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Different Geo-Climatic Conditions and Its Effect on Active Constituents of the Cultivated Prishniparni (*Uraria Picta*), Evaluated by Using HPLC and HPTLC

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Abstract: *Uraria picta* Desv. of family Fabaceae is an important plant species in traditional system of medicine. It is one of the important ingredient for many Ayurveda formulations like Chyawanprash, Dashmoolarishta, Mahanarayan tail etc. In this study, the effect of different geo-climatic conditions on active constituent of cultivated Prishnaparni was evaluated for quality assurance. The extracts were prepared from whole plant parts collected from different geographic locations by using ethanol and water solvent combinations. The ethanol extract was subjected to Diaion HP 20 column, leading to isolation of compound. The structure of the compound was elucidated by spectroscopic techniques. The HPTLC densitometric analysis was also carried out using CAMAG HPTLC system. The HPLC chromatographic separation was performed using a Hyperclone BDS C18 LC column. The solvent system consisted of mixtures of water with 0.1% o-phosphoric acid and acetonitrile and signal was monitored at 267 nm. In this study, a new method for isolation and purification of Rhoifolin was developed from Prishnaparni. The highest Rhoifolin content (0.8%) was found in raw material of *Uraria picta* collected from Hardoi district, UP. Extractive values show the highest yield (12.4%) in hydro-alcoholic extract. The current study showed that the raw materials collected from different locations shows significant presence and percentage of Rhoifolin. Thus, it can be consider as a chemical marker for the standardization and quality control of *Uraria picta* plant. This will help to identify the genuine raw drugs of *Uraria picta* plant parts and this study will help to identify the suitable site either for commercial cultivation or conservation.

Keywords: *Uraria picta* • Cultivation • Isolation • Rhoifolin • quantification • HPLC • HPTLC

Introduction

Ayurveda is an oldest health care science and is an important part of Indian health care system since ages. Medicinal and aromatic plants are the backbone of Ayurveda and other traditional health care systems. Recently global demand of Ayurveda has increased significantly due to its natural way of treating diseases. In COVID- pandemic also Ayurveda treatment proven to be very effective which increased demand of medicinal plants to many folds.

In India, more than 300 species of medicinal and aromatic plants are being utilized in major herbal formulations including plant parts e.g., root, stem, bark, leaves, flowers, seeds and fruits. These plants produce in the form of raw drugs/raw materials has come through sustainable sources and many of them cultivated in own farms and with the farmer groups or by the stakeholders (Rana et al., 2022). The cultivated fields are mostly developed by stakeholders near nature or actual habitats of the medicinal and aromatic plants. Although, *in-situ* and *ex-situ* cultivation are being properly in scientific manner and productivity of the medicinal and aromatic plants (MAPs). In India, use of the medicinal plants to cure and abate certain diseases and ailments is an age-old practice starting from a situation when life-saving herbs from the wild habitats provided the shelters during

emergencies and trauma. Indian forests have rich biodiversity, where medicinal and aromatic plants (MAPs) make up a large part and largely deeds as reservoir for sourcing of the raw material. It is estimated that more than 90% raw material supply for herbal industries is coming through wild collections only. Most of the time this wild collection involves destructive harvesting methods which are responsible for depletion of natural resources of these important medicinal plant species which resulted that many species are on the verge of extinction. If this continues further, then we may permanently lose some important plant species from some confined habitats. Hence it is very important to conserve these species through cultivation and sustainable harvesting methods for future of our indigenous Ayurveda health care system. The indiscriminate collection of medicinal plants has led to many of them becoming rare, threatened, or endangered. Cultivation is the best solution for the species for the fulfilment the industrial demands are using this species for various lifesaving drugs. Commercial cultivation is the best method of conservation for medicinal plants as it will reduce pressure on available natural resources for raw material supply to AYUSH industries and it will ensure sustainable supply of genuine species in future which ultimately helps to maintain therapeutic value of finished products (Rana et al., 2022).

Prishniparni (*Uraria picta*) is one the important medicinal plant mentioned in Ayurveda which is highly consumed by AYUSH industries. It is one of the important ingredient for many Ayurveda formulations like Chyawanprash, Dashmoolarishta, Mahanarayan tail etc. As per Ayurveda Prishniparni is useful in Fever, Burning sensation in body, asthma/difficulty in breathing, Diarrhea with blood/dysentery, Cough, Excessive thirst, Aphrodisiac and Fracture (Kirtikar and Basu, 1935, Yadav et al., 2009). About 20 species in tropical Africa, S.E. Asia, Indomalasia and Australia; 9 species are found in India. The most common species are *Uraria picta, U. rufescens, U. lagopoides* and *U. lagopus*. It is found in grassy localities, scrub jungles. Throughout India ascending to 1500 m.asl. *Uraria picta* is an erect small shrub of almost 90 cm height. Every part of the plant shows specific medicinal properties like root decoction used for cough, cold, chills, general healing, and antiseptic, leaves part used as diuretic, aphrodisiac, general antiseptic, oral sores etc, whole plant part used as an anti-venom, gynaecological disorder and treatment of gonorrhea (Allen, 1981; Hooker, 1872; Neill, 2005; Burkil, 1985; Jain and Defillips, 1991, Kirtikar and Basu, 1995).

Flavonoids are the main chemical constituents of this plant which includes flavones and isoflavones, triterpenes and steroids sare other chemical constituents reported from *Uraria picta*. The major biologically active constituent reported in *U. picta* is Rhoifolin which exhibits a wide range of biological activities it includes anti-inflammatory, anti-thrombotic, anti-hepatic properties and is used in treatment of Alzheimer's disease due to their free radical scavenging activity (Saija et al., 1995; Akdemir and Taltli 2001, Occuhiuto and Limardi, 1994, Calderone and Chericoni 2004; Occuhiuto and Circosta, 1990; Rahman and Gibbons 2007). Prishniparni is required in huge quantity by Ayurveda industries and at present majorly sourced from different suppliers where the chances of the collection of the raw material may be mixed with other species. In the current market scenario, it is very difficult to get 100% genuine raw material of Prishniparni and it always found to be mixed with other species (Anonymous). This may alter desired therapeutic benefits of finished products also continuous extractions in huge quantity from wild will also questions about its sustainability in future. Considering the above issues there is dire need to introduce this species in cultivation which will resolve issues about its genuinity and sustainability.

We have already started cultivation of genuine species of Prishniparni (*Uraria picta*) in Uttar Pradesh from more than 10 years and now able to achieve self-sufficiency in fulfillment of its requirement through cultivation. Geo-climatic conditions play an important role in quality of produce for any agriculture or horticulture crops. The same applicable for medicinal plants as well. As medicinal plants are not in traditional cultivation practices, it is important to find out the best geo-climatic conditions for further propagation of medicinal plant species. This study aims towards finding the best geo-climatic conditions for medicinal plant Prishniparni to produce good quality raw material in terms of high quantity of active phytochemical (Rhoifolin). This study was to assess the level of Rhoifolin content in the *ex-situ* cultivation and to assess the genetic diversity among them. These compounds were found abundant in cultivated plants. The study of the species can be considered as a model for the conservation of other important threatened species (Prasad et al., 2022). Also, in this study we have reported new isolation and purification method of Rhoifolin from the whole plant of *Uraria picta* and different extracts prepared by using solvent combination of ethanol and water. Rhoifolin content was estimated in all extracts and raw material collected from different geographic regions of UP and Bihar by HPLC. HPTLC fingerprinting of all extracts and raw materials were developed along with isolated compound. Phenotype and Genotype diversity is considered important for welfare of human beings. In view of the emphasis on chemical features, intra-species genetic diversity attains greater importance for medicinal and aromatic plants. As a result, this study will contribute to the understanding, authentication, quality control and conservation of Prishnaparni. As a result, this study will contribute to the understanding, authentication and quality control of Prishnaparni as raw materials or raw drugs.

Material and Methods

Plant Collection and Identification:

The plant specimens of *Uraria picta* (Prishnaparani) were collected from different cultivated sources of Uttar Pradesh (Kushinagar, Deoria and Hardoi) and Bihar (Motihari). Collected plant species made into herbarium specimens as per standard method and given in-house voucher/field collection number. The plant species were identified by consulting the Herbarium of Botanical Survey of India, (BSD) Dehradun, Forest Research Institute (DD) Dehradun and Garhwal University Herbarium (GUH) Srinagar Garhwal, Uttarakhand. Raw materials (RM) of the same has also been identified through powder microscopy and anatomical section as per standard methods. The whole plant parts of the plants (as a raw material) along with herbarium specimens (accession number-116635) have been kept in the museum as reference sample or as an in-house standard for future references (**Fig. 1**).

Leaves of the plants **Flowers of the plants** **Habit of the plants**

Fig. 1 Photographs of the Plants (*Uraria picta*)

Chemicals and Reagents

HPLC grade methanol, acetonitrile (ACN) and ortho phosphoric acid used in the study were obtained from Merck. HPLC grade water was obtained from PALL life sciences water purification system. Durapore PVDF 0.45 µm membrane filter was obtained from Merck. Solvent used for extraction and column chromatography is AR grade procured from Rankem. Diaion HP 20 used for chromatographic separation procured from Ipsum Lifesciences LLP, Mumbai. Rhoifolin was isolated in the lab. The identity and purity were confirmed by chromatographic methods (HTLC, HPLC), spectroscopic data (1D-NMR and HR-ESI-MS) and by comparison with published spectral data. The purity of standard compound was calculated to be 97%. For the HPTLC study solvents used were of chromatography grade and all the chemicals used were of analytical reagent grade.

Preparation of alcohol extract

Dried whole plant part of *Uraria picta* (100 gm) were pulverised to get coarse ground powder. Coarse ground powder was taken in round bottom flask connected with condenser and it was refluxed for 3 hrs using ethanol solvent (4 volume w.r.t. batch size) at 80^0C. Then extracted solution was filtered through Whatman filter paper 4 (125 mm) to get the filtrate. The same process repeated twice for 3 hrs by using fresh solvent till the drug appeared exhaust. The obtained filtrate was concentrated by rotary evaporator under vacuum to get fine dried powder. The same process was implemented for all remaining four raw materials to get alcohol extracts.

Preparation of hydroalcoholic extract

Dried whole plant part of *Uraria picta* (100 gm) were pulverised to get coarse ground powder. Coarse ground powder taken in round bottom flask connected with condenser and refluxed for 3 hrs using alcohol-water (70:30) as solvent ratio at 80^0C, solvent used four volumes w.r.t. batch size for extraction. Then filtered through Whatman filter paper 4 (125 mm) to get the filtrate. The process repeated twice for 3 hrs by using fresh solvent till the drug appeared exhaust. The obtained filtrate was concentrated by rotary evaporator under vacuum to get fine powder. The same process was implemented for all four raw materials to get hydro-alcoholic extract (Saini et al., 2023).

Preparation of aqueous extract

Dried whole plant part of *Uraria picta* (100 gm) were pulverised to get coarse ground powder. Coarse ground powder taken in round bottom flask fitted with condenser and refluxed for 3 hrs using water (4 volume w.r.t. batch size) as solvent at 90^0C. Then filtered through Whatman filter paper 4 (125 mm) to get the filtrate. The process repeated twice for 3 hrs by using fresh solvent till the drug appeared exhaust. Obtained filtrate was concentrated by rotary evaporator under vacuum to get fine powder. The same process was implemented for all four raw materials to get aqueous extract. All the extracts were

stored in controlled condition and airtight container till further use (Saini et al., 2023).

Isolation and Characterization of Rhoifolin from *Uraria picta*

Column chromatographic separation was performed using Diaion HP 20 and the TLC experiment was performed with pre-coated Merck silica gel 60 PF254 aluminum sheets; the spots were visualized under UV light at 254 nm. The 400 MHz NMR (Bruker, Switzerland) was used to record spectra in DMSO with tetramethyl silane (TMS) as internal standard. ^{13}C NMR spectra were recorded at 400 MHz. Chemical shifts are given in parts per million. LC-MS system (Agilent Technologies) was used for mass spectra. The hydro- alcoholic extract (10 gm) of *Uraria picta* was chromatographed over a Diaion HP 20 resin column and eluted with gradient solvent system of water– ethanol to give six fractions. Fraction 6 (water–ethanol, 40:60 v/v) has resulted in the isolation of the desired marker which was further purified using crystallization with methanol. The structure elucidation of Rhoifolin (**Fig. 2**) was performed with the help of UV, $^{1}HNMR$, ^{13}C NMR, and mass (LC-MS) analysis and confirmed as reported earlier (Yadav et al., 2009; Aoki et al., 2017; Sawant and Kachwala, 2012).

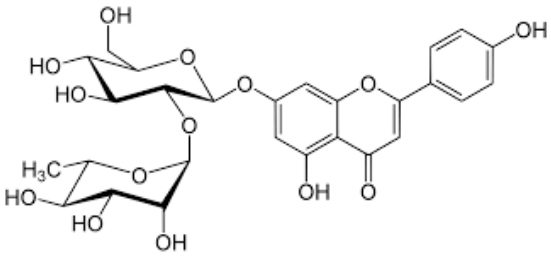


Fig. 2 Structures of isolated Rhoifolin of *Uraria picta*

Chromatographic methods

HPTLC Analysis

Preparation of Standard Solution

Stock solution of Rhoifolin compound was prepared at a concentration of 2.0mg/25mL in methanol. From which 20µL were injected in HPTLC system for making standard curve.

Sample Preparation

Dried and finely coarse ground plant materials (5.0 g) of *Uraria picta* were extracted with the hot reflux and sonication in 50mL methanol. The supernatant was transferred and collected to a flask. The procedure was repeated thrice, and pooled extract was concentrated under vacuum by using and final volume adjusted to 50.0mL with analytical grade methanol. The solution was filtered through 0.45 μm membrane filter before analysis.

HPTLC Instrumentation and Conditions

A CAMAG HPTLC system equipped with automatic TLC Sampler 4, visualizer, densitometer and vision CATS software was used. HPTLC was performed on 100 mm × 100 mm aluminum backed plates coated with 0.2 mm layers of silica gel 60 F254 (Merck, Mumbai, India). Standard solutions of Rhoifolin and sample solutions were applied to the plates as bands 12.0 mm wide, 10.0 mm apart, and 15.0 mm from the bottom edge of the same chromatographic plate. Ascending development to a distance of 80 mm was performed at room temperature (28 ± 2°C), with ethyl acetate– methanol–water–glacial acetic acid 75.0:15.0:10.0:5.0 (v/v), as mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 30 min. After development, the plates were dried in air and then visualized at 254 and 366 nm using Camag TLC Visualizer.

HPLC Analysis

Preparation of Standard Solution

Stock solution of Rhoifolin compound was prepared at a concentration of 2.0mg/25mL in methanol. From which 20µL were injected in HPLC system for making standard curve.

Sample Preparation

Dried and coarse ground plant materials (5.0 g) of *Uraria picta* were extracted with the hot reflux and sonication in 50mL methanol. The supernatant was transferred to a flask. The procedure was repeated thrice, and combined extract was concentrated under vacuum by

using rota- evaporator and final volume adjusted to 50.0mL with methanol. Solution was filtered through 0.45 μm membrane filter before analysis. Similarly, 20- 30 mg of dry extracts were dissolved in 25 ml methanol (HPLC grade) to get 1 mg/ml solution, filtered through 0.45 µm membrane filter and injected into waters HPLC system.

HPLC Instrumentation and Conditions

HPLC analysis was performed by using Waters Alliance 2695 (Millford, MA, USA) system which is connected to Waters 2996 photodiode array detector (DAD). The chromatographic separation was performed using a Hyperclone BDS C18 LC column (250 × 4.6 mm, 5 μm, Phenomenex, USA) at 25^{0}C. The solvent system consists of isocratic mixtures of water with 0.1% o-phosphoric acid (solvent A) and acetonitrile (solvent B). The final optimized HPLC condition is 80: 20 (A: B) for 30 mins. The HPLC solvents were degassed before being delivered into the system. All samples selected for HPLC analysis were filtered through a 0.45 μm membrane filter. The flow rate was 1.5 mL/min. The injection volume was 20 μL. Signal was monitored at 267 nm.

Results

Extract yield:

Hydro-alcohol extract of *Uraria picta* whole plant shows higher percentage of yield as compared to other solvent extracts. In this study, ethanol extract yield was found in the range of 3.76 to 6.26, hydroalcoholic extract yield found 6.62 to 12.4% and water extract yield found 6.5 to 11.28% respectively (Table 1).

Table 1 Percentage of yield of different extracts of *Uraria picta*

Plant name	Part used	Collection region	Name of Solvent	Weight of plant material (g)	Weight of dried extract (g)	Extractive value (%)
Uraria picta	Whole plant	Kushinagar	Ethanol	100	6.26	6.26
			Ethanol: Water (70:30)		12.4	12.4
			Water		11.28	11.28
		Hardoi	Ethanol	100	3.76	3.76
			Ethanol: Water (70:30)		6.62	6.62
			Water		6.50	6.50

Isolation and Characterization of compound from *Uraria picta*

The hydro-alcoholic extract was subjected to a Diaion HP20 column, leading to the isolation of compound 1. Compound 1 was obtained as pale-yellow amorphous powder from water–ethanol (40:60) elute. The authenticity of the Rhoifolin was confirmed by its spectral characteristic. The molecular formula of compound 1 was assigned as $C_{27}H_{30}O_{14}$ from the molecular ion peak at m/z: 579.4 (M^+) in the EIMS. ^{1}H NMR (400 MHz, DMSO) sugar moieties δ: 1.21 (d, *J*=6.4 Hz, 3H), 3.21- 3.79 (m, 10H), 5.13 (s, 1H), 5.38 (d, *J*=4.8 Hz, 1H); 6.38 (d, *J*=2.4 Hz, 1H), 6.80 (d, *J*=2.0 Hz, 1H), 6.89 (s, 1H), 6.96 (d, *J*=9.2 Hz, 2H), 7.96 (d, *J*=8.8 Hz, 2H), 10.44 (s, 1H), 12.98 (s, 1H). ^{13}C NMR (400 MHz, DMSO) δ: 18.5, 60.9, 68.8, 70.0, 70.8, 70.9, 72.3, 76.7, 77.4, 77.6, 94.9, 98.2, 99.7, 100.9, 103.6, 105.8, 116.4, 121.4, 129.0, 157.4, 161.5, 161.8, 162.9, 164.7, 182.4. Based on the above data and comparison with literature data, the name of compound 1 is given Rhoifolin (**Fig. 3**).

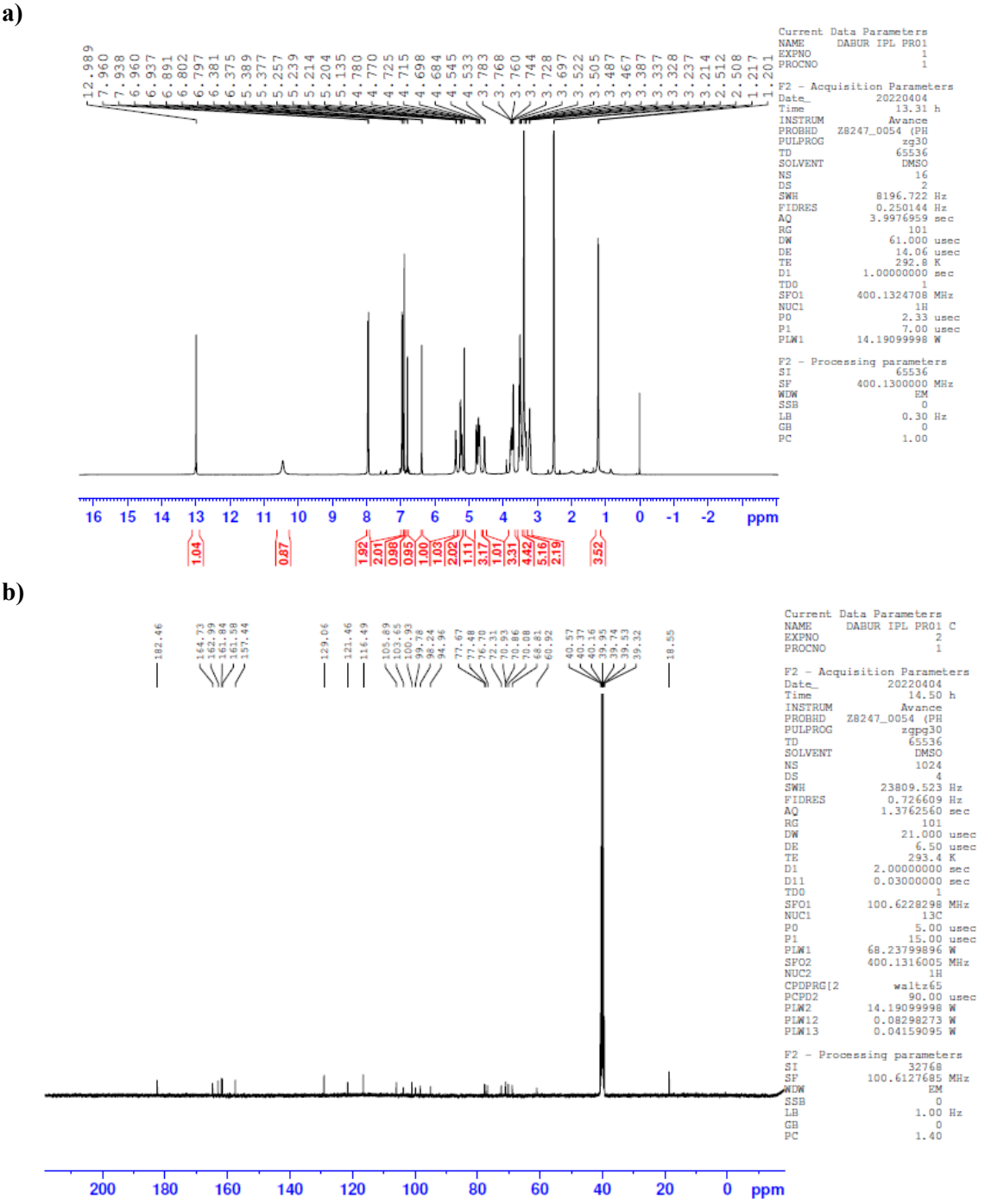


Fig. 3 The spectra of the isolated compound 1 from *Uraria picta*: a) ^{1}H-NMR, b) ^{13}C-NMR

HPTLC Analysis

Initial trial experiments were conducted to select a suitable mobile phase for accurate analysis of the Rhoifolin in extracts. Of the various mobile phases tried, ethyl acetate–methanol–water–glacial acetic acid 75.0:15.0:10.0:5.0 (v/v), gave the best resolution of Rhoifolin (R_F 0.57). The identity of the Rhoifolin band from the extracts of the whole plant powder of *Uraria picta* was confirmed by overlaying their UV absorption spectra with respect to standard. The HPTLC analysis of *Uraria picta* revealed the presence of Rhoifolin and various other phytochemicals as illustrated (**Fig. 4**). The chromatogram was obtained upon scanning at UV 254 nm and 366 nm, were also generated.

HPLC Analysis

Initially various mobile phases were tried in attempts to obtain the best separation and resolution of Rhoifolin in extracts. The mobile phase consisting of isocratic elution of water with 0.1% o-phosphoric acid (solvent A) and acetonitrile (solvent B) was found to be an appropriate mobile phase allowing adequate separation of Rhoifolin using Hyperclone BDS C18 LC column column at a flow rate of 1.5 ml/min. The retention time for Rhoifolin was 10.21 min. The injection volume was 20 μL. Signal was monitored at 267 nm. Under this system, the chromatogram of Rhoifolin and extracts of *Uraria picta* is also revealed (**Fig. 5**).

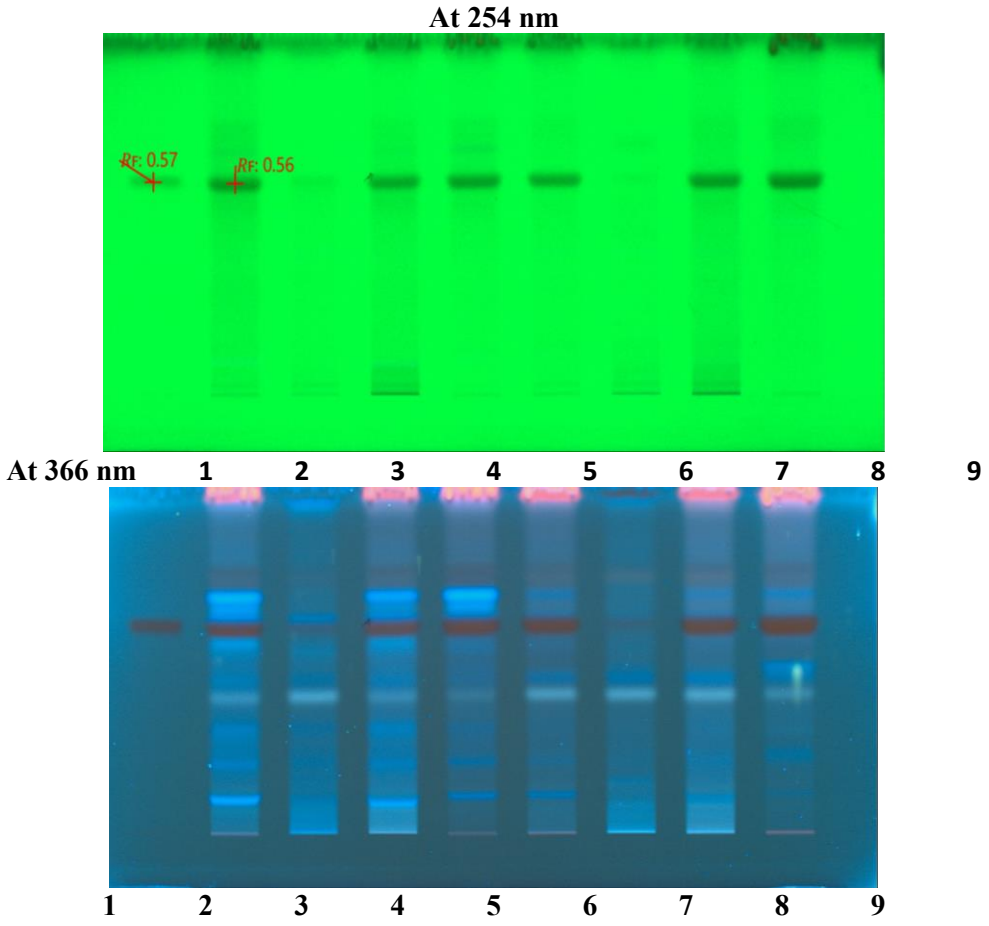
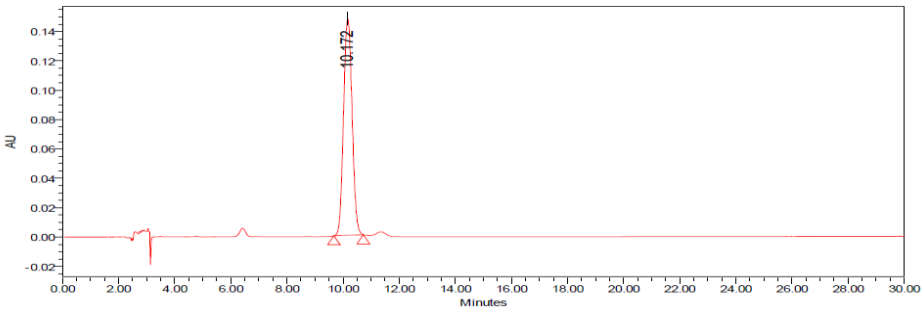
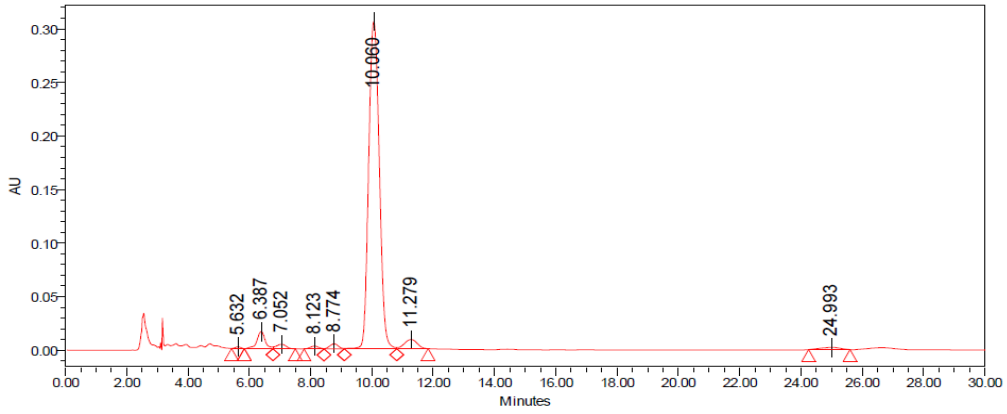


Fig. 4 HPTLC fingerprint profile of isolated compound Rhoifolin, raw material and extracts of Prishnaparni.T1: Rhoifolin T2: RM from Kushinagar, T3- T5: Water, HA & Alcohol extracts of Kushinagar region RM, T6: RM from Hardoi, T7- T9: Water, HA & Alcohol extracts of Hardoi region

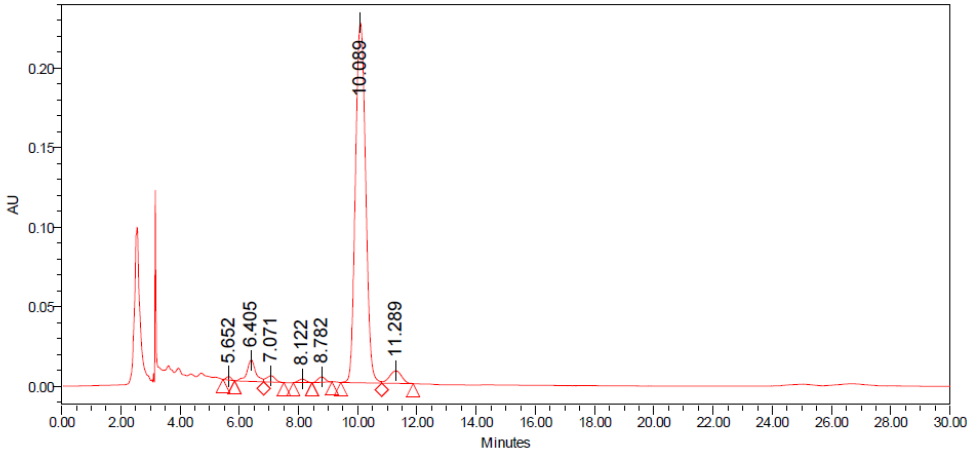
A)



B)



C)



D)

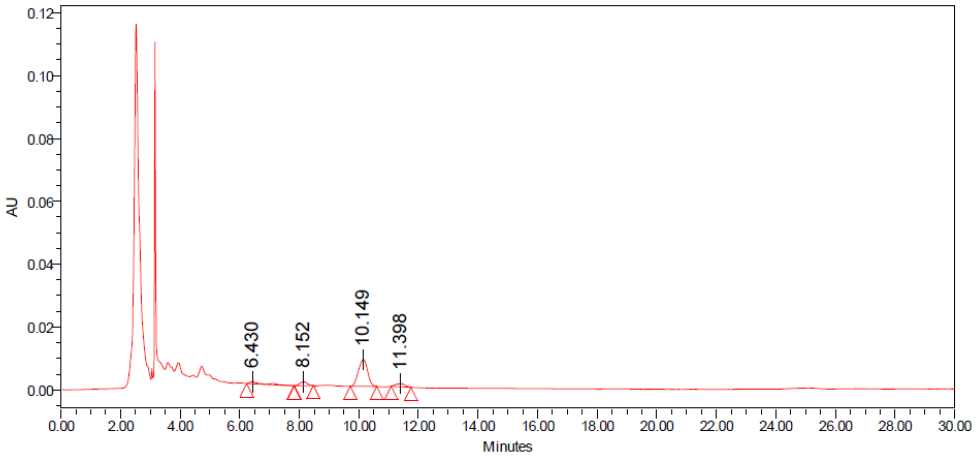


Fig. 5 HPLC chromatogram of isolated compound Rhoifolin **(A)**, Extracts prepared from *Uraria picta* collected from Hardoi district; Alcohol extract **(B)**, Hydro-alcoholic extract **(C)**, Water extract **(D)**.

Quantitative estimation of Rhoifolin in different extracts of *Uraria picta* by HPLC

Rhoifolin compound and extracts sample solutions were run in isocratic mobile phase systems mentioned in HPLC methodology section. Highest Rhoifolin content (13.88%) found in ethanol extract of *Uraria picta* collected from Hardoi region. In comparison with all four herbal raw materials of *Uraria picta* whole plant, the highest Rhoifolin content (0.8%) found in raw material of *Uraria picta* collected from Hardoi district (**Table 2**).

Table 2 Rhoifolin content in raw materials and extracts of *Uraria picta* collected from different regions

Plant Name	Sample	Region	%Results (w/w)[a]
Uraria picta (Whole plant)	Dried whole plant	Kushinagar, UP	0.49
	Dried whole plant	Motihari,Bihar	0.46
	Dried whole plant	Deoria , UP	0.40
	Dried whole plant	Hardoi, UP	0.81
	Alcohol extract	Kushinagar	8.65
	Hydro-alcoholic extract		5.73
	Water extract		0.71
	Alcohol extract	Hardoi	13.88
	Hydro-alcoholic extract		10.43
	Water extract		0.47

[a] The results (%w/w) presented in the above table are the average values of 'n' concentrations of sample solutions (n =2).

Discussion

The extraction, isolation, purification, HPTLC fingerprint profiling, and HPLC quantification of Rhoifolin were carried out in herbal raw material collected from different regions of Indian states. The current study attempts to extraction of whole plant part of *Uraria picta* by using different polarity solvents like ethanol, hydro-alcoholic and aqueous, isolation, purification and characterization of isolated compound by using LCMS, NMR spectroscopy, HPTLC profiling of raw material, extracts and HPLC quantification of Rhoifolin in different raw material and extracts prepared from *Uraria picta* whole plant part.

Comparative study has been carried out for the herbal raw material collected from different regions of India. Extraction yield recorded for raw material collected from Kushinagar and Hardoi regions. In comparison with extract yield hydro-alcoholic solvent found higher yield than alcohol. Rhoifolin content found maximum in *Uraria picta* whole plant collected from Hardoi district (Latitude: 27° 23' 48.8076". Longitude: 80° 7' 46.8912"), Uttar Pradesh. Ethanol extract found the highest content of Rhoifolin. Ethanol extract subjected for column chromatography by using Diaion HP 20 resin with different combination of solvents ethanol and water. Fractions collected from column chromatography are pulled based on TLC profiling and mixed fractions concentrated and kept for crystallization with methanol. Fraction water–methanol (40:60) gave single spot compound after crystallization. The purity was checked by HPLC and submitted for Mass and NMR analysis. The structure of compound of *Uraria picta* was elucidated by spectroscopic methods (^{1}H NMR, ^{13}C NMR, and MS) and compared with the earlier literature (Billore et al., 2004). The ^{1}H NMR spectra of compound 1 revealed that the presence of two hydroxyl groups, which displayed signals at 12.98 (s, 1H) and 10.44 (s, 1H), respectively and two doublets δ_H at 6.38 (d, J=2.4 Hz, H-6) and δ_H at 6.80 (d, J=2.0 Hz, H-8) on the A-ring; A_2B_2-type aromatic δ_H at 7.96 (d, J=8.8 Hz, H-2', H-6') and δ_H at 6.96 (d, J=9.2 Hz, H-3', H-5'), together with an olefinic δ_H at 6.89 (s, H-3) on a flavone C-ring. ^{1}H NMR data matching exactly with previously reported data in the literature. Besides this, glycosidic δ_H at 5.38 (d, J=4.8 Hz, H-1"), 5.13 (s, H-1'"), δ_C at 100.93 (C-1") and δ_C at 98.24 (C-1'") were evident in the 1^H and 13^C NMR spectra. The multiplet δ_H at 3.21–3.79 (10H, m, H-2"–H-6", H-2'"–H-5'") was assignable to the coupling between protons and methylene protons of the glucosyl ring. The spectrum displayed singlet at 1.21 (s, 3H) showing the presence of one

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methyl group. The ^{13}C NMR of compound 1 recorded the presence of 27 carbons, including a two-sugar moiety at δ_C (100.9, 98.2, 77.6, 76.7, 72.3, 70.9, 70.8, 70.0, 68.8, and 60.9) and one peak at δ 182.4, which was assigned to the carbonyl group. The ^{13}C NMR spectrum also exhibited the presence of δ_C (161.5, 99.7, 162.9, and 94.9) for the A-ring, δ_C (164.7, 103.6, 182.4, 157.4, and 105.8) for the C-ring, and δ_C (121.4, 129.0, 116.4, and 161.8) for the B-ring of the flavone. The mass spectrum of compound 1 displayed a molecular ion peak at m/z 578 corresponding to its molecular formula $C_{27}H_{30}O_{14}$. Therefore, the structure of the purified compound was elucidated as Rhoifolin, in accordance with the reported data in the literature. The HPTLC performed on all extracts and raw material of *Uraria picta* collected from different regions showed the presence of Rhoifolin and other phytoconstituents as shown in Fig 4. Understanding of genetic diversity (within existing populations) and genetic structure/differentiation (among existing populations) has become a core issue of conservation genetics and is a prerequisite for developing an effective conservation strategy. The present study reported low genetic diversity within the studied accessions of *Uraria picta* (Sork and Smouse, 2006).

Conclusion

Thus, there is an immediate need to protect the populations of the species along with *in situ* and *ex situ* conservation cum commercial cultivation strategies. The new isolation and purification method of Rhoifolin was developed from *Uraria picta* whole plant by using Diaion HP 20 resin. The isolated compound was characterized by using Mass and NMR spectroscopy. The extraction process was developed by using different combinations of ethanol and water solvents. Based on the study, the highest Rhoifolin content was found in ethanol extract however highest % yield found in hydro-alcoholic extract. HPTLC profiling shows presence of Rhoifolin in all samples of herbal raw materials and extracts developed. Rhoifolin content found maximum in Hardoi district of the Uttar Pradesh. It may be due to suitable habitats or natural climatic condition of the *Uraria picta*. Thus, in the present study, Rhoifolin isolated from the whole plant part of the *Uraria picta* could be consider as a chemical marker for the standardization and quality control of *Uraria picta* plant in the form of raw drugs or raw materials for Ayurvedic drugs.

Abbreviations

HPLC: High-performance liquid chromatography; HPTLC: High-performance thin-layer chromatography; RT: Retention time; Rf: Retention factor; TLC: Thin-layer chromatography; UV: Ultraviolet rays; DMSO: Dimethyl sulfoxide; LC-MS: Liquid Chromatography and Mass Spectroscopy; UP: Uttar Pradesh, NMR: Nuclear Magnetic Resonance; PCR: Polymerase Chain Reaction.

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