MORPHOLOGICAL, ANATOMICAL AND PHYTOCHEMICAL PROFILING OF *WITHANIA SOMNIFERA* (L.) DUNAL CULTIVATED IN GIA LAI, VIETNAM

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Received May 1st, 2026; Accepted June 18th, 2026

Abstract: *Withania somnifera (L.) Dunal is a medicinal species extensively investigated for its pharmacological activities; however, region-specific pharmacognostic data remain limited, particularly for materials cultivated in Southeast Asia. This study aimed to establish a comprehensive diagnostic profile of W. somnifera cultivated in Gia Lai Province, Vietnam, integrating morpho-anatomical and preliminary phytochemical analyses. Macroscopic, anatomical, and powder microscopic characteristics were systematically investigated, alongside preliminary phytochemical screening using a modified Ciuley fractionation approach. The Vietnamese specimens exhibited typical species-specific traits, including pubescent aerial organs, swollen roots, and characteristic orange-red berries. Anatomical analysis revealed abundant calcium oxalate crystals, branched multicellular trichomes, dorsiventral mesophyll organization, and the presence of intraxylary phloem, all of which represent key diagnostic features. Powder microscopy further confirmed the presence of characteristic vessel elements, starch granules, and crystal morphotypes. Phytochemical screening revealed an organ-specific distribution of secondary metabolites, with sterol/triterpenoid-like constituents broadly detected, alkaloids predominant in roots, and carotenoids abundant in leaves and fruits. Flavonoids, saponins, tannins, and cardiac glycosides were distributed across multiple organs. These findings provide a robust set of region-specific diagnostic markers supporting authentication, quality control, and future standardization of Vietnamese-cultivated W. somnifera, thereby contributing valuable data to the global pharmacognostic database.*

Keywords: *Withania somnifera, pharmacognosy, anatomy, morphology, phytochemical screening.*

1. INTRODUCTION

Withania somnifera (L.) Dunal (Ashwagandha, Indian ginseng or winter cherry) is an accepted medicinal species of the Solanaceae family. It is generally described as a perennial subshrub or shrub with pubescent aerial parts, ovate to elliptic leaves, greenish to yellowish axillary flowers, and orange-red berries enclosed by a persistent enlarged calyx [1, 2]. According to taxonomic databases, the species has a broad natural distribution extending from southern Europe to Central China and from Africa to Myanmar, and it has been widely cultivated in dry and semi-arid regions, particularly in India, for medicinal use [1 – 3]. In cultivation, the plant is commonly harvested at about 150 – 210 days after planting; drying of leaves and reddening of berries are considered practical indicators of crop maturity, while the roots represent the principal medicinal material [3].

W. somnifera has long been used in traditional Ayurvedic medicine, mainly in the form of root powder or root extract. Reported major bioactive groups include steroidal lactones, especially withanolides such as withaferin A and withanolide A, together with alkaloids, sitoindosides, sterols/triterpenoids, flavonoids, and other phenolic constituents. Published experimental and clinical studies have described several biological activities of *W. somnifera* preparations and isolated constituents, including adaptogenic, anti-stress/anxiolytic, anti-inflammatory, antioxidant, immunomodulatory, and neuroprotective potentials [4 – 6]. These findings provide the pharmacological background for the continued interest in this medicinal plant, although the chemical profile and diagnostic characters may vary with plant organ, cultivation conditions, geographical origin, and post-harvest handling.

For medicinal plant materials, pharmacognostic characterization remains essential for authentication, quality control, and standardization. Macroscopic, anatomical, powder microscopic, and preliminary phytochemical data provide practical diagnostic criteria for identifying raw materials and detecting possible substitution or adulteration. As *W. somnifera* has recently been cultivated in the Central Highlands of Vietnam, region-specific diagnostic data are needed to support the evaluation and future standardization of Vietnamese-cultivated material. Therefore, this study aimed to establish a diagnostic profile of *W. somnifera* cultivated in Gia Lai

Province, Vietnam, integrating macroscopic, anatomical, powder microscopic, and preliminary phytochemical analyses.

2. MATERIALS AND METHODS

2.1. Plant material

Representative whole plants of *Withania somnifera* (L.) Dunal (n = 6 individual plants; cultivation age = 6 months; developmental stage: flowering and fruiting) were collected from cultivated fields in Gia Lai Province, Central Highlands, Vietnam (14°N, 108°E) in July 2025. The plant material included roots, stems, leaves, flowers, fruits, and seeds.

The cultivated material was derived from seeds originally obtained from India under the name *Withania somnifera* (L.) Dunal. The seeds were propagated and cultivated under local field conditions in Gia Lai Province. Species identity was supported by the seed origin and subsequent comparison of the cultivated plants with accepted taxonomic descriptions of *W. somnifera* [1, 2], particularly the pubescent aerial parts, ovate to elliptic leaves, greenish to yellowish axillary flowers, orange-red berries enclosed by a persistent calyx, and swollen roots.

Fresh samples were used for macroscopic and anatomical analyses, while portions were shade-dried at ambient temperature (25 – 30°C) for powder microscopy and phytochemical screening.

2.2. Macroscopic studies

Diagnostic features were documented photographically. Macroscopic characteristics of fresh plant organs were examined according to the World Health Organization (WHO) guidelines for quality control of herbal materials [7]. Parameters including size, shape, color, surface features, texture, fracture characteristics, and organoleptic properties were recorded. Representative photographs were taken under standardized lighting conditions to document diagnostic features.

2.3. Anatomical studies

Transverse sections of fresh roots, stems, petioles, and leaves were prepared manually using a sharp razor blade. Sections were double-stained with iodine green and carmine red to differentiate lignified and non-lignified tissues. Stained sections were mounted in glycerin – water (1 : 1, v/v) and examined under a light microscope (Olympus CX23, Japan) equipped with a digital imaging system. Diagnostic anatomical features, including epidermal structure, vascular organization, trichomes, calcium oxalate crystals, and starch grains, were observed and recorded. Representative micrographs were captured for documentation.

2.4. Powder microscopy

Shade-dried plant organs were pulverized and passed through an 80-mesh sieve to obtain uniform powder samples. Powdered materials were treated with chloral hydrate solution for clearing and mounted in glycerin for microscopic observation. Diagnostic elements such as vessel types (spiral, pitted), fibers, cork cells, starch granules, calcium oxalate crystals, trichomes, and parenchyma fragments were identified and described according to standard pharmacognostic procedures.

2.5. Preliminary phytochemical screening

Preliminary phytochemical screening was performed using a modified Ciuley fractionation method [8]. Briefly, the dried powdered samples of each plant organ were first defatted with petroleum ether to remove non-polar constituents. The petroleum ether extract was collected as fraction A and used for the detection of lipophilic compounds, including carotenoids and volatile oil-like constituents.

The defatted plant residue was subsequently extracted with ethanol. The ethanolic extract was concentrated under reduced pressure, then dissolved in **2% hydrochloric acid solution** and filtered to obtain an acidified aqueous solution. This solution was successively partitioned with **diethyl ether** to obtain fraction B, which was used for the detection of coumarins, anthranoids, flavonoids, and triterpenoid/sterol-like compounds.

The remaining acidic aqueous layer was then alkalinized with **ammonia solution to pH 9 - 10** and extracted with **chloroform** to obtain fraction C, which was used as the alkaloid fraction and tested with Mayer's, Dragendorff's, and Bouchardat's reagents.

After removal of the organic layer, the residual aqueous phase was retained as fraction D. This fraction was used for the detection of saponins, tannins, reducing sugars, organic acids, and cardiac glycosides by appropriate qualitative reactions, including the foam test, ferric chloride test, Fehling's test, and specific color reactions for cardiac glycosides. All reactions were performed in triplicate, and results were recorded based on reproducible color changes or precipitate formation.

2.6. Data documentation and reproducibility

All observations were conducted under controlled laboratory conditions. Microscopic analyses were performed on multiple sections or samples to ensure reproducibility.

Qualitative phytochemical results were expressed semi-quantitatively as absent (–), present (+), moderately positive (++), or strongly positive (+++), based on the intensity of observed reactions.

3. RESULTS

3.1. Macroscopic Morphology

Withania somnifera cultivated in Gia Lai Province, Vietnam, is an erect shrub ranging from 40 to 100 cm in height. The aerial parts are uniformly pubescent. Young stems are green and become greyish-white at maturity, with distinct lenticels. Roots are cylindrical, pale whitish to yellow, and characteristically swollen at the crown region, which represents a key diagnostic feature. Leaves are simple, ovate to elliptic (4.3 – 7.0 × 2.5 – 3.6 cm), with a cuneate and slightly unequal base and a rounded to slightly acute apex. Venation is pinnate and more prominent on the abaxial surface. Leaves are arranged alternately on lower nodes and oppositely near the apex. Both leaf surfaces and the cylindrical petiole (1.1 – 1.3 cm) are covered with fine hairs. Flowers are actinomorphic, bisexual, and pentamerous, occurring singly or in small axillary clusters (4 – 6 per node). The calyx is campanulate (~2 mm), with five triangular lobes and a pubescent outer surface. The corolla is light green to yellow, tubular, and 3 – 4 mm in length. Five stamens are inserted on the corolla tube. The ovary is superior, globose, and bilocular, with a single style (~2 mm). Fruits are smooth, globose berries (9 – 11 × 6 – 8 mm), enclosed within a persistent calyx that becomes papery and brown upon maturation. The fruits turn from green to orange-red when ripe. Seeds are yellowish, kidney-shaped to sub-round, approximately 2 mm in diameter.

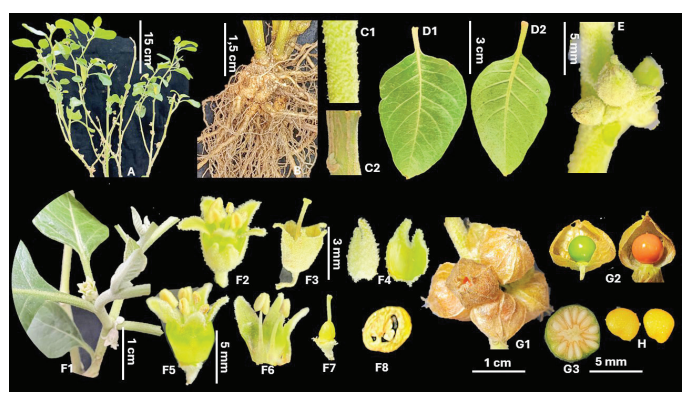

Figure 1. *Macroscopic morphology of Withania somnifera cultivated in Vietnam. Morphology of Withania somnifera; (A) Whole plant; (B) swollen root; (C1 – C2) young and mature stem; (D1 – D2) adaxial and abaxial leaf surface; (E) flower buds; (F1 – F8) floral structures including solitary flower, fully opened flower, calyx, corolla, stamens, pistil, and ovary (cross-section); (G1 – G3) fruit cluster, immature and mature fruits, and transverse section of young fruit; (H) seed.*

3.2. Anatomical Features

Root: The transverse section of the root is nearly circular. The outermost layer consists of a cork (phellem) composed of 3 – 4 layers of rectangular, suberized cells. Beneath the cork, 2 – 3 layers of loosely arranged cortical parenchyma are present. A distinct endodermis with a Casparian strip surrounds 1 – 4 layers of pericycle cells. The primary phloem is compressed, whereas the secondary phloem forms 6 – 8 radial rows of thin-walled cells. Numerous calcium oxalate crystals are distributed throughout the cortex and phloem. The secondary xylem occupies the central region and consists of lignified vessels and medullary rays (1 – 3 cells wide) arranged radially.

Stem: The stem transverse section is circular. The epidermis is single-layered with a cutinized outer wall and bears multicellular branched non-glandular trichomes. In mature stems, partial exfoliation of the epidermis is observed. A developing periderm composed of 2 – 3 layers of suberized cells is present beneath the epidermis. The cortex consists of 4 – 5 layers of angular collenchyma followed by 3 – 4 layers of loosely arranged parenchyma. Scattered sclerenchyma cells are observed within the cortex. The vascular cylinder comprises a discontinuous sclerenchymatous pericycle (1 – 3 layers), collapsed primary phloem, and well-developed secondary phloem (6 – 7 layers) containing calcium oxalate crystals. The secondary xylem is prominent and consists of large lignified vessels, whereas smaller primary xylem vessels exhibit centrifugal differentiation. Intraxylary phloem is clearly present as discrete clusters separated from the primary xylem by 1 – 2 layers of parenchyma. The

pith consists of loosely arranged parenchymatous cells containing scattered crystals.

Petiole: In transverse section, the petiole is nearly circular with a slightly undulated adaxial surface. The epidermis is single-layered with a thick cuticle and bears numerous branched multicellular non-glandular trichomes. Collenchyma occurs discontinuously (5 – 7 layers), mainly in the protruded regions, followed by cortical parenchyma. Subepidermal layers contain chloroplast-bearing cells. A single dominant arcuate vascular bundle is located centrally, with xylem positioned adaxially and phloem abaxially. The xylem consists of approximately 30 – 32 radial rows of lignified vessels separated by xylem parenchyma. The primary phloem forms 5 – 6 layers of thin-walled cells, and an internal phloem is also present. Two smaller accessory vascular bundles are observed laterally above the main bundle.

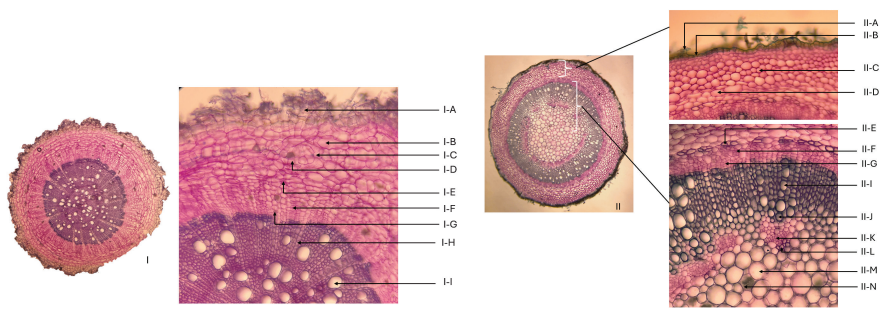

Figure 2. *Anatomical features of root and stem of Withania somnifera.*

(I) Root transverse section: (I-A) phellem (cork); (I-B) cortical parenchyma; (I-C) endodermis; (I-D) pericycle; (I-E–I-F) primary and secondary phloem; (I-G) calcium oxalate crystals; (I-H) medullary rays; (I-I) secondary xylem.

(II) Stem transverse section: (II-A) protective trichomes; (II-B) epidermis; (II-C) angular collenchyma; (II-D) cortical parenchyma; (II-E) sclerenchymatous pericycle; (II-F–II-G) primary and secondary phloem; (II-I–II-J) secondary and primary xylem; (II-K) intraxylary phloem; (II-L) sclerenchyma; (II-M) pith; (II-N) calcium oxalate crystals.

Leaf lamina: In transverse section, the midrib is convex on both surfaces, more prominent on the abaxial side, and 4 – 5 times thicker than the lamina. The upper and lower epidermises are single-layered with cutinized walls and bear numerous branched multicellular non-glandular trichomes. Collenchyma forms 4 – 5 layers on the adaxial side and 2 – 3 layers on the abaxial side, followed by loosely arranged ground parenchyma. The main vascular bundle is arcuate and structurally similar to that of the petiole. The lamina proper exhibits a dorsiventral mesophyll organization, consisting of a single layer of palisade parenchyma and 3 – 4 layers of spongy parenchyma. Stomata are more abundant on the abaxial surface, indicating an amphistomatic leaf. Sand-type calcium oxalate crystals are scattered within the mesophyll. Obliquely cut secondary veins are also observed.

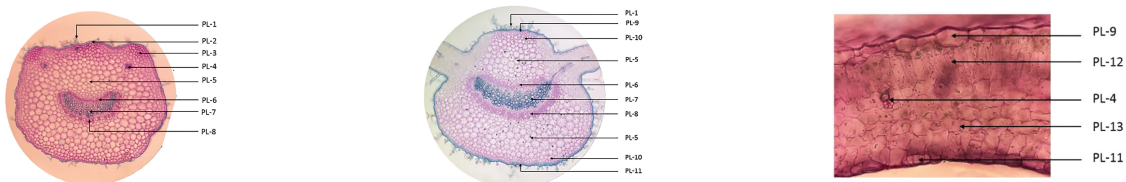

Figure 3. *Transverse sections of the petiole and leaf lamina of Withania somnifera.*

(PL-1) Non-glandular trichome; (PL-2) epidermis; (PL-3) collenchyma; (PL-4) accessory vascular bundle; (PL-5) cortical parenchyma; (PL-6) internal phloem; (PL-7) xylem; (PL-8) primary phloem; (PL-9) upper epidermis; (PL-10) angular collenchyma; (PL-11) lower epidermis; (PL-12) palisade parenchyma; (PL-13) spongy parenchyma.

3.3. Powder microscopic characteristics

Powdered materials of *Withania somnifera* exhibited distinct organ-specific diagnostic features. Root powder is ivory-white with a faint characteristic odor. It contains abundant parenchymatous fragments bearing numerous starch granules and calcium oxalate crystals (predominantly sand-type). Vessel elements with bordered pits are frequently observed. Stem powder appears fine and slightly greenish. It is characterized by fragments of epidermis bearing multicellular

branched non-glandular trichomes, along with spiral and pitted vessels. Sclerenchymatous elements and pigmented tissues are occasionally present. Leaf powder is dark green and composed of epidermal fragments with stomata surrounded by 3 – 4 subsidiary cells, indicating a typical stomatal complex. Numerous branched multicellular trichomes are observed. Fragments of mesophyll tissue, including palisade and spongy parenchyma, as well as spiral vessels, are also present. Fruit powder is orange-yellow with a slightly pungent odor. It consists predominantly of pigmented parenchymatous tissue fragments, spiral vessels, and granular materials.

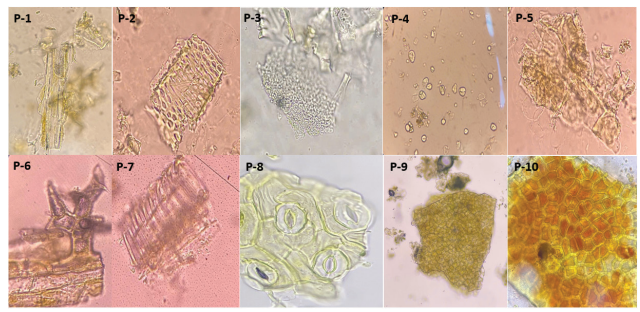

Figure 4. *Microscopic characteristic of powdered Withania somnifera .*

(P-1) Tissue fragment containing calcium oxalate crystals; (P-2) pitted vessel element; (P-3) calcium oxalate crystals; (P-4) starch granules; (P-5) parenchymatous tissue fragment; (P-6) branched non-glandular trichomes and epidermis; (P-7) spiral vessel element; (P-8) epidermal fragment with stomata; (P-9) pigmented leaf tissue fragment; (P-10) fruit tissue fragment.

3.4. Preliminary phytochemical screening

Preliminary phytochemical screening revealed a distinct organ-dependent distribution of secondary metabolites in *Withania somnifera* (Table 1).

Sterol-like constituents were detected in all examined organs, with stronger reactions observed in roots. Alkaloids showed a marked predominance in roots (+++), while moderate levels were detected in fruits and lower levels in aerial parts. Carotenoids were most abundant in leaves and fruits (+++), with lower levels in stems and absence in roots. Volatile oil-like constituents were weakly detected in all examined organs. Coumarins and tannins were consistently present across plant organs. Flavonoids were detected in all plant parts, with stronger reactions in leaves and fruits. Saponins were present in all organs, showing higher intensity in leaves and fruits. Cardiac glycosides were also detected in all tested organs, with moderate to strong reactions. Anthranoids were not detected in any of the analyzed samples, while organic acids were only weakly detected in fruits. These findings are consistent with the semi-quantitative data presented in Table 1.

Table 1. *Qualitative phytochemical profile of different plant organs of Withania somnifera cultivated in Vietnam*

Compound class	Leaves	Stems	Roots	Fruits
Sterols	++	++	+++	++
Carotenoids	+++	++	–	+++
Volatile oils	+	+	+	+
Coumarins	+	+	+	+
Anthranoids	–	–	–	–
Flavonoids	+++	+	++	+++
Triterpenoids	+	+	+	++
Alkaloids	+	+	+++	++
Saponins	++	+	+	++
Tannins	+++	++	++	+++
Organic acids	–	–	–	+
Cardiac glycosides	++	+	++	++

(–) not detected; (+) present; (++) moderately positive; (+++) strongly positive.

4. DISCUSSION

The present study established a comprehensive pharmacognostic profile of *Withania somnifera* cultivated in Gia Lai Province, Vietnam, integrating macroscopic, anatomical, powder microscopic, and preliminary phytochemical data. The observed morpho-anatomical features, including sand-type calcium oxalate crystals, abundant starch granules, spiral and pitted vessel elements, branched multicellular non-glandular trichomes, and pigmented tissues, are consistent with those reported in standard pharmacognostic references and previous studies from India and Iran, thereby confirming the botanical authenticity of the investigated material. Notably, minor variations were observed, including increased trichome density and slightly reduced lamina dimensions. These differences may be attributed to environmental and

ecological factors such as altitude, temperature, and soil conditions in the Central Highlands of Vietnam. Such variations are consistent with the concept of phenotypic plasticity commonly reported in medicinal plants and highlight the importance of region-specific characterization for accurate identification and quality control. The phytochemical screening results demonstrated a broad distribution of secondary metabolites across plant organs, generally consistent with previous reports on *W. somnifera*. Triterpenoid/sterol-like constituents were detected in all organs, in agreement with the widespread occurrence of withanolides as key chemotaxonomic markers of the species. Alkaloids showed a marked predominance in roots, supporting their recognized role in the pharmacological activity of the plant. Carotenoids were most abundant in leaves, reflecting their association with photosynthetic tissues. The detection of flavonoids, saponins, tannins, and cardiac glycosides across multiple organs further supports the complex phytochemical composition of *W. somnifera*. However, as the present analysis was based on qualitative screening, the intensity of reactions should be interpreted as indicative rather than quantitative. In addition, variations in metabolite distribution compared with previous studies may reflect differences in extraction procedures, assay sensitivity, or environmental conditions affecting metabolite biosynthesis. Overall, the integration of morpho-anatomical and phytochemical characteristics provides a robust set of diagnostic criteria for authentication of *W. somnifera*. These findings contribute valuable region-specific data to the global pharmacognostic database and provide a scientific basis for future quantitative analyses, chromatographic profiling, and standardization of Vietnamese-cultivated material. These results may also support future chemotaxonomic and pharmacological investigations of regionally cultivated materials.

5. CONCLUSION

This study established a comprehensive pharmacognostic profile of *Withania somnifera* cultivated in Vietnam, encompassing macroscopic, anatomical, and powder microscopic characteristics. Key diagnostic markers, including sand-type calcium oxalate crystals and branched multicellular non-glandular trichomes, provide reliable criteria for identification and quality control of the plant material. Preliminary phytochemical screening demonstrated an organ-dependent distribution of secondary metabolites, with sterol/triterpenoid-like constituents widely distributed, alkaloids predominantly in roots, carotenoids abundant in leaves and fruits, and flavonoids, saponins, tannins, and cardiac glycosides distributed across multiple organs. These qualitative findings offer complementary chemical markers that support morpho-anatomical identification. Overall, the results contribute region-specific pharmacognostic data for Vietnamese-cultivated *W. somnifera* and provide a scientific basis for future quantitative analysis, chromatographic profiling, and standardization.

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