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*Original Research Article*

# Comparative Morpho-Anatomical Structure of the Leaflet and Stem and its Initial Chemical Analysis of the *Tribulus* Species (Zygophyllaceae) from the South Central Coast of Vietnam

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ABSTRACT

This article provides a comprehensive account of the morphology, micromorphology, and phytochemical composition of two *Tribulus* species in Vietnam, *Tribulus cistoides* L. and *Tribulus terrestris* L.. The study aims to describe and compare the morphological and anatomical characteristics, as well as phytochemical composition in the stems and leaves of the two species for potential applications in the pharmaceutical field, using the double-staining method and preliminary phytochemical screening based on characteristic reactions. The results show that *T. cistoides* and *T. terrestris* are similar in habitat, the oblong-lanceolate leaflets, the ovate-lanceolate stipules, but are distiguished by the flowers and fruits shape. Micromorphology, of both species is similar in the structrure of the midrib of the leaf (one upper and lower epidermis layer, palisade mesophyll, vascular bundles, sclerenchyma, parenchyma), the leaflet blade (dorsoventral with one upper, lower epidermis, palisade and spongy mesophyll, and vascular bundles with Kranz- type sheaths), and stem (one epidermis layer parenchymatous cortex, pericylic bundles, vascular bundles, and pith). It can be identified based on the xylem row count in leaflet vascular bundles, the spongy mesophyll of the leaf blades, and stem vascular bundle features. In the preliminary qualitative screening, both species were found to contain important compounds such as alkaloids, saponins, and flavonoids, with polyphenols, tannins, coumarins, quinones, terpenoids, amino acids, and carbohydrates being reported for the first time in *T. cistoides*. These findings support accurate identification and standardization of medicinal materials, conservation evaluation, and provide a foundation for future pharmacological and biological studies.

***Keywords*:** Anatomy, Characterization, Plant Taxonomy, Phytochemical, Zygophyllales.

## Introduction

Zygophyllaceae is a family of trees, shurbs and herbs, opposite or spirally arranged leaves, the flowers 5-merous and fruits are commonly capsules or schizocarps, estimated about 22 genera and 285 accepted species distributing to tropical and subtropical areas characterized by arid or semi-arid conditions.[1,2] The *Tribulus* genus is belonging to Zygophyllaceae family, including 30 species.[2] Species in the *Tribulus* genus are prostrte annual or perennial herbs with pinnately compound leaves bearing 3-10 pairs of sessile leaflets. Flowers are solitary, pentamerous, with 5-10 stamens and a 5-locular ovary. Fruits splits into 5 indehiscent mericarps, each containing 2-5 horizontally arranged seeds.[3] In Vietnam, the *Tribulus* genus has two species, *T. cistoides* L. and *T. terrestris* L., distributed in the central coastal region, arid areas.[4,5] Currently, medicinal plants are widely used in folk practice.

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Quality control is an essential factor to ensure the correct identification of herbal drugs, in which the description of anatomical structures, the detection of individual components in powdered drugs by microscopy, as well as the qualitative analysis of phytochemicals, are widely applied. *Tribulus terrestris* has been extensively studied in terms of anatomy, taxonomy, and phytochemistry, support proper identification and reduce substitution of genuine medicinal materials[6-9] *Tribulus terrestris* (Puncturevine), commonly known as the "plant Viagra", is among the most extensively studied aphrodisiac plants, with its therapeutic effects largely attributed to furostanol saponins. Protodioscin, the principal phytochemical in *T. terrestris*, has been reported to stimulate testosterone production in men, while women with hypoactive sexual desire disorder (HSSD) treated with this species showed elevated testosterone levels.[10,11] In contract, *T. cistoides* remains poorly studied, especially taxonomy, anatomy, and medicinal uses.[12] In Vietnam, both species frequently co-occur and exhibit highly similar morphological traits, making field identification challenging. Therefore, this study aims to characterize and compare the morphological and anatomical features of *T. terrestris* and *T. cistoides*, alongside a preliminary phytochemical analysis of these two species, to support accurate identification and taxonomic resolution within the genus *Tribulus* in Vietnam.

## Materials and Methods

*Collection and Identification of plant materials.*
The leaves and stems of *Tribulus terrestris* and *T. cistoides* were collected from Phan Rang – Thap Cham city, Ninh Thuan Province

(Now is Ninh Chu Ward, Khanh Hoa Province) the South Central Coast Vietnam in February 2024. A voucher specimen (Code: *V.T.E. Quach TE01* & *V.T.E. Quach TE02* was deposired at the Faculty of Biology, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.

*Macro-morphology*

The studied materials were collected from Ninh Thuan provice, and preserved in Ethanol 70°. Using Canon EF–S 60 mm f/2.8 Marco USM lens captured all photograghs of the species. Morphological terminology follows Beentje (2012).[13] The scientific name of the species was determined by comparing standard relevant morphological literatures and from digital images of specimens accessed through digitalized herbarium (GBIF, BM, JSTOR).[3,4,14 - 17]

*Micro-morphology*

Anatomical analysis was performed using the Carmine-Methylene Blue double staining method described by Tran (1981) and was carried out at the Faculty of Biology, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam.[18] The different parts (petiole, leaflets, young stems, and mature stems) of the studied samples were cut into appropriate sections or pieces and then cut into thin slices using a razor blade. These thin cross-section samples were soaked in Sodium hypochlorite (8%) for 15 minutes to clear cellular contents, then neutralized with Acetic Acid (1%) for 2 minutes. The cleared specimens were subsequently stained with Carmine (10%) for 15 minutes, followed by Methylene Blue (1%) for 2 minutes. After each step, the excess chemicals were removed with distilled water. The slices were observed and photographed under an optical microscope Nikon Eclipse E100 (Japan) at 4x, 10x, 40x magnifications.

*Preliminary phytochemical screening*

The study was conducted at the Faculty of Biology, Ho Chi Minh University of Education, Ho Chi Minh City, Vietnam. Leaves and stems were collected, washed, and dried at 50-65°C until they reached a constant mass, then ground into the powder. The dried and powdered plant material was extracted with methanol in a Soxhlet apparatus. The crude extract was fractionated using solvents of increasing polarity, including *n*-hexane, ethyl acetate, and ethanol. Five grams of powdered plant material were extracted with methanol using a Soxhlet apparatus for 4 hours. The extract was then concentrated to dryness. The resulting residue was dissolved in 20 mL of distilled water and transferred to a separatory funnel. Subsequently, 20 mL of *n*-hexane was added, shaken thoroughly, and the *n*-hexane fraction was collected. The remaining aqueous layer was then extracted with 20 mL of ethyl acetate (EA); the ethyl acetate fraction was collected. The final residual aqueous extract was subsequently dissolved in 20 mL of ethanol. Preliminary quantitative phytochemical screening was performed using characteristic chemical reactions, following the method of Nguyen (2007), Nguyen (2020), and Vietnamese Pharmacopoeia V (Ministry of Health, 2017), showing 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*. Perennial herbs, prostrate to ascending. Branches 0.3–1.5 m long, dense pubescent when young, pubescent to glabrous when old. Stipules ovate-lanceolate, 4–8 mm long, apex acute to acuminate, pubescent to puberulent on both sides, margin ciliate. Leaves 5–11 cm long with 5–10 pairs leaflets, petiole dense puberulent; leaflets oblong to oblong-lanceolate, 6–16 x 3–6 mm, adaxial leaflets surface puberulent to glabrous, adaxial leaflets surface villous to glabrous, margin ciliolate or entire, apex acute to rounded, base oblique round–cordate. Flower solitary, axillary, 2–4 cm in diam., 5-merous, pedicel slender, 2–4 cm long, puberulent. Calyx 5 lobes, lobes narrowly lanceolate to lanceolate, green, 6–12 mm long, outer surface puberulent, inner surface glabrous, margin ciliolate or entire. Corolla 5 lobes, lobes obovate, thin, yellow, 11–18 mm long, apex rounded. Stamens 10 in 2 whorls; filaments 3–5 mm long, slender, glabrous; anthers ovate-oblong, 1.2–1.5 mm long, apex rounded, dorsifixed. Ovary with 10 young spines in the middle, 5-locular, ca. 2 mm long, dense villous; style 1–1.5 mm long, glabrous, stigma 5-lobed. Fruit schizopcarp, murications, 15–22 mm in diam. (including spines), stalk 2–5.5 cm long; mericarp semi-circular, 9–11 mm long, lateral spines 2, 2–4 mm long, villous to glabrous, basal spines 2, ca. 1 mm long.

Phenology**.** Flowering and fruiting most of the year.

Distribution and habitat**.** The species occurs in the central coastal region, arid areas of Africa, Australia, Pacific islands, Oceania, Tropical America, and Asia.[2] In Vietnam, it is distributed in Khanh Hoa, 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 is Ninh Chu Ward, Khanh Hoa Province), along the railway track, alt 0-10m, 11°36'10"N 108°56'51"E, 12 February 2024, *V.T.E. Quach TE01* (kept at the Faculty of Biology, Ho Chi Minh University of Education).

***Tribulus terrestris* L.,** Sp. PI. 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*. Annual herbs, prostrate to ascending. Branches 0.3–0.8 m long, dense pubescent when young, pubescent to glabrous when old. Stipules ovate-lanceolate, 3–5 mm long, apex acute to acuminate, dense puberulent to villous on both side, margin dense long ciliate. Leaves 7–10 cm long with 5–7 pairs leaflets, petiole dense puberulent; leaflets oblong to oblong–lanceolate, 4–9 x 3–5 mm, adaxial leaflets surface puberulent to glabrous, adaxial leaflets surface villous to glabrous, margin ciliolate or entire, apex acute to rounded, base oblique. Flower solitary, axillary, 1.1–2 cm in diam., 5-merous, pedicel slender, 1–1.5 cm long, dense puberulent. Calyx 5 lobes, lobes narrowly lanceolate to linear lanceolate, green, 4–8 mm long, outer surface puberulent to villous, inner surface glabrous, margin ciliolate or entire. Corolla 5 lobes, lobes obovate, thin, yellow, 8–11 mm long, apex rounded. Stamens 10 in 2 whorls; filaments 2–3 mm long, slender, glabrous; anthers ovate-oblong, 1.2–2 mm long, apex rounded, dorsifixed. Ovary 5-locular, 1.5–1.8 mm long, dense villous; style ca. 1 mm long, glabrous, stigma 5-lobed. Fruit schizopcarp, murications, 15–20 mm in diam. (including spines), stalk 1–1.7 cm long; mericarp semi-circular with contracted toward the apex; ca. 7 mm long, lateral spines 2, 5–7 mm long, villous to glabrous, basal spines 2, ca. 2 mm long.

Phenology**.** Flowering and fruiting from February to June.

Distribution and habitat**.** The species is distributed across tropical and subtropical regions worldwide.[2] In Vietnam, it has been recorded in dry zones along the central coast in Khanh Hoa, 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 is Ninh Chu Ward, Khanh Hoa Province), along the railway track, alt 0-10m, 11°36'10"N 108°56'51"E, 12 February 2024, *V.T.E. Quach TE02* (kept at the Faculty of Biology, Ho Chi Minh University of Education).

Based on morphological analysis, *Tribulus terrestris* is similar to *T. cistoides* in leaflet obliquely oblong, stipules triangular, but differs in having shorter pedicels (1–1.5 cm vs. 2–4 cm long), smaller flowers (1.1–2 cm vs. 2–4 cm in diam.), shorter fruit stalks (1–1.7 cm vs. 2–5.5 cm long), and longer fruit spines (5–7 mm vs. 2–4 mm). A detailed morphological comparion between the two species is shown in Table 2.

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*Anatomical Characteristics*
*Midrib leaflet-anatomy.*
The anatomical structure of the leaflet midrib is generally similar between the two species (Figure 3A & 3B). The midrib length near the base of the leaflet is greater in *Tribulus cistoides* (Fig. 3B1) than in *T. terrestris* (Figure 3A1). Trichomes are present on both adaxial and abaxial surfaces. The upper and lower epidermis in both species consist of a single layer of compactly arranged cells of unequal size, covered by a thick cuticle. The palisade mesophyll comprises a single layer of rectangular cells with uniform size in *T. terrestris* and

**Table 1:** Preliminary phytochemical tests for plant extracts using in this study

| Phytoconstiuents | Test | Observation |
|---|---|---|
| Polyphenol | $FeCl_3$ | Dark green precipitate |
| | $Pb(CH_3COO)_2$ | White precipitate |
| Tannins | Gelatin NaCl | Cloudy white precipitate |
| Flavonoids | Mg/HCl | Solution turns red color |
| Courmarins | lacton ring reaction | A white precipitate appears after the lactone ring of coumarins is reopened by acidification following alkaline treatment. |
| Alkaloids | Wagner | Reddish-brown precipitate |
| | Mayer | White precipitate |
| Quinones | HCl (Cs) | Solution turns green color |
| Saponins | Foam test | Stable foam for 15 minutes |
| Terpenoids | Chloroform + $H_2SO_4$ (Cs) | Solution turns brick-red/green color |
| Amino acids | $Na_2CO_3$ 5% | Stable foam |

**Table 2:** Morphological comparisons between *Tribulus cistoides* and *T. terrestris*

| Character | *T. cistoides* | *T. terrestris* |
|---|---|---|
| Leaflets | 5–10 pairs | 5–7 pairs |
| Flower | 2–4 cm in diam. | 1.1–2 cm in diam. |
| Pedicel | 2–4 cm long | 1–1.5 cm long |
| Calyx lobes | 6–12 mm long | 4–8 mm long |
| Corolla lobes | 11–18 mm long | 8–11 mm long |
| Styles | 1–1.5 mm long | ca. 1 mm long |
| Fruit | 9–11 mm long, 15–22 mm diam. (including spines) | ca. 7 mm long, 15–20 mm in diam. (including spines) |
| Mericarp | semi-circular | semi-circular with contracted toward the apex. |
| Stalk | 2–5.5 cm long | 1–1.7 cm long |
| Spines | 2–4 mm long | 5–7 mm long |

**Table 3:** Phytochemical sreening of n-hexane, Ethyl aceatate and Ethanol extracts of *Tribulus cistoides* and *T. terrestris*

| Chemical constituents | Test | Extracts | | | | | |
|---|---|---|---|---|---|---|---|
| | | *T. cistoides* | | | *T. terrestris* | | |
| | | n-hexane | Ethyl acetate | Ethanol | n-hexane | Ethyl acetate | Ethanol |
| Polyphenol | $FeCl_3$ | – | + | + | – | + | + |
| | $Pb(CH_3COO)_2$ | – | – | + | – | – | + |
| Tannins | Gelatin NaCl | – | – | + | – | + | + |
| Flavonoids | Mg/HCl | – | + | + | – | + | + |
| Courmarins | lacton ring reaction | – | + | + | – | – | + |
| Alkaloids | Wagner | + | + | – | + | + | – |
| | Mayer | + | + | – | + | + | – |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Quinones | HCl (Cs) | + | + | – | + | + | – |
| Saponins | Foam test | – | – | + | – | – | + |
| Terpenoids | Chloroform + $H_2SO_4$ (Cs) | + | + | – | + | + | – |
| Amino acids | $Na_2CO_3$ 5% | – | + | + | – | + | + |

Note: Cs: Concentrated solution, (+) Presence, and (–) Absence.

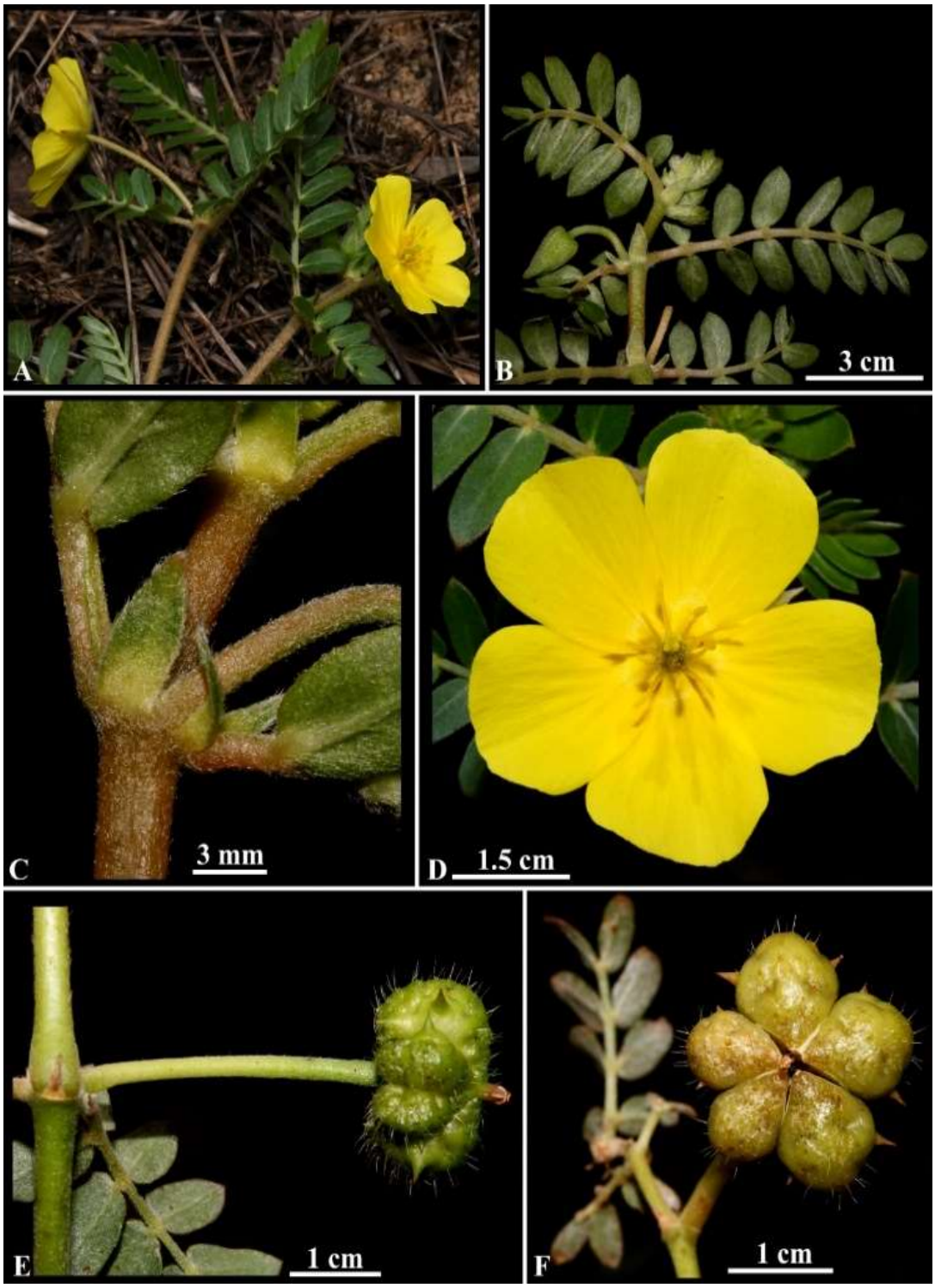


**Figure 1:** *Tribulus cistoides* L. **(A)** Flowering plant, **(B)** Leaves with leaflets (Abaxial surface), **(C)** Stipules, **(D)** Flower close up in front view, **(E, F)** Fruit in different view. *V.T.E. Quach TE01*.

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**Figure 2:** *Tribulus terrestris* L. **(A)** Flowering plant, **(B)** Leaves with leaflets (Abaxial surface), **(C)** Stipules, **(D)** Flower close up in front view, **(E, F)** Fruit in different view. *V.T.E. Quach TE02.*

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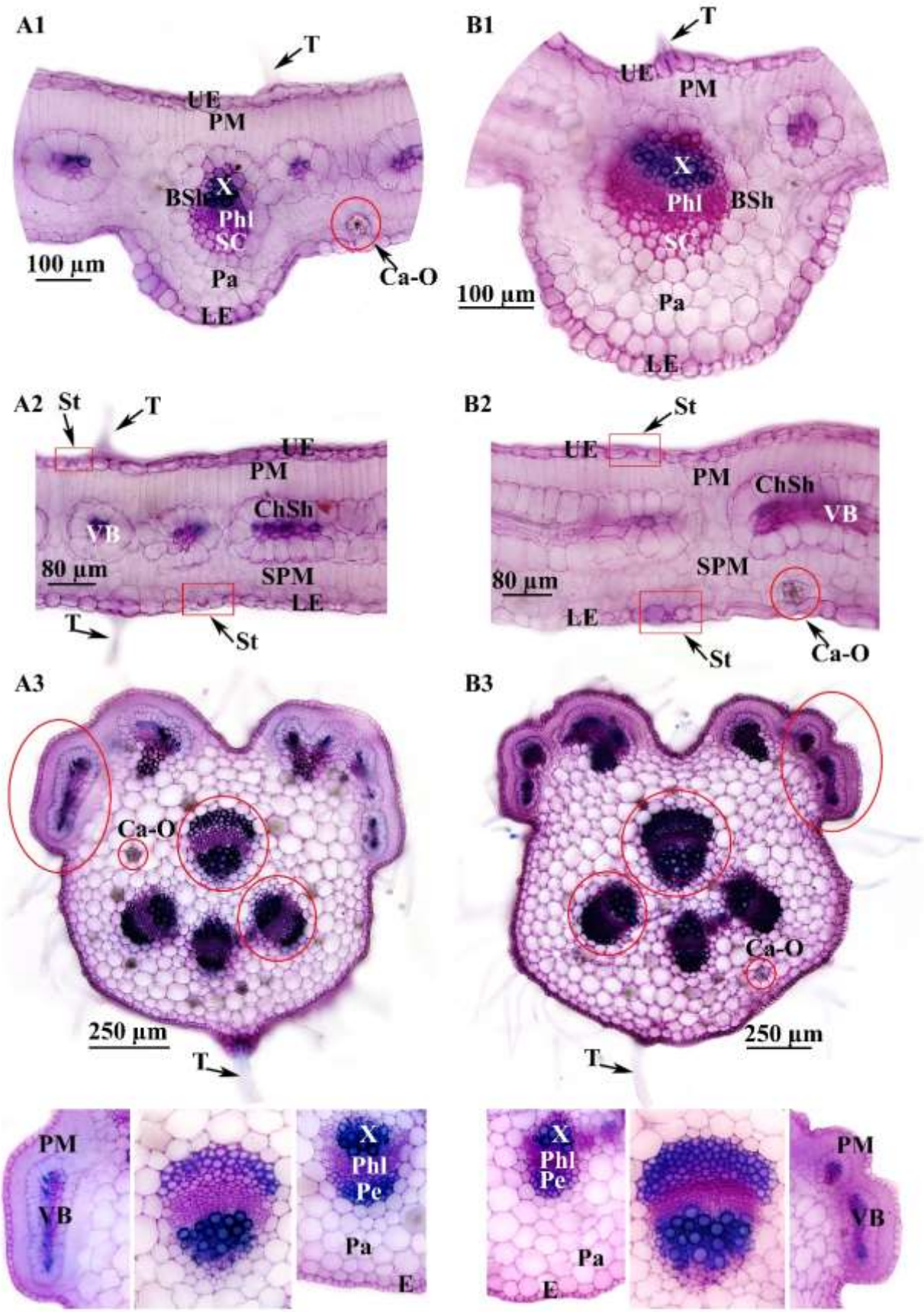


**Figure 3:** Microscopic characteristics of leaf cross-sections of *T. terrestris* **(A)** and *T. cistoides* **(B)**. (1) Midrib of leaflet, (2) Leaflet blade, (3) Petiole of leaf. (BSh) Bundle sheath, (Ca-O) Calcium oxalate crystal, (LE) Lower epidermis, (Pa) Parenchyma, (Pe) Pericycle, (Phl) Phloem, (PM) Palisade mesophyll, (SC) Sclerenchyma, (St) Stomata, (T) Trichomes, (UE) Upper epidermis, (VB) Vascular bundle, (X) Xylem.

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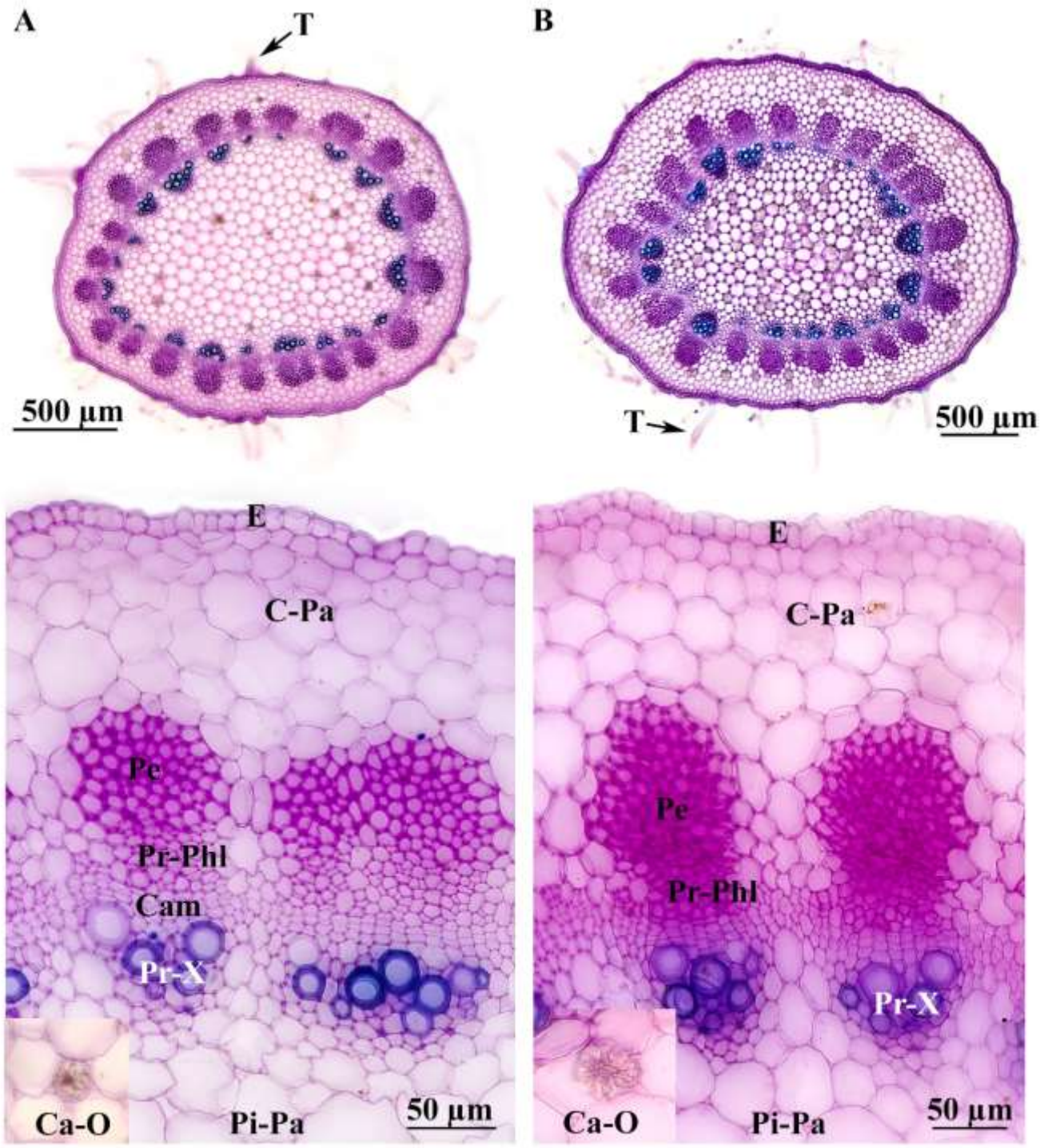


**Figure 4:** Microscopic characteristics of young stem cross-sections of *T. terrestris* **(A)** and *T. cistoides* **(B)**. (Cam) Cambium regions, (Ca-O) Calcium oxalate crystal, (C-Pa) Cortex parenchyma, (E) Epidermis, (Pe) Pericycle, (Pi-Pa) Pith parenchyma, (Pr-Phl) Primary phloem, (Pr-X) Primary xylem, (T) Trichomes.

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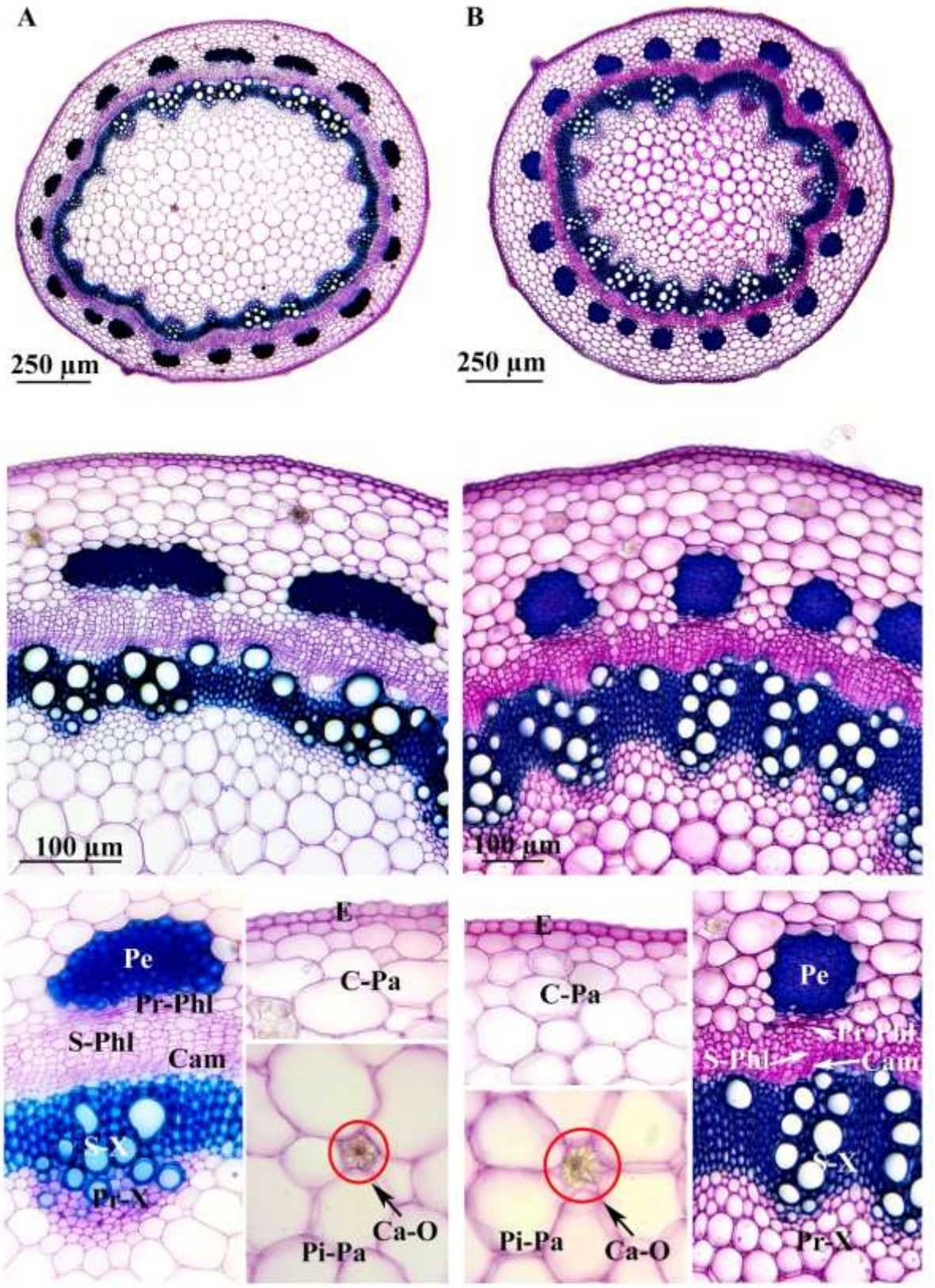


**Figure 5:** Microscopic characteristics of mature stem cross-sections of *T. terrestris* **(A)** and *T. cistoides* **(B).** (Cam) Cambium regions, (Ca-O) Calcium oxalate crystal, (C-Pa) Cortex parenchyma, (E) Epidermis, (Pe) Pericycle, (Pi-Pa) Pith parenchyma, (Pr-Phl) Primary phloem, (Pr-X) Primary xylem, (S-Phl) Secondary phloem, (S-X) Secondary xylem.

variable size in *T. cistoides*. The vascular bundle includes xylem located adaxially, consisting of lignified cells with large vessels arranged in radial rows (3–5 rows in *T. terrestris* and 7–9 rows in *T. cistoides*), and phloem located abaxially, composed of irregularly arranged cells with thicked intercellular spaces (3–5 layers in *T. terrestris*, and 5–7 layers in *T. cistoides*). The sclerenchyma is made up of thick–walled cells of varying sizes, irregular arranged without intercellular spaces (2 layers in *T. terrestris* and 4 layers in *T. cistoides*). Surrounding the vascular bundle is a bundle sheath composed of large cells with cellulose walls. The ground parenchyma consists of large, thin–walled cells of different sizes, arranged loosely with intercellular spaces (it comprises 2 layers in *T. terrestris* and 3–4 layers in *T. cistoides*).

*Leaflet blade-anatomy.*

The leaflet blade exhibits a dorsoventral structure (Fig. 3A2 & 3B2). Anatomically, both the adaxial and abaxial epidermis consist of a single layer of closely packed cells of unequal size, without intercellular spaces. Non–glandular trichomes are present on both surfaces, with a higher density on the abaxial side. Epidermis cells are covered by a thick cuticle. The palisade mesophyll consists of a single layer of elongated rectangular cells, with slightly undulating walls, arranged perpendicularly to each other. It is thicker in *Tribulus terrestris* than in *T. cistoides*. The spongy mesophyll is composed of several layers of parenchyma cells of varying sizes with cellulose walls and loosely arranged to form intercellular spaces (2–4 layers in *T. terrestris* and 2–3 in *T. cistoides*). Kranz–type chlorenchyma sheaths envelop the vascular bundles. Stomata are distributed on both surfaces in both species. In addition, calcium oxalate crystals are scattered within the mesophyll tissue of both species.

*Petiole-anatomy.*

The petiole anatomy of both species consists of a single layer of polygonal epidermal cells, tightly packed without intercellular spaces (Fig. 3A3 & 3B3). Non–glandular trichomes are evenly distributed on the petiole surface. The ground parenchyma is composed of large, thin–walled cells with cellulose walls, arranged closely with small intercellular spaces. The vascular bundle system is characteristically divided into four discrete vascular bundles: one large cental bundle and three smaller peripheral bundles. Each vascular bundle consists of centrally located xylem with lignified cells and large vessels arranged in radial rows and phloem located peripherally, composed of several layers of irregularly arranged, wrinkled–walled cells. Above the phloem lies a pericycle region with lignified cells of uneven size. *Tribulus terrestris* possesses fewer xylem vessels, phloem layers, and pericycle cell layers in the petiole compared to *T. cistoides*. Additionally, the calcium oxalate crystals are scattered throughout the parenchyma tissue of the petiole in both species.

*Stem-anatomy*.

The primary stem exhibits an almost circular cross–sectional outline (Fig. 4A1 & 4B1). The epidermis consists of a single layer of tightly packed cells of uneven size. Non–glandular trichomes are unicellular and occasionally multicellular. The cortical parenchyma comprises 3–5 layers of irregularly sized cells with small intercellular spaces. The pericycle is organized into discontinuous strands located above the vascular bundles, comprised of multiple layers of irregularly arranged cells with thick cellulose walls. The vascular bundle system consists of primary xylem and phloem, with fewer vascular bundles in *Tribulus terrestris* (22–24 stem vascular bundles) than in *T. cistoides* (25–27 stem vascular bundles). The xylem is positioned internally, with primary xylem composed of relatively uniform vessels. The phloem is external, with irregularly arranged primary phloem cells. The cambium region consists of 2–3 layers of rectangular, uniformly sized cells with cellulose walls in *T. terrestris*, and 4–6 layers in *T. cistoides*. The pith is composed of large, thin–walled parenchyma cells.

The secondary structure of the stem largely resembles that of the primary stem (Fig. 5A & 5B). Secondary xylem and phloem are formed as a result of cambium region activity. The secondary xylem comprises several radial rows of lignified cells, with *Tribulus terrestris* having 5–7 rows and *T. cistoides* having 3–5 rows. The xylem vessels are large and arranged in small groups, and the primary xylem is pushed inward. The xylem parenchyma consists of numerous small cells with thick cellulose walls (2–3 layers in *T. terrestris* and 4–5 layers in *T. cistoides*). The secondary phloem is composed of small, irregular cells with cellulose walls. As it develops, it pushes the primary phloem outward. The pericycle cells in the secondary stem are lignified and thick–walled. In addition, the calcium oxalate crystals are scattered in the cortical and pith parenchyma of the and primary and secondary stems.

This study successfully provides detailed morphological and anatomical data, along with a preliminary phytochemical screening, for two *Tribulus* species, *Tribulus terrestris* and *T. cistoides*, found in Vietnam. This is particularly significant as *T. cistoides* has been poorly studied in Vietnam, despite both species frequently co-occurring and exhibiting highly similar morphological traits, making field identification challenging. Our results address this gap by elucidating clear distinguishing features at both macroscopic and microscopic levels.

These anatomical distinctions have been effectively integrated into an identification key, providing an invaluable tool for distinguishing the two species, especially when external morphological traits are ambiguous. The ubiquitous presence of calcium oxalate crystals within the mesophyll, petiole, and stem parenchyma of both species is a common feature within the genus, potentially playing a role in plant defense or ion regulation.[22, 23]

*Key based on the morphological leaflet and stem of taxonomic identification of Tribulus genus in Vietnam*

1a. The midrib vascular bundle xylem 7–9 radial rows, 4 sclerenchyma layers; 2–4 spongy mesophyll in leaflet blade; 25–27 stem vascular bundles, 4–6 cambium layers, 3–5 xylem radial rows .....
.....

1b. The midrib vascular bundle xylem 3–5 radial rows, 2 sclerenchyma layers; 2–3 spongy mesophyll in leaflet blade; 22–24 stem vascular bundles, 2–3 cambium layers, 5–7 xylem radial rows .....
.....

*Preliminary Analysis of The Chemical Composition Of Tribulus terrestris and Tribulus cistoides Powder*

Phytochemical sceening of *n*-hexane, ethyl acetate and ethanol extracts of the *Tribulus terrestris* and *T. cistoides* plants are shown in Table 2. In *n*-hexane extract of two species revealed the presence of alkaloids, quinones, and terpenoids, while in ethyl acetate extract are found flavonoids, coumarins (*T. cistoides*), alkaloids, quinones, terpenoids, and amino acids. The polyphenols, tanins, coumarins, saponins, and amino acids are found in both ethanol extracts (Table 3).

Our preliminary screening confirmed the presence of commonly reported compounds such as alkaloids, saponins, and flavonoids in both *T. terrestris* and *T. cistoides*. This aligns with previous phytochemical studies on *T. terrestris*.[24,25,26] Critically, this study marks the first report of phenolics, tannins, coumarins, quinones, terpenoids, amino acids, and carbohydrates in *T. cistoides*. This novel finding holds significant implications for future research into the biological activities and pharmacological potential of *T. cistoides*.[12,27] The presence of these compound classes suggests that *T. cistoides* may possess antioxidant (phenolics, flavonoids), anti-inflammatory (coumarins), or other bioactivities associated with terpenoids and alkaloids. This is particularly relevant in the context of traditional medicine and the development of natural product-based pharmaceuticals. The qualitative analysis across different solvent extracts (*n*-hexane, ethyl acetate, ethanol) also provides initial insights into the polarity and extractability of these compound groups.

## Conclusion

In conclusion, the morphological and anatomical characteristics of two *Tribulus* spcies and the premilinary chemical analysis of *T.*

*cistoides* are reported for the first time in this study. In terms of morphology, two *Tribulus* species are nearly identical, they differ in they are distiguished by number of xylem rows in vascular bundle of leaflets, spongy mesophyll of leaflets blade, vascular bundle of stems. In the preliminary qualitative sceening, both species were found to contain important compounds such as alkaloids, saponins, flavonoids, especially polyphenols, tannins, coumarins, quinones, terpenoids, and amino acids were also recorded in *T. cistoides*.

## Conflict of Interest
The authors declare no conflict of interest.

## Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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the structure of flowers shape, fruits shape and fruits spines. Anatomically, the stem and leaf structure of both species are similar,

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