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Morpho-Anatomical and Preliminary Phytochemical Comparison of *Tribulus cistoides* and *T. Terrestris* (Zygophyllaceae) from South Central Vietnam

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Abstract

This paper offers a detailed examination of the morphological, micromorphological, and phytochemical profiles of two Tribulus species found in Vietnam: Tribulus cistoides L. and Tribulus terrestris L. The research seeks to outline and contrast the morphological and anatomical traits, together with the phytochemical content in the stems and leaves of these species, to explore their possible uses in pharmaceuticals. This was achieved through the double-staining technique and initial phytochemical screening via specific color reactions. Findings indicate that T. cistoides and T. terrestris share similarities in habitat, oblong-lanceolate leaflets, and ovate-lanceolate stipules, yet differ noticeably in flower and fruit morphology. Regarding micromorphology, both species exhibit comparable structures in the leaf midrib (single upper and lower epidermal layers, palisade mesophyll, vascular bundles, sclerenchyma, and parenchyma), leaflet blade (dorsiventral with single upper and lower epidermis, palisade and spongy mesophyll, plus vascular bundles featuring Kranz-type sheaths), and stem (single epidermal layer, parenchymatous cortex, pericyclic bundles, vascular bundles, and pith). Differentiation is possible through the number of xylem rows in leaflet vascular bundles, the spongy mesophyll layers in leaf blades, and characteristics of stem vascular bundles. Preliminary qualitative analysis revealed the presence of key compounds, including alkaloids, saponins, and flavonoids, in both species, while polyphenols, tannins, coumarins, quinones, terpenoids, amino acids, and carbohydrates were detected for the first time in T. cistoides. These results facilitate precise identification and standardization of medicinal resources, aid in conservation assessments, and lay the groundwork for subsequent pharmacological and biological investigations.

Keywords: Anatomy, Characterization, Plant taxonomy, Phytochemical, Zygophyllales

Introduction

The Zygophyllaceae family comprises trees, shrubs, and herbs with opposite or spirally arranged leaves, 5-merous flowers, and fruits typically in the form of capsules or schizocarps. It includes approximately 22 genera and 285 accepted species, primarily distributed in tropical and subtropical regions with arid or semi-arid climates [1, 2]. The genus Tribulus belongs to this family and encompasses 30 species [2]. Members of Tribulus are prostrate annual or perennial herbs possessing pinnately compound leaves with 3-10 pairs of sessile leaflets. The flowers are solitary and pentamerous, equipped with 5-10 stamens and a 5-locular ovary. Fruits are divided into 5 indehiscent mericarps, each housing 2-5 seeds arranged horizontally [3]. In Vietnam, the genus is represented by two species, T. cistoides L. and T. terrestris L., occurring in the central coastal zones and dry regions [4, 5]. Medicinal plants continue to play a significant role in traditional practices.

Citation. Quach TVE, Pham THN, Nguyen QB. Comparative Morpho-Anatomical Structure of the Leaflet and Stem and its Initial Chemical Analysis of the Tribulus species (Zygophyllaceae) from the South

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Central Coast of Vietnam. Trop J Nat Prod Res. 2025; 9(11): 5284–5293 https://doi.org/10.26538/tjnpr/v9i11.4
Quality control plays a critical role in ensuring accurate identification of herbal medicines. This involves detailed anatomical descriptions, microscopic detection of components in powdered samples, and qualitative phytochemical evaluation. Tribulus terrestris has been thoroughly investigated regarding its anatomy, taxonomy, and chemical constituents, which helps in correct identification and prevents adulteration [6-9]. Known commonly as "plant Viagra," T. terrestris is one of the most researched aphrodisiac herbs, with its benefits mainly linked to furostanol saponins. Protodioscin, its primary active compound, is noted for boosting testosterone levels in men, and treatments with this plant have resulted in increased testosterone among women suffering from hypoactive sexual desire disorder (HSDD) [10, 11]. By contrast, T. cistoides has received limited attention in terms of taxonomy, anatomy, and therapeutic potential [12]. In Vietnam, the two species often grow together and display closely resembling external features, complicating field distinction. Accordingly, the present work focuses on describing and comparing the morphological and anatomical properties of T. terrestris and T. cistoides, combined with an initial phytochemical evaluation, to improve identification accuracy and clarify taxonomic relationships within the Tribulus genus in Vietnam.

Materials and Methods

Collection and identification of plant materials

Leaves and stems of Tribulus terrestris and T. cistoides were gathered from Phan Rang–Thap Cham city, Ninh Thuan Province (currently Ninh Chu Ward, Khanh Hoa Province), located in the South Central Coast of Vietnam, during February 2024. Voucher specimens (Codes: V.T.E. Quach TE01 and V.T.E. Quach TE02) were deposited at the Faculty of Biology, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.

Macro-morphology

Specimens were obtained from Ninh Thuan Province and preserved in 70% ethanol. Photographs of the species were taken using a Canon EF–S 60 mm f/2.8 Macro USM lens. Morphological descriptions adhere to the terminology outlined by Beentje (2012) [13]. Species names were confirmed by cross-referencing with relevant morphological references and digital images of herbarium specimens available through online databases (GBIF, BM, JSTOR) [3, 4, 14-17].

Micro-morphology

Anatomical examinations employed the Carmine-Methylene Blue double-staining procedure as detailed by Tran (1981) [18], conducted at the Faculty of Biology, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam. Various plant parts (petiole, leaflets, young stems, and mature stems) were sectioned appropriately and sliced thinly by hand with a razor blade. These cross-sections were bleached in 8% sodium hypochlorite for 15 minutes to remove cell contents, then neutralized in 1% acetic acid for 2 minutes. Staining involved immersion in 10% carmine for 15 minutes followed by 1% methylene blue for 2 minutes, with rinsing in distilled water after each stage to eliminate excess reagents. Prepared slides were examined and imaged using a Nikon Eclipse E100 optical microscope (Japan) at magnifications of 4×, 10×, and 40×.

Preliminary phytochemical screening

The analysis was performed at the Faculty of Biology, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam. Fresh leaves and stems were cleaned, dried at 50-65°C to constant weight, and pulverized. Dried powder was extracted with methanol via the Soxhlet apparatus. The crude methanolic extract was sequentially partitioned with solvents of rising polarity: n-hexane, ethyl acetate, and ethanol. Specifically, 5 grams of powder were extracted with methanol for 4 hours in a Soxhlet extractor, then concentrated to dryness. The residue was resuspended in 20 mL of distilled water and subjected to liquid-liquid partitioning: first with 20 mL of n-hexane, then with 20 mL of ethyl acetate from the remaining aqueous phase, and finally, the aqueous residue was taken up in 20 mL of ethanol. Qualitative phytochemical tests were carried out using standard color reactions according to methods described by Nguyen (2007), Nguyen (2020), and the Vietnamese Pharmacopoeia V (Ministry of Health, 2017), as summarized in **Table 1** [19-21].

Results and Discussions

Taxonomic treatment

Tribulus cistoides L., Sp. Pl. 1: 387. 1753 **(Figure 1).**
Tribulus terrestris var. cistoides (L.) Oliv. in Fl. Trop. Afr. 1: 284. 1868.

Type: Habitat in America calidiore, Hermann, Paradisus Batavus: 236, pl. 236. 1698 (lectotype, designated by Wijnands, D.O., Bot. Commelins: 203. 1983). = Tribulus taiwanense T.C.Huang & T.H.Hsieh in Taiwania 39: 63. 1994. (other synonyms can be found in POWO (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:873411-1)).

Description. Multi-year herbaceous plants growing flat or slightly upright. Stems reach 0.3–1.5 m, covered thickly with hairs when new, later sparsely hairy or smooth. Stipules shaped ovate-lanceolate, measuring 4–8 mm, tip pointed or tapering, hairy to finely hairy on both faces, edges fringed with hairs. Leaves measure 5–11 cm, having 5–10 pairs of leaflets, stalk thickly finely hairy; leaflets shaped oblong to oblong-lanceolate, sized 6–16 × 3–6 mm, top face finely hairy to smooth, bottom face long-hairy to smooth, edges lightly fringed or smooth, tip pointed to blunt, base slanted, rounded to heart-shaped. Blooms single in leaf axils, diameter 2–4 cm, five-parted, on thin stalks 2–4 cm long, finely hairy. Sepals five, narrow lanceolate to lanceolate, green, 6–12 mm, outside finely hairy, inside smooth, edges lightly fringed or entire. Petals five, reverse-ovate, fragile, yellow, 11–18 mm, tip blunt. Stamens ten in two circles; threads thin, smooth, 3–5 mm; anthers ovate-oblong, 1.2–1.5 mm, tip blunt, attached at the back. Ovary with ten developing spines centrally, five chambers, roughly 2 mm, thickly long-hairy; style 1–1.5 mm, smooth, stigma divided into five parts. Fruit a splitting carp with a bumpy surface, 15–22 mm across (with spines), supporting stalk 2–5.5 cm; each segment half-round, 9–11 mm, two side spines 2–4 mm (long-hairy to smooth), two bottom spines about 1 mm.

Phenology. Produces flowers and fruits nearly year-round.

Distribution and habitat. Found in dry coastal zones across Africa, Australia, Pacific islands, Oceania, tropical America, and Asia [2]. Within Vietnam, present in Khanh Hoa and Lam Dong provinces.

Vernacular name (Vietnamese name). Gai ma vương to, Quỉ kiến sầu to.

Specimens examined: Vietnam, Phan Rang–Thap Cham city, Ninh Thuan Province (now Ninh Chu Ward, Khanh Hoa Province), beside railway tracks, elevation 0-10 m, 11°36'10"N 108°56'51"E, 12 February 2024, V.T.E. Quach TE01 (stored at the Faculty of Biology, Ho Chi Minh University of Education).

Tribulus terrestris L., Sp. Pl. 1: 387. 1753 **(Figure 2).**

Type: Europe; Netherlands. Herb. Clifford 160 (Tribulus 1) (lectotype: BM000558734 [image!], designated by Burtt B. L., Kew Bull. 9: 398. 1954). = Tribulus lanuginosus L. in Sp. Pl.: 387. 1753. (other synonyms can be found in POWO (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77226367-1)).

Description. Single-year herbaceous plants growing flat or slightly upright. Stems 0.3–0.8 m, thickly hairy when new, later sparsely hairy or smooth. Stipules ovate-lanceolate, 3–5 mm, tip pointed or tapering, thickly finely hairy to long-hairy on both faces, edges with thick, long fringes. Leaves 7–10 cm with 5–7 pairs of leaflets, stalk thickly finely hairy; leaflets oblong to oblong-lanceolate, 4–9 × 3–5 mm, top face finely hairy to smooth, bottom face long-hairy to smooth, edges lightly fringed or entire, tip pointed to blunt, base slanted. Blooms single in leaf axils, diameter 1.1–2 cm, five-parted, on thin stalks 1–1.5 cm long, thickly finely hairy. Sepals five, narrow lanceolate to linear-lanceolate, green, 4–8 mm, outside finely hairy to long-hairy, inside smooth, edges lightly fringed or entire. Petals five, reverse-ovate, fragile, yellow, 8–11 mm, tip blunt. Stamens ten in two circles; threads thin, smooth, 2–3 mm; anthers ovate-oblong, 1.2–2 mm, tip blunt, attached at the back. Ovary five chambers, 1.5–1.8 mm, thickly long-hairy; style roughly 1 mm, smooth, stigma divided into five parts. Fruit a splitting carp with a bumpy surface, 15–20 mm across (with spines), supporting stalk 1–1.7 cm; segments half-round, narrowing to the tip, about 7 mm, two side spines 5–7 mm (long-hairy to smooth), two bottom spines about 2 mm.

Phenology. Produces flowers and fruits between February and June.

Distribution and habitat. Occurs widely in tropical and subtropical areas around the world [2]. In Vietnam, it is noted in the arid coastal regions of Khanh Hoa and Lam Dong provinces.

Vernacular name (Vietnamese name). Gai ma vương nhỏ, Quỉ kiến sầu nhỏ.

Specimens examined: Vietnam, Phan Rang–Thap Cham city, Ninh Thuan Province (now Ninh Chu Ward, Khanh Hoa Province), beside railway tracks, elevation 0-10 m, 11°36'10"N 108°56'51"E, 12 February 2024, V.T.E. Quach TE02 (stored at the Faculty of Biology, Ho Chi Minh University of Education).

From external features, Tribulus terrestris shares with T. cistoides the slanted oblong leaflets and triangular stipules, yet stands apart with reduced stalk lengths for

flowers (1–1.5 cm against 2–4 cm), reduced bloom sizes (1.1–2 cm against 2–4 cm), reduced fruit supporting stalks (1–1.7 cm against 2–5.5 cm), and extended side spines on fruits (5–7 mm against 2–4 mm). An in-depth external feature contrast for both is provided in **Table 2**.

Anatomical characteristics

Midrib leaflet anatomy.

Cross-sections of the leaflet central vein display overall resemblance across the pair of species **(Figures 3a and 3b)**. The central vein appears extended close to the leaflet base for Tribulus cistoides **(Figure 3b1)** relative to T. terrestris **(Figure 3a1)**. Hairs appear on the upper and lower faces. For each, the upper and lower epidermal layers feature one row of closely fitted cells varying in size, protected by a dense cuticle. Palisade tissue includes one row of elongated, uniform cells in T. terrestris and cells varying in dimensions in T. cistoides. Central conducting tissue features xylem positioned toward the upper side, formed by thickened cells containing wide conduits aligned in radial series (3–5 rows in T. terrestris and 7–9 rows in T. cistoides), while phloem lies toward the lower side, built from randomly placed cells having expanded gaps between them (3–5 layers in T. terrestris, and 5–7 layers in T. cistoides). Supporting tissue contains heavily thickened cells differing in dimensions, placed irregularly without gaps (2 layers in T. terrestris and 4 layers in T. cistoides). Encircling the conducting tissue is a covering layer made of big cells with cellulosic boundaries. Basic ground tissue includes spacious, delicate-walled cells differing in dimensions, placed with gaps (forming 2 layers in T. terrestris and 3–4 layers in T. cistoides).

Table 1. Preliminary phytochemical tests for plant extracts used in this study

Phytoconstituent	Test Method	Observation
Polyphenols	$FeCl_3$	Formation of dark green precipitate
	$Pb(CH_3COO)_2$	Formation of white precipitate
Tannins	Gelatin + NaCl	Cloudy white precipitate observed
Flavonoids	Mg + HCl	Solution changes to red color
Coumarins	Lactone ring reaction	White precipitate appears after lactone ring reaction
Alkaloids	Wagner's test (acidification after alkaline treatment)	Reddish-brown precipitate formed
Quinones	Mayer's test + HCl (Cs)	White precipitate formed; solution turns green
Saponins	Foam test	Stable foam lasting 15 minutes
Terpenoids	Chloroform + H_2SO_4 (Cs)	Solution changes to brick-red/green color
Amino acids	Na_2CO_3 5%	Stable foam formed

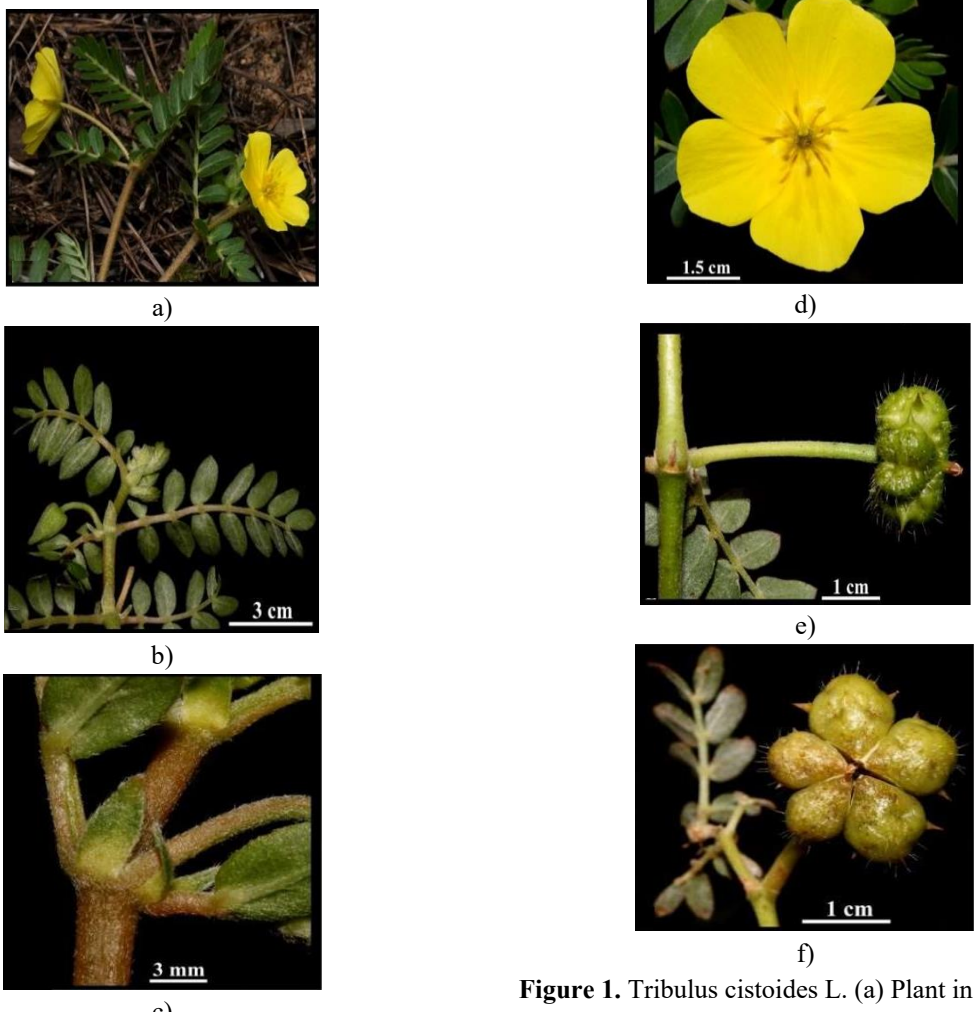
Table 2. External feature contrasts between Tribulus cistoides and T. terrestris

Character	T. terrestris	T. cistoides
Leaflets	5–7 pairs	5–10 pairs
Flower diameter	1.1–2 cm	2–4 cm
Pedicel length	1–1.5 cm	2–4 cm
Calyx lobe length	4–8 mm	6–12 mm
Corolla lobe length	8–11 mm	11–18 mm
Style length	approximately 1 mm	1–1.5 mm
Fruit size (including spines)	approximately 7 mm long, 15–20 mm in diameter	9–11 mm long, 15–22 mm in diameter
Mericarp shape	Semi-circular, tapering toward the apex	Semi-circular
Stalk length	1–1.7 cm	2–5.5 cm
Spine length	5–7 mm	2–4 mm

Table 3. Chemical constituent detection in n-hexane, ethyl acetate, and ethanol fractions from Tribulus cistoides and T. terrestris

Chemical Constituent	Test	n-Hexane	T. terrestris Ethyl Acetate	T. terrestris Ethanol	n-Hexane	T. cistoides Ethyl Acetate	T. cistoides Ethanol
Polyphenols	$FeCl_3$	–	+	+	–	+	+
	$Pb(CH_3COO)_2$	–	–	+	–	–	+
Tannins	Gelatin + NaCl	–	+	+	–	–	+
Flavonoids	Mg + HCl	–	+	+	–	+	+
Coumarins	Lactone ring reaction	–	–	+	–	+	+
Alkaloids	Wagner	+	+	–	+	+	–
	Mayer	+	+	–	+	+	–
Quinones	HCl (Cs)	–	–	+	+	–	+
Saponins	Foam test	–	+	–	–	+	–
Terpenoids	Chloroform + H_2SO_4 (Cs)	+	+	–	+	+	–
Amino acids	Na_2CO_3 5%	–	+	+	–	+	+

Note: Cs: Concentrated solution, (+) Detected, and (–) Not detected.



a) b) c) d) e) f)

Figure 1. Tribulus cistoides L. (a) Plant in bloom, (b) Leaf showing lower leaflet faces, (c) Pair of stipules, (d) Detailed frontal flower view, (e, f) Fruit from various angles. V.T.E. Quach TE01.

a)

b)

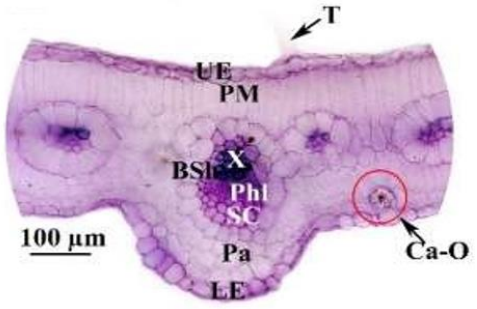
c)

d)

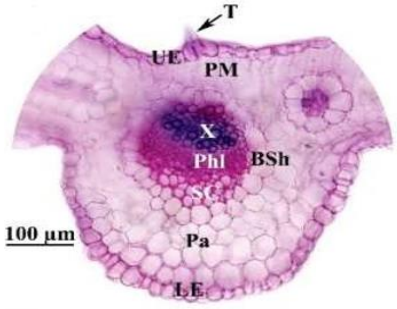
e)

f)

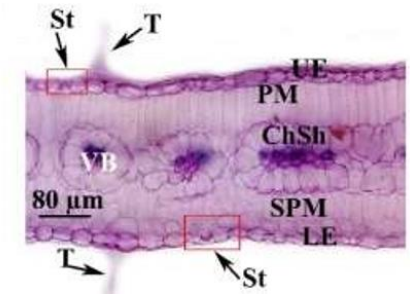
Figure 2. Tribulus terrestris L. (a) Plant in bloom, (b) Leaf showing lower leaflet faces, (c) Pair of stipules, (d) Detailed frontal flower view, (e, f) Fruit from various angles. V.T.E. Quach TE02.



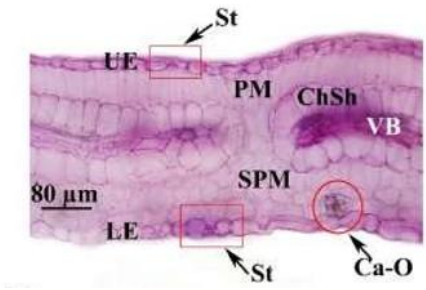
a1)



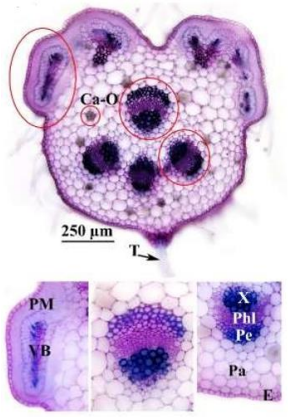
b1)



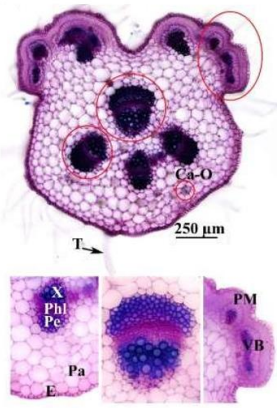
a2)



b2)

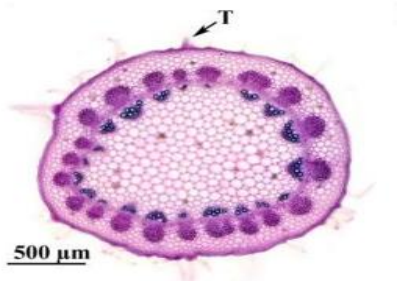


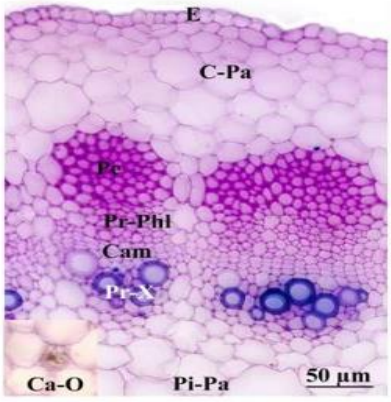
a3)



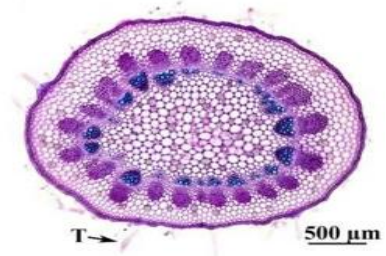
b3)

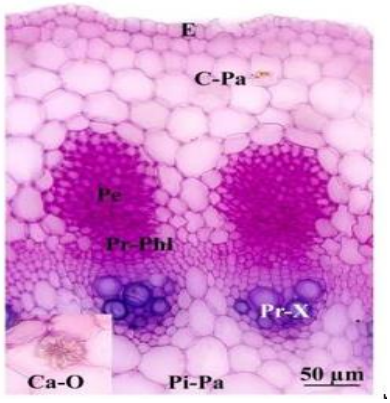
Figure 3. Transverse sections through leaves revealing microscopic details for T. terrestris (a) and T. cistoides (b). (1) Central vein of leaflet, (2) Leaflet lamina, (3) Leaf stalk. (BSh) Bundle sheath, (Ca-O) Calcium oxalate crystal, (LE) Lower epidermis, (Pa) Parenchyma, (Pe) Pericycle, (Phl) Phloem, (PM) Palisade mesophyll, (SC) Sclerenchyma, (St) Stomata, (T) Trichomes, (UE) Upper epidermis, (VB) Vascular bundle, (X) Xylem.





a)





b)

Figure 4. Transverse sections through immature stems showing microscopic details for T. terrestris (a) and T. cistoides (b). (Cam) Cambium regions, (Ca-O) Calcium oxalate crystal, (C-Pa) Cortex parenchyma, (E) Epidermis, (Pe) Pericycle, (Pi-Pa) Pith

parenchyma, (Pr-Phl) Primary phloem, (Pr-X) Primary xylem, (T) Trichomes.

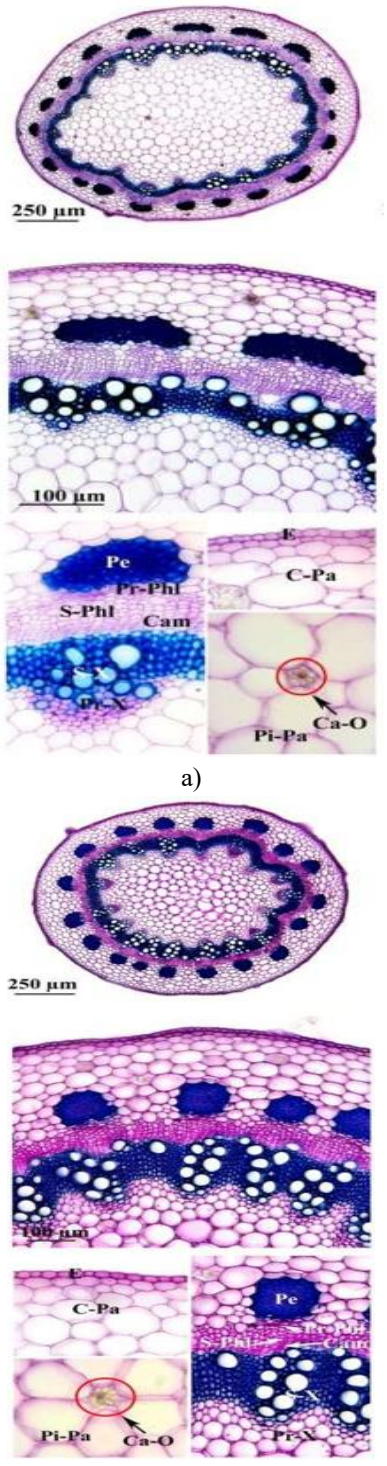


Figure 5. Transverse sections through fully developed stems showing microscopic details for T. terrestris (a) and T. cistoides (b). (Cam) Cambium regions, (Ca-O) Calcium oxalate crystal, (C-Pa) Cortex parenchyma, (E) Epidermis, (Pe) Pericycle, (Pi-Pa) Pith parenchyma, (Pr-Phl) Primary phloem, (Pr-X) Primary xylem, (S-Phl) Secondary phloem, (S-X) Secondary xylem.

Leaflet blade anatomy.

The lamina displays a bifacial organization **(Figures 3a2 and 3b2)**. Both upper and lower covering layers contain one row of tightly fitted cells differing in dimensions, lacking gaps. Simple hairs appear on each face, more numerous on the lower one. Covering cells bear a heavy outer coating. Columnar tissue forms one row of stretched rectangular units, showing minor wavy boundaries, oriented at right angles. This tissue appears thicker in Tribulus terrestris compared to T. cistoides. Loose tissue consists of multiple rows of ground cells varying in dimensions with cellulosic boundaries and spaced apart, creating air pockets (2–4 layers in T. terrestris and 2–3 in T. cistoides). Special photosynthetic covering surrounds the conducting tissues. Breathing pores occur on each face in the two plants. Furthermore, calcium oxalate formations are dispersed inside the ground tissue of the two plants.

Petiole anatomy

Cross-sections of the leaf stalk in the two plants reveal one layer of many-sided covering cells, fitted closely with no gaps between them **(Figures 3a3 and 3b3).** Simple hairs spread evenly across the stalk exterior. Basic ground tissue builds from spacious, delicate-walled units with cellulosic boundaries, positioned tightly yet allowing minor air pockets. Conducting tissue splits into four separate units: one major central unit plus three minor outer units. Every unit places thickened xylem cells with wide conduits in radial series at the core, while surrounding phloem forms from multiple rows of randomly placed, crinkled-walled units. Directly over the phloem sits a supporting ring of thickened cells varying in dimensions. Tribulus terrestris shows reduced counts of xylem conduits, phloem rows, and supporting ring units in the stalk relative to T. cistoides. Moreover, calcium oxalate formations disperse across the ground tissue in the stalk of the two plants.

Stem anatomy.

Young stem sections appear nearly round in outline **(Figures 4a1 & 4b1)**. Outer covering forms one row of snugly arranged units differing in dimensions. Simple

hairs are mostly single-celled, sometimes multi-celled. Outer ground tissue includes 3–5 rows of randomly sized units with tiny air pockets. Supporting ring appears as broken strands positioned over conducting units, built from several rows of irregularly placed units having heavy cellulosic boundaries. Conducting system contains initial xylem and phloem, with lower counts in Tribulus terrestris (22–24 stem conducting units) compared to T. cistoides (25–27 stem conducting units). Xylem lies inward, initial xylem showing fairly even conduits. Phloem sits outward, initial phloem units placed randomly. Growth zone forms 2–3 rows of elongated, even-sized units with cellulosic boundaries in T. terrestris, versus 4–6 rows in T. cistoides. Central ground tissue builds from spacious, delicate-walled units.

Mature stem sections closely match young ones **(Figures 5a and 5b)**. Additional xylem and phloem develop from growth zone activity. Additional xylem includes multiple radial series of thickened units, with Tribulus terrestris displaying 5–7 series and T. cistoides showing 3–5 series. Xylem conduits are spacious and grouped small, while initial xylem shifts inward. Xylem ground tissue contains many tiny units with heavy cellulosic boundaries (2–3 rows in T. terrestris and 4–5 rows in T. cistoides). Additional phloem builds from tiny, random units with cellulosic boundaries. During growth, it shifts initial phloem outward. Supporting ring units in mature stems become thickened and heavy-walled. Furthermore, calcium oxalate formations spread through outer and central ground tissue in both young and mature stems.

Present work delivers thorough external and internal feature details, plus initial chemical constituent checks, for the pair of Tribulus plants, Tribulus terrestris and T. cistoides, occurring in Vietnam. This carries special weight since T. cistoides lacked prior detailed examination in Vietnam, even though the pair often grow together and share closely matching external traits, complicating on-site recognition. Current outcomes fill this void by highlighting reliable separating traits at visible and tiny scales.

These internal differences support the construction of a recognition guide, offering a vital aid for separating the pair, particularly where visible traits prove unclear. Widespread calcium oxalate formations inside ground tissue of leaves, stalks, and stems represent a shared trait across the genus, possibly aiding defense or mineral balance [22, 23].

Identification guide using leaflet central vein and stem features for Tribulus plants in Vietnam

1a. Central vein conducting unit xylem 7–9 radial series, 4 supporting tissue rows; 2–4 loose tissue in leaflet lamina; 25–27
stem conducting units, 4–6 growth zone rows, 3–5 xylem radial series

1b. Central vein conducting unit xylem 3–5 radial series, 2 supporting tissue rows; 2–3 loose tissue in leaflet lamina; 22–24
stem conducting units, 2–3 growth zone rows, 5–7 xylem radial series

Initial chemical constituent review of tribulus terrestris and tribulus cistoides material

Chemical detection in n-hexane, ethyl acetate, and ethanol fractions from Tribulus terrestris and T. cistoides appears in **Table 2**. N-hexane fractions from the pair indicated alkaloids, quinones, and terpenoids, whereas ethyl acetate fractions contained flavonoids, coumarins (T. cistoides), alkaloids, quinones, terpenoids, and amino acids. Polyphenols, tannins, coumarins, saponins, and amino acids appeared in the ethanol fractions of the pair **(Table 3).**

Current initial checks verified typical constituents like alkaloids, saponins, and flavonoids across T. terrestris and T. cistoides. This matches earlier chemical reports on T. terrestris [24-26]. Importantly, the present work first documents phenolics, tannins, coumarins, quinones, terpenoids, amino acids, and carbohydrates within T. cistoides. This fresh discovery opens major paths for upcoming studies on the biological effects and medicinal value of T. cistoides [12]. Detection of these groups hints that T. cistoides could offer antioxidant action (phenolics, flavonoids), inflammation-reducing effects (coumarins), or further benefits tied to terpenoids and alkaloids. This proves highly useful for folk healing practices and the creation of plant-derived medicines. Fraction checks across varied solvents (n-hexane, ethyl acetate, ethanol) further give early clues on compound polarity and recovery ease.

Conclusion

Overall, external and internal traits of the pair of Tribulus plants plus initial chemical review of T. cistoides appear here for the first time. Externally, the pair appears highly alike, yet separate via xylem series counts in leaflet conducting units, loose tissue in leaflet lamina, and stem conducting units. Initial detection revealed key constituents like alkaloids, saponins, flavonoids in the

pair, notably with polyphenols, tannins, coumarins, quinones, terpenoids, and amino acids also noted in T. cistoides.

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