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Original Research Article

Microanatomical and Phytochemical Characterization of *Amaranthus tricolor* L.

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ABSTRACT

Amaranthus tricolor L. is a plant of significant botanical diversity and richness. In recent years, it has emerged as a versatile medicinal plant material, with numerous studies highlighting its considerable pharmacological effects. This study provides a comprehensive micromorphological and phytochemical characterization of *A. tricolor*. The study uses the Ciuley method, a rigorous and scientific approach that has been improved and modified by the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam. The results, based on *A. tricolor* collected in District 8, Ho Chi Minh City, Vietnam, were consistent with descriptions published in previous studies. The herbal powder contained distinctive microscopic constituents such as reticulate vessel elements, scalariform vessel elements, and spherical and cuboidal calcium oxalate crystals. The phytochemical characterization revealed the presence of various chemical groups in *A. tricolor*, including lipids, carotenoids, triterpenoids, flavonoids, tannins, saponins, organic acids, reducing agents, and polyuronic compounds. The macroscopic, microscopic, and chemical characteristics of *A. tricolor* are crucial for its medicinal use. Macroscopic studies ensure accurate botanical identification, while microscopic analysis reveals internal structures that improve quality control. Chemical characterization identifies active compounds responsible for the documented pharmacological effects. This comprehensive understanding enhances the efficacy, safety, and therapeutic potential of *A. tricolor*, supporting its optimal use in herbal medicine and paving the way for discovering new therapeutic agents.

Keywords: *Amaranthus tricolor*, Microanatomical, Phytochemical characterization, Root microanatomy, Leaf microanatomy, Stem microanatomy

Introduction

According to the International Diabetes Federation (IDF), approximately 537 million adults aged 20-79 were living with diabetes worldwide in 2023. This number is projected to rise to 643 million by 2030 and 783 million by 2045. [1] Diabetes and its complications result in approximately 6.7 million deaths annually, accounting for about 11.3% of global deaths in this age group. [1] The economic burden of diabetes is substantial, with global direct healthcare expenditure reaching USD 966 billion in 2021, representing a 316% increase from 2000. [2] Common complications associated with diabetes include cardiovascular diseases, kidney damage, nerve damage, and eye damage. Approximately 50% of individuals with type 2 diabetes will develop at least one major complication within 10 years. [3] Diabetes mellitus is an endocrine disorder related to insulin deficiency or resistance, leading to elevated blood sugar levels. This condition can cause multiple complications affecting organs such as the kidneys, retina, nerves, and cardiovascular system. [4]

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One mechanism contributing to these complications is the increased generation of free radicals due to elevated blood sugar levels. [5] Free radicals can damage cellular components such as DNA, proteins, and lipids. They can also induce inflammation, increase cellular sensitivity to apoptosis (programmed cell death), and impair the immune system. [6] Consequently, the relationship between diabetes and free radicals is bidirectional: diabetes increases free radical production, while free radicals exacerbate the risk and severity of diabetes complications. [5] *Amaranthus tricolor*, a member of the Amaranthaceae family, is distinguished by its striking reddish-purple leaves. This plant species is widespread and primarily cultivated in the Indian subcontinent and tropical regions worldwide. *A. tricolor* is regarded as a leafy vegetable in Africa. It is considered a promising crop due to its unique resilience to heat, drought, pests, and diseases, as well as the high nutritional value of its seeds and leaves. The name "*A. tricolor*" in Greek means "The plant that never fades", symbolizing the resilience and survival ability of this species in harsh environments. *A. tricolor* can thrive and grow well under harsh climatic conditions and in poor soil. [7] *A. tricolor* has been used in traditional medicine in Vietnam for centuries to treat conditions such as hemorrhoids, blood clotting disorders, bladder pain, toothache, and dysentery, with effects on mucosal contraction, diuresis, and liver protection [7]. Recent studies have also demonstrated that *A. tricolor* exhibits various pharmacological effects, including blood sugar reduction, [8] antioxidant, [9]antibacterial, [10] anti-inflammatory, [11] liver protection, [12] anticancer, [13] antiviral [14] and gastric protection. [15] Previous studies have identified several compounds in *A. tricolor*, such as flavonoids, phenolic acids, betalain pigments, [16] carbohydrates, tannins, glycosides, saponins, [17] alkaloids, terpenoids, [18] amino acids, steroids, fatty acids and organic acids. [19] Among these, phenolic compounds and betacyanins are the most prevalent components in the plant. [10]

However, detailed descriptions and comprehensive studies on the botanical anatomy and powder characteristics of each part of *A. tricolor* are lacking. The novelty of this research lies in its detailed investigation into the botanical anatomy and characteristics of *A. tricolor* powder, an area that has not been extensively studied. By conducting a thorough morphological and histological analysis, this study aims to fill existing gaps in the scientific

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literature and provide valuable insights into the pharmacological potential of *A. tricolor*. The research methods employed are highly relevant to the study's objectives. Utilizing advanced morphological and histological techniques ensures accurate and detailed observations of the plant's characteristics. This methodological approach reinforces the research novelty by providing comprehensive data that enhances the understanding and application of *A. tricolor* in both traditional and modern medicine. This comprehensive understanding will improve scientific knowledge of *A. tricolor's* unique properties and support its optimal use in herbal medicine. The detailed investigation into the botanical anatomy and characteristics of *A. tricolor* powder will address existing gaps in the scientific literature and offer valuable insights into its pharmacological potential.

Materials and Methods

Sample collection and authentication

The whole plant of *A. tricolor* was collected in December 2022, District 8, 10°42'08.1"N 106°36'31.6"E, Ho Chi Minh City, Vietnam. After collection, the medicinal materials went through morphological examination based on descriptive documents. Subsequently, the materials were cleaned, dried, dehydrated, roughly ground, and stored at the Faculty of Pharmacy, Nguyen Tat Thanh University with the voucher specimens (accession NTT-DL-023). The sample of *A. tricolor* was identified using the *mat*K gene sequencing methods. [20] The BLAST analysis results showed 100% similarity with the *Amaranthus tricolor* species in the GenBank database. [21]

Leaf, root, and stem microanatomy

Fresh leaves, roots, and stems of *A. tricolor* were sectioned to obtain a thin transverse section, and the microanatomy was studied. Using a double staining method of Carmine – iodine green and microscopic observation, by using the protocol of Vietnamese Pharmacopoeia V. [22, 23]

The plant materials were cleansed and rinsed with water, placed in the shade, and dried until sliced into pieces. The dried whole plant was ground into a rough powder. Before being tested, the powdered materials were put in a clean, sealed, and properly labeled vial.

Microscopic and organoleptic characteristics of powdered A. Tricolor

The plant material comprises of roots, stems, and leaves that have been dried at a temperature not exceeding 60 °C. Next, the sample is finely ground into separate powders of each plant part. The powders are then assessed organoleptically for color, odor, and taste. Subsequently, the powdered plant parts are placed on microscope slides, a drop of water is added, and a coverslip is applied. Finally, the samples are observed under a microscope (CarlZeiss-PrimoStar, Germany) to identify the characteristic microscopic constituents. [24]

Preliminary phytochemical screening

Phytochemical screening of the *A. tricolor* plant was conducted utilizing standard analytical methods. These procedures followed a methodology that was developed and subsequently modified based on the Ciuley approach displayed in Table 1. [25]

Table 1. Preliminary phytochemical tests for plant extracts

Phytoconstituents	Test	Observation
Lipids	Spot on paper	Translucent spot
Carotenoids	Carr-Price	Blue turns to red
	H_2SO_4	Blue or green turns to blue-green
Triterpenoids	Liebermann-Burchard	Reddish-brown to purple, upper layer green
Flavonoids	Mg/HCl	Alkaline solution pink to red
Alkaloids	General alkaloid reagents	Precipitation
Tannins	$FeCl_3$ solution	Green-black or blue-black (polyphenols)
Saponins	Liebermann reagent	Purple-brown ring
Organic acids	Na_2CO_3	Effervescence
Reducing agents	Fehling's reagent	Brick-red precipitate

Results and Discussion

Leaf, root, and stem microanatomy

Morphological characteristics

A. tricolor is an herbaceous plant growing upright, 30 cm tall, with many opposing branches at the base. Tap roots usually have one tap root and many surrounding secondary roots. The young stem has a nearly round cross-section, purple-red color, and is soft and succulent. The old body is solid and has a rounded cross-section. Leaves are simple and grow diagonally opposite each other. The leaf blade is entire, broad, triangular, 6 cm long, 4 cm wide. The tip of the leaf blade is blunt and has a forked tip. The angle of the leaf blade is tapered, and the upper surface is darker purple than the lower surface. The web-shaped leaf veins are visible on the underside. The leaf petiole is 4 cm long, purple-pink in color, trough-shaped, and slightly expanded at the base (Figure 1).

Microanatomy characteristics

Root microanatomy

Root microanatomy of *A. tricolor* exhibits a circular cross-section.

Cortex: Occupies approximately 1/6 of the phloem area, while the vascular tissue system region occupies 5/6. The cortex consists of 3-5 layers of rectangular cells, nearly uniform in size, arranged radially, with thin walls often flaking off. The cortical parenchyma comprises 5-8 layers of flattened oval cells, arranged haphazardly, surrounded by calcium oxalate crystals.

The vascular tissue system consists of 3-4 rings of phloem-xylem. The phloem-xylem ray in the central region is divided into two fan-shaped branches, each containing an upper phloem and a lower xylem. The primary phloem ray is polygonal in shape, with small sizes, arranged in small clusters. The secondary phloem ray consists of multiple layers of nearly round polygonal cells arranged radially. Xylem 2 contains numerous large vessels, unevenly sized and arranged irregularly. Xylem 1 forms multiple bundles surrounding the central region beneath the medullary rays, each bundle comprising 4-6 small, unevenly sized vessels oriented centripetally.

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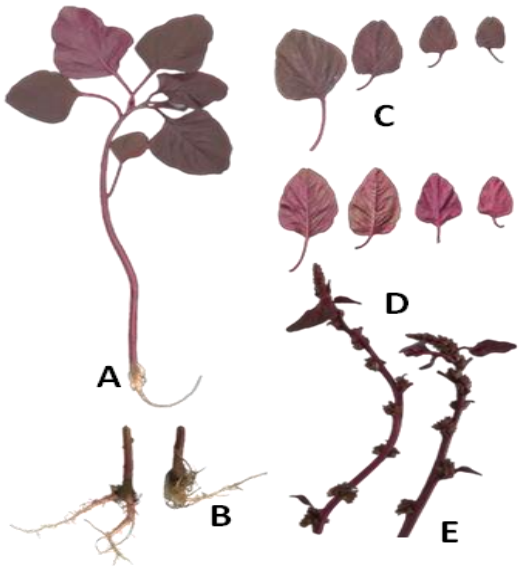

Figure 1. Morphological characteristics *A. tricolor*
(A) Whole plant of *A. tricolor*, (B) Root, (C) Upper surface of the leaf, (D) Lower surface of the leaf, (E) Inflorescence

The medullary rays extend through the xylem region and widen in the phellem region, consisting of elongated rectangular or polygonal cells arranged in radial rows. Calcium oxalate crystals are abundant in the phellem parenchyma cells (Figure 2).
Microscopic examination of the powder: The root powder is yellow, odorless, and has a bland taste. The microscope shows reticulated vessel fragments, cuboidal calcium oxalate crystals, epidermis with glandular trichomes, scalariform vessels, cork fragments, and colored fibers. Some components are shown in Figure 3.

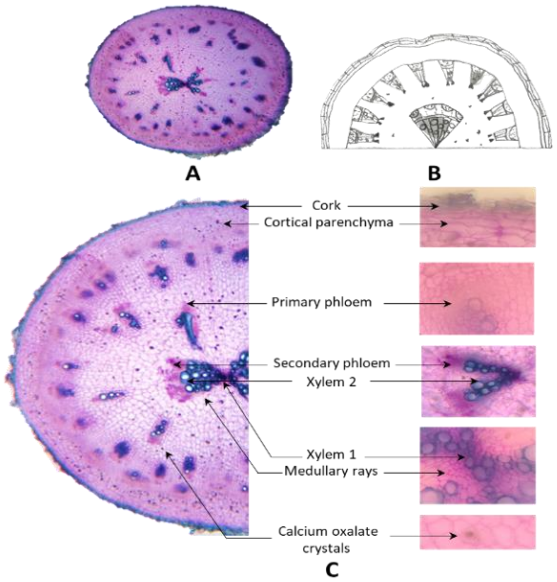

Figure 2. Root microanatomy of *A. tricolor* (A) General root microanatomy, (B) Diagram of root microanatomy (C) Detailed root microanatomy

Stem microanatomy
Epidermal peeling: Peeling the epidermis of the stem reveals numerous glandular trichomes. The glandular trichomes have an oval, elongated head and are concentrated near the leaf base.
Stem microanatomy is oval-shaped. The epidermis consists of a layer of polygonal cells nearly uniform in size and arranged closely together. Next are 3-5 layers of thick angular collenchyma cells arranged irregularly. The cortical parenchyma comprises polygonal or nearly round cells of varying sizes. The vascular tissue system begins with a ring of conducting tissue. The phloem-xylem bundle consists of 4-6 layers of primary phloem with nearly uniform sizes, closely packed together to form an almost circular shape. The secondary phloem comprises polygonal cells, uniformly sized, stacked on top of xylem 2.

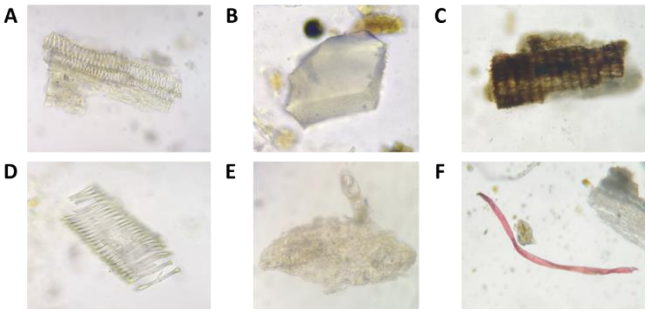

Figure 3. Microscopic constituents in the root powder of *A. tricolor* (with magnifications 40X). (A) Reticulated vessel fragments, (B) Cuboidal calcium oxalate crystals, (C) Cork fragments, (D) Scalariform vessels, (E) Epidermis with glandular trichomes, (F) Fibers

Xylem 1 consists of separate vessels, centripetally oriented within the pith parenchyma region. The pith parenchyma has polygonal or nearly round shapes, varying in size. Scattered throughout are calcium oxalate crystals (Figure 4).
Microscopic examination of the powder: Stem powder has a purplish-green color, a fragrant odor, and a slightly bitter taste. Under the microscope, it reveals the presence of reticulate vessel elements, glandular trichomes, scalariform vessel elements, epidermis containing vascular bundles, spiral vessels, pitted vessel elements, parenchyma fragments, spherical raphide crystals of calcium oxalate, and pigment masses. Some of the components found in the stem powder are illustrated in Figure 5.

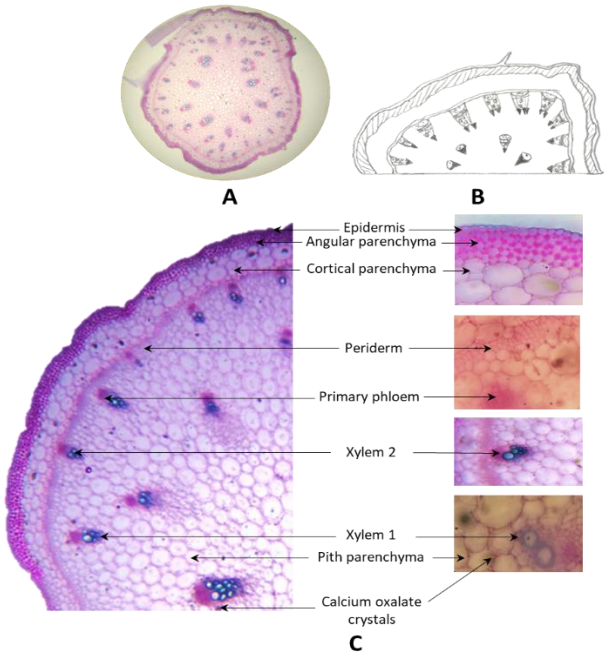

Figure 4. Stem microanatomy of *A. tricolor* (A) General stem microanatomy, (B) Diagram of stem microanatomy, (C) Detailed stem microanatomy

Leaf microanatomy
Midrib: The upper surface is concave, and the lower surface is convex.
The epidermis consists of an oval-shaped layer of cells covered with multicellular hairs. Next are 3-4 layers of thick, angular collenchyma cells. Surrounding, there are calcium oxalate crystals. The vascular tissue system consists of 5-8 bundles arranged in a circular pattern, with the primary phloem composed of 6-8 layers of small cells stacked on top of the primary xylem. The secondary xylem forms 3-5 layers of radially differentiated circular or polygonal vessels. The pith parenchyma region comprises polygonal cells of uneven sizes.

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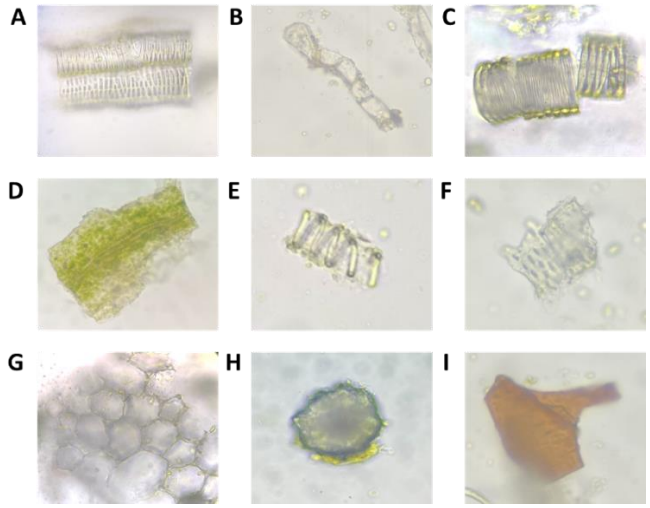

Figure 5. Microscopic constituents in the stem powder of *A. tricolor* (with magnifications 40X). (A) Reticulate vessel elements, (B) Glandular trichomes, (C) Scalariform vessel elements, (D) Epidermis containing vascular bundles, (E) Spiral vessels, (F) Pitted vessel elements, (G) Parenchyma fragments, (H) Spherical raphide crystals of calcium oxalate, (I) Pigment masses

Leaf blade: The upper and lower epidermis consists of a single layer of oval cells with uneven sizes, with the upper epidermis cells being more significant than those of the lower epidermis. Both epidermal layers have numerous protective hairs, and the lower epidermis contains many anomocytic stomata. Beneath each upper epidermal cell are 1 to 3 tightly packed palisade mesophyll cells, forming dense clusters of palisade mesophyll. The spongy mesophyll tissue consists of numerous uniform-sized polygonal cells arranged into intercellular spaces (Figure 6).

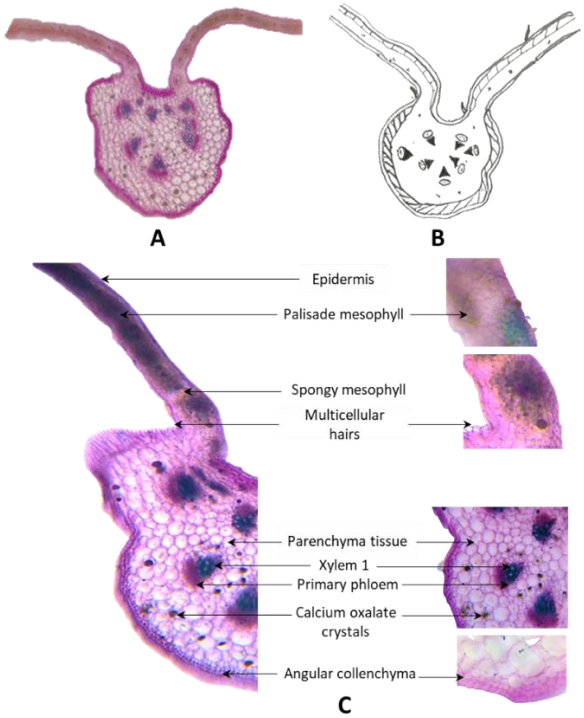

Figure 6. Leaf microanatomy of *A. tricolor* (A) General leaf microanatomy, (B) Diagram of leaf microanatomy (C) Detailed leaf microanatomy

Microscopic examination of the powder: Leaf powder has a purplish-green color, a fragrant odor, and a slightly bitter taste. Under the microscope, it reveals the presence of glandular trichomes, pitted vessel elements, multicellular covering trichomes, spherical raphide crystals of calcium oxalate, cuboidal calcium oxalate crystals, and parenchyma fragments (Figure 7).

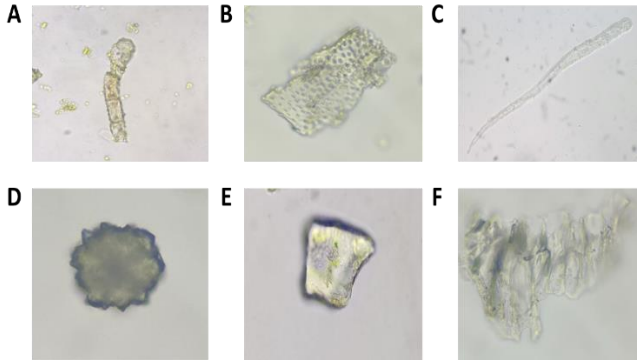

Figure 7. Microscopic constituents in the leaf powder of *A. tricolor* (with magnifications 40X). (A) Glandular trichome, (B) Pitted vessel elements, (C) Multicellular covering trichomes, (D) Spherical raphide crystals of calcium oxalate, (E) Cuboidal calcium oxalate crystals, (F) Parenchyma fragments

The preliminary phytochemical survey results presented in Table 2 indicate that the *A. tricolor* species contain a variety of compounds such as lipids, carotenoids, triterpenoids, flavonoids, tannins, saponins, organic acids, reducing agents, polyuronic compounds and may also contain alkaloids.

Table 2. Preliminary phytochemical screening of *A. tricolor*

Phytochemical constituents	**Results**		
	Ether Extract	Ethanol Extract	Aqueous extract
Lipids	+	-	-
Carotenoids	+	-	-
Triterpenoids	+	+	+
Flavonoids	-	+	+
Alkaloids	-	+	+
Tannins	-	+	+
Saponins	-	+	+
Organic acids	-	+	+
Reducing agents	-	+	+

Conclusion

The study characterized *A. tricolor* microanatomically and phytochemically. Macroscopic and microscopic features aligned with previous literature. Notable microscopic constituents included reticulate and scalariform vessel elements, and calcium oxalate crystals. Phytochemical analysis revealed lipids, carotenoids, triterpenoids, flavonoids, tannins, saponins, organic acids, reducing agents, and polyuronic compounds. Understanding these characteristics is vital for accurate identification, quality control, and optimal use in medicine. It is recommended to continue the *in vitro* bioactivity, safety, efficacy of *A. tricolor* in the further research.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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