/substitution though chemical fingerprinting, thereby ensuring the safety and efficacy of finished formulations.

Materials and Methods

Plant materials

The tubers of *Pueraria tuberosa* (Willd.) DC. were collected from three natural locations of India, and judicious sampling was done, following GCP (Good collection practices) guidelines[14]. The samples were identified by Dr. Sharad Srivastava (Chief Scientist and Head, Pharmacognosy division, CSIR- NBRI, Lucknow, Uttar Pradesh, India). Dried specimens were deposited in raw drug repository of the institute and voucher number was assigned for each collection. Additionally, samples were procured from twelve different herbal markets *viz*., Kota, Delhi, Lucknow, Trivandrum, Dehradun, Jaipur, Guwahati, Indore, Amritsar, Chennai, and Odisha of the country (India) and geographical locations were documented (Table 1). The collected plant material were washed under running water, chopped, shade dried followed by air drying (25ºC±2), and pulverized to coarse powder (40 mesh, IP).

Chemicals and reagents

Standards *viz*., gallic acid (≥97%), puerarin (≥97%), daidzein (≥97%), genistein (≥97%), p-coumaric acid (≥97%), biochanin A (≥97%), were procured from Sigma-Aldrich (Mumbai, India). C_{18} RP-HPLC column (4.6×250 mm internal diameter, 5 μm particle size) was procured from SunFire (USA). HPLC-grade solvents (acetonitrile, water, methanol, orthophosphoric acid) and other analytical grade solvents (toluene, ethyl acetate, formic acid) were also purchased from Merck (Mumbai, India).

Evaluation of pharmacognostical parameters

Physicochemical parameters *viz*., moisture content, ash values (total ash, water-soluble ash and acid insoluble ash), and extractive values (water soluble, ethanol soluble and hexane soluble) were evaluated using standard protocols described in the Ayurvedic

Table 1 — Geographical location details of collected and marketed samples

S. No.	Sample code	Plant name	Location	Voucher number	Latitude	Longitude	Altitude (m)
1	PT-1	*Pueraria tuberosa* (Willd.) DC.	Bhimtal, Uttarakhand	305487	20°40'60"N	79°33'17"E	1434
2	PT-2	*Pueraria tuberosa* (Willd.) DC.	Chitrakoot, Madhya Pradesh	305488	25°10'15"N	80°51'22"E	162
3	PT-3	*Pueraria tuberosa* (Willd.) DC.	Gandhmardhan hills, Odisha	305489	20°52'26"N	80°50'34"E	990
4	PT-4	Vidarikand	Kerala market	353257	10°09'47"N	76°38'28"E	79
5	PT-5	Vidarikand	Kota market	353288	25°12'49"N	75°51'53"E	278
6	PT-6	Vidarikand	Lucknow market	353620	26°51'10"N	80°54'54"E	151
7	PT-7	Vidarikand	Delhi market	353280	28°39'30"N	77°13'14"E	249
8	PT-8	Vidarikand	Trivandrum market	353604	8°29'38"N	76°56'51"E	58
9	PT-9	Vidarikand	Dehradun market	353657	30°19'12"N	78°02'06"E	678
10	PT-10	Vidarikand	Jaipur market	353673	26°54'59"N	75°49'30"E	475
11	PT-11	Vidarikand	Guwahati market	353332	26°10'45"N	91°45'10"E	74
12	PT-12	Vidarikand	Indore market	353366	22°43'01"N	75°51'03"E	581
13	PT-13	Vidarikand	Amritsar market	353373	31°37'02"N	74°52'21"E	258
14	PT-14	Vidarikand	Chennai market	353404	13°05'20"N	80°15'16"E	30
15	PT-15	Vidarikand	Odisha market	353303	22°11'13"N	86°38'41"E	116

Pharmacopeia of India (API)[15]. Quantitative evaluation of different phytochemicals, *viz*., total sugar content[16], total starch content[16], total phenolic content[17], and total flavonoid content[18] were analysed using spectrophotometric methods.

Preparation of plant extract

Accurately weighed (2 g) of each powdered sample was subjected to cold maceration with HPLC grade methanol (40 mL), with continuous shaking for 6 h, then allowed to stand at room temperature (27± 3ºC) for 18 h. The solution was filtered, and the residue was resuspended in fresh solvent. The procedure was repeated three times, and the pooled filtrate was then concentrated in rotatory evaporator (Buchi, Switzerland) under standard conditions of reduced temperature (35±2°C) and pressure (40 mbar) and concentrated extract was finally lyophilized and stored at 4ºC for further analysis.

Chemical fingerprinting & quantification through RP-HPLC

Preparation of standards and samples

Stock solutions of standards *viz*., gallic acid, daidzein, puerarin, genistein, p-coumaric acid, and biochanin A of strength 1 mg mL^{-1} was freshly prepared in HPLC grade methanol (1 mL) and stored at 4ºC until analysis. Working standard solutions were further diluted in methanol to a concentration of 0.1 mg mL^{-1}. Samples were prepared by dissolving a known quantity of lyophilized methanolic extract to obtain a working solution of 10 mg mL^{-1}. Working solutions were filtered through 0.22 µm Millipore membrane filter (USA) prior to chromatographic analysis.

Instrumentation and chromatographic conditions

Separation and quantification of targeted metabolites was done on a reverse phase high-pressure liquid chromatography (RP-HPLC) system of Waters (Massachusetts, USA), consisting of a pump for delivering mobile phase (Waters- 1525), autosampler (Waters- 2707) with a 30 µL loop for sample injection, photodiode array detector (Waters-2998) and column heater (Waters- 1500) for maintaining the optimum temperature of the column. All these units were operated, controlled, and monitored on the Empower software. The separations of targeted metabolites were achieved on the C_{18} RP-HPLC column (4.6×250 mm internal diameter, 5 µm particle size) supplied by SunFire. The injection volume was 10 µL for all analytes and detection was done at 254 nm. Identification of peaks was done by comparing the retention time (R_t) of standard peaks and the R_t of sample peaks, along with the spectral information provided by photodiode array (PDA) detector operated over the range of 190-400 nm.

Statistical analysis

Results are represented as mean ± standard deviation (S.D.) of three technical replicates (XLSTAT, 2010, Microsoft Corporation, USA).

Results and Discussion

Botanical studies

Morphologically, the tubers of *P. tuberosa* are big pot like, with a hard brownish, and thick outer surface (Supplementary Fig. S1 a), while the inner portion is soft, fibrous, white to creamy, starchy and sweet in taste. The identification of fresh tubers is easy, but in herbal markets the samples (Supplementary Fig. S1 b-m) is sold in pieces, making morphological examination difficult and insufficient for ensuring correct identification. For example, the sample procured from the Lucknow market (Supplementary Fig. S1 d) resembles *Cycas* sp., due to the presence of leaf scars on its surface. Similarly, the sample procured from Trivandrum (Supplementary Fig. S1 f) appears similar to *Ipomoea* sp., because of its tuber shape and the presence of concentric rings. Therefore, the evaluation of pharmacognostical parameters, along with chemical profiling through different analytical techniques, was undertaken to verify the authenticity of the marketed samples.

Pharmacognostical standardization

The samples were evaluated for physicochemical (Supplementary Table S1) and phytochemical (Supplementary Table S2) parameters required for quality control, as per API regulatory guidelines. The total ash content of the collected samples ranges from 10 to 17%, aligning with the values mentioned in the API for *Vidarikand*, whereas in the procured samples it ranged from 2.87 to 5.66%, indicating lower organic content which may be due to adulteration/substitution. The water-soluble ash content in the collected samples (7.96 to 14.86%) was also higher than that in the procured samples (1.8 to 4.16%), although the acid-insoluble ash values were comparable. Additionally, moisture content was found to be higher in the procured samples (3.11 to 9.75%) compared to the collected samples (4.98 to 6.61%). The samples were extracted with different solvents

from low polarity to high polarity. Hexane-soluble extractive values were found similar in both collected and procured samples. However, the water-soluble extractive values of the collected samples (14.2 to 23.2%) were corresponded to the API values, whereas the procured samples show lower values (6.26 to 13.48%). Similarly, the alcohol extractive values of the collected samples (3.05 to 5.73%) were higher than those of the procured samples (0.1 to 1.71%) (Fig. 1). A similar previous study reported physicochemical characterization of authentic *P. tuberosa* sample in comparison to marketed samples from Delhi, Lucknow, and Mumbai. The marketed samples were found to have lower total ash content, acid insoluble ash, water extractive, and alcohol extractive value than the authentic *P. tuberosa* sample, similar to the findings observed in our study[19]. The differences in physicochemical values in authentic and procured *Vidarikand* samples indicate possible adulteration in the marketed samples. However, these are preliminary tests that need to be further confirmed by chromatographic analysis.

The total phenolic content of the collected samples (0.24 to 0.75%) was higher than that of the procured samples (0.01 to 0.2%). However, the total flavonoid content and total sugar content were comparable between the collected and procured samples. The total starch content, was higher in the procured samples (0.09 to 1.22%) compared to the collected samples (0.09 to 0.23%) (Fig. 2).

Chemical fingerprinting and metabolite content in collected and marketed samples

Quantification of metabolites through RP-HPLC

The separation of targeted metabolites was achieved under 0.1% orthophosphoric acid in water (A) and acetonitrile (B), eluted in an isocratic manner (65:35% v/v; A: B), with a flow rate of 0.7 mL min^{-1},

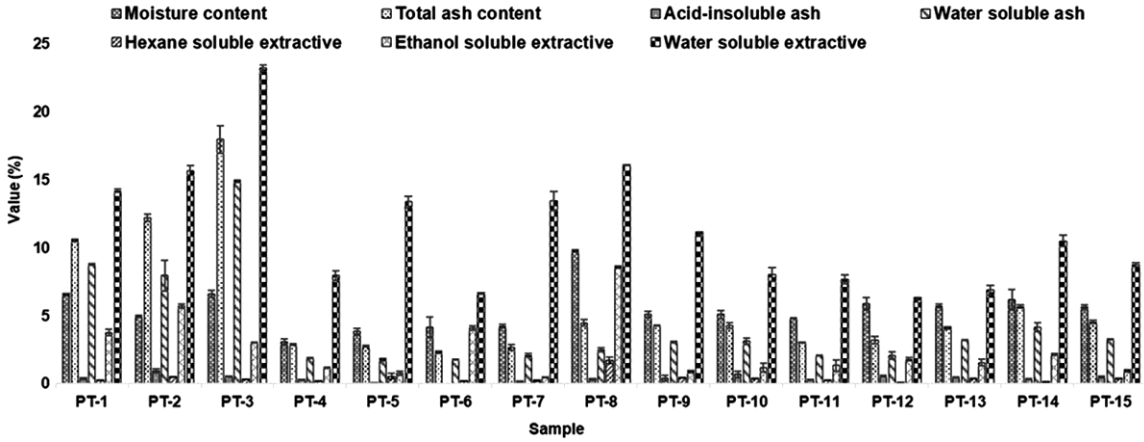

Fig. 1 — Physicochemical parameters of *Pueraria tuberosa* (Willd.) DC. collected and marketed samples. Error bar represents standard deviation, n=3

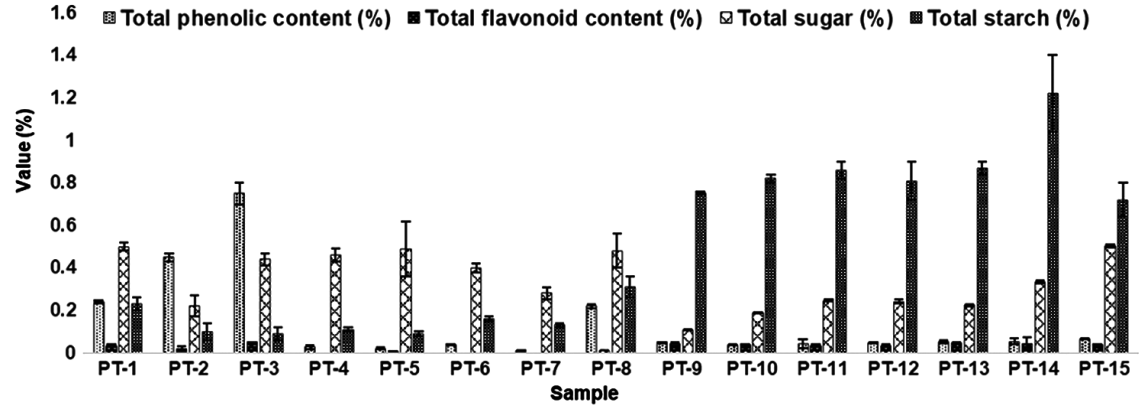

Fig. 2 — Phytochemical parameters of *Pueraria tuberosa* (Willd.) DC. collected and marketed samples. Error bar represents standard deviation, n=3

thermostated at 35ºC and the total run time was 20 min. The bioactive markers *viz*., gallic acid, p-coumaric acid, puerarin, biochanin A, daidzein, and genistein were identified at retention times (R_t) of 4.42±0.02, 5.81±0.08, 6.36±0.12, 7.76±0.14, 8.77±0.09, and 15.73±0.15 min respectively (Fig. 3). The presence of targeted metabolites in the plant extract was confirmed by matching the retention time and absorption spectra with those of the standards. The quantification of metabolites was done at absorption maxima of 254 nm. The data indicate significant variation in metabolite content among the collected and procured samples (Table 2). The HPLC chromatogram (Fig. 4) depicted 14 characteristic peaks in collected samples, which were used for the comparison between collected and procured samples (Supplementary Table S3). The HPLC chromatogram of procured samples is entirely different from the authentic samples as in procured samples only limited number of metabolite peaks was observed. Peak number 1, 2, 4 & 6 is present in all the collected and procured samples, so they cannot be used for identification. However, peak number 3, 5, 7, 8, 9, 10, 11, 12 & 14 were exclusive to collected samples and can be used to identify of *Vidarikand* adulteration under the developed HPLC conditions. Interestingly peak number 13 (R_t 14.21) was only detected in procured sample that can also be used for identification.

In authentic samples (PT-1 to PT-3), all targeted metabolites were detected and quantified. Gallic acid ranged from 2.84-4.95 µg/mg, while p-coumaric acid varied between 0.60-2.27 µg/mg. Among the isoflavonoid, puerarin was the major constituent (12.18-33.48 µg/mg), whereas biochanin A (0.35-0.53 µg/mg), daidzein (0.49-0.87 µg/mg), and genistein (0.24-0.35 µg/mg) were present in minimal amounts. In contrast, among the procured samples (PT-4 to PT-15), only gallic acid and p-coumaric acid were

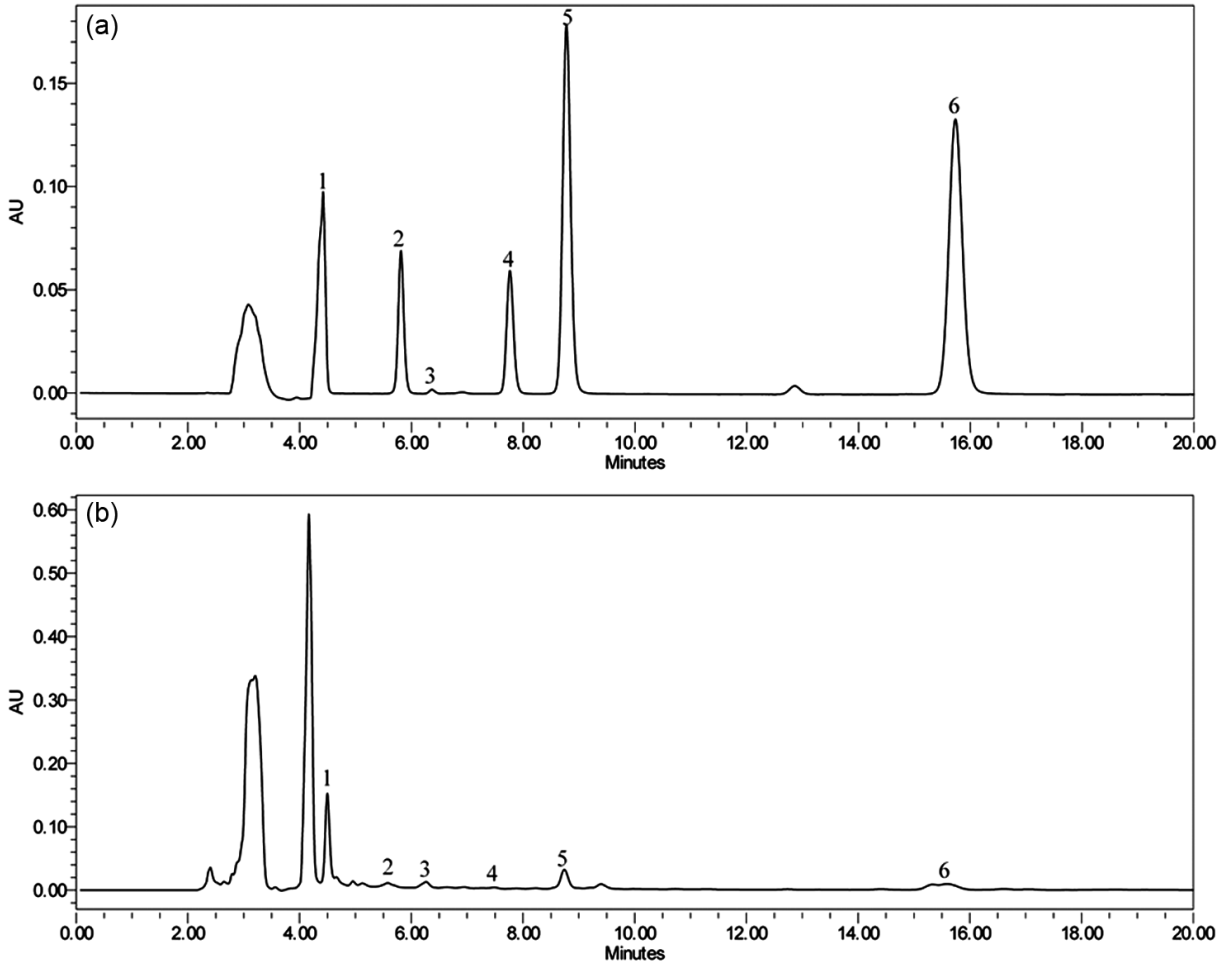

Fig. 3 — RP-HPLC chromatogram of mixture of marker compounds (gallic acid (1), p- Coumaric acid (2), puerarin (3), biochanin A (4), daidzein (5), and genistein (6)) (a) and collected sample (PT-1) (b)

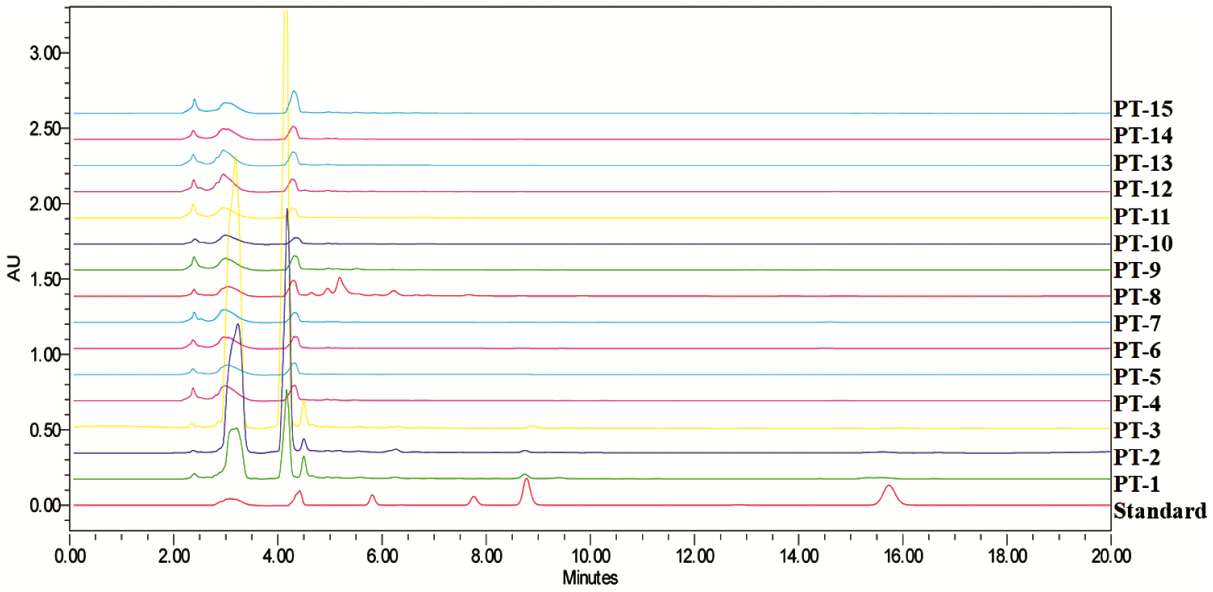

Fig 4 — 3D densitogram of methanolic extract of *Pueraria tuberosa* (Willd.) DC. collected and procured (market) samples scanned at 254 nm

Table 2 — Metabolites content in methanolic extract of *Pueraria tuberosa* (Willd.) DC. collected and marketed samples

S.No.	Sample code	Metabolite content (µg/mg) *					
		Gallic acid	P-coumaric acid	Puerarin	Biochanin A	Daidzein	Genistein
1.	PT-1	4.06±0.05	1.82±0.04	16.52±0.20	0.47±0.02	0.87±0.02	0.35±0.03
2.	PT-2	2.84±0.07	2.27±0.02	33.48±0.14	0.53±0.04	0.49±0.04	0.31±0.05
3.	PT-3	4.95±0.09	0.60±0.07	12.18±0.13	0.35±0.04	0.51±0.01	0.24±0.04
4.	PT-4	0.14±0.01	0.22±0.06	Nd	Nd	Nd	Nd
5.	PT-5	0.24±0.02	Nd	Nd	Nd	Nd	Nd
6.	PT-6	0.18±0.04	0.23±0.01	Nd	Nd	Nd	Nd
7.	PT-7	1.57±0.03	Nd	Nd	Nd	Nd	Nd
8.	PT-8	2.46±0.05	0.55±0.05	Nd	0.59±0.07	Nd	Nd
9.	PT-9	2.27±0.09	0.28±0.06	Nd	Nd	Nd	Nd
10.	PT-10	1.36±0.02	0.28±0.02	Nd	Nd	Nd	Nd
11.	PT-11	1.56±0.03	Nd	Nd	Nd	Nd	Nd
12.	PT-12	0.30±0.01	0.28±0.02	Nd	Nd	Nd	Nd
13.	PT-13	0.24±0.01	0.26±0.01	Nd	Nd	Nd	Nd
14.	PT-14	0.19±0.02	0.21±0.05	Nd	Nd	Nd	Nd
15.	PT-15	0.30±0.05	0.25±0.06	Nd	Nd	Nd	Nd

* Values are mean ± S.D, Nd: Not detected under the developed HPLC conditions

detected in varying concentrations, with no major metabolites present except for biochanin A in PT-8 (Trivandrum market). However, the presence of gallic acid and p-coumaric acid in market samples does not provide any significance, as these metabolites are widely distributed across plant species. Previous phytochemical studies have largely focused on isoflavonoid, with puerarin reported at 42.1 µg/mg[13] and 9.28–3.02% w/w[1,20], while genistein and daidzein were detected in comparatively high concentrations (55.7 mg/g and 68.7 mg/g, respectively) in ethanolic extracts of procured tubers[12]. In contrast, our study revealed considerably lower levels of these compounds in authentic samples. Such differences may be attributed to geographical variation[21] and differences in extraction methods. These findings indicate that puerarin, daidzein, and genistein, together with the characteristic chromatographic profile, can serve as reliable biomarkers for the authentication of Vidarikand tubers and for the detection of adulterants.

Conclusion

In this study, pharmacognostical parameters, and RP-HPLC quantification of bioactive metabolites *viz.*, gallic acid, p-Coumaric acid, puerarin, biochanin A,

daidzein, and genistein in collected and marketed *Vidarikand* samples was done. This study demonstrates that authentic *Pueraria tuberosa* tubers possess distinct pharmacognostical traits and a characteristic RP-HPLC profile with the presence of puerarin, daidzein, and genistein, whereas most marketed samples lacked these key metabolites, indicating widespread adulteration. Notably, none of the marketed samples matched or were identified as authentic *P. tuberosa*. The integration of pharmacognostical parameters with chemical fingerprinting confirms that these isoflavonoid, along with the chromatographic profile, serve as reliable biomarkers for the authentication of *Vidarikand* and the detection of adulteration in commercial trade. This highlights the urgent need for strict quality control procedures and market regulations to ensure the authenticity and efficacy of herbal drugs.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_24(11)(2025)1074-1081_SupplData.pdf

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Conflict of Interest

Two of the authors are affiliated with the funding agency, the research work including study design, data collection, analysis, and interpretation was carried out independently to ensure impartiality and avoid any potential bias.

Author Contributions

AT: Methodology, Formal analysis, Writing-original draft; MKC: Formal analysis, Writing-original draft; AM: Writing- review & editing; VR: Formal analysis, Writing- review & editing; RS: Formal analysis; SKL: Resources; NS: Resources; RA: Resources, Writing- review & editing; SS: Conceptualization, Supervision, Writing- review & editing.

Ethics statement

Not applicable

Data Availability

Data will be made available on request.

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