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(RESEARCH ARTICLE)

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Anatomical characterization and evaluation of antioxidant activity of *Ficus hispida* L. f.

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Abstract

Ficus hispida L.f. (Moraceae) is widely distributed in Asian countries and has been traditionally used in folk medicine for the treatment of various inflammatory and digestive disorders. This study aimed to investigate the morphological and anatomical characteristics of the roots, stems, and leaves, and to evaluate the antioxidant activity of the fruit extract. Plant samples were collected in Phan Đình Phùng Ward, Thai Nguyen, Viet Nam. Morphological and anatomical analyses were conducted using standard botanical methods and observations under a light microscope. The results showed that *Ficus hispida* L.f. exhibits typical dicotyledonous characteristics, including well-developed secondary tissues, open collateral vascular bundles, abundant laticifers, and clearly differentiated palisade and spongy mesophyll in the leaf. The antioxidant activity of the ethanolic fruit extract of *Ficus hispida* L.f. was determined using the DPPH free radical scavenging assay. The extract demonstrated strong antioxidant activity with an IC_{50} value of 20.36 µg/mL, compared with 12.71 µg/mL for vitamin C. These findings indicate the potential of *Ficus hispida* fruits as a natural source of antioxidants for pharmaceutical and functional food applications.

Keywords: *Ficus hispida* ; Morphology; Anatomy; Antioxidant activity; DPPH assay.

1 Introduction

Ficus hispida L.f., belonging to the family Moraceae, is a small to medium-sized tree widely distributed in tropical and subtropical regions of Asia, including Vietnam, India, Bangladesh, China, and Thailand [1]. Various parts of the plant, such as the fruits, leaves, stems, and bark, have long been used in traditional medicine for the treatment of diarrhea, inflammation, skin diseases, dysentery, ulcers, jaundice, and digestive disorders [2].

The fruits of *Ficus hispida* grow in clusters and are covered with fine hairs; when ripe, they turn reddish-purple to dark purple in color. The fruit pulp contains numerous small seeds and exhibits a slightly astringent taste [3]. This species is rich in biologically active compounds, including phenolics, flavonoids, triterpenoids, alkaloids, glycosides, and sterols, which are associated with antioxidant, anti-inflammatory, antimicrobial, and glucose-regulating activities [4], [5].

In vitro studies have demonstrated that methanolic extracts of *Ficus hispida* fruits possess strong antioxidant activity, as evidenced by their ability to scavenge DPPH and ABTS free radicals in a concentration-dependent manner, which correlates positively with total phenolic and flavonoid contents [1], [4]. In animal models, these extracts have also been reported to reduce serum lipid levels and enhance the activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and catalase [4].

According to traditional practices in India, Vietnam, and other Southeast Asian countries, the fruits and other parts of *Ficus hispida* are commonly used in fresh or dried forms or processed into herbal decoctions to support digestive

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health and improve general well-being [2]. However, comprehensive scientific studies focusing on the morphological and anatomical characteristics of the roots, stems, and leaves, as well as the biological activities of *Ficus hispida* fruits in Vietnam, remain limited [5].

Therefore, the present study aims to investigate the morphological and anatomical features of the roots, stems, and leaves, and to evaluate the antioxidant activity of fruit extracts from *Ficus hispida* L.f., thereby providing a scientific basis for the sustainable utilization and development of this species in pharmaceutical and functional food applications.

2 Materials and methods

2.1 Materials

Fruits of *Ficus hispida* L.f. were collected in March 2025 in Phan Đình Phùng Ward, Thai Nguyen, Viet Nam, and were used as raw materials for ethanol extraction. In addition, other plant parts including roots, stems, and leaves were collected from the same location for morphological and anatomical studies.

The plant samples were taxonomically identified by Assoc. Prof. Dr. Sỹ Danh Thường, Thai Nguyen University of Education – Thai Nguyen University, based on morphological characteristics observed during sample collection to ensure scientific accuracy for the study. Voucher specimens were prepared and deposited at the Biological Museum of the Faculty of Biology, Thai Nguyen University of Education, under accession number TNUE-FH2025-01.

2.2 Methods

2.2.1 Sample collection and preparation

Plant materials of *Ficus hispida* L.f., including roots, stems, leaves, and fruits, were collected under dry weather conditions. Immediately after collection, the samples were thoroughly washed with clean water to remove dirt and impurities, then air-dried. For preservation and subsequent analyses, the samples were immersed in 90% ethanol and stored in a well-ventilated laboratory.

The fruit samples were subsequently used for ethanol extraction following the method described by Nguyen Nghia Thin (2008) [6].

2.2.2 Methods for morphological and anatomical studies

Morphological study: The morphological characteristics of *Ficus hispida* L.f. were investigated and described based on standard botanical taxonomic references, including the works of Tran Van Ba and Hoang Thi San (1998) [7] and Hoang Thi San (2002) [8]. The investigation focused on the external features of roots, stems, leaves, and fruits to record diagnostic traits and general morphological characteristics of the species.

Anatomical study: Roots, stems, and leaves were used to prepare temporary anatomical sections, which were observed under a light microscope. The anatomical structures of each organ were recorded, analyzed, and described in detail following the guidelines of Tran Van Ba and Hoang Thi San (1998) [7] and Kixeleva (1998) [8], with emphasis on secretory structures, tissue layers, and characteristic cellular organization of the species.

2.2.3 Microscopic imaging method

Microscopic slides were observed and photographed using a STE-1 CAM light microscope (China) coupled with Motic software. Images were captured at different magnifications to facilitate detailed analysis and description of the histological structures of roots, stems, and leaves.

2.2.4 Preparation of ethanol extract

Preparation and extraction of ethanol extract from *Ficus hispida* L.f. fruits: After collection, the fruits were washed thoroughly to remove dirt and impurities, air-dried, and then oven-dried at 50 °C for 48 hours. The dried fruits were ground into a fine powder to facilitate extraction. The extract was obtained using an ultrasonic-assisted maceration method, in which 10 g of fruit powder was soaked in 30 mL of 90% ethanol for 3 days. After extraction, the solution was filtered through Whatman No.1 filter paper (Merck, Germany) and concentrated under reduced pressure using a Rotavapor R100 (Buchi, Switzerland) to obtain the ethanol extract. The resulting extract was stored at 4 °C for subsequent analyses [10].

2.2.5 Determination of antioxidant activity of Ficus hispida L.f. fruit extract by the DPPH free radical scavenging assay

Evaluation of antioxidant activity using the DPPH method: The antioxidant activity of the *Ficus hispida* L.f. fruit extract was determined using the DPPH free radical scavenging assay, following the method described by Tabart et al. (2009) [11]. Briefly, 100 µL of the extract at various concentrations (0.5, 1, 2, 4, 8, 16, 32, 64, and 128 µg/mL) was transferred into test tubes, followed by the addition of 2.9 mL of 0.1 mM DPPH solution prepared in methanol. The reaction mixture was vortexed thoroughly and incubated for 30 minutes at room temperature in the dark. The absorbance of the resulting solution was measured at 517 nm using a Shimadzu UV-Vis 1800 spectrophotometer. The DPPH radical scavenging activity was calculated using the following equation: DPPH scavenging activity (%) = 100 x (Ac - As)/Ac where AcAc is the absorbance of the control sample and AsAs is the absorbance of the extract sample. The antioxidant activity of the extract was expressed as the EC5050 value, defined as the concentration of the extract required to scavenge 50% of DPPH free radicals.

3 Results and discussion

3.1 Morphological characteristics of *Ficus hispida* L.f. in Thai Nguyen Province

The results of morphological investigation and description showed that *Ficus hispida* L.f. (locally known as Sung ngai), belonging to the family Moraceae, is a small- to medium-sized woody tree. In the study area, plant height ranged from 3 to 10 m, while some well-developed individuals could reach greater heights. The trunk is cylindrical, with bark ranging from gray to grayish-brown. At the juvenile stage, the trunk and branches are densely covered with stiff hairs, gradually becoming glabrous as the plant matures. When injured, the plant exudes a milky white latex.

Young branches are grayish-brown and densely covered with coarse, rigid hairs; these hairs gradually disappear on older branches. Leaves are opposite, simple, with leaf blades that are ovate, elliptic, or obovate in shape. The leaf base is rounded or slightly cordate, while the apex is acute to long-acuminate; the leaf margin is irregularly serrate. The lamina is thick and rough, densely covered with stiff hairs on both surfaces, measuring 11–20 cm in length and 5–12 cm in width. Leaf venation consists of three primary veins arising from the base and 5–6 pairs of lateral veins, which are prominently raised on the leaf surface. Petioles are 15–30 mm long and pubescent. Stipules are triangular, 15–25 mm in length, and deciduous.

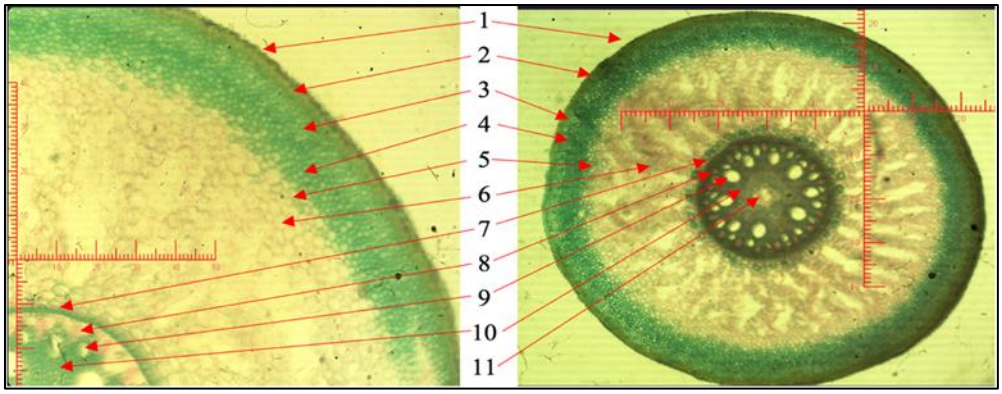
Flowers and fruits develop as syconia, usually occurring in clusters on sparsely leaved branches and occasionally appearing directly on the trunk or older branches (cauliflory). The fruits are globose or subglobose, with a diameter of approximately 1–2.5 cm; the fruit surface is rough and pubescent, turning yellow or yellowish-green at maturity. *Ficus hispida* is a dioecious species, with distinct male and female plants. Male flowers are small, consisting of three tepals and one stamen, whereas female flowers possess an ovary enclosed by the perianth.

Comparison with the description of *Ficus hispida* reported by Pham Hoang Ho (2000) [12] for populations distributed across various regions of Vietnam revealed a high degree of similarity in trunk, leaf morphology, branching pattern, and floral and fruit characteristics. These findings confirm that the collected specimens exhibit the typical morphological traits of *Ficus hispida* under the ecological conditions of the study area.

3.2 Evaluation of Antioxidant Activity

3.2.1 Anatomical characteristics of the root

The anatomical features of the transverse section of the root of *Ficus hispida* L.f. are described from the outermost layer to the innermost structures, as illustrated in Figure 1.



1. Cork; 2. Cork cambium; 3. Chlorenchyma and cortical parenchyma; 4. Laticifer; 5. Vascular cambium; 6. Cortical parenchyma; 7. Sclerenchymatous cortex; 8. Phloem; 9. Large vessel; 10. Pith parenchyma; 11. Pith (observed at 200× magnification).

Figure 1 Transverse section of the root anatomy of *Ficus hispida* L.f.

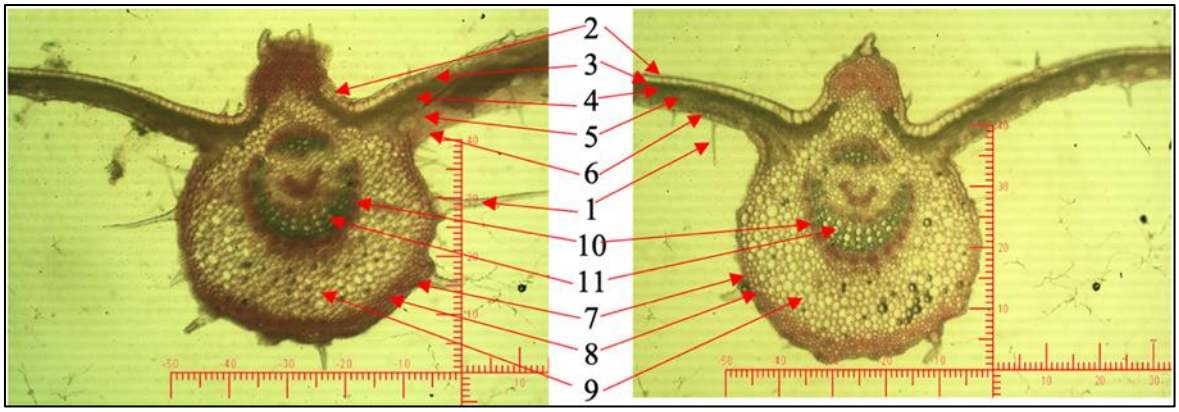
The outermost layer of the root is the cork (1), consisting of several tightly packed cell layers with suberized cell walls that stain blue with dye, serving to protect the inner tissues. Just beneath it is the cork cambium (2), composed of small, living cells with thin cellulose walls. These cells divide tangentially; the outer derivatives differentiate into cork cells, while the inner derivatives form the chlorenchyma and cortical parenchyma (3). The chlorenchyma and cortical parenchyma consist of several layers of large parenchymatous cells, relatively loosely arranged with numerous intercellular spaces. Scattered within the cortical parenchyma are laticifers (4), which are characteristic secretory cells of the mulberry family (Moraceae) and contain latex. Inside the cortical parenchyma is a sclerenchymatous ring (7), composed of thick, lignified cells that provide mechanical support for the root. Next is the phloem (9), formed from the outer side of the vascular cambium (5). It consists of polygonal, thin-walled cells that are closely arranged and stain pink with carmine; phloem cells differentiate centripetally. The vascular cambium (5) is a thin meristematic layer composed of slightly elongated rectangular living cells that divide tangentially; it produces secondary phloem outward and secondary xylem (6) inward. The secondary xylem differentiates centrifugally and contains numerous distinct large vessels (8), which function in the conduction of water and mineral salts. At the center of the root are the pith parenchyma (10) and the pith (11), composed of large, thin-walled parenchymatous cells that serve a storage function.

Thus, in the secondary structure of the root of *Ficus hispida* L.f., the xylem and phloem are arranged in an open collateral vascular bundle pattern, which is characteristic of dicotyledonous roots.

3.2.2 Anatomical characteristics of the leaf

The transverse anatomical structure of the leaf of *Ficus hispida* L.f. is described from the outermost layer inward, corresponding to the annotations presented in Figure 2.

The outermost layer of the leaf blade consists of epidermal hairs (1), which are protective trichomes distributed over the leaf surface, functioning in protection and reducing water loss. Just beneath is the upper epidermis (2), composed of tightly arranged rectangular cells whose outer walls are covered with a well-developed cuticle; these cells generally lack chloroplasts. Below the upper epidermis lies the assimilation tissue (3), which is well developed and consists of elongated cylindrical cells arranged relatively close together. These cells contain numerous chloroplasts and serve as the primary photosynthetic tissue of the leaf. Beneath this layer is the spongy mesophyll (5), composed of irregularly shaped cells loosely arranged with large intercellular spaces, facilitating gas exchange. Spongy mesophyll cells also contain chloroplasts, but at a lower density than the palisade mesophyll. Within the leaf blade, closed vascular bundles (4) are present, consisting of xylem and phloem arranged in a closed collateral pattern. The phloem (11), located toward the lower epidermis, is responsible for transporting photosynthetic products. Surrounding the vascular bundle are supporting tissue (8) and sclerenchyma (10), composed of thick-walled cells that enhance the mechanical strength of the leaf veins. Between the vascular bundles and within the midrib is parenchyma tissue (9), consisting of large, thin-walled fundamental parenchyma cells that function in storage and tissue connection. The lowermost layer of the leaf blade is the lower epidermis (6), made up of tightly arranged rectangular cells with numerous stomata, responsible for gas exchange and transpiration.



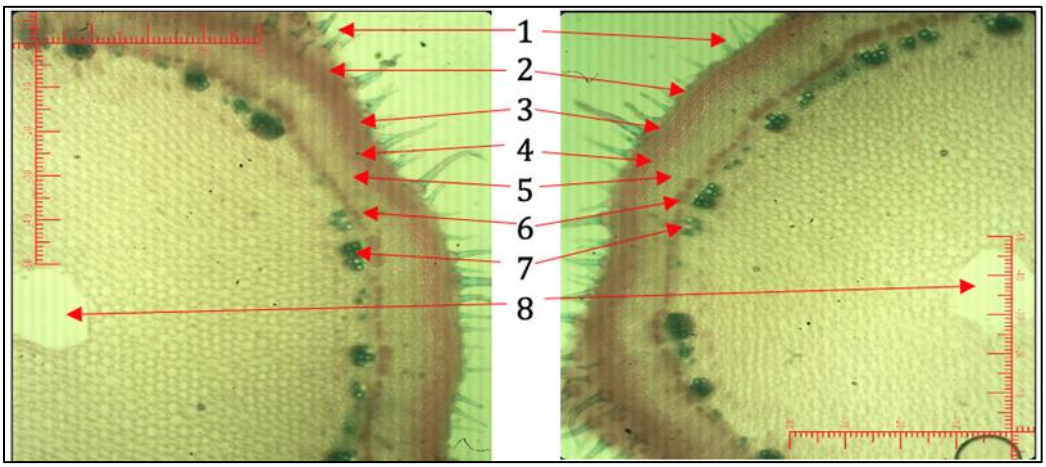
1. Epidermal hair (trichome); 2. Upper epidermis; 3. Assimilation tissue (palisade mesophyll); 4. Closed vascular bundle in the leaf blade; 5. Spongy mesophyll; 6. Lower epidermis; 7. Epidermis; 8. Supporting tissue; 9. Parenchyma; 10. Sclerenchyma; 11. Phloem (observed at 200× magnification).

Figure 2 Transverse section of the leaf anatomy of *Ficus hispida* L.f.

The anatomical structure of the leaf of *Ficus hispida* L.f. clearly exhibits the typical characteristics of a dicotyledonous leaf, with well-differentiated assimilation tissue and a well-developed vascular bundle system.

3.2.3 Anatomical characteristics of the stem

The transverse anatomical structure of the stem of *Ficus hispida* L.f. is described from the outside inward as shown in Figure 3.



1. Epidermal hair; 2. Cork; 3. Cork cambium; 4. Chlorenchyma and cortical parenchyma; 6. Secondary phloem; 7. Laticifer and xylem vessel; 8. Cavity. (Observed at 200× magnification)

Figure 3 Transverse section of the stem of *Ficus hispida* L.f.

The transverse section of the stem shows a clearly differentiated secondary structure typical of dicotyledonous plants. From the outside inward, the tissues are arranged sequentially as follows: (1) Epidermal hairs, which function in protection and help reduce water loss. (2) The cork layer, composed of tightly packed cells with suberized walls, protecting the inner tissues from mechanical damage and pathogen invasion. (3) The cork cambium, a meristematic layer responsible for producing cork outward and cortical tissues inward. (4) The cortex, including chlorenchyma and cortical parenchyma, consisting of relatively large, thin-walled cells arranged with intercellular spaces; these tissues function in photosynthesis (in young stems), storage, and internal transport. (6) The secondary phloem, located inward from the cortex, composed of thin-walled living cells responsible for transporting photosynthetic products. (7) Laticifers and xylem vessels, scattered within the phloem region, are characteristic features of species in the mulberry family (Moraceae); they contain latex and contribute to conduction. (8) Cavities, located within the vascular tissues arranged in a continuous ring; the central part of the stem consists of a large pith region, where cavities or intercellular spaces can be observed, associated with storage and internal aeration.

Overall, the anatomical features observed in this transverse section confirm the typical stem structure of *Ficus hispida* , with well-developed secondary tissues and the presence of laticifers characteristic of the mulberry family (Moraceae).

3.3 Evaluation of Antioxidant Activity

The antioxidant activity of the wormwood ethanol extract was assessed using the DPPH assay. The results demonstrated a clear dose-dependent relationship between extract concentration and the percentage of free radical scavenging (Figure 1).

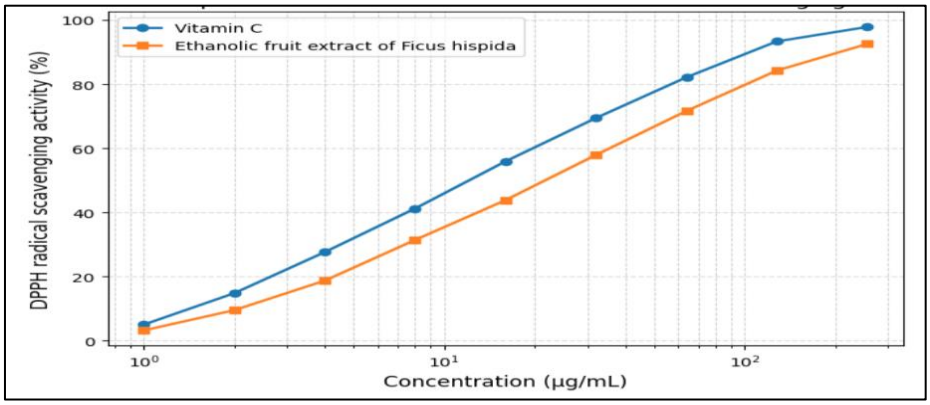


Figure 4 Graph illustrating the relationship between extract concentration and DPPH radical scavenging (%) of *Ficus hispida* L.f. leaf extract and Vitamin C.

The present study demonstrated that the ethanolic fruit extract of *Ficus hispida* L.f. exhibited an IC_{50} value of 20.36 µg/mL in the DPPH free radical scavenging assay, whereas the reference standard, vitamin C, showed an IC_{50} of 12.71 µg/mL under the same conditions. Based on the commonly accepted antioxidant activity classification — where IC_{50} < 50 µg/mL is considered strong or very strong, 50–100 µg/mL moderate, and >100 µg/mL weak — the *Ficus hispida* fruit extract can be categorized as possessing strong antioxidant activity, although its activity does not surpass that of vitamin C. Furthermore, previous research has documented significant antioxidant potential in extracts from *Ficus hispida* fruits in DPPH assays, suggesting this species as a promising source of natural antioxidants.

It is important to note that the DPPH assay principally reflects direct free radical scavenging ability and does not fully characterize the overall antioxidant mechanisms or the relative superiority of botanical extracts over well-established standards such as vitamin C. Comprehensive biochemical assays (e.g., ABTS, FRAP, ORAC) and profiling of major bioactive compounds — including flavonoids, phenolic acids, and other phytochemicals — are necessary to elucidate the mechanistic basis of the observed antioxidant effects in *Ficus hispida* fruit extracts [13].

4 Conclusion

The results of morphological and anatomical analyses showed that *Ficus hispida* L.f. clearly exhibits the fundamental characteristics of dicotyledonous plants, including well-developed secondary tissues, open collateral vascular bundles, abundant characteristic laticifers, and distinctly differentiated palisade and spongy mesophyll in the leaves. Evaluation of the antioxidant activity of the ethanolic fruit extract using the DPPH assay demonstrated significant free radical scavenging capacity, with an IC_{50} value of 20.36 µg/mL, comparable to that of vitamin C (IC_{50} = 12.71 µg/mL). These findings further strengthen the scientific basis for the application and utilization of *Ficus hispida* fruits as a natural antioxidant source in pharmaceutical and functional food development.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All the authors hereby declare that no conflicts of interest exist related to this article.

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