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Research Article

Pharmacognostical and phytochemical research profile of Astilbe rivularis Buch. Ham.

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Abstract

Background: *Astilbe rivularis* Buch. -Ham., a folklore rhizomatous perennial herb of the Saxifragaceae family, is traditionally used in the Himalayan regions of India to treat a range of infectious and non- infectious diseases. Despite its ethnomedicinal significance, a comprehensive pharmacognostical profile of this plant has not been updated. Objective: To establish a detailed pharmacognostical and phytochemical profile of the whole plant of *Astilbe rivularis* Buch. Ham, along with its *Rasanirdharana*. Materials and Methods: Pharmacognostical evaluation included organoleptic assessment, morphological description, transverse sections and powder microscopy. Phytochemical studies comprised preliminary qualitative screening of major secondary metabolites, physicochemical analysis such as extractive values, ash content, loss on drying (LOD) & pH and HPTLC profiling. *Rasanirdharana* was conducted using 25 healthy volunteers using a structure proforma to identify the primary and secondary tastes. Results: Distinct morphological and microscopical features were observed in roots, rhizomes, stems and leaves of *Astilbe rivularis.* Phytochemical screening confirmed the presence of carbohydrates, alkaloids, glycosides, reducing sugars, tannins, phenols and saponins. The HPTLC chromatogram revealed multiple peaks at various wavelengths. *Rasanirdharana* evaluation indicated *Kashaya Rasa* as *Pradhana Rasa* and *Tikta Rasa* as *Anurasa*. Conclusion: The integrated pharmacognostical and phytochemical findings provide a robust framework for the identification, quality control and further pharmacological study of *Astilbe rivularis* Buch. -Ham.

Keywords: *Astilbe rivularis* Buch. Ham., Folklore plant, Pharmacognostical study, Phytochemical screening, HPTLC, *Rasanirdharana*

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Introduction

The Himalayas, a biodiversity hotspot, is home to many endemic medicinal plants, with traditional practices in the region still largely unexplored (1). One such plant is *Astilbe rivularis* Buch. Ham., belongs to family Saxifragaceae. A folklore medicine is found mostly in the Eastern Himalayan regions of India. It is commonly called as River Astilbe and false buck's beard in English, *Buro-okhati, Gosy, Bansupari, Padah, Padum, Pothee* in India (2).

The leaves of this plant are eaten raw for toothache and also given for blood purification (3). The root bark is used in body ache and menstrual disorder by the tribal communities of Sikkim (4). It is commonly and traditionally used in treatment of swelling, spasm, gastric ulcer, diarrhea and bleeding (5). Root powder of this plant is believed to be an abortifacient hence, is used to cause the abortion (6). Root paste is applied externally and bandaged for curing bone fractures and dislocation, also mixed with honey and taken to control post-partum diarrhea and dysentery Roots are dried and cut into small pieces and taken during cough, cold and body pain once a day (7,8). It is also used as the neuroprotective agent as per the recent pharmacological research (9).

Major chemical components reported from this species are aesculatin, astilbic acid, astilbin, aticoside, bergenin, dimethylaesculatin, daucosterol, eucryphin, palmitine, peltoboykinoleic acid, scopoletin, sitosterol, and stilbene (10).

The recent review of the research drug, specifically from last five years revealed that, detailed scientific research has been carried out in the field of pharmacology and Chemical characterization of the plant *Astilbe rivularis* Buch. Ham., However, these documents have a lack of basic standard identity and purity of the research plant. These lacuna causes limited use of the drug *Astilbe rivularis* in scientific manner and for making formulations to treat disease conditions of the patient.

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Due to the unavailability of a detailed Pharmacognsotical study and a comprehensive analytical evaluation of the different parts of *Astilbe rivularis* Buch. Ham. creates the drug susceptible to adulteration and misidentification. Additionally, due to the lack of *Rasa* (Taste) identity, produces uncertainty of classical therapeutic mechanism of the drug. Hence, this research study addresses such type of critical complications by providing detailed pharmacognostical, analytical and *Rasanirdharana* based standardization of *Astilbe rivularis,* thereby establishing essential quality control parameters and validating its ethnobotanical applications.

Materials and Methods

Collection and authentication of the drug

The whole plant of *Astilbe rivularis* Buch. Ham., was collected from Darjeeling and its peripheral areas on 17th February 2025 and authenticated by the Dravyaguna department of I.T.R.A, with the help of subject expert. The herbarium specimen was also deposited in the same with Specimen no: ITRA/DG/Herbarium/8. (Figure 1)

Figure 1: Herbarium specimen of the research plant - *Astilbe rivularis* Buch. Ham.

Macroscopical evaluation / Organoleptic evaluation

The specimen was observed with naked eyes as well as with the help of dissecting microscope. Evaluation of the drug was done by their various characters like fractures, textures, odor, taste and touch (11).

Microscopic evaluation

Thin free hand sections and peelings of root, rhizome, stem and leaves i.e., petiole, mid-rib, upper and lower epidermis of the plant were taken and cleared with chloral hydrate. They were first mounted in distilled water

Staining procedure

The mentioned free hand sections were added with 1-2 drops of the phloroglucinol solution ensuring the entire sections immersed. Allow this solution to sit for 2 to 3 minutes, that provide time to phloroglucinol to penetrate the cell wall. Add 1 drop of the conc. HCl solution directly on the sections. Photographs were taken of sections to note down the colour change of transverse sections and powder characteristics of the samples (11). The powder microscopy of the whole plant was also carried out to exact identity of the powder characters.

Histochemical evaluation

Histochemical tests were performed, for starch, section was treated with iodine, for calcium oxalate/carbonate crystal and lignified elements, treated with phloroglucinol-HCl and for tannin, treated with $FeCl_3$ (12).

Physiochemical analysis

Various physiochemical tests as mentioned in Ayurvedic Pharmacopeia of India were carried out such as loss on drying (LOD) (13,14), total ash, acid insoluble ash, water soluble extractive values, alcohol soluble extractive values (15) and pH value (16) (13).

Extraction Methodology

This methodology is essential to describe in detail as methanolic extract of the sample drug was used for the HPTCL study. This method determines the number of active constituents extracted with solvents from a given amount of medicinal plant materials.

a) Determination of Water-soluble Extractive value:

About 5gm accurately weighed sample powder was macerated in a glass-stopper conical flask. 100ml chloroform water was added and macerated for 6 hrs., shaking frequently and then allowed to stand for 18 hrs. Then after 24hr it was filtered rapidly and 20ml of the filtrate was transferred in a tarred flat bottom evaporating dish with a pipette and evaporated to dryness on a boiling water bath. Then evaporating dish was dried at 105oC for 6 hrs. and then cooled and weighed. From the weight of the residue the percentage of water-soluble extractive was calculated with reference to air dried sample.

Calculation:

$$\text{Percentage of Water-soluble Extract} = \frac{\text{Weight of residue x Vol. makeup}}{\text{Weight of Sample x Vol. taken}} \text{ X 100 \%W/W}$$

b) Determination of Alcohol soluble extractive:

About 5gm accurately weighed powder was macerated with 100ml of alcohol (methanol) in a closed conical flask for 24hr, shaking frequently during 6 hr. and allowed to stand for 18 hours. It was filtered rapidly to prevent loss of solvent and 20ml of the filtrate was evaporated to dryness in a tarred flat bottom evaporating dish and dried at 105oC to constant weight. From the weight of the residue the percentage of alcohol soluble extractive value was calculated with reference to air dried sample.

Calculation:

$$\text{Percentage of Alcohol-soluble Extract} = \frac{\text{Weight of residue x Vol. makeup}}{\text{Weight of Sample x Vol. taken}} \text{ X 100 \%W/W}$$

Preliminary phytochemical investigation

Qualitative tests are used to detect the presence of functional groups, responsible for the biological activity of the substance. Qualitative tests were carried out by using the methanol and water-soluble extracts of the sample. The extracts were used for preliminary phytochemical screening with the use of different chemical like Hager's, Dragendorff's, Mayer's, and Wagner's tests for alkaloids; ferric chloride, shinoda test, potassium dichromate and dilute iodine tests for tannins and phenolics. (17)

HPTLC (High Performance Thin Layer Chromatography)

High-Performance Thin-Layer Chromatography (HPTLC) is an advanced and automated version of traditional Thin-Layer Chromatography (TLC). It serves as a crucial tool for quality assessment in the evaluation of botanical materials. HPTLC enables the efficient and cost-effective analysis of a wide range of

compounds. Additionally, it allows for the simultaneous analysis of numerous samples in a single run, thereby reducing analytical time. The technique employs various wavelengths of light for analysis and facilitates easy comparison with standards (18), (19). The different types of instrument requirements and specification of the HPTLC mentioned below. The observations and results of the HPTLC study provided in the related headings.

Instrument Criteria

➢ Camag Linomat 5 S/N:290678
➢ Software Server HPTLC, version 4.0.24047.1
➢ TLC Scanner 4 S/N:290783
➢ Stationary phase Supelco, HPTLC Silica gel 60 F254
➢ Plate format 200 x 100 mm.
➢ Application type User
➢ Application Position Y: 8.0 mm, length: 6.0 mm, width: 0 mm
➢ Track First position X: 12.0 mm, distance: 10.4 mm
➢ Solvent front position 70 mm
➢ Sample solvent type: methanol
➢ Dosage speed: 150 nL/s
➢ Pre dosage volume: 0.20 µL
➢ Instrument diagnostics: Valid diagnostics

Scan applied plate TLC Scanner 4 (S/N: 290783)

➢ Scanner type: Single λ
➢ Optimization for: Light (sensitivity)
➢ Measurement mode: Absorbance
➢ Filter: n/a
➢ Detector mode: Automatic
➢ Profile representation: Classic
➢Scanning speed: 20 mm/s
➢ Data resolution: 100 µm/step
➢ Slit: 5 x 0.45 mm, micro
➢ Partial scan: No
➢ Lamp Deuterium
➢ Wavelength(s): 305 nm
➢ Instrument diagnosties: Valid diagnostics

Spectrum Scan applied plate TLC Scanner 4 (S/N: 290783)

➢ Scanner type: Spectrum
➢ Optimization for: Resolution
➢ Measurement mode: Absorbance
➢ Filter: n/a
➢ Detector mode: Automatic

Mobile Phase

Toluene: Ethyl acetate: Formic acid: Methanol (6:4:0.1:1)

LC-MS (Liquid Chromatography-Mass Spectrometry)

Liquid Chromatography-Mass Spectrometry (LC-MS) is a powerful analytical technique that combines the separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. This combination allows for the detailed analysis of complex mixtures, providing both qualitative and quantitative information about the components present. (20)

LC-MS was performed by injecting 10 µL of methanolic extract from rack vial 2, position F6 into an ultra-performance liquid chromatograph using method UPLC_NP_01, which run for 17 minutes. Detection occurred via both MS total ion current and PDA at 220 nm, ensuring both mass and UV data. The raw data was processed with the same UPLC_NP_01 protocol as part of sample set 28052025_LCMS (21).

Rasanirdharana

Rasa is the only quality manifested by substances that makes a gustatory appeal. Taste can only be considered as the nearest non-satisfactory equivalent for the term *Rasa* in the context of Ayurvedic pharmacology since taste perception and taste sensibility are considered as complex bio-physical and psychological events (22). Some previous studies have also drawn attention to the Ayurvedic concept of use of *Rasa* for drug identification, drug action and new drug discovery (23). After taking the approval of departmental higher authority of ITRA institute for proceeding the *Rasanirdharana* activity, this research work has been carried out to discovery the *Rasa* of *Astilbe rivularis (Buro-okhati)* by following procedure to proper identification of drug.

Selection of volunteers

The whole plant powder of the research drug was used for the *Rasanirdharana.* Total 25 healthy volunteers were selected as this number of sample size for the stated study was done by the researcher and provide adequate data for *rasa* identification (24). For data analysis using Chi-square test, a minimum sample size 20-30 is recommended (25). The volunteers were selected includes MD, PhD, UG and other students of ITRA Institutes.

Inclusion criteria

The participants who volunteered for the study were explained about the study and their role in the study. These all are healthy volunteers aged between 18 years to 45 years of either gender and have a fundamental familiarity with the concept of *Shadarasa* (the six tastes).

Exclusion criteria

The participates with history of smoking, tobacco chewing and alcohol consumption. Those suffering from chronic disease condition of oral cavity, stomatitis, loss of tastes & smell. The persons taking medication known to alter sensory perception.

Procedure: A specially designed proforma was used to assess the *Rasa* perceived by the volunteers. The tests were conducted 2 to 3 hours post-lunch. Volunteers were instructed to rinse their mouths with RO water 20 minutes prior to the test to ensure a neutral oral environment. The identity of the test substance was kept confidential to eliminate bias. A standardized quantity of the test drug 3 gm was placed on the anterior portion of each volunteer's protruded tongue. Participants were then asked to perceive and record the taste sensation.

Their sensory responses of the volunteers were recorded using a structured questionnaire proforma, based on the characteristics of individual *Rasa* as described in classical *Ayurvedic* texts (26). This recorded data was subjected to statistical evaluation by using a Chi-square test. This determines the statistical significance of the dominant taste perceived by the volunteers compared to an expected equal distribution among the six tastes. A P-value of <0.01 was considered statistically significant.

Results

Macroscopical evaluation / Organoleptic evaluation

Morphological and organoleptic observation are shown in the figure 2 and table no 1 respectively and they exhibited following characters mainly.

1. Habit- a perennial rhizomatous herb, reaching heights of 1-1.5 meters.

2. Root- tap roots emerges abundantly from the basal regions of rhizome nodes, wiry arising in many as groups. It is brown, smooth to touch with characteristic odor.

3. Rhizome- creeping fleshy underground stem with nodes and internodes, dark brown fibrous surface, nodes covered with hairy persistent scales at the outer side.

4. Stem- branched herbaceous emerging from underground rhizomes usually reddish brown to reddish green colored mostly covered with scattered long brown hairs which gives them a rough surface.

5. Leaf- Compound, tripinnate, imparipinnate leaf with a reticulate venation, petiolate, leaflets lanceolate, narrowly to broadly ovate, oblique, obtuse base, acuminate apex with serrate margins. Ranches and petiole are covered with very long thin brown hairs especially at the base of the petiole.

Table 1: Organoleptic characters of the *Astilbe rivularis* Buch. Ham. (whole plant)

	Root	Rhizome	Stem	Leaf
Touch	Smooth	Fibrous	Hairy	Rough
Color	Brown	Brown	Reddish green	Green
Taste	Astringent	Astringent	Astringent, bitter	Bitter, Astringent
Oduor	Characteristic	Characteristic	Characteristic	Characteristic

Microscopic evaluation

Microscopy of Root: The transverse section of the root is almost circular in outline. For accurate botanical identification, several key diagnostic microscopic features were identified (Figure 3). The outermost region is characterized by a distinctly two-zoned cork: an outer dark brown zone (9-12 layers) and inner transparent zone of thin-walled, tangentially elongated cell (6-9 layers). Beneath this lies a narrow cortex made up of 10-13 layers of thick walled, oval-to-round parenchyma. The vascular bundle serves as another major diagnostic marker, featuring a broad phloem region that encircles a central woody xylem core. The xylem is distinctly characterized by vessels arranged in prominent groups interspersed with fibers, accompanied by distinct multiseriate medullary rays.

Microscopy of Rhizome: The transverse section of the rhizome exhibits a circular to irregular outline, starting with an outer 3–5 layered compressed brown cork and a broad, thick-walled parenchymatous cortex. A 1–2 layered endodermis separates the cortex from the central vascular bundles, which feature a broken ring of phloem alternating with patches of sclerenchyma fibers and multiseriate medullary rays. The innermost pith consists of thick-walled, rounded-to-oval parenchyma. Key microscopic diagnostic features for identification include abundant rosette and cluster calcium oxalate crystals scattered throughout the tissues, dense simple starch grains concentrated specifically in the pith, and the prominent vascular landmarks of sclerenchyma patches and medullary rays (Figure 4).

Microscopy of Stem: The transverse section of stem circular to molar shaped in outline. A single layered cuticularized epidermis often pierced with stomata and covered with long thread like brown coloured simple to multicellular hairs followed with single layered hypodermis. Cortex are 9-12 layered thick walled loosely arranged spongy parenchyma and a wide ring of sclerenchyma fibres appearing as somewhat a wheel like structure followed with pockets of phloem as broken ring. Xylem made up of fibres, vessels and parenchyma alternating with mostly biseriate medullary rays. Pith appearing as wider zone made up of thick-walled spongy parenchyma, simple starch grains and rosette and cluster crystals of calcium oxalate found throughout the section. (Figure 5)

Microscopy of Petiole and Leaf: The transverse sections of the petiole and leaf display distinct, well-organized structural characteristics. The petiole is broadly oval to angular, comprising a cuticularized epidermis, a thick-walled parenchymatous cortex and a central pith. The leaf itself is distinctly dorsiventral, featuring a broadly convex lower midrib embedded in thick-walled parenchyma. Both the petiole and the leaf midrib contain centrally located collateral vascular strands that are uniquely encircled by sclerenchyma fibers and radially traversed by biseriate medullary rays. Additionally, the leaf lamina consists of an upper palisade zone of 2–3 cell layers and a loosely arranged lower spongy parenchyma of 6–9 layers.

The epidermis of both the petiole and leaf is densely interspersed with stomata and distinctive long, brownish, thread-like trichomes (both unicellular and multicellular), while the leaf lamina specifically bears unique sessile glandular hairs. Furthermore, the tissues of the petiole are characterized by an abundant, scattered distribution of rosette and cluster calcium oxalate crystals alongside simple starch grains. These specific cellular inclusions, combined with the defining wedge-shaped collateral vascular strand of the petiole, provide definitive microscopic fingerprints for this species (Figure 6).

Powder microscopy

The powder microscopy of *Astilbe rivularis* whole plant showed annular, pitted and spiral vessels, rosette and cluster crystals, fragments of cork, stomata, fibres, simple starch grains and trichomes. (Figure 7)

Histochemical test

Histochemical tests performed on the section of the plant detect the presence of starch grains, crystals, tannin and lignified elements.

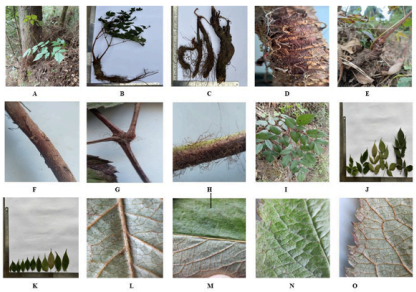
Physicochemical analysis

The whole plant powder of *Astilbe rivularis* was tested for various physicochemical parameters. The observed results are depicted in table 3. The water extractive value 18.80 % w/w, alcohol soluble extractive value 22.83 % w/w, moisture content (7% w/w), ash value (4.34 % w/w) and acid insoluble ash (0.29%) represent the quality of drugs.

Preliminary qualitative investigation

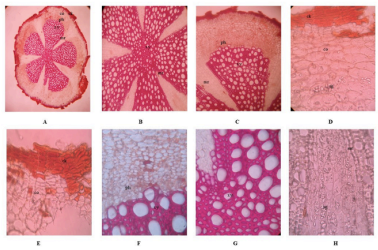
Preliminary qualitative chemical test for water and methanolic extract of the whole plant powder of *Astilbe rivularis* Buch. Ham. were carried out following standard procedure. The observed results are presented in table 4.

Figure 2: Morphological characters of *Astilbe rivularis* Buch. Ham.



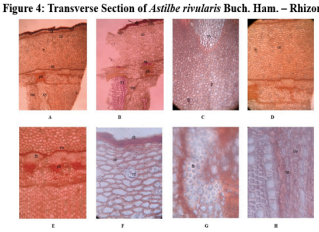
(A) Natural habit (B) Uprooted plant (C) Variable sizes of rhizomes (D) Scales on rhizomes (E) Roots protruding from rhizome (F) Enlarged view of root (G) Pulvinus base (H) Enlarged view of stem (I) Phyllotaxy of leaf (J) Various sizes of leaf (K) Various sizes of leaflets (L) Midrib (M) Surfaces of leaf (N) Lower surface of leaf (O) Upper surface of leaf

Figure 3: Transverse Section of *Astilbe rivularis* Buch. Ham. – Root



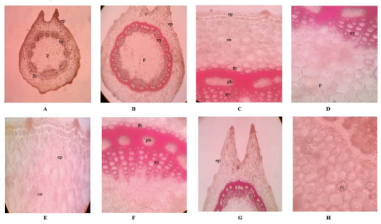
(A) Lignified diagrammatic section (10x) (B) Detailed section (10x) (C) Enlarged view of T.S (10x) (D) Cork and cortex in surface view (40x) (E) Enlarged cork and cortex region (40x) (F) Phloem region (40x) (G) Xylem region (40x) (H) Medullary rays with simple starch grains (40x) [ck- cork, co- cortex, ph- phloem, xy- xylem, mr- medullary rays, sg- starch grains]

Figure 4: Transverse Section of *Astilbe rivularis* Buch. Ham. – Rhizome



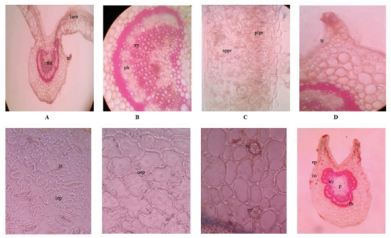
(A) Diagrammatic section (10x) (B) Lignified Diagrammatic section (10x) (C) Xylem and pith region (10x) (D) Cortex with crystals (10x) (E) Endodermis with fibres and phloem (10x) (F) Rosette crystals in cortex region (40x) (G) Fibres (40x) (H) Medullary rays with starch grains (40x) [ck- cork, co- cortex, en- endodermis, ph- phloem, mr- medullary rays, xy- xylem, p- pith, fb- fibres, rc- rosette crystals, sg- starch grains]

Figure 5: Transverse Section of *Astilbe rivularis* Buch. Ham. - Stem



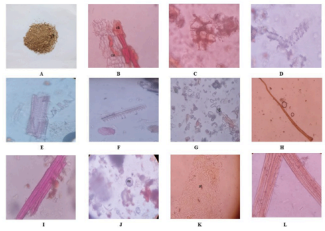
(A) Diagrammatic section (10x) (B) Lignified diagrammatic section (10x) (C) Cortex region with epidermis (40x) (D) Spongy parenchyma in pith region (40x) (E) Cortex region (40x) (F) xylem long with phloem and fibres (40x) (G) Angular view of the stem (10x) (H) Rosette crystals (40x) [ep- epidermis, p- pith, fb- fibres, xy- xylem, ph- phloem, co- cortex, rc- rosette crystals]

Figure 6: Transverse Section of *Astilbe rivularis* Buch. Ham. – Leaf



(A) Lignified diagrammatic section (10x) (B) Lignified midrib section (10x) (C) Lamina (10x) (D) Trichomes (10x) (E) Lower epidermis with stomata (40x) (F) Upper epidermis (40x) (G) Rosette and cluster crystals (40x) (H) Lignified diagrammatic section of petiole (10x) [lam- lamina, tr- trichomes, mid- midrib, ph- phloem, xy- xylem, plpr- palisade parenchyma, sppr- spongy parenchyma, st- stomata, lep- lower epidermis, uep- upper epidermis, rc- rosette crystals, cc- cluster crystals, p- pith, fb- fibres, co- cortex, ep- epidermis, fb- phloem]

Figure 7: Powder characters of the Whole plant – *Astilbe rivularis* Buch. Ham.



(A) Powder of the whole plant (B) Cork and cortex (40x) (C) Fragments of cork in surface view (40x) (D) Spiral vessels (40x) (E) Pitted vessels (40x) (F) Annular vessels (40x) (G) simple starch grains (40x) (H) Simple multicellular trichomes (10x) (I) Lignified Fibres (40x) (J) Rosette crystals (40x) (K) Fragments of epidermis with stomata (40x) (L) Enlarged trichomes (40x) [ck- cork, co- cortex, sg- starch grains, rc- rosette crystals, st- stomata]

Table 2: Results of histochemical tests of *Astilbe rivularis* Buch. Ham. (whole plant)

Sl. No.	Test for	Test	Observation	Result
1	Starch	Powder + Iodine solution	Starch turned blue in colour	+
2	Calcium oxalate crystals	Powder + HCl	Dissolved	+
3	Lignified elements	Powder + HCl + Phloroglucinol	Lignified tissues turned pink in colour	+
4	Tannin	Powder+ $FeCl_3$	Deep blue coloured	+

Table 3: Physicochemical parameters of *Astilbe rivularis* Buch. Ham. (whole plant powder)

Sl. No	Test name	Sample
1	Loss on drying	7 % w/w
2	Ash value	4.34 % w/w
3	Acid insoluble ash	0.29 % w/w
4	Water soluble extractive value	18.80 % w/w
5	Alcohol soluble extractive value	22.83 % w/w
6	pH value	6.42

Table 4: Preliminary qualitative chemical tests of various extracts of whole plant

Sl. No	Functional group	Test performed	Methanolic Extract	Observation	Water Extract	Observation
1	Alkaloid	Meyer's test	+	Creamy White coloured precipited formed	+	Cream coloured pprecipitate observed
2	Cardiac glycoside	Legal's test	+	Pinkish red colour observed	-	Brown colour observed
3	Cyanogenetic glycoside	Guinard reaction	-	No any colour change observed of filter paper	-	No any colour change observed of filter paper
4	Anthraquinone glycoside	Bontrager's reaction	-	No pink or red colour observed	-	No pink or red colour observed
5	Coumarin glycoside	Fluorescence test	-	Solution remain unchaged	-	Solution remain unchaged
6	Tannin	Lead acetate test	+	Formation of white precipitate observed	+	Formation of white precipitate observed
		Ferric chloride test		Greenish colour solution observed		Dark greenish colour solution observed

7	Protein	Biuret test	-	Solution remains unchanged	-	Solution remains unchanged
		Protein containing Sulphur test		Solution remains unchanged		Solution remains unchanged
8	Amino acid	Ninhydrin test	-	No any changes are observed	-	No any changes are observed
9	Carbohydrate	Molish's test	+	Reddish -violet ring observed	+	\Violet ring observed
10	Reducing sugar	Fehling's test	+	Yellow to red precipitate observed	+	Yellow to red precipitate observed
11	Flavonoid	Shinoda test	+	Light Pink colour observed	+	Pinkish colour observed
12	Steroids	Salkowski reaction	+	Red and greenish fluorescent observed	-	No any colour changed observed
13	Phenols	Ferric chloride test	+	Greenish colout observed	+	Purplish colur observed

HPTLC Fingerprinting

HPTLC fingerprinting carried out for the methanolic extract of the whole plant *Astilbe rivularis* Buch. Ham. to detect and quantify compounds across multiple wavelengths. The detailed densitometric analysis yielding the following results based on the detected Rf values and their corresponding percentage areas. (Table 5 and Figure 8)

Analysis at 254 nm: The chromatogram revealed a well determined profile consisting of 10 distinct peaks. The most prominent separated compound was detected at a maximum Rf of 0.085, which accounted for a major 30.62% of the total peak area. Other highly concentrated constituents were observed at Rf 0.121 (18.37% area), Rf 0.037 (13.90 % area) and Rf 0.426 (12.58% area).

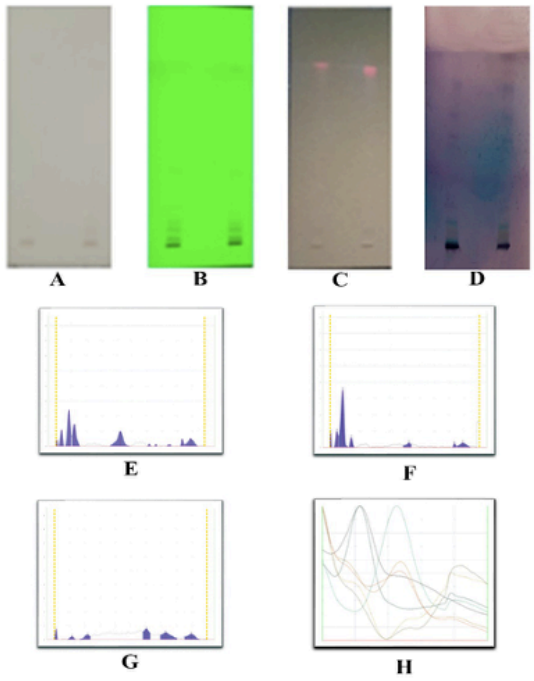
Analysis at 366 nm: Under this wavelength 7 specific peaks were detected. The densitometric scan showed excellent separation of a dominant fluorogenic compound at rf 0.085, representing a significant 51.57% of the total peak area. Additional notable separated compounds were identified at Rf 0.047 (14.44% area) and 0.005 (13.52% area).

Analysis at 540 nm: Post derivatization scanning at 540 nm resolved 6 clear peaks. The two most abundant compounds separated at this wavelength were located at Rf 0.011 (25.58% area) and Rf 0.603 (25.33% area). Further well-resolved bands were observed at Rf 0.727 (16.93% area) and Rf 0.897 (13.21% area).

Table 5: Observed Rf Values at different wavelength through HPTLC fingerprinting of *Astilbe rivularis* Buch. Ham. whole plant

Peaks(spo	Max Rf	Area	Max Rf	Area	Max Rf	Area
1	0.006	5.63	0.005	13.52	0.011	25.58
2	0.037	13.90	0.047	14.44	0.116	7.25
3	0.085	30.62	0.085	51.57	0.218	11.68
4	0.121	18.73	0.114	8.81	0.603	25.33
5	0.426	12.58	0.527	4.00	0.727	16.93
6	0.615	2.03	0.834	3.49	0.897	13.21
7	0.660	1.83	0.889	4.17		
8	0.752	1.70				
9	0.834	6.28				
10	0.889	6.72				

Figure 8: HPTLC profile of methanolic extract of *Astilbe rivularis* Buch. Ham. whole plant powder



(A) HPTLC plate at UV 254 nm (B) HPTLC plate at UV 366 nm (C) HPTLC plate at UV 540 nm (D) HPTLC plate after derivatization (E) HPTLC at 254 nm (F) HPTLC at 366 nm (G) HPTLC at 540 nm (H) Wavelengths of all tracks

LC-MS profiling

The untargeted LC-MS analysis of the *Astilbe rivularis* Buch. Ham whole plant methanolic extract was conducted to establish a basic chemical profile and identify probable candidate phytoconstituents.

The resulting base peak chromatograms illustrated the intensity of the compounds within an auto-scaled chromatogram. The LC-MS profiling including primary m/z values, retention times and peak area percentage is detailed in Table 6 and Figure 9.

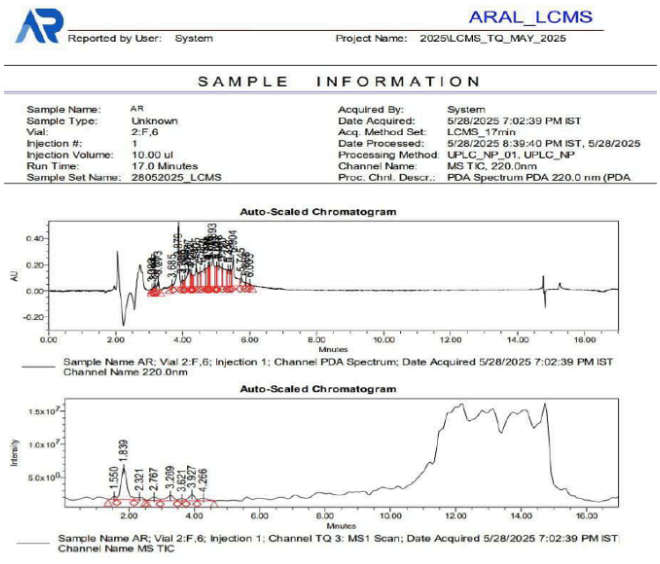
Notably, the extract was dominated by two major components eluting at retention times 1.839 minutes and 1.856 minutes, representing peak areas of 53.31% and 48.83%, respectively. Other significant signals included peaks at 4.963 minutes (14.37% peak area), 3.963 minutes (12.92% peak area) and 5.564 minutes

(12.17% area). Due to the unavailability of a standard reference library during this specific analytical run, the primary m/z values were used strictly to deduce probable molecular candidates.

Table 6: LC-MS profile of *Astilbe rivularis* Buch. Ham whole plant methanolic extract

Retention	Peak	m/z MS	Probable candidates
1.544	8.27	137.14	-
1.550	3.51	188.93	Benzylpiperazine
1.839	53.31	365.26	Acacetin 7-
1.856	48.83	387.28	-
2.321	4.93	549.38	Pheophytin-a
2.769	7.39	199.37	Acacetin(C16H12O5)
3.269	11.84	303.39	Fenoterol(C17H21NO4) Ellagic acid(C12H6O6)
3.621	3.19	222.47	Pheophorbide-b
3.718	3.43	1221.28	Pheophorbide-b,
3.927	10.26	178.25	Taxifolin(C15H12O7)
3.963	12.92	327.24	-
4.266	5.59	130.25	Tartaric acid(C4H6O6) Threonicacid(C4H8O5) Mucic acid(C6H10O8)
4.963	14.37	593.30	Astaxanthin,
5.564	12.17	447.41	Isoquercetin(C21H20O12) Kaemferol(C15H10O6) Kaempferol3-O-hexoside

Figure 9: LC-MS profile of *Astilbe rivularis* Buch. Ham whole plant methanolic extract

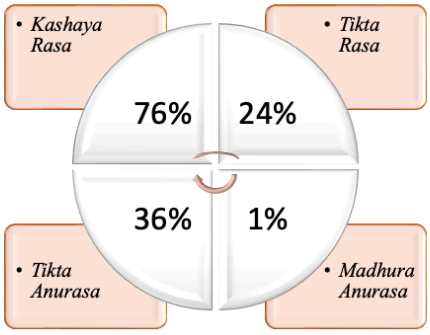


Rasanirdharana

In *Rasanirdharana* study of *Astilbe rivularis* conducted on 25 healthy volunteers, 19 participants (76%) perceived *Kashaya Rasa* and 6 (24%) participants perceived *Tikta Rasa* as *Pradhana Rasa.* A Chi-square goodness-of-fit test was conducted to compare these observations against an expected equal distribution among the six tastes (*Shadarasa)*. The distribution was highly significant (χ^2 = 70.28, p<0.001), represents that the percentage of *Kashaya Rasa* as the primary taste was not due to chance.

Regarding the *Anurasa* (secondary taste), 9 volunteers (36%) reported *Tikta Rasa,* while 1 volunteer perceived *Madhura Rasa.* During the evaluation process, specific subjective sensations were also documented: 13 volunteers (52%) experienced a cleansing and drying effect in the mouth, 4 volunteers (16%) reported abundant salivation along with a cleansing sensation, 5 volunteers (20%) noted a moistening and softening of the tongue and oral cavity, 3 volunteers (12%) experienced a shrinking sensation in the eyebrows and eyelids.

Figure 10: *Rasanirdharana* of *Astilbe rivularis* Buch. Ham. whole plant powder.



Discussion

Astilbe rivularis, commonly called *Budho-Okhati/ Thulo Aushadhee* in Nepali which literally translates as “old medicine” or “great medicine is recognized in traditional healing practices (27).

Pharmacognostical study of the plant is a pivotal part for standardization and correct identification of the medicinal plant species. The plant *Astilbe rivularis* is identified as a rhizomatous perennial herb covered with long brown hairs especially in aerial parts. Rhizomes are variable in shape and size with fibrous hairy and scaly surface. The stems are somewhat angled; hairy and leaves are rough to touch due to the presence of trichomes on both the surfaces (Figure 1 & 2 and Table 1).

Microscopically the plant revealed several distinct tissue features; roots displayed centrally placed xylem (Figure 3); the rhizome displayed phloem fibres and starch- and crystal-rich central pith (Figure 4); stems had a broad central pith surrounded by xylem, phloem, and fibres, with multicellular trichomes embedded in the epidermis (Figure 5); petioles showed wedge-shaped vascular bundles flanked by biseriate medullary rays and unicellular trichomes; and leaves were dorsiventral with hosting simple glandular trichomes and stomata (Figure 6). The detail microscopic characters are highly required to meet the pharmacopeial standards of the drug. These findings help to correct identity of the plant.

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. This method has been developed for qualitative and quantitative analysis for the cellular components, including proteins, carbohydrates, lipids, nucleic acids and the range of ionic elements occurring in the cells (28). This study is carried out on the research drug shows in table no 2, the presence of starch. Lignified cells, tannin and crystals. This represents the antimicrobial activity of the plant due to presence of tannin (29).

The physicochemical results establish essential quality bench mark for the *Astilbe rivularis.* A lesser amount of moisture 7.00% w/w satisfies the standard safety parameters, indicating sufficient dehydration to prevent the drug from rancidity and quality degradation; the 4.34% w/w total ash reflects the baseline physiological mineral content, whereas the minimal 0.29% w/w acid-insoluble ash confirms proper post-harvest washing by ruling

out external earthy or silicious contamination. Furthermore, the higher yield of alcohol-soluble extractive matter (22.83% w/w) over the water-soluble portion (18.80% w/w) reveals that the therapeutic secondary metabolites are mostly lipophilic or semi-polar, which validates the plant's rich density of condensed tannins and polyphenols while guiding appropriate solvent selection for pharmaceutical extraction. Ultimately, the weak acidity (pH 6.42) acts as a unique biochemical fingerprint indicating an abundance of organic phenolic compounds (Table 3).

The presence of secondary metabolites (Table 4) such as alkaloids, cardiac glycosides, flavonoids, tannins, steroid, saponin, carbohydrates and reducing sugar. It showed absence of amino acids, proteins and coumarin glycosides in alcoholic extract of the samples. This result exhibits different types medicinal properties of the plant. For instance, Tannin compounds give effect on GIT, including anti-oxidant, anti-microbial, anti-viral and anti-mutagenic activities (30).

A chromatographic fingerprint of plant extracts using the HPTLC represents a chromatographic pattern of pharmacologically active or chemically specific constituents available in the plants (31). The chromatogram of methanolic extract of *Astilbe rivularis* Buch. Ham. whole plant was developed using the mobile phase solvent system - Toluene: Ethyl acetate: Formic acid: Methanol– 6:4:0.1:1 (v/v).

The characteristic Rf values, combined with their respective percentage peak area. Provide a highly reliable and quantifiable basis for comparison and identification. For instance, the major compound identified at Rf 0.085, which represents 30.62 % of the peak area at 254 nm and 51.57% at 366 nm – can serve as a specific primary marker for this extract. Furthermore, the varied absorption profiles at different wavelength (254 nm, 366 nm and 540 nm) highlight the complex phytochemical makeup of the extract. By establishing these clear, well-determined marker bands and documenting their precise percentage area, this HPTLC profiling directly facilitates the development of standardized, reproducible and effective herbal medicines form *Astilbe rivularis.*

The LC-MS analysis (Table 6 and Figure 9) of *Astilbe rivularis* Buch. Ham. yielded base peak chromatograms, highlighting the most intense ions detected during the analysis. The extract revealed several prominent peaks, suggesting that can be presence of flavonoid, glycosides, quercetine, Acacetin, Gorsypin like compounds. Base peak intensities indicated that the present compounds with m/z values can be corresponding to known flavonoid aglycones and their glycosidic forms. For instance, a peak with m/z 199.37 is characteristic of Acacetin-7-o-Glucoside, m/z 447.41 is characteristic of Iso-quercetin (32), while m/z 593.30 corresponds to Astaxanthin, pheophorbide-a (33). These findings are consistent with previous studies identifying similar flavonoid glycosides in plant extracts.

These flavonoids, glycosides and Quercetin are known for their antioxidant, anti-inflammatory, Cytotoxic, phytotoxic, antimicrobial and antioxidant effect and anticancer properties (34), highlighting the therapeutic potential of *Astilbe rivularis* Buch. Ham. However, further analyses, such as MS/MS fragmentation and comparison with authentic standards, are recommended to confirm the identities of these compounds and to explore their pharmacological activities.

The characteristics (*Lakṣaṇas*) for the identification of each *Rasa* is well documented in Ayurvedic classics (35, 36). A recent study on the ayurvedic concept of *Abhāvapratinidhidravya* (drug substitution) establishes that similarity in *rasa* may be used as one of the tools to establish substitute drugs (37). This study shows in the figure 9 and result of *Rasanirdharana*, the research plant has *Kasaya Rasa* and *Tikta Rasa.* The combination of *Kashaya Rasa* and *Tikta Rasa* in the plant corelate well with its traditional uses for *Pittaja* and *Kaphaja roga*, gastrointestinal problems, skin disease and other parasitic diseases (38).

Conclusion

The comprehensive evaluation of *Astilbe rivularis* Buch. Ham. *(Budho-okhati)* successfully established its standard identity, purity and essential quality control parameters. By integrating a detailed pharmacognostical study, analytical evaluation and *Rasanirdharana* (Taste identity) – this research provides the necessary framework to clearly understand the plant. The confirmed physicochemical stability and rich alcohol soluble phytochemical profile effectively justify the plant's extensive ethnobotanical applications. This validates its folklore use in many diseases conditions. Most importantly, by establishing these rigorous standardization parameters along side unique HPTLC chromatographic maps, this study actively mitigates the risks of adulteration and misidentification. This integrated foundational research will support the scientifically sound utilization of *Astilbe rivularis* in the development of standardized, safe and highly effective herbal formulations.

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