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Research Article

Pharmacognostical Evaluation of *Bhringaraja* (*Eclipta Alba* Hassk): An Ayurvedic Drug

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ABSTRACT

This study explores the pharmacognostical properties of *Bhringaraja* (*Eclipta alba Hassk*) *churna*, an herb highly valued in Ayurvedic medicine for its therapeutic benefits. The primary aim is to authenticate the identity of *Bhringaraja churna* and assess its quality parameters through comprehensive macroscopic, microscopic, and physicochemical, phytochemical evaluations**. Macroscopic Analysis**: The macroscopic examination confirmed the characteristic features of *Bhringaraja*, such as its yellowish-brown color, fine texture, and characteristic earthy aroma and bitter taste. **Microscopic Analysis:** Leaf microscopy showed upper and lower epidermis, cuticle, hypodermis, collenchyma, simple trichome, vascular bundle phloem, proto and meta xylem. Affirming the herb's identity and physicochemical parameters: Various physicochemical parameters were measured, including moisture content, ash values, extract values, and pH. These parameters ensure the stability, and quality of the *Churna*. This study underscores the importance of pharmacognostical and phytochemical research in enhancing the understanding and application of traditional herbal medicines in contemporary healthcare.

INTRODUCTION

Bhringaraja, scientifically known as *Eclipta alba Hassk* and commonly referred to as False Daisy[1], is a cornerstone herb in the traditional Indian system of medicine. Ayurveda Revered for its multifaceted therapeutic properties, *Bhringaraja* has been traditionally used to promote hair growth, liver and skin health. In Ayurvedic texts, Acharya Sharangadhara while explaining *Rasayana* according to age, mentions *Bhringaraja* as *Rasayana* to prevent skin aging.[2] *Bhringaraja* is acclaimed for its *Rasayana* (rejuvenating) properties, making it a vital component in formulations aimed at revitalizing and restoring balance in the body.

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***Bhringaraja* Vernacular Names[3]**

Language	Name
Sanskrit	Kesaraja, Bhrnga, Markava
English	Trailing eclipta
Hindi	Bhangra
Kannada	Garugada
Malayalam	Kayyonni
Marathi	Maka
Telugu	Guntagalagara.
Tamil	karisalankanni
Bengali	Bheemraja
Gujarati	Bhangro

Taxonomic Classification[4]

Kingdom: Plantae
Subkingdom: Viridaeplantae
Infrakingdom: Streptophyta
Division: Tracheophyta
Subdivision: Spermatophytina
Infradivision: Angiospermae
Class: Magnoliopsida
Superorder: Asteranae

Order: Asterales

Family: Asteraceae

Genus: Eclipta L.

Species*: alba*

Habitat

Bhringarāja. Commonly seen in South Indian states like Karnataka, Kerala, Andhra Pradesh, Tamilnadu and grows abundantly in Uttar Pradesh, Gujarat, Maharashtra, West Bengal etc especially nearby Marshy places as a weed.

Varieties[5]

Rāja Nighanṭu - 3 types

1. *Śweta Bhringarāja* - Eclipta alba Hassk
2. *Pita Bhringarāja* - Wedelia calendulacea Less
3. *Nila Bhringarāja*

Bapalal Vaidya mentioned another variety called *Rakta Bhringaraja* based on colour of the flower and botanically identified as Flaviera rependa Lag.

Rasadi panchaka[6]

Rasa	*Katu, Tikta*
Guna	*Laghu, Ruksa*
Vipaka	*Katu*
Virya	*Ushna*
Karma	*Kaphavatahara, Kesya, Twachya, Krimghna, Swasahara, Kasahara, Sothahara, Panduhara, Dantya, Rasayana, Balya, Netrya*

Morphology

Habit - Annual herb.

Root-Branched, tap root.

Stem-Herbaceous, aerial, erect, cylindrical, branched, solid, glabrous, hairy.

Leaf - Ramal and cauline, simple, opposite, decussate, sub-sessile, exstipulate, elliptical, acute, reticulate venation.

Inflorescence- Capitulum: ray florets (peripheral flowers) are ligulate, and disc florets (Central flowers) are tubular; involucre of bracts present.

Fruit-Cypsela

Useful parts- *Panchanga* (Leaves, stem, flower, roots and fruits)

Bhringaraja* (*Eclipta alba* Hassk*) herb

Bhringaraja* (*Eclipta alba* Hassk*) churna

As per WHO norms, botanical standards are proposed as a protocol for identification of the herbal drug. The phytochemical studies of drugs were done by making use of various parameters help in standardizing the drug and authenticate it.

Dried whole herb with flowers and fruits; stems, cylindrical up to 4mm thick, reddish brown in colour; leaves, long lanceolate, narrowed at both ends, having a rough surface of dark green colour which is covered with white appressed hair, flower heads are in pairs, axillary or terminal, with white ray florets and yellow or black disc (when ripe). It has mild odour and bitter taste.

Pharmacognostical and Phytochemical Analysis of *Bhringaraja Churna*

MATERIALS AND METHODS

Pharmacognostical Study includes 1) Plant identification 2) Drug collection 3) Organoleptic study 4) Physicochemical study 5) Phytochemical study 6) TLC 7) HPTLC.

The correct identity of the species and its morphological characters, authenticated by comparing them with the characters mentioned in various Ayurvedic texts and API. In the present study *Bhringaraja* whole plant was collected from its natural

habitat from the surroundings of sheshachalam forest area Tirupati in Andhra Pradesh. Physicochemical Study: It includes foreign matter, moisture content, total ash, acid insoluble ash and water-soluble, alcohol soluble, petroleum, ether extract. Phytochemical Analysis: By performing different qualitative tests of a sample, we can get an idea about the type of Phytoconstituents present in the sample. TLC: Thin layer chromatography[7] is a quantitative technique used to identify different phytochemicals present in a drug and works on the principle of separation. HPTLC: High performance thin layer chromatography[8] is an invaluable quality assessment tool for the evaluation of botanical materials.

All Pharmacognostical tests carried out in Shri B. M Kankanawadi Ayurveda Mahavidyala, Belgum, Karnataka. Here are the results.

Table 1: Organoleptic Characters of *Bhringaraja*

S.No	Characteristics	*Bhringaraja* Whole Powder
1	Texture	Fine powder
2	Odour	Aromatic
3	Colour	Yellowish brown
4	Taste	Slight bitter

Table 2: Physicochemical Constituents of *Bhringaraja*[9]

S.No	Constituents	*Bhringaraja* powder
1	LOD	4.059%
2	Ash value	20.225%
3	Acid insoluble ash	10.505%
4	Water soluble extractive	19.372%
5	Alcohol soluble extractive	9.136%
6	pH	6.65

Soxhlet Extraction Apparatus

Table 3: Phytochemical Analysis of *Bhringaraja*[10]

Tests	Aqueous Extract	Alcohol Extract	Chloroform	Petroleum Ether
Test for carbohydrates	+	+	+	+
Test for sugars	+	+	-	-
Test for monosaccharides	+	+	+	-
Test for pentose sugar	-	-	+	-
Test for non-reducing sugar	-	-	-	-
Test for hexose sugar	-	-	-	-
Text for proteins	+	-	-	-
Test for amino acids	-	-	-	-
Test for steroids	-	+	-	-
Test for flavonoids	+	+	+	-
Test for alkaloids	-	-	-	-
Test for tannins	+	+	-	-
Cardiac Glycosides	-	-	+	+
Anthraquinone Glycosides	+	+	-	-
Saponin Glycosides	-	-	-	-

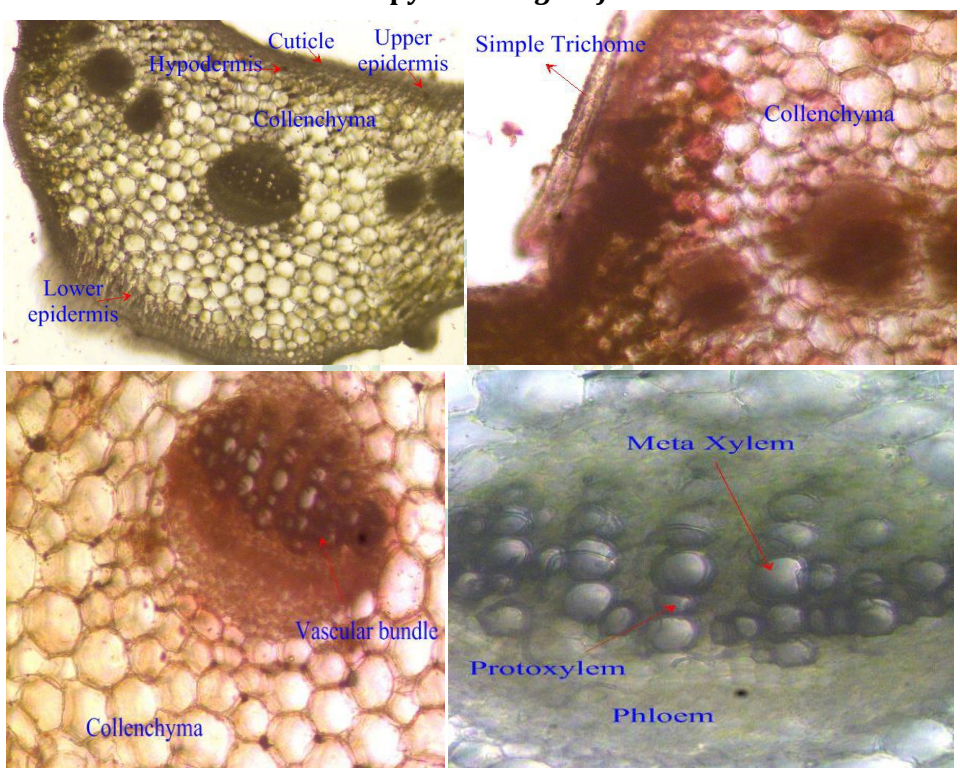
The phytochemicals present in aqueous extract of *Bhringaraja* whole plant powder contains carbohydrates, Reducing sugar, monosaccharides, proteins, flavonoids, tannins, anthraquinone glycosides. The phytochemicals present in alcohol extract of *Bhringaraja* whole plant powder carbohydrates, reducing sugar, monosaccharides, steroids, flavonoids, tannins, Anthroquinone glycosides. The phytochemicals present in chloroform extract of *Bhringaraja* whole plant powder

Carbohydrates, monosaccharides, pentose sugars, flavonoids, cardiac glycosides. The phytochemicals present in petroleum ether extract of *Bhringaraja* whole plant powder carbohydrates, cardiac glycosides.

Microscopic

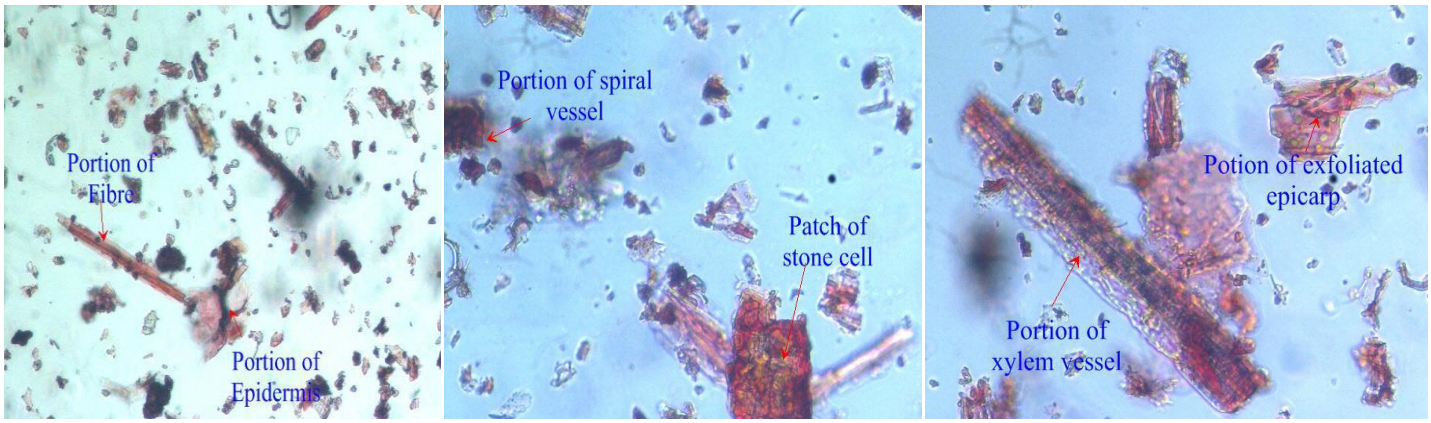
Leaf Petiole - shows single layered upper and lower epidermis consisting of tubular cells, covered with striated cuticle; trichomes of two types, non-glandular, uniseriate, 1-5 celled, warty, and with pointed apical cell; epidermis followed by wide cortex, consisting of 2-5 layered collenchyma on both, upper and lower side with distinct angular thickening; parenchyma 4-6 layered on upper side and 5-8 layered on lower side consisting of isodiametric, thin-walled cells with intercellular spaces; vascular bundles central one largest while others small flanking to either side of central bundle, consists of xylem on dorsal side and phloem on ventral side, xylem vessels arranged in radial rows traversed by xylem rays. Midrib - cut at basal region shows both upper and lower single layered epidermis, externally covered with cuticle, a few epidermal cells elongate outwards to form uniseriate hairs; epidermis followed by cortex, consisting of 3-5 layered collenechymatous cells on both sides; section cut at middle region shows 3-4 layered collenchymatous cells on dorsal and 1-3 layered on ventral side, while the section cut at apical region, shows 2 layered collenchymatous cells on both sides, in the basal region section shows vascular bundle similar to that of petiole while in the section cut at middle and apical region section shows 4 smaller bundles shifting towards lamina.

Microscopy of *Bhringaraja* Leaf



Bhringaraja Leaf Microscopy Showed: Upper & Lower Epidermis, Cuticle, Hypodermis, Collenchyma (a) Simple Trichome, Collenchyma (b), Vascular Bundle (c) Phloem, Proto & Meta xylem (d)

Powder Microscopy - Dark green; shows vessels in large groups or single broken pieces with pitted walls, numerous fibres entire or in pieces, trichomes entire or in pieces, warty, a few attached with epidermal and subsidiary cells, anomocytic and anisocytic stomata.



Bhringaraj Microscopy shows: Portion of Fibre & Portion of epidermis (a) Portion of spiral vessel & Patch of Stone cell (b) Portion of Exfoliated Epicarp & Portion of Xylem vessel (c)

TLC: TLC of the water and alcohol extract was performed using specially developed and optimized mobile phase so as to avoid any variability in the pattern due to change in mobile phase. The TLC was performed to get the finger print of the crude extract and to study it qualitatively.

Developing a Thin-Layer Chromatography (TLC) method for *Bhringaraja* (*Eclipta alba*) whole plant powder:

Materials

- TLC plates (silica gel 60 F254, 10 x 10cm)
- Solvents: Methanol.
- *Bhringaraja* whole plant powder
- UV lamp

Procedure

1. **Preparation of Samples**
   - Weigh accurately about 1 gram of *Bhringaraja* whole plant powder and extract with 10ml of methanol.
   - Sonicate the mixture for about 15 minutes.
   - Filter the solution through a 0.45µm filter to remove particulates.
2. **Preparation of Mobile Phase**
   - Mix Toulene and Ethyl acetate 7:3 ratio.
3. **Application of Samples**
   - Apply 10µL of the sample solution and reference standard solutions onto the TLC plate using a micropipette.
   - Allow the spots to dry.
4. **Development of Chromatogram**
   - Place the TLC plate in a development chamber containing the mobile phase (Toulene: Ethyl alocohol 7:3) until the solvent front reaches the top of the plate.
   - Remove the plate and allow it to dry.
5. **Detection**
   - Visualize the chromatogram under a UV lamp at 254nm and 366nm.
   - Alternatively, spray the plate with a suitable detecting reagent (e.g., anisaldehyde-sulfuric acid reagent) and heat it to develop the spots.
6. **Documentation and Analysis**
   - Document the chromatogram by photographing it under UV light or after derivatization.
   - Measure the Rf values and compare them with reference standards to identify the compounds.

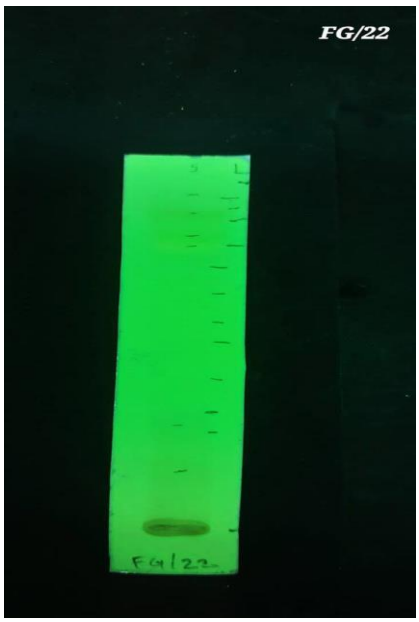
Bhringaraja whole plant powder mobile phase:

Alcoholic extract: Toluene: Ethyl Acetate - 7:3

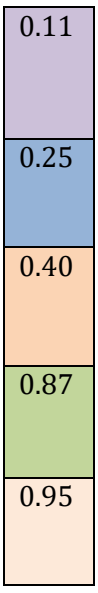
Rf Values of Methanolic extracts of *Bhringaraja* whole plant powder

Type of Wave	(TLC) Alcohol extract
Short wave	0.11, 0.25, 0.40, 0.87, 0.95
Long wave	0.08, 0.10, 0.17, 0.25, 0.36, 0.41, 0.49, 0.55, 0.60, 0.65, 0.70, 0.82, 0.88, 0.95

Short Wave TLC Plate

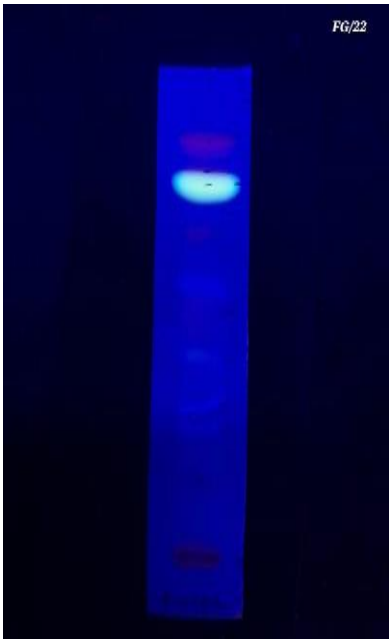


Rf



Short wave: 0.11,0.25.0.40.0.87.0.95

Long Wave TLC Plate



Rf

0.08
0.10
0.17
0.25
0.36
0.41
0.49
0.55
0.60
0.65
0.70
0.82
0.88
0.95

Long wave:
0.08,0.10,0.17,0.25,0.36,0.41,0.49,0.55,0.060,
0.65,0.70,0.82.0.88,0.95

HPTLC of *Bhringaraja*

Developing a High-Performance Thin-Layer Chromatography (HPTLC) method for *Bhringaraja* (*Eclipta alba Hassk*) whole plant powder:

Materials:

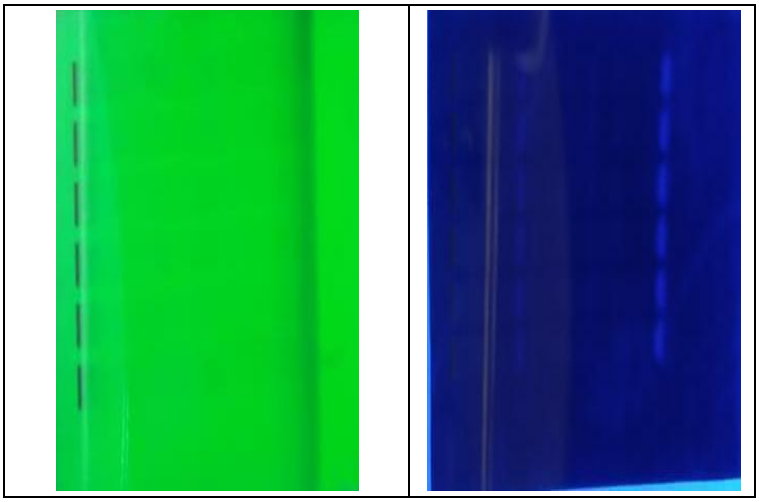
- HPTLC plates (silica gel 60 F254, 100 x 100 cm)
- Solvents: methanol, toluene, ethyl acetate.
- *Bhringaraja* whole plant powder
- Reference standards
- CAMAG Server DESKTOP -JL5ATRO, Version 4.0.24047.1 software
- UV lamp

Procedure

1. **Preparation of Samples**
   - Weigh accurately about 1mg of *Bhringaraja* powder and dissolve it in 10ml of methanol.
   - Filter the solution through a 0.45µm filter to remove particulates.
2. **Preparation of Mobile Phase**
   - Mix Toulene and Ethyl acetate in the ratio of 7:3 (v/v).
3. **Application of Samples**
   - Apply 2µL of the sample solution and reference standard solutions onto the HPTLC plate using a micropipette.
   - Allow the spots to dry.
4. **Development of Chromatogram**
   - Place the HPTLC plate in a development chamber containing the mobile phase (Toluene and Ethyl acetate in the ratio of 7:3 (v/v).) until the solvent front reaches the top of the plate.
   - Remove the plate and allow it to dry.
5. **Detection**
   - Visualize the chromatogram under a UV lamp at 366nm and 370nm.
   - Alternatively, scan the plate using a densitometer with a deuterium lamp at 350nm.
6. **Documentation and Analysis**

- Document the chromatogram using CAMAG Server DESKTOP -JL5ATRO, Version 4.0.24047.1 software

Measure the Rf values and compare them with reference standards to identify the compounds. Quantify the compounds using the densitometer readings.



Picture of HPTLC plates

In HPTLC studies of alcohol extract of *Bhringaraja* in Toluene: Ethyl acetate mobile phase showed that separation of 8 spots with Rf values as follows 0.018, 0.069, 0.18, 0.23, 0.30, 0.60, 0.78 and 0.85 with concentration of 19.21%, 15.00%, 35.21%, 5.90%, 4.77%, 7.71%, 9.19% & 3.02% respectively. This can be considered as in house quality standards for *Bhringaraja*.

HPTLC Report of *Bhringaraja*

Table 1: HPTLC Automatic TLC applicator conditions:

Scanner type	**Multiple λ**
Optimization for	Light (sensitivity)
Measurement mode	Fluorescence
Filter	K400
Detector mode	Automatic
Profile representation	classic
Scanning speed	20mm/s
Data resolution	100µm /step
Slit	5 x0.2 mm, micro
Partial scan	No
Lamp	Deuterium & Tungsten
Wavelength (s)	370nm
Instrument diagnostics	Valid daignostics

Table 2: TLC Plate and development chamber conditions

Tank	**TTC 20 x 10**
Mobile phase	Toluene: Ethyl acetate (7:3 v/v)
Saturation time	20 min
Use saturation pad	True
Use smart ALERT	False
Volume front through	10 mL
Volume rear through	20 mL
Drying time	5 min
Drying temperature	Room temperature

Table 3: Track Assignment for TLC plate Application:

Track	Vial ID	Description	Volume	Type
1	2	*Bringaraja*	2.0 µL	Sample
2	2	*Bringaraja*	2.0 µL	Sample
3	2	*Bringaraja*	2.0 µL	Sample
4	2	*Bringaraja*	2.0 µL	Sample
5	2	*Bringaraja*	2.0 µL	Sample
6	2	*Bringaraja*	2.0 µL	Sample

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BHRINRAJ_20240511_100144 visionCATS

Analysis: BHRINRAJ_20240511_100144

Path: Home/AYUSH

Based on method: BHRINRAJ

Created	11-May-2024 10:01:48	BSRC
Modified	13-May-2024 11:20:40	BSRC
Last HPTLC log	13-May-2024 11:20:40	Analysis modified
Explorer notes		

Track Assignment

Track	Vial ID	Description	Volume	Type
1	2	BR	2.0 µL	Sample
2	2	BR	2.0 µL	Sample
3	2	BR	2.0 µL	Sample
4	2	BR	2.0 µL	Sample
5	2	BR	2.0 µL	Sample
6	2	BR	2.0 µL	Sample
7				

Track Assignment notes

A track marked with ⚠ means: the application type is overwritten in some evaluation(s).

System setup:

Software	Server DESKTOP-JL5ATRO, version 4.0.24047.1
Chamber	N/A
Linomat 5	S/N:251214 (Linomat 5)
TLC Scanner 4	S/N:250407 (Scanner 4)

Chromatography

Plate settings:

Stationary phase	Supelco, HPTLC Silica gel 60 F_{254}
Plate batch number	
Plate format	100 x 100 mm
Application type	Band
Application	Position Y: 8.0 mm, length: 8.0 mm, width: 0 mm
Track	First position X: 15.0 mm, distance: 11.4 mm
Solvent front position	70 mm
Notes	

Application 1 - Linomat 5 (S/N: 251214):

Sample solvent type	methanol
Dosage speed	150 nL/s
Pre dosage volume	0.20 µL
Instrument diagnostics	Valid diagnostics
Notes	

Development 1 - Chamber:

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BHRINRAJ_20240511_100144 visionCATS

Tank	TTC 20x10
Mobile phase	
Saturation time	20 min
Use saturation pad	true
Use smartALERT	false
Volume front through	10 mL
Volume rear through	20 mL
Drying time	5 min
Drying temperature	Room temperature
Notes	

Scan developed plate 1a - TLC Scanner 4 (S/N: 250407):

Scanner type	Single λ
Optimization for	Light (sensitivity)
Measurement mode	Fluorescence
Filter	K400
Detector mode	Automatic
Profile representation	Classic
Scanning speed	20 mm/s
Data resolution	100 µm/step
Slit	5 x 0.2 mm, micro
Partial scan	No
Lamp	Deuterium & Tungsten
Wavelength(s)	370 nm
Instrument diagnostics	Valid diagnostics
Documentation step label	
Notes	

Scan developed plate 1b - TLC Scanner 4 (S/N: 250407):

Scanner type	Multiple λ
Optimization for	Light (sensitivity)
Measurement mode	Fluorescence
Filter	K400
Detector mode	Automatic
Profile representation	Classic
Scanning speed	20 mm/s
Data resolution	100 µm/step
Slit	5 x 0.2 mm, micro
Partial scan	No
Lamp	Deuterium & Tungsten
Wavelength(s)	370 nm
Instrument diagnostics	Valid diagnostics
Documentation step label	
Notes	

System suitability tests:

SST settings:

SST tracks	

Substance BHRINRAJ

CAMAG® World Leader in Planar Chromatography

BHRINRAJ_20240511_100144 visionCATS

Substance name	BHRINRAJ
R_F	0.000
ΔR_F	0.010
Step	
λ	
Min. height	
Status	Not computed
Description	

Impurity test:

Data acquisition

Application 1 - Linomat 5 (S/N: 251214):

Executed	11-May-2024 10:55:00 BSRC

Development 1 - Chamber:

Executed	11-May-2024 10:57:13 BSRC

Scan developed plate 1a - TLC Scanner 4 (S/N: 250407):

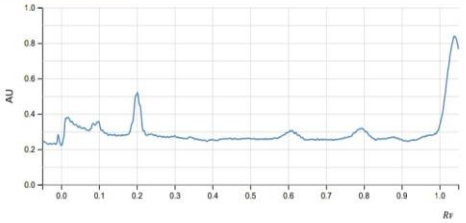
Executed	11-May-2024 11:00:14 BSRC

Scan:

Wavelength	370 nm

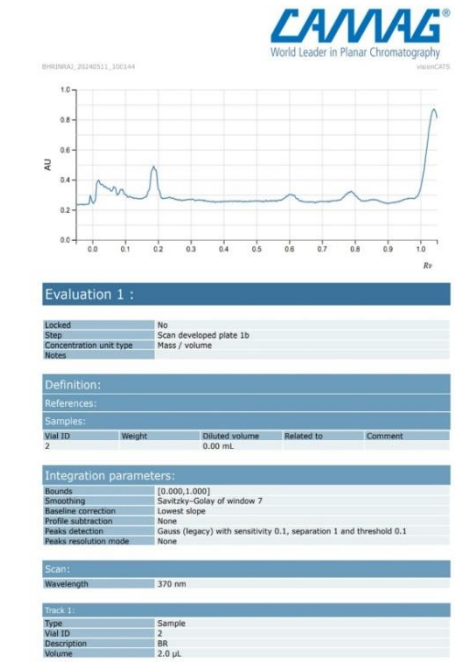
Track 1:

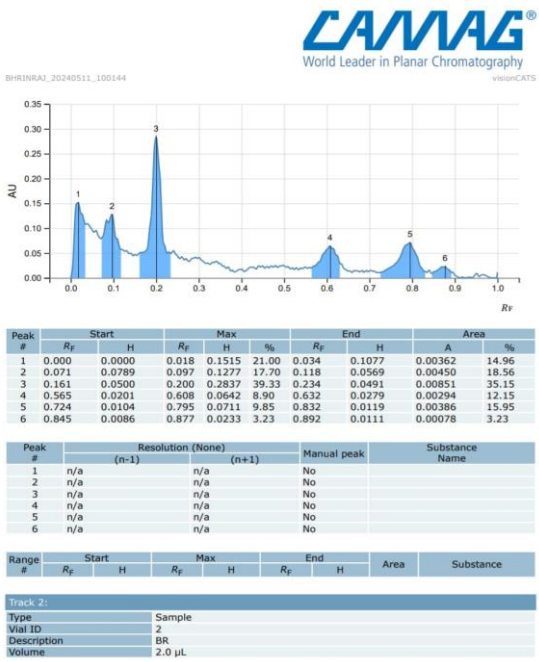
Type	Single λ

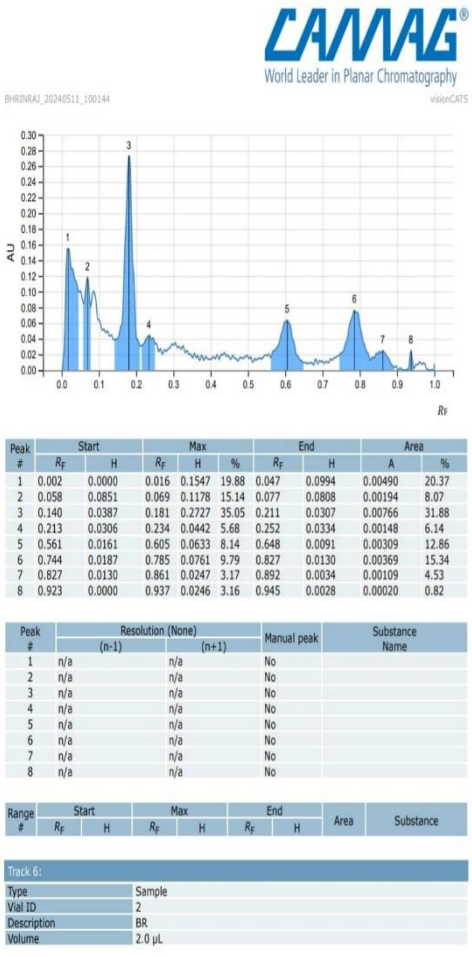


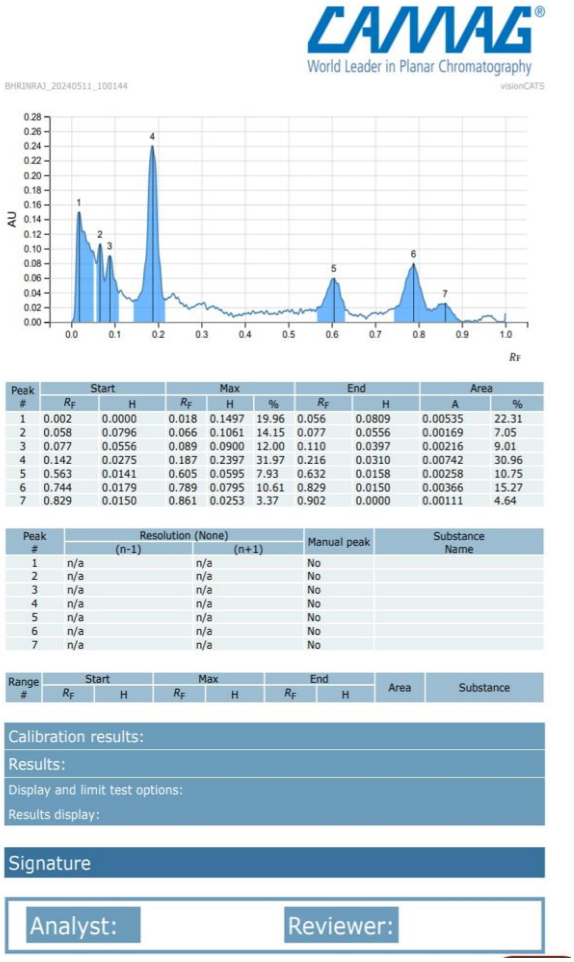
Track 2:

Type	Single λ









DISCUSSION

The present study provides a comprehensive analysis of the pharmacognostical and phytochemical characteristics of *Bhringaraja* (*Eclipta alba Hassk*), a traditional Ayurvedic herb. The findings of this study have significant implications for the quality control and standardization of *Bhringaraja*, which is essential for its safe and effective use in traditional medicine and pharmaceutical applications. The pharmacognostical analysis revealed that *Bhringaraja* has distinctive macroscopic and microscopic features, including its leaf morphology and microscopy. These characteristic features can be used to authenticate *Bhringaraja* and distinguish it from other herbs. The presence of trichomes on the leaves of *Bhringaraja* also

suggests that the herb may have adaptogenic properties, which enable it to thrive in a variety of environmental conditions, The phytochemical analysis identified several bioactive compounds in *Bhringaraja*, flavonoids, tannins, Cardiac glycosides, anthraquinone glycosides these compounds have been reported to exhibit a range of biological activities, including anti-inflammatory, antioxidant, and hepatoprotective effects. The presence of these compounds in *Bhringaraja* provides a scientific basis for its traditional use in Ayurvedic medicine, particularly for the treatment of liver disorders and hair loss. The findings of this study also have implications for the development of new herbal formulations and pharmaceutical products.

CONCLUSION

In conclusion, the present study provides a comprehensive analysis of the pharmacognostical and phytochemical characteristics of *Bhringaraja*. The findings of this study have significant implications for the quality control and standardization of *Bhringaraja*, and for the development of new herbal formulations and pharmaceutical products. Further studies are needed to fully explore the therapeutic potential of *Bhringaraja* and its bioactive compounds. The bioactive compounds present in *Bhringaraja* could be isolated and purified for use in the development of new drugs, or they could be used as lead compounds for the synthesis of new pharmaceutical agents.

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