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Histological Differentiation of *Swertia* species: An Implication for Raw material Authentication to Avert Adulteration

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Abstract**:** *Swertia chirayita* (Roxb. ex Fleming) Karst., a critically valued species of the family Gentianaceae, is the principal source of the Ayurvedic drug "chirata" and is extensively utilized in traditional medicine systems due to antimalarial, hepatoprotective, antipyretic, and appetite-stimulant properties. However, increasing demand coupled with overharvesting, habitat degradation, and limited cultivation has rendered the species critically endangered, leading to its frequent adulteration with morphologically similar *Swertia* species. This study aimed to establish a robust histological framework to distinguish *S. chirayita* from common adulterants through comparative anatomical analysis. Key diagnostic features such as stomatal type, epidermal cell structure, and transverse sections of leaf and stem tissues were examined. The identified histological markers offer reliable, reproducible criteria for species authentication, particularly in fresh or partially processed samples, where morphological identification may be ambiguous. These findings contribute substantially to the development of standardized protocols for the authentication and quality assurance of *S. chirayita*, thereby supporting its safe and effective use in phytomedicines.

Keywords**:** *Swertia chirayita*, Histological differentiation, Species authentication, Adulteration, Herbal medicine, Quality control

Swertia chirayita (Roxb. ex Fleming) Karst., a member of the family Gentianaceae, is a high-altitude medicinal herb indigenous to the temperate regions of the Himalayas, extending from Kashmir to Bhutan and the Khasi Hills of Meghalaya, thriving between 1200 and 3000 meters above mean sea level (Tandon et al., 2010, Bajaj et al., 2017, Mazumder et al., 2023). It is recognized as one of the 32 high-priority medicinal plants by the National Medicinal Plants Board (Kala and Sajwan 2007) and has gained international recognition for its diverse pharmacological activities, including tonic, febrifuge, laxative, stomachic, hypoglycemic, hepatoprotective, anti-inflammatory, antimicrobial, and immunomodulatory effects (Alam et al., 2011, Verma et al., 2013, Ali et al., 2017, Khan et al., 2018). The therapeutic potential of *Swertia chirayita* is primarily attributed to its bitter principles, notably amarogentin and swertiamarin, alongside flavonoids, xanthones, iridoids, and secoiridoid glycosides (Pant et al., 2000, Sharma et al., 2010, Negi et al., 2011, Kumar and Chandra 2013, Kumar and Staden 2016). Swertiamarin has been reported for its hepatoprotective (Wang et al., 2011), anti-diabetic (Vaidya et al., 2013), and anticancer activities (Kavimani and Manisenthlkumar 2000), while amarogentin exhibits potent anti-diabetic properties (Phoboo et al., 2013), anti-cancer (Pal et al., 2012) and anti-arthritic properties (Saravanan et al., 2014). In traditional medical systems, including Ayurveda, Unani, and Siddha, *S. chirayita* holds a prominent position. Historically was included in the Edinburgh Pharmacopoeia (1839) and continues to be recognized in the Indian, British, and American pharmacopeias (Joshi and Dhawan 2005). *S. chirayita* is widely used in the treatment of malaria, hepatic disorders, gastrointestinal ailments, diabetes, and skin diseases (Keil et al., 2000, Joshi 2008, Pant et al., 2010). Additionally, it is employed in managing chronic fever, malaria, anemia, bronchial asthma, hepatotoxic disorders, liver disorders, hepatitis, gastritis, inflammatory conditions, and various digestive disorders, constipation, dyspepsia, skin diseases, anemia, worms, ulcers, epilepsy, ulcers, scanty urine, hypertension, melancholia, and certain types of mental disorders, secretion of bile, blood purification, and diabetes (Banerjee et al., 2000, Rai et al., 2000, Saha et al., 2006, Bhatt et al., 2006, Chen et al., 2011). In addition to its antiviral properties, its anti-hepatitis B virus (anti-HBV) potential has been validated in recent studies, reinforcing its versatility as a therapeutic agent (Zhou et al., 2015). *S. chirayita* is commonly administered in various forms, including concentrated infusions, tinctures, powders, and fluid extracts (Joshi 2008, Phoboo et al., 2008). Its extracts are widely utilized in the cosmetic industry, where they serve as active ingredients in products like facial creams, cleansers, scrubs, hair oils, and skin tonics, including Safi. Herbal formulations such as Ayush-64, Diabecon, Mensturyl syrup, and Melicon V ointment (Edwin and Chungath 1988, Mitra et al., 1996) contain *Swertia chirayita* extract in different concentrations for its antipyretic, hypoglycaemic, antifungal, and antibacterial properties. Ayush-64, incorporates *S. chirayita* and has shown significant effectiveness in managing COVID-19. The formulation was repurposed for mild to moderate cases of COVID-19, showing promising results in

clinical trials (Panda et al., 2022). Moreover, *S. chirayita* also played a vital role as a key ingredient in Traditional Chinese Medicine (TCM) formulations used against SARS-CoV-2 during the early stages of the pandemic in China (Gyawali et al., 2020, Omokhua-Uyi and Staden 2021, Ahmad et al., 2021, Shereen et al., 2021).

Despite its significant medicinal value, *S. chirayita* faces severe threats in its natural habitat due to overexploitation, habitat fragmentation, low seed viability and slow propagation rates and is classified as critically endangered in its native range (Joshi and Dhawan 2005). Overharvesting and widespread habitat destruction have severely depleted wild populations of the species, leading the Government of India to prohibit wild harvesting and prioritize conservation initiatives (Ved and Goraya 2007). In addition, its restricted geographic distribution (Bhat et al., 2013), coupled with persistent seed viability challenges (Badola and Pal 2002, Joshi and Dhawan 2005), further worsens its vulnerability to extinction, underscoring the urgent need for targeted and sustainable conservation interventions.

The rising market demand for *S. chirayita* has led to widespread adulteration, with the species often being substituted by morphologically similar species such as *S. angustifolia, S. cordata, S. alata*, *S. paniculata*, and even unrelated species like *Andrographis paniculata* (Girach et al., 1994, Brahmachari et al., 2004, Latif and Rehman 2014). Such practices compromise therapeutic efficacy, jeopardize consumer safety, and undermine the integrity of herbal formulations. Given the limitations of traditional macroscopic and phytochemical identification methods particularly in processed or powdered materials there is an urgent need for more reliable diagnostic approaches.

Histological profiling, based on stable microanatomical features such as stomatal type, epidermal architecture, mesophyll organization, and vascular arrangement, offers a reproducible and robust tool for precise species authentication. As these anatomical traits largely withstand post-harvest processing, they hold significant potential for pharmacognostic applications. The present study aims to elucidate the detailed histological features of *S. chirayita* and differentiate it from commonly substituted adulterants *viz. S. alata, S. angustifolia, S. cordata* and *S. purpurascens.* By establishing a diagnostic anatomical key, this research seeks to strengthen authentication protocols, enhance quality control standards, and promote the development of safe, standardized herbal formulations.

MATERIAL AND METHODS

Plant materials: Plant samples, including cultivated *S. chirayita* from the University farm in Shilly, *S. alata* and *S. angustifolia* from Dharon Ki Dhar district Solan, Himachal Pradesh (30.881417° N, 77.178488° E) and *S. purpurascens* and *S. cordata* from Hatu district Shimla, Himachal Pradesh (31.244087° N, 77.501339° E (were collected from their respective natural habitats for anatomical studies. The specimens were processed using standard anatomical techniques to examine various morphological features. The study focused on epidermal cell morphology, leaf trichomes, stomatal types and dimensions, and the transverse sections (T.S.) of stems and vertical sections (V.S.) of leaves across these five *Swertia* species.

Sample fixation and preservation: Fresh leaf and stem samples from each species were immediately fixed in a formalin-acetic acid (FAA) solution (ethyl alcohol: acetic acid: formaldehyde in a ratio of 90:5:5) and preserved at room temperature for further analysis.

Sample preparation and staining procedure: Thin sections of leaves and stems were carefully prepared using a sharp razor blade and mounted on slides for microscopic examination. The prepared sections were initially hydrated in distilled water, followed by sequential dehydration in 25 and 50% ethyl alcohol solutions for 5 minutes each. Sections were then stained with aqueous safranine for 5 minutes, followed by dehydration in 75% ethyl alcohol. Afterward, the sections were stained with fast green for 5 minutes. The stained sections were mounted with cover slips and examined under microscope for microscopic analysis.

Microscopic analysis*:* To examine the epidermal cell morphology, fresh leaf peels from the lower epidermis were stained with cotton blue and cleared in lactophenol. The morphological characteristics of the epidermal cells were then documented under a microscope.

Stomatal characteristics: Stomatal size was measured using ocular and stage micrometers on sections from the lower parts of the leaf epidermis. Stomatal types were classified according to system by Fahn (1967). The stomatal index (SI) was calculated from at least 25 readings using the formula:

$$SI = \frac{S}{(E+S)} \times 100$$

Where

SI = Stomatal index

S = No. of stomata/unit area

E = No. of epidermal cells in the same area

RESULTS AND DISCUSSION

The, comparative histological evaluation of five *Swertia* species revealed distinct anatomical markers, thereby strengthening the diagnostic framework for authenticating genuine “chirayita.”

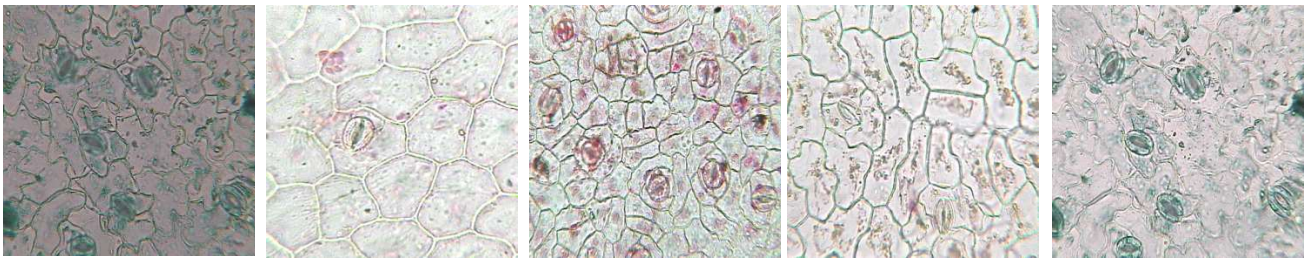
Anatomical Characters

Epidermal cell: Epidermal cell morphology and surface features revealed distinct variations across the studied species (Fig. 1). *S. angustifolia* and *S. purpurascens* displayed prominently wavy epidermal cell outlines, while *S. cordata* showed moderately wavy cells; in contrast, *S. chirayita* and *S. alata* featured angular epidermal cells.

Among these, *S. angustifolia* possessed the largest epidermal cells, serving as a distinguishing anatomical marker. In addition, trichomes both unicellular and multicellular were consistently present among all species, though their density and structural variations further contributed to species differentiation. Similar patterns of taxonomically significant epidermal and trichome variation have been reported by Al-Shami et al. 2022 in *Veronica* species, where detailed comparative analyses of stems, leaves, and petioles revealed distinctive epidermal cell shapes and trichome structures useful for species authentication. These observations are further supported by Chen et al. (2008), and highlighted epidermal cell morphology and trichome characteristics as reliable diagnostic traits for species authentication in medicinal plants.

Stomata type: Stomatal characteristics also play a significant role in differentiation, with *S. chirayita* exhibiting anisocytic stomata, distinct from the anomocytic stomata observed in other species (Table 1). This observation is consistent with previous studies reinforcing the role of stomatal morphology in the authentication of *S. chirayita* (Xue et al., 2000). The stomatal index exhibited variability across species, with *S. angustifolia* demonstrating the highest index and *Swertia cordata* the lowest. *S. chirayita* and *S. purpurascens* displayed similar indices, albeit with slight deviations from previously reported values (Natrajan and Prasad 1972).

These variations emphasize the necessity for diverse methodologies to accurately interpret stomatal indices for species differentiation and quality assessment in medicinal plants. The findings of our study are further substantiated by the work of Jiachun et al. (1993) which presents a comprehensive analysis of the microscopic structure of *Swertia* species. Balekundri and Mannur (2020) emphasized the importance of incorporating robust diagnostic parameters, including anatomical features, to ensure the authenticity of herbal products and mitigate the risk of adulteration and underscores the complementary role of anatomical characterization in maintaining species integrity and quality control in herbal formulations. Li et al. (2013) examined both microscopic and macroscopic traits across eight *Swertia* species, identifying diagnostic features such as epidermal cell wall sinuosity, fiber presence in sepals, and stomatal distribution. These anatomical markers have proven valuable for species authentication and standardization in herbal medicines. Integrating these insights with advanced anatomical techniques, current study refines the criteria for accurately identifying *S. chirayita*, further supporting its authenticity in herbal preparations (Kshirsagar et al., 2019). Rashid et al. (2019) highlighted significant anatomical and phytochemical differences between *S. cordata* and its common adulterant, *S. paniculata*, emphasizing the importance of accurate anatomical authentication. This reinforces the critical role of



(a) *S. alata* (b) *S. angustifolia* (c) *S. chirayita* (d) *S. cordata* (e) *S. purpuras cens*

Fig. 1. Epidermal cells of *Swertia* species

Table 1. Stomatal characteristics of different *Swertia* species

Parameters	Tetramerous species			Pentamerous species	
	S. chirayita	*S. alata*	*S. angustifolia*	*S. purpurascens*	*S. cordata*
Stomata length	32.630.031m	33.770.050m	33.850.037m	34.220.038m	38.980.032m
Stomata breadth	19.630.032m	19.380.032m	21.590.023m	20.940.025m	25.670.023m
Stomata type	Anisocytic	Anomocytic	Anomocytic	Anomocytic	Anomocytic
Stomatal index	23.470.346	20.580.098	31.980.420	24.520.117	18.690.200

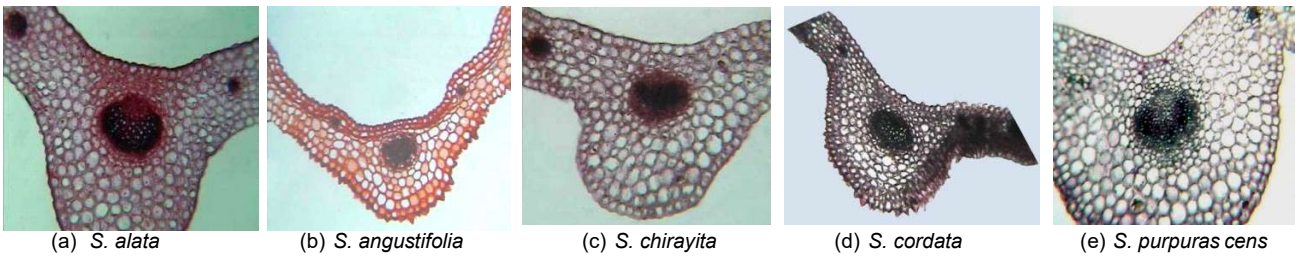
detailed anatomical profiling in ensuring species authenticity, improving quality control, and preventing adulteration in herbal medicine.

Anatomy of leaf: Vertical sections of leaves across all *Swertia* species studied, expressed a consistent anatomical feature: the mesophyll remained undifferentiated, lacking distinct palisade and spongy tissues, and displayed small intercellular spaces (Fig. 2). Bisht et al. (2011) also reported loosely arranged spongy mesophyll cells in *S. chirayita*., *S. angustifolia* exhibited a distinct anatomical characteristic the presence of a well-developed collenchymatous layer which serves as an important marker distinguishing it from *S. chirayita* and other related species.

Additionally, the vascular bundles in *S. angustifolia* were positioned closer to the upper epidermis, contrasting with the more centrally located vascular bundles observed in other *Swertia* species. Such anatomical variations highlight the diagnostic significance of leaf structural traits in species differentiation. The importance of employing simple macroscopic and microscopic evaluations for the authentication of plant materials has also been emphasized by Arathy and Sandhya (2021), particularly in distinguishing genuine *Cinnamomum verum* from its adulterants. Similarly, the unique anatomical features identified in *S. angustifolia* underscore its importance in ensuring accurate identification and maintaining the quality and authenticity of herbal drugs derived from *Swertia* species.

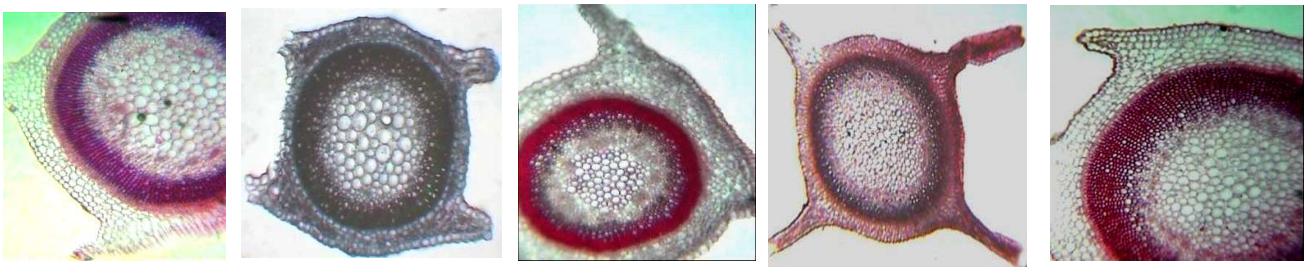
Anatomy of stem: Transverse sections of stem tissues revealed both conserved and distinct anatomical features that are essential for species-level differentiation (Fig. 3). All species exhibited a single layered epidermis, a characteristic feature within the *Swertia* genus, as reported in previous studies (Bisht et al., 2011). A well-developed amphiphloic siphonostele was consistently observed across all taxa, with *Swertia chirayita* displaying the most pronounced configuration, thus reinforcing its role as the reference species for 'Chirata'.

However, in contrast to earlier studies reporting acicular crystals in the cortical region of *Swertia chirayita* (Anonymous 1998, Bisht et al., 2011), no such crystals were detected in the present study. This discrepancy may be attributed to environmental or developmental factors influencing crystal formation. Ergastic cell contents, such as starch grains and prismatic crystals, serve as valuable diagnostic markers in herbal drug identification, including for stem tissues. These features, observed in the stem sections, enhance the reliability of anatomical diagnostics and contribute to distinguishing species based on both macroscopic and microscopic traits (Kumar et al., 2018). Quantitative anatomical parameters, such as tissue thickness in transverse sections (Hassan et al., 2015), cell dimensions, and the size of starch grains, cortical cell and epidermal cells in *Fritillaria cirrhosa* (Singh et al., 2020), further substantiate these diagnostic methods. The presence of a distinct endodermal layer in *S. chirayita* further distinguishes it from other species, potentially reflecting specialized solute transport mechanisms. In contrast, *S. cordata* exhibited radially thickened pericyclic cell walls and enlarged pith



(a) *S. alata* (b) *S. angustifolia* (c) *S. chirayita* (d) *S. cordata* (e) *S. purpuras cens*

Fig. 2. V. S. of leaf (closeup of mid rib region of different *Swertia* species)



(a) *S. alata* (b) *S. angustifolia* (c) *S. chirayita* (d) *S. cordata* (e) *S. purpuras cens*

Fig. 3. T. S. of stem of different *Swertia* species

relative to the cortical parenchyma, providing clear structural markers for identification. *S. angustifolia* displayed a collenchymatous hypodermis, a unique feature that enhances its diagnostic utility. These anatomical distinctions are consistent with the findings of Rashid et al. (2019), who reported significant divergence between *S. cordata* and *S. paniculata*. The frequent misidentification of *Swertia chirayita* (Gentianaceae) and *Andrographis paniculata* (Acanthaceae), two unrelated species with distinct therapeutic profiles, underscores the critical need for reliable diagnostic methods. The comparative anatomical study by Kumar et al. (2022) on raw herbal drugs of *Abrus precatorius* and *Glycyrrhiza glabra* emphasizes the importance of integrating both macroscopic and microscopic characteristics for distinguishing closely related species. Similarly, the anatomical profiling of *Swertia chirayita*, incorporating both exomorphic and endomorphic traits, provides essential data for accurate species delimitation and ensures the authenticity and integrity of raw materials used in herbal formulations (Selvam 2011).

CONCLUSIONS

The study provides a useful approach for detecting adulteration in fresh leaf and stem samples of *Swertia chirayita*. However, its applicability may be limited when dealing with dry and thoroughly mixed samples, where distinguishing morphological or anatomical features becomes difficult. In such cases, additional methods or modifications would be required for reliable purity assessment. Future research should focus on evaluating and adapting the methodology for dry samples, as this would significantly enhance the practical utility of the study for trade and quality control of medicinal plants.

AUTHOR'S CONTRIBUTION

Aruna Mehta: Sample collection, histological studies, analyzed microscopic observations and manuscript drafting; Sunil Marpa: data interpretation, manuscript writing and review; Kumari Bandana: literature review, data interpretation and manuscript preparation; Yourmila Kumari: discussion and manuscript editing; Garima: manuscript writing, formatting and referencing; Kashish: figure organization and manuscript proofreading.

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