

STANDARDIZATION OF TALISHPATRA (*ABIES WEBBIANA*) BASED ON CLASSICAL PHARMACOGNOSTIC PARAMETERS AND MODERN ANALYTICAL TECHNIQUES (HPLC-LCMS)

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ABSTRACT

Introduction: *Talishpatra* (*Abies webbiana*) is an important Ayurvedic drug extensively used in classical formulations for respiratory and digestive disorders. Due to increasing demand and restricted natural distribution, the drug is highly prone to substitution and adulteration, necessitating scientific standardisation of authentic material. **Objective:** The present study was undertaken to establish comprehensive pharmacognostical, physicochemical, phytochemical, and chromatographic standards for a certified sample of *Talishpatra* to ensure its identity, purity, and quality.

Materials and Methods: A certified sample of *Talishpatra* leaves was collected from Joginder Nagar (Himachal Pradesh) and authenticated botanically. Standard pharmacognostical evaluation including macroscopic, microscopic, powder microscopy, physicochemical analysis,

preliminary phytochemical screening, TLC profiling, HPLC fingerprinting, and LC-MS analysis was carried out as per API guidelines. **Results:** The certified sample exhibited characteristic macroscopic features such as linear-lanceolate needles, glossy green surface, and aromatic odor. Microscopy revealed diagnostic features including sunken stomata, resin canals, tracheids with bordered pits, and sclerenchymatous tissues. Physicochemical parameters were found within prescribed limits. Phytochemical screening confirmed the

presence of flavonoids, terpenoids, tannins, and glycosides. TLC and HPLC profiles showed consistent and reproducible fingerprints, while LC–MS confirmed the presence of marker phytoconstituents specific to *Abies webbiana*. **Conclusion:** The established pharmacognostical and analytical parameters can serve as reliable reference standards for authentication and quality control of *Talishpatra* in Ayurvedic pharmaceutics.

KEYWORDS: *Talishpatra*, *Abies webbiana*, Pharmacognosy, Standardization, HPLC, LC–MS.

INTRODUCTION

Herbal medicines constitute the backbone of Ayurvedic therapeutics, where the success of treatment is fundamentally dependent on the authenticity, purity, and quality of the raw materials employed. Unlike synthetic drugs, herbal formulations are complex mixtures of multiple phytoconstituents, and any variation in botanical identity or quality of the source material can significantly influence therapeutic outcomes.^[1]

Talishpatra, botanically identified as *Abies webbiana* (family Pinaceae), is a well-known Himalayan medicinal plant traditionally described in Ayurvedic classics for its *Kaphahara*, *Shwasahara*, and *Deepana* properties. It is a key ingredient in widely used formulations such as *Talishadi Churna* and *Sitopaladi Churna*, which are prescribed in conditions like *Kasa*, *Shwasa*, *Pratishyaya*, and digestive disorders.^[2]

Despite its extensive therapeutic utility, *Talishpatra* is naturally confined to specific Himalayan regions and is largely sourced from wild populations. Increased commercial demand, coupled with inadequate regulatory surveillance, has resulted in frequent substitution with other coniferous leaves that closely resemble *Talishpatra* morphologically. Such adulteration not only compromises therapeutic efficacy but also raises concerns regarding safety and reproducibility of clinical outcomes.^[3]

In this context, pharmacognostical standardisation supported by physicochemical, phytochemical, and chromatographic profiling emerges a scientific approach for ensuring identity, purity, and consistency of herbal raw drugs. The present study aims to comprehensively standardise a certified sample of *Talishpatra*, thereby establishing reference parameters for future quality evaluation and routine surveillance.

AIM

The aim of the present study was to establish comprehensive pharmacognostical and analytical standards for *Talishpatra* (*Abies webbiana*) using an authenticated certified sample.

OBJECTIVES

The objectives of the present study were to evaluate the macroscopic, microscopic, physicochemical, phytochemical, and chromatographic characteristics of *Talishpatra* using a certified sample, and to confirm its botanical authenticity through LC-MS analysis, thereby establishing reliable reference standards for quality control and detection of adulteration.

MATERIALS AND METHODS

Collection and Authentication

A certified sample of *Talishpatra* leaves was obtained from RIISM, Joginder Nagar, Himachal Pradesh, a recognized institutional source of genuine Himalayan medicinal plants. Botanical authentication was carried out by taxonomists, confirming the identity of the sample as *Abies webbiana*. The collected leaves were shade dried, pulverized into coarse powder, and stored in airtight containers for further analysis.

Macroscopic Evaluation

Macroscopic evaluation of the *Talishpatra* leaves was carried out in accordance with the guidelines prescribed in the Ayurvedic Pharmacopoeia of India (API). The intact leaves were examined visually and organoleptically for diagnostic characters including colour, size, shape, surface characteristics, margin, apex, texture, odour, and fracture. These parameters were carefully observed under normal daylight to record distinctive morphological features such as the needle-like form, surface lustre, and characteristic aromatic odour, which are useful for preliminary identification and detection of gross substitution. The macroscopic characters thus obtained served as primary criteria for confirming the authenticity and uniformity of the raw drug prior to detailed microscopic and analytical evaluation.^[4]

Microscopic Evaluation

The dried leaves of *Talishpatra* were finely powdered and subjected to detailed microscopic examination to identify characteristic diagnostic features. A small quantity of

the powdered drug was cleared, stained, and mounted on glass slides using standard pharmacognostical techniques. The powder microscopy revealed the presence of distinctive anatomical elements such as elongated tracheids with bordered pits, well-defined resin canals, thick-walled sclerenchymatous cells, and characteristic sunken stomatal structures. These microscopic features are considered diagnostic of *Abies webbiana* and play a crucial role in confirming botanical identity, as well as in differentiating the genuine drug from closely related species and common adulterants.^[5]

Physicochemical Analysis

Physicochemical evaluation of the certified *Talishpatra* sample was carried out to assess its purity, quality, and suitability for medicinal use, following the standard procedures prescribed in the Ayurvedic Pharmacopoeia of India (API). Parameters such as loss on drying were determined to evaluate the moisture content and drying adequacy of the drug, as excessive moisture may lead to microbial growth and deterioration. Total ash value was estimated to assess the total inorganic content, while acid-insoluble ash was measured to determine the presence of siliceous matter such as sand and soil. Extractive values, including water-soluble and alcohol-soluble extractives, were calculated to estimate the amount of active phytoconstituents soluble in respective solvents, thereby providing an indication of the drug's chemical nature and extraction efficiency. These parameters collectively serve as important quality control indices for standardization and detection of adulteration.^[6]

Phytochemical Screening

Qualitative phytochemical screening of the certified *Talishpatra* sample was carried out to identify the presence of major classes of bioactive phytoconstituents using standard chemical tests. The powdered drug and its extracts were subjected to specific reagents and reactions to detect alkaloids, flavonoids, tannins, terpenoids, glycosides, steroids, carbohydrates, proteins, and phenolic compounds. The appearance of characteristic colour changes or precipitates in the respective tests indicated the presence or absence of these constituents. This preliminary phytochemical evaluation provides essential information regarding the chemical nature of the drug and supports its therapeutic potential, as well as serving as a supportive parameter for quality control and standardization.^[6]

Chromatographic Analysis

Thin Layer Chromatography (TLC)

TLC was performed using silica gel plates and appropriate solvent systems. The developed plates were visualized under UV light at 254 nm and 366 nm, followed by derivatization with NP and NP-PEG reagents. R_f values were recorded.^[7]

High Performance Liquid Chromatography (HPLC)

Methanolic extract of the certified sample was analyzed using HPLC to obtain a characteristic chromatographic fingerprint. Retention times of major and minor peaks were recorded.^[8]

Liquid Chromatography–Mass Spectrometry (LC–MS)

LC–MS analysis was performed using electrospray ionization (ESI) in negative ion mode to identify marker compounds and confirm botanical authenticity.^[9]

OBSERVATIONS AND RESULTS

Physicochemical Parameters of Certified *Talishpatra* Sample

The certified reference sample of *Talishpatra* collected from RIISM, Joginder Nagar complied with all quality parameters prescribed under the Ayurvedic Pharmacopoeia of India (API). Exact numerical values are presented below.

Table 1: Physicochemical parameters of certified *Talishpatra* sample.

Parameter	Certified sample value	API reference / Interpretation
Foreign matter (%)	1.8	Within limit (<2%)
Loss on drying (%)	6.2	Acceptable, indicates proper drying
Total ash (%)	5.2	Within API limit (<6%)
Acid-insoluble ash (%)	14.5	Complies with API
Water-soluble extractive (%)	23.9	Indicates high polar constituents
Alcohol-soluble extractive (%)	0.4	Within acceptable range

Interpretation

The certified sample shows acceptable physicochemical quality with foreign matter, loss on drying, total ash, and acid-insoluble ash all within API limits, indicating good purity and proper processing

TLC Fingerprinting of Certified Sample

TLC analysis of the certified sample showed distinct and reproducible Rf values, confirming chemical uniqueness.

Table 2: TLC Rf values of certified *Talishpatra* sample.

Parameter	Rf values	Observation
UV 254 nm	0.63, 0.60	Distinct quenching zones
UV 366 nm	0.63, 0.60	Pink-red fluorescence
After NP derivatization	0.63	Stable flavonoid band
After NP-PEG derivatization	0.60, 0.63	Characteristic fluorescence

Interpretation

The TLC analysis showed clear and reproducible Rf values with characteristic fluorescence and quenching patterns, indicating a stable phytochemical profile. The response after NP and NP-PEG derivatization confirmed the presence of flavonoids. Overall, the TLC fingerprint supports the authenticity and chemical consistency of the *Talishpatra* sample.

HPLC Fingerprinting

HPLC analysis of the certified *Talishpatra* sample revealed a chemically rich and diverse chromatographic profile.

Table 3: HPLC characteristics of certified *Talishpatra* sample.

Parameter	Observation
Major peak retention time (Rt)	12.241 min
Secondary peaks	Multiple minor peaks present
Interpretation	Indicates genuine phytochemical diversity

Interpretation

The HPLC profile showed a prominent peak at 12.241 minutes with multiple minor peaks, indicating the presence of diverse phytoconstituents. The consistent chromatographic fingerprint confirms genuine phytochemical diversity and authentic *Abies webbiana*.

LC-MS observation

LC-MS analysis conclusively established the identity of the certified sample.

Table 4: LC-MS marker compound of certified *Talishpatra*.

Marker compound	Ion mode	m/z observed	Mass accuracy	Inference
4-Methoxy Quercetin	(M-H) ⁻	195.0512	$\Delta = 0.0002$	Authentic <i>Abies webbiana</i>

Interpretation

LC-MS analysis detected 4-Methoxy Quercetin in negative ion mode with high mass accuracy ($\Delta = 0.0002$), indicating precise molecular identification. The presence of this compound is considered a characteristic marker of *Abies webbiana*. Thus, the LC-MS findings conclusively confirm the botanical authenticity of the certified *Talishpatra* sample.

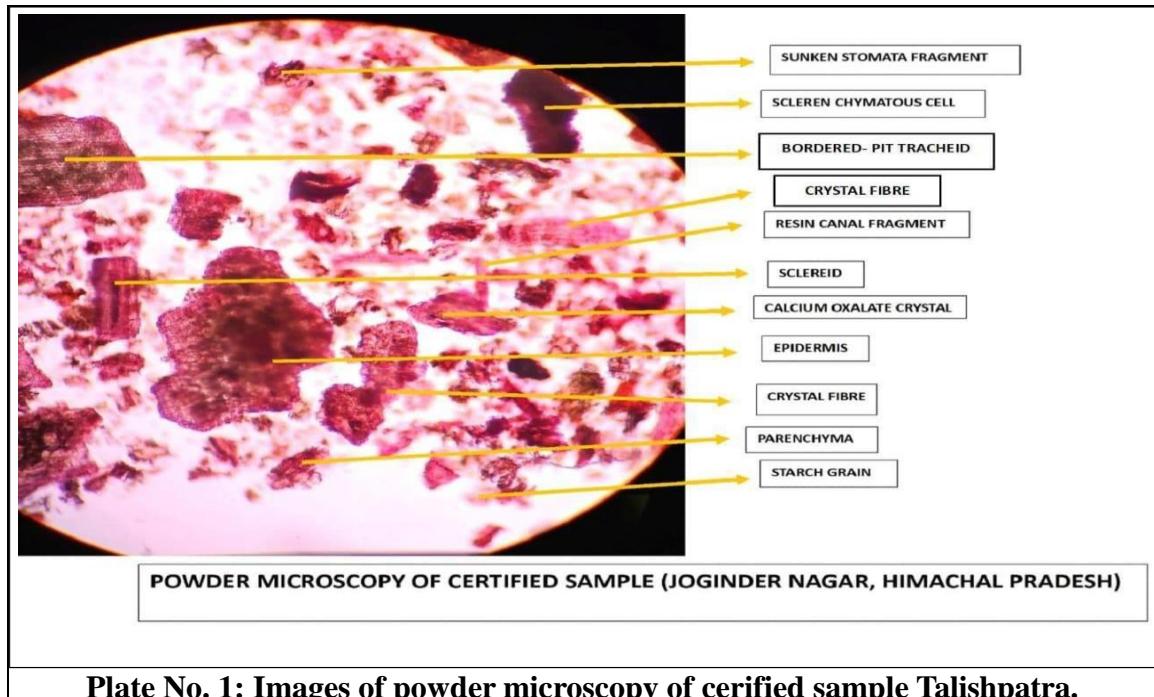


Plate No. 1: Images of powder microscopy of cerified sample *Talishpatra*.

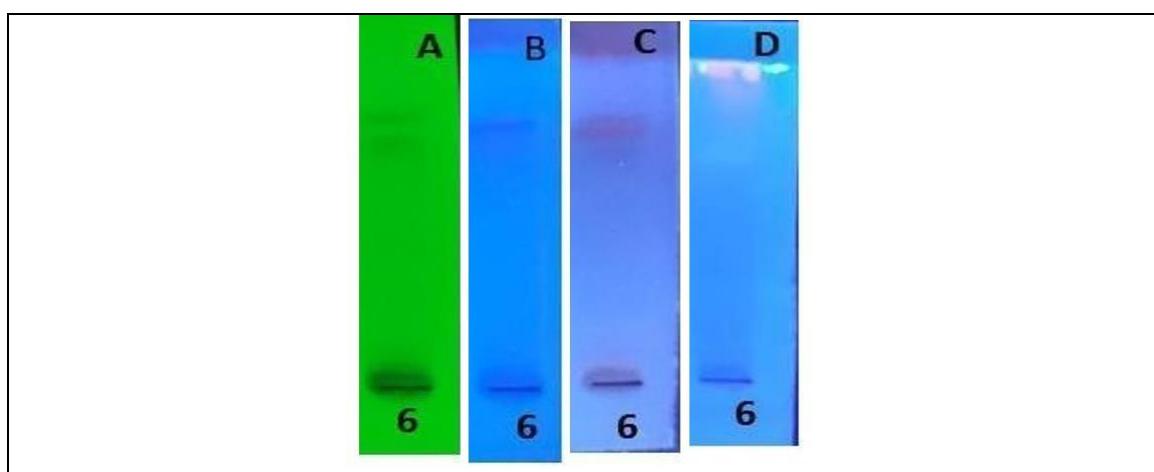


Plate 2:- TLC profiling of certified sample of *Talishpatra*.

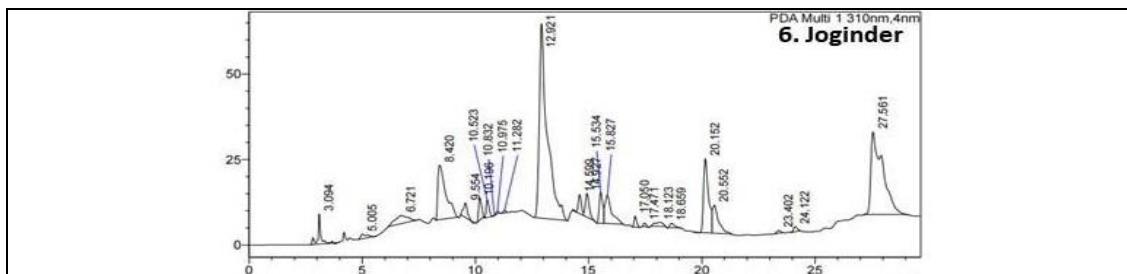


Plate 3:- HPLC-PDA fingerprinting of methanolic extract of certified *Talishpatra* at 310nm.

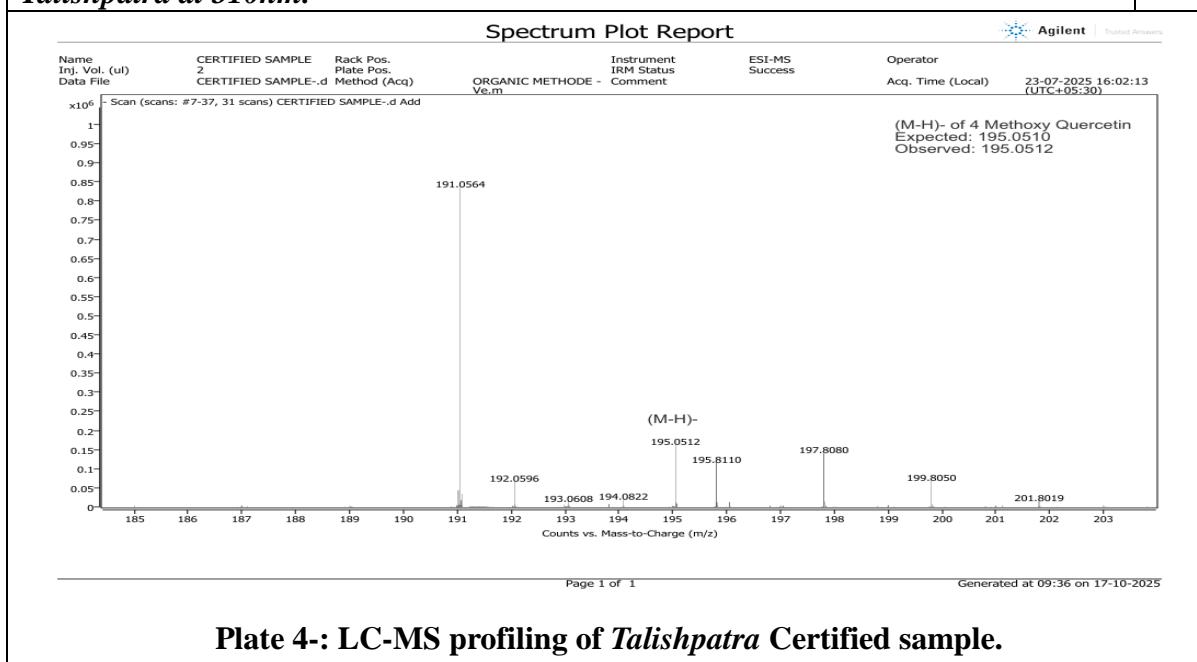


Plate 4:- LC-MS profiling of *Talishpatra* Certified sample.

DISCUSSION

The certified *Talishpatra* sample consistently fulfilled classical, pharmacognostical, physicochemical, phytochemical, chromatographic, and molecular criteria of authentic *Abies webbiana*. Low moisture content, acceptable ash values, and high water-soluble extractive values indicate superior quality and proper post-harvest handling. TLC and HPLC fingerprints revealed chemical stability and diversity, while LC-MS confirmed the presence of 4-Methoxy Quercetin, a definitive marker of *Abies webbiana*, absent in all market samples. These findings reinforce the necessity of using certified reference standards for raw drug authentication and routine quality surveillance.

CONCLUSION

The present study successfully establishes comprehensive pharmacognostic, physicochemical, phytochemical, and advanced chromatographic standards for *Talishpatra* using an authenticated certified reference sample of *Abies webbiana*. The integration of macroscopic and microscopic diagnostic features with physicochemical constants,

qualitative phytochemical profiling, and reproducible TLC, HPLC, and LC-MS fingerprints provides a scientifically validated framework for accurate identification and quality evaluation of the raw drug. These systematically generated parameters serve as reliable reference standards for routine quality control, detection of adulteration and substitution, and assurance of batch-to-batch consistency. Consequently, the findings offer a strong scientific basis for the standardization of *Talishpatra*, supporting its safe, effective, and reproducible use in Ayurvedic practice and the herbal pharmaceutical industry.

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