

Morphology, micromorphology and physicochemical properties of *Annona squamosa* L. (Annonaceae) leaves from Vietnam

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ABSTRACT

Introduction: In everyday life, nature has given us countless useful medicinal plants. Among the common plants in Vietnam, the custard apple (*Annona squamosa* L.) has not been mainly used as a fruit but also traditionally as a medicine. However, studies on morphological characteristics remain limited in Vietnam as well as around the world. **Objective:** Determine the morphological and microscopic characteristics, and physicochemical features of *A. squamosa* leaves. **Materials and methods:** *A. squamosa* leaves, morphological research process (analysis and photography), microscopic analysis process (staining leaf microscopic specimens and microscopy), physicochemical properties (described in Vietnamese Pharmacopoeia V). **Results:** The characteristics of *A. squamosa* leaves were described in detail such as morphology, cross-sections, leaf powder composition, and purity of herb. **Conclusion:** The morphological, microscopic components and components in medicinal powder will help create the basis for building testing standards for this medicinal plant in the future as well as support further research on *A. squamosa* species in Vietnam.

Keywords: *Annona squamosa*, morphological, micro-morphological, powder feature

1. INTRODUCTION

Herbal plants have been considered one of the important sources of medicine since the beginning of human civilization. The demand for natural health products such as pharmaceuticals, functional foods, and cosmetics is increasing.

Natural-derived drugs are recognized to be effective and play a very important role in human disease treatment methods. In daily life, nature has provided us with countless useful medicinal plants. Some plants are both a source of food and have remarkable healing and disease-prevention properties. Among the very popular plants in Vietnam, Custard-Apple (*Annona squamosa* L.) is a plant mainly used for fruit, but folk medicine also uses it as a medicine [1].

It is noteworthy that according to Indian folk medicine, the young leaves of *A. squamosa* have antidiabetic activity. This medicine is widely used by men of tribes around the Aligarh district of Uttar

Pradesh, India, and the Chotanagpur district of Bihar, India. Villagers in these places take a mixture of 4-5 new young leaves combined with 5 black peppercorns (*Piper nigrum*) chopped in the early morning to treat diabetes with good results [2].

Many documents and published articles on the extract and isolated active ingredients from *A. squamosa* leaves have hypoglycemic, hepato-protective, antioxidant, antitumor, and anti-lice effects. These effects of this medicinal plant are related to the presence of alkaloids, carbohydrates, essential oils, tannins, and phenolic compounds in the different parts of the plant [3].

In Vietnam, there have not been many published studies in detail on the morphology of this species as well as in the world. Therefore, this study provides information on morphological, micromorphological features to identify *A. squamosa* harvested in Vietnam precisely. Also, this

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study determined some physicochemical parameters of the herbs collected in Vietnam, including moisture content, total cash value, and acid-soluble ash value [4]. These results are a reliable document to support the precise identification of *A. squamosa* in Vietnam to set a foundation for selecting the correct species as medicinal and motivate further research on isolating purified compounds and determining the pharmacological activities of this medicinal plant.

2. MATERIALS AND METHODS

2.1. Materials

Medium aged leaves *A. squamosa* L. collected at the foot of Ba Den Mountain, Tan Hung Commune, Tan Chau District, Tay Ninh Province in September 2023. The leaves are dried and ground into coarse powder and stored in a cool, dry place. A voucher specimen was deposited at the Department of Pharmacognosy - Faculty of Pharmacy - University of Medicine and Pharmacy, Ho Chi Minh City.

2.2. Methods

2.2.1. Procedures of morphological study

Samples were observed, morphological characteristics were described and photos were taken. Determine the scientific name of this species based on documents [1, 5, 6].

2.2.2. Procedures of micro-morphological study

The micro-morphological characteristics of the samples were determined according to the guidelines of the Vietnamese Pharmacopoeia V [4] via the double staining method of iodine green carmine. Leaves of *A. squamosa* were chopped and cut into segments/pieces with a razor. The samples

were then manually cut horizontally into thin slices (approximately 10-20 μm thick) with a razor blade. Next, the thin finished cross-sections were bleached with 5.0% (w/v) chloramine-T detergent and followed by 50% (v/v) chloral hydrate for 10 min. Those slices were neutralized with 1.0% (w/v) acetic acid for 2 min before being double stained with 0.3% (w/v) Iodine Green and 1.0% (w/v) Carmine, in which the sample was immersed in Iodine Green for 5s and in Carmine for 10s (until the samples became clearer). After each step, excess bleach, reagents, and dyes were removed using double distilled water (ddw). Samples were placed on slides with 1-2 drops of the glycerin-water mixture (50:50, v:v) pre-existing and covered with a coverslip. The samples were observed and photographed under an optical microscope (Labomed, USA) at 4 $\times$, 10 $\times$, and 40 $\times$ magnifications. Similarly, the leaf powder features were also observed under the microscope with 10X and 40X magnification (Labomed, USA) [4].

2.2.3. Physicochemical parameters

The indicators are determined according to the guidelines of the Vietnamese Pharmacopoeia V [4]:

- The moisture content is determined by the method of determining weight loss due to drying using an external moisture drying balance (Sartorius) in Appendix 9.6 (page PL-182).
- Acid insoluble ash value and total ash value in Appendix 9.7 and 9.8 (pages PL-182 and PL-183).

All purity test parameters are taken as the average value of 3 independent tests, with stable results.

3. RESULTS

3.1. Morphological characteristics

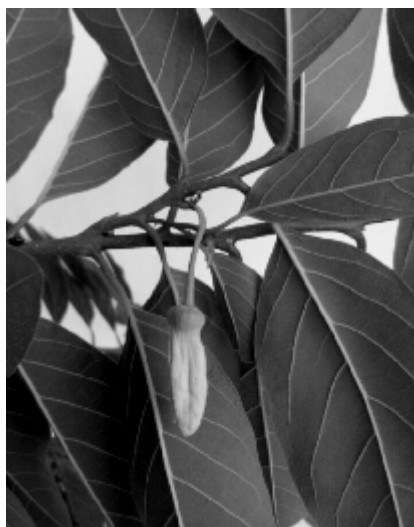


Figure 1. Morphological characteristics of leaves of *A. squamosa* L.

Morphological characteristics of leaves: Leaves are simple, whole, grow alternately; Leaf blades are lanceolate, 9-13 cm long, 3-5 cm wide, darker green on top than bottom, feather-shaped leaf veins clearly visible on the underside, 7-9 pairs of secondary veins; Leaf petioles are cylindrical, nearly round, 0.8-1 cm long, swollen at the bottom and darker green; no stipule.

3.2. Microbiological characteristics of *A. squamosa* leaves

Leaf midrib region: The upper surface is concave, the lower surface is convex polygonal. The upper and lower epidermis consist of single layer, polygonal cells, cellulose walls; the cuticle layer is thin and flat. The upper and lower collenchyma tissue is the angular collenchyma, 2-3 layers, polygonal cells, cellulose walls, arrangement random. The cortical parenchyma is angular parenchyma, multi-layered, cellulose walls, arrangement random. The sclerenchyma tissue forms a continuous ring, 3-4 layers, polygonal

cells. The arc-shaped conduction system consists of xylem above and phloem below. The xylem vessel are circular polygonal cells, lignified cell walls, and arranged in rows of 3-5 vessels; the parenchymatous xylem are polygonal cells, lignified cell walls, arranged in 1-4 rows, alternating with rows of the proto-xylem vessels. The phloem are polygonal cells, cellulose walls. The parenchymatous pith is angular parenchyma, multi-layered, lignified cell walls, arrangement random.

Leaf blade region: The upper and lower epidermis consist of single layer, polygonal cells, cellulose walls; the cuticle layer is thin and flat; stomata are concentrated in the lower epidermis. The palisade mesophyll consists of single layer, under each upper epidermal cell there are 1-2 palisade mesophyll cells. The spongy mesophyll region is 2 times thicker than the palisade mesophyll region, cells have variable shapes.

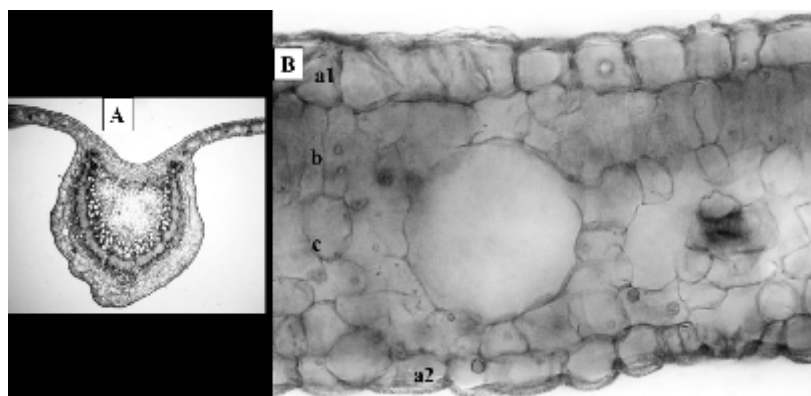


Figure 2. The features of cross-sectioned leaves of *A. squamosa* L.

A. The features of cross-sectioned leaf, B. The leaf blade region, a. epidermis (1. upper, 2. lower), b. palisade mesophyll, c. spongy mesophyll

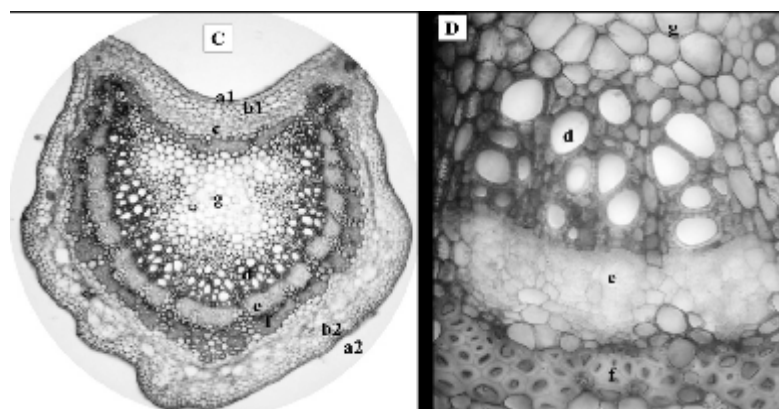


Figure 3. The features of cross-sectioned leaves of *A. squamosa* L.

C. Leaf midrib region, D. A part of leaf midrib region
a. epidermis (1.upper, 2.lower), b. collenchyma (1.upper, 2.lower), c. cortical parenchyma, d. Proto-xylem, e. Proto-phloem, f. sclerenchyma, g. parenchymatous pith

3.3. Characteristics of leaf powder

Leaf powder: The components of leaf powder, including the fragment of upper epidermal cells, the fragment of lower epidermal cells, the

stomata have a anomocytic type, the fragment of palisade mesophyll, the fiber, the fragment of spiral xylem vessel, the fragment of scalariform xylem vessel.

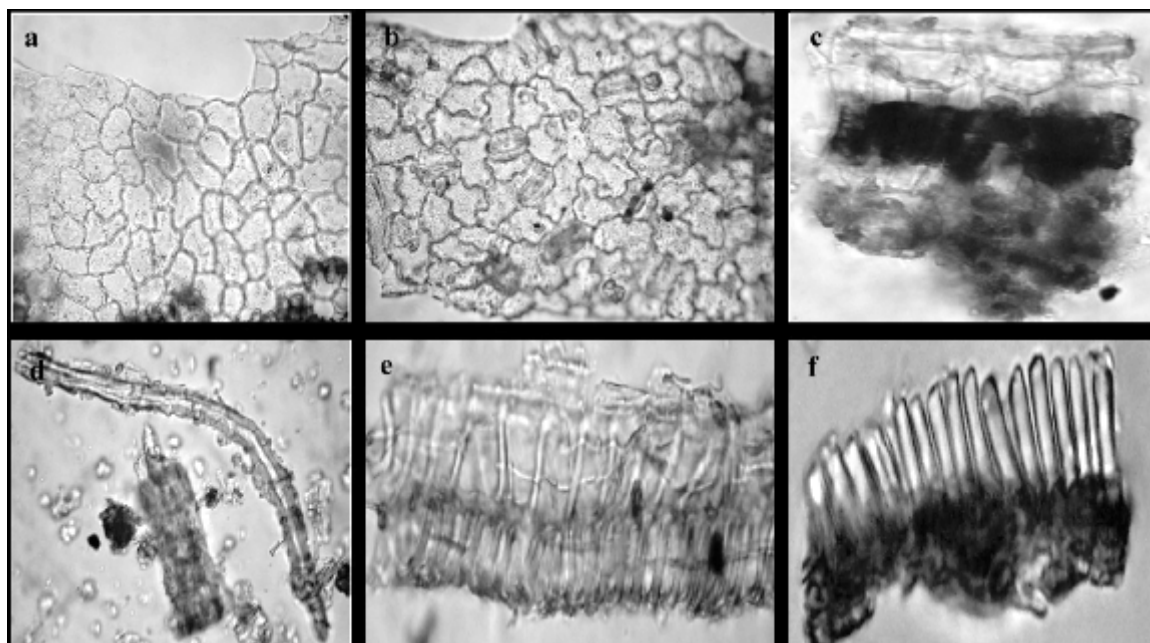


Figure 4. The features of leaf powder of *A. squamosa* L.

a. Fragment of upper epidermal cells, b. Fragment of lower epidermal cells, c. Fragment of palisade mesophyll, d. fiber, e. Fragment of spiral xylem vessel, f. Fragment of scalariform xylem vessel

3.4. Physicochemical parameters

According to the guidelines of the Vietnamese Pharmacopoeia V [4], Appendix 9.6; 9.7; 9.8; test

are taken as the average value of 3 independent tests, with stable results. The results are recorded in Table 1.

Table 1. Results of purity test of *A. squamosa* leaf powder

Test criteria	1	2	3	Mean
Moisture content (%)	7.2	7.11	7.15	7.15
Total ash value (%)	8.63	8.62	8.6	8.61
Acid insoluble ash value (%)	0.34	0.31	0.32	0.32

Comment: Moisture content leaf of *A. squamosa*: 7.15%, total ash value: 8.61%, acid insoluble ash value: 0.32%

4. DISCUSSION

The authors analyzed morphological characteristics, using morphological methods to compare and contrast with documents [1, 5-7]. *A. reticulata* and *A. squamosa* have similar shapes, colors, and surface textures but have some differences in morphology. The mature leaves of *A. reticulata* have an acuminate tip and grow up to 23 cm in length and 5.5 cm in breadth, whereas on maturity, the leaves of *A. squamosa* grow only up to 9-13 cm in length

and 3-5 cm in width and have an acute tip. However, the leaves of both the drugs have more or less similar dimensions when young. *A. reticulata* has 9-18 pairs of lateral veins, which in comparison are more than the 8-11 pairs of *A. squamosa* [5]. Custard apple leaves collected at the foot of Ba Den mountain, Tay Ninh province have the scientific name *Annona squamosa* L. (Annonaceae). The study examined the morphological characteristics in more detail with color illustrations. The anatomical micro-morphological of leaves and petioles are described in detail with illustrations. The physicochemical parameters also showed that the purity of the raw materials was consistent when

compared with other studies [6]. In addition, micro-morphological components in medicinal powder will help create the basis for building testing standards for this medicinal plant in the future as well as support further research on *Annona squamosa* species in Vietnam.

5. CONCLUSION

Botany of *A. squamosa* leaves: Description of the

botanical characteristics of *A. squamosa* leaves shows similarities with specialized botanical documents. Microbiology of *A. squamosa* leaves: The microscopic characteristics of leaves and powder of *A. squamosa* leaves have been determined. Physicochemical properties of leaves: Moisture content leaf of *A. squamosa* (7.15%), total ash value (8.61%), acid insoluble ash value (0.32%).

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Đặc điểm hình thái, vi phẫu và thông số lý hóa của dược liệu lá Na *Annona squamosa* L. (Annonaceae) ở Việt Nam

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TÓM TẮT

Đặt vấn đề: Trong cuộc sống đời thường, thiên nhiên đã ban tặng cho chúng ta vô số cây thuốc hữu ích. Trong số những loại cây rất phổ biến ở Việt Nam, mãng cầu (*Annona squamosa* L.) là loại cây chủ yếu được dùng để lấy quả nhưng còn được dùng trong y học dân gian làm thuốc chữa bệnh. Tuy nhiên, ở Việt Nam chưa có nhiều nghiên cứu được công bố chi tiết về hình thái của loài này cũng như trên thế giới. **Mục tiêu:** Xác định các đặc điểm hình thái, vi phẫu, bột dược liệu và các thông số lý hóa của lá *A. squamosa*. **Đối tượng và phương pháp nghiên cứu:** Lá *A. squamosa*, quy trình nghiên cứu hình thái (phân tích và chụp hình), quy trình nghiên cứu vi hình thái (nhuộm các tiêu bản vi phẫu lá và xem trên kính hiển vi), độ tinh khiết (được thực hiện theo ĐĐVN V). **Kết quả:** Các đặc điểm của lá *A. squamosa* được mô tả chi tiết như đặc điểm hình thái, vi phẫu, cấu tử bột lá và thông số lý hoá của dược liệu. **Kết luận** các thành phần hình thái, vi phẫu và

cấu tử trong bột dược liệu sẽ giúp tạo cơ sở xây dựng tiêu chuẩn khảo nghiệm cho cây thuốc này trong tương lai cũng như hỗ trợ các nghiên cứu sâu hơn về loài *A. squamosa* ở Việt Nam.

Từ khóa: *Annona squamosa*, đặc điểm hình thái, đặc điểm vi phẫu, cấu tử bột

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