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# Anatomical characterization and evaluation of antioxidant activity of *Ficus auriculata* Lour. In Việt Nam

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## Abstract

*Ficus auriculata* (Moraceae), commonly known as "Vả" in Vietnam, is widely distributed in tropical and subtropical regions of Asia and has long been used in traditional medicine and as a functional food source. 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 Province. Morphological and anatomical analyses were performed using standard botanical methods and light microscopic observations. The results showed that *Ficus auriculata* possesses typical dicotyledonous characteristics, including well-developed secondary tissues, collateral vascular bundles, abundant laticifers, and clearly differentiated palisade and spongy mesophyll tissues in the leaf. The antioxidant activity of the ethanolic fruit extract was evaluated using the DPPH free radical scavenging assay. The extract exhibited strong antioxidant activity with an $IC_{50}$ value of 22.45 µg/mL, compared with 12.71 µg/mL for vitamin C. These findings suggest that the fruits of *Ficus auriculata* are a promising natural source of antioxidant compounds with potential applications in pharmaceutical and functional food industries.

**Keywords:** Anatomy; Antioxidant activity; DPPH assay; *Ficus auriculata*; Morphology

## 1. Introduction

*Ficus auriculata*, commonly known as the Roxburgh fig or "Vả" in Vietnam, belongs to the family Moraceae and is widely distributed in tropical and subtropical regions of Asia, including Vietnam, India, Nepal, Bangladesh, China, and Thailand [1]. The species commonly grows in humid forests, along streams, and in mountainous regions. In several Asian countries, the fruits and young leaves are traditionally consumed as food and used in folk medicine for improving digestion and supporting general health [2].

The tree of *Ficus auriculata* is characterized by its large cordate leaves, short trunk, and clustered syconium fruits that develop directly on the trunk and major branches. The fruits are globose to pear-shaped and become reddish-purple when ripe [1]. Previous phytochemical studies demonstrated that the fruits and stem bark of the species contain phenolics, flavonoids, tannins, triterpenoids, and sterols, which are associated with antioxidant, antimicrobial, anti-inflammatory, and other biological activities [2], [3].

Several in vitro studies reported that extracts from *Ficus auriculata* exhibited strong antioxidant activity through DPPH and ABTS radical scavenging assays, which positively correlated with total phenolic and flavonoid contents [3], [4]. In addition, optimized extraction of the fruit showed high levels of phenolic compounds and reducing power activity, suggesting its potential application as a natural antioxidant source in pharmaceutical and functional food industries [3]. Comparative studies on different drying methods also revealed that the antioxidant and phenolic contents of the fruits were significantly influenced by processing conditions [4].

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Metabolomic investigations using 1H-NMR and UHPLC-MS/MS further identified diverse phytochemical constituents in different fractions of *Ficus auriculata* fruits, including phenolic acids, flavonoids, and other bioactive metabolites that may contribute to its medicinal properties [5]. Moreover, recent studies demonstrated that the fruits possessed considerable digestive bioaccessibility and potential cytotoxic and antioxidant activities, supporting their application in functional food and pharmaceutical industries [6].

However, studies focusing on the morphological and anatomical characteristics of the roots, stems, and leaves, together with antioxidant evaluation of fruit extracts collected in Vietnam, remain limited. 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 auriculata*, thereby providing scientific evidence for the sustainable utilization and development of this species in pharmaceutical and functional food applications.

## 2. Materials and methods

### 2.1. Materials

Fresh fruits of *Ficus auriculata* were harvested in March 2026 from naturally growing plants in Thai Nguyen province, Viet Nam, and subsequently used for ethanol extraction and antioxidant analysis. Additional samples including roots, stems, and leaves were simultaneously collected from the same population to investigate morphological and anatomical characteristics.

The collected plant materials were authenticated by Assoc. Prof. Dr. Sỹ Danh Thường, Thai Nguyen University of Education – Thai Nguyen University, through comparative evaluation of diagnostic morphological features. Representative voucher specimens were preserved and archived at the Biological Museum, Faculty of Biology, Thai Nguyen University of Education, under the reference code TNUE-FA2026-01.

### 2.2. Methods

#### *2.2.1. Sample collection and preparation*

Samples of *Ficus auriculata*, including roots, stems, leaves, and fruits, were collected during periods of dry weather to minimize excess moisture contamination. After harvesting, all materials were rinsed carefully with clean water to eliminate adhering soil and foreign particles, followed by natural air-drying at room temperature. The prepared samples were then preserved in 90% ethanol and kept under well-ventilated laboratory conditions prior to morphological, anatomical, and phytochemical analyses.

Fruit materials were further processed for ethanol extraction according to the procedure reported by Nguyen Nghia Thin (2008) [7].

#### *2.2.2. Methods for morphological and anatomical studies*

Morphological analysis: The external morphology of *Ficus auriculata* was examined using conventional botanical classification methods in accordance with the descriptions provided by Tran Van Ba and Hoang Thi San (1998) [8] and Hoang Thi San (2002) [9]. Observations were conducted on vegetative and reproductive organs, including roots, stems, leaves, and fruits, in order to identify characteristic taxonomic features and document the general morphology of the species.

Anatomical analysis: Fresh roots, stems, and leaves were sectioned manually to obtain temporary microscopic slides for anatomical observation under a light microscope. The internal structures of each organ were carefully examined and comparatively described following the methods proposed by Tran Van Ba and Hoang Thi San (1998) [8] and Kixeleva (1998) [10]. Particular attention was paid to the arrangement of tissues, secretory elements, vascular organization, and other distinctive anatomical characteristics of the species.

#### *2.2.3. Microscopic imaging method*

Prepared anatomical sections were examined under a STE-1 CAM optical microscope (China) integrated with Motic imaging software. Micrographs were obtained at various magnification levels to support detailed observation, documentation, and analysis of the anatomical features of the roots, stems, and leaves.

*2.2.4. Preparation of ethanol extract*

Preparation and ethanol extraction of *Ficus auriculata* fruits: Fresh fruits were cleaned thoroughly with water to eliminate adhering impurities and dust particles, followed by natural air-drying. The materials were subsequently dried in a hot-air oven at 50 °C for 48 h until a constant weight was achieved. The dried fruits were pulverized into fine powder prior to extraction.

For extraction, 10 g of fruit powder was immersed in 30 mL of 90% ethanol and subjected to ultrasonic-assisted maceration for 72 h. The obtained mixture was filtered using Whatman No.1 filter paper (Merck, Germany). The filtrate was then concentrated under reduced pressure with a Rotavapor R100 system (Buchi, Switzerland) to yield the crude ethanol extract. The extract samples were preserved at 4 °C until further phytochemical and antioxidant analyses were performed [11].

*2.2.5. Determination of antioxidant activity of Ficus auriculata fruit extract by the DPPH free radical scavenging assay*

Determination of antioxidant activity by DPPH radical scavenging assay: The antioxidant potential of the fruit extract obtained from *Ficus auriculata* was evaluated using the DPPH free radical scavenging method according to the procedure of Tabart et al. (2009) [12]. Different concentrations of the extract (0.5, 1, 2, 4, 8, 16, 32, 64, and 128 µg/mL) were prepared, and 100 µL of each concentration was mixed with 2.9 mL of freshly prepared 0.1 mM DPPH solution in methanol.

The mixtures were shaken thoroughly using a vortex mixer and incubated at ambient temperature in dark conditions for 30 min to allow the reaction to occur completely. Absorbance values were subsequently recorded at 517 nm using a Shimadzu UV–Vis 1800 spectrophotometer. The free radical scavenging capacity was calculated according to the following formula: DPPH scavenging activity (%) = 100 x (Ac - As)/Ac.

where AcAc represents the absorbance of the control reaction and AsAs corresponds to the absorbance of the tested extract. Antioxidant activity was expressed as the IC50 value, indicating the concentration of extract required to neutralize 50% of DPPH radicals.

## 3. Results and discussion

### 3.1. Morphological characteristics of *Ficus auriculata* in Thai Nguyen Province

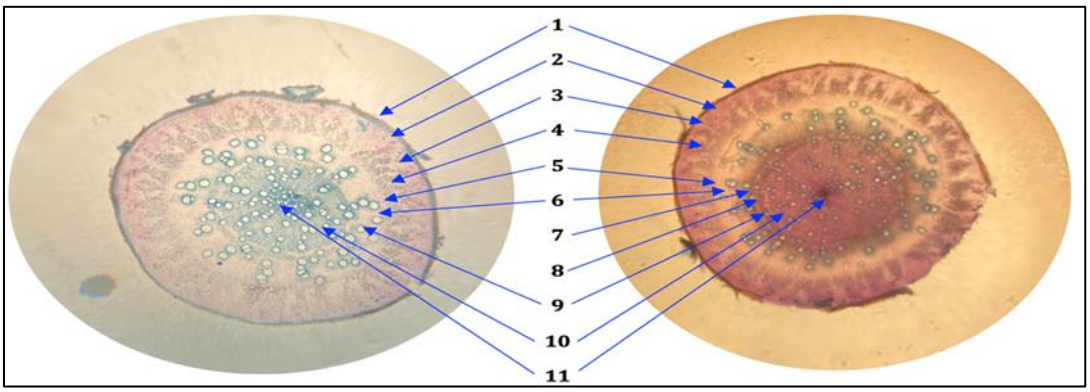
The morphological observations indicated that *Ficus auriculata* (commonly known as "Vả" in Vietnam), belonging to the family Moraceae, is a medium-sized woody tree widely distributed in tropical and subtropical regions. In the investigated area, mature individuals commonly reached heights of 5–12 m, with a broad canopy and short trunk. The bark surface was grayish-brown to dark brown, relatively smooth in young plants and becoming slightly fissured with age. Similar to many members of the genus *Ficus*, injured tissues exuded abundant white latex. Young branches were thick, greenish-brown, and sparsely pubescent when immature, gradually becoming glabrous during development. Leaves were simple, alternate, and notably large, broadly ovate to suborbicular in shape. The leaf blade measured approximately 15–35 cm in length and 12–30 cm in width, with a cordate base, entire margin, and obtuse to shortly acuminate apex. The adaxial surface was dark green and slightly rough, whereas the abaxial surface was paler and prominently veined. Venation was pinnate with several pairs of conspicuous lateral veins originating from the midrib. Petioles were stout and densely pubescent, ranging from 5–12 cm in length. Stipules were large, lanceolate, and caducous, leaving distinct scars after abscission [13], [1]. The syconia developed in clusters directly on the trunk and older branches (cauliflorous habit), which is considered one of the characteristic features of the species. Young fruits were green and pubescent, gradually turning reddish, orange-red, or purplish-red upon maturation. Mature fruits were depressed-globose to pyriform, approximately 3–6 cm in diameter, with a fleshy receptacle containing numerous small seeds [13], [14]. The species is monoecious, bearing both male and female flowers within the same syconium. Floral structures are highly reduced and enclosed within the fleshy receptacle, consistent with the typical reproductive morphology of the genus *Ficus* [13].

Comparison of the collected specimens with previous taxonomic descriptions provided by Corner (1965) [1] and Pham Hoang Ho (2000) [13] demonstrated a high degree of agreement in vegetative and reproductive characteristics, including leaf morphology, fruit arrangement, pubescence, and cauliflorous fruiting habit. These observations confirmed that the investigated samples possessed the typical morphological features of *Ficus auriculata* under the ecological conditions of Thai Nguyen Province.

### 3.2. Evaluation of Antioxidant Activity

*3.2.1. Anatomical characteristics of the root*

The transverse anatomical structure of the root of *Ficus auriculata* is organized into distinct tissue regions extending from the outer protective layers toward the central vascular tissues, 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).

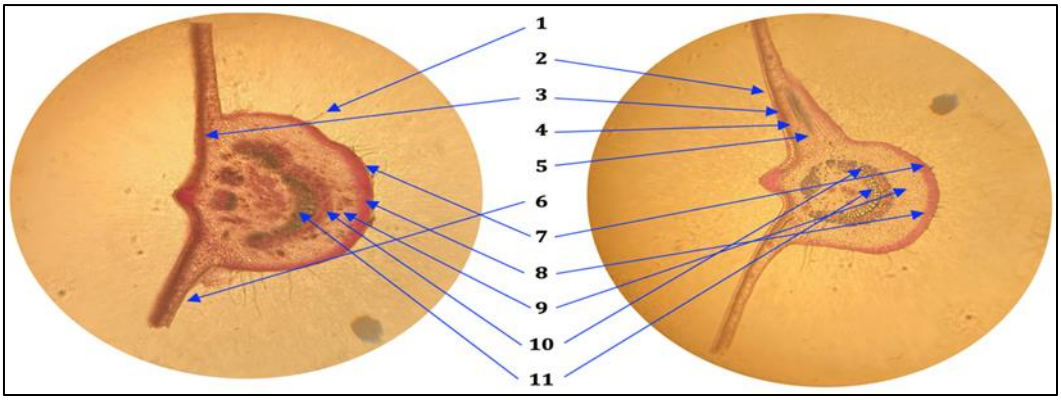
**Figure 1** Transverse section of the root anatomy of *Ficus auriculata*

The outermost region of the root consists of the cork layer (1), composed of several rows of compactly arranged suberized cells that function as a protective barrier against mechanical injury and water loss. Beneath the cork lies the cork cambium (2), a secondary meristematic tissue formed by small, thin-walled living cells capable of tangential division. The derivatives produced outward differentiate into cork cells, whereas the inward derivatives contribute to the cortical tissues. The cortex (3) is composed mainly of large parenchymatous cells loosely arranged with noticeable intercellular spaces. Numerous laticifers (4), characteristic secretory structures commonly found in members of the Moraceae family, are distributed throughout the cortical region and contain milky latex. In some areas, groups of sclerenchymatous cells (7) with thick lignified walls are present, contributing to the mechanical support and rigidity of the root structure. Toward the inner region, the vascular tissues are clearly differentiated. The vascular cambium (5) appears as a narrow layer of actively dividing meristematic cells situated between the secondary phloem and secondary xylem. Secondary phloem (9), located external to the cambium, is composed of thin-walled living cells arranged compactly and responsible for the transport of organic nutrients. Secondary xylem (6), produced inward by the cambium, occupies a large proportion of the root and contains numerous well-developed vessel elements (8) specialized for water and mineral conduction. At the center of the root lies the pith region (10, 11), consisting of large thin-walled parenchyma cells involved primarily in storage functions.

The overall anatomical organization of the root demonstrates the typical secondary growth pattern of dicotyledonous plants, characterized by open collateral vascular bundles and a well-developed cambial zone. Similar anatomical characteristics have previously been reported for several species of the genus *Ficus* and other representatives of the family Moraceae [15], [16].

*3.2.2. Anatomical characteristics of the leaf*

The transverse section of the leaf of *Ficus auriculata* revealed a relatively typical dicotyledonous structure, with tissues clearly differentiated from the outermost layer inward, corresponding to the annotations shown in Figure 2.




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 auriculata*

The outer surface of the leaf is covered by numerous epidermal hairs (1), which function in reducing water loss and protecting the leaf against environmental stress. Beneath this layer is the upper epidermis (2), composed of a single layer of compactly arranged rectangular cells covered externally by a thin cuticular layer. The epidermal cells generally lack chloroplasts and primarily function in protection. Directly below the upper epidermis is the palisade mesophyll (3), consisting of one to two layers of elongated cylindrical cells arranged closely together and containing abundant chloroplasts, representing the principal photosynthetic tissue of the leaf. Toward the lower portion of the lamina lies the spongy mesophyll (5), formed by irregularly shaped parenchymatous cells loosely distributed with large intercellular spaces that facilitate gaseous exchange. These cells also contain chloroplasts, although less densely than those of the palisade tissue. Embedded within the mesophyll are collateral vascular bundles (4), in which xylem occupies the upper position while phloem (11) is oriented toward the lower epidermis. The vascular bundles are surrounded by mechanical supporting tissues, including collenchyma and sclerenchyma cells (8, 10), contributing to the structural rigidity of the leaf. The midrib region contains well-developed parenchyma tissue (9), composed of large thin-walled cells involved in storage and tissue connection. The lower epidermis (6) consists of tightly arranged cells interspersed with numerous stomata responsible for transpiration and gas exchange. Similar anatomical characteristics have been reported for species of the genus *Ficus*, particularly regarding the differentiation of mesophyll tissues, the presence of laticiferous structures, and the arrangement of collateral vascular bundles in leaves of Moraceae members [15], [3].

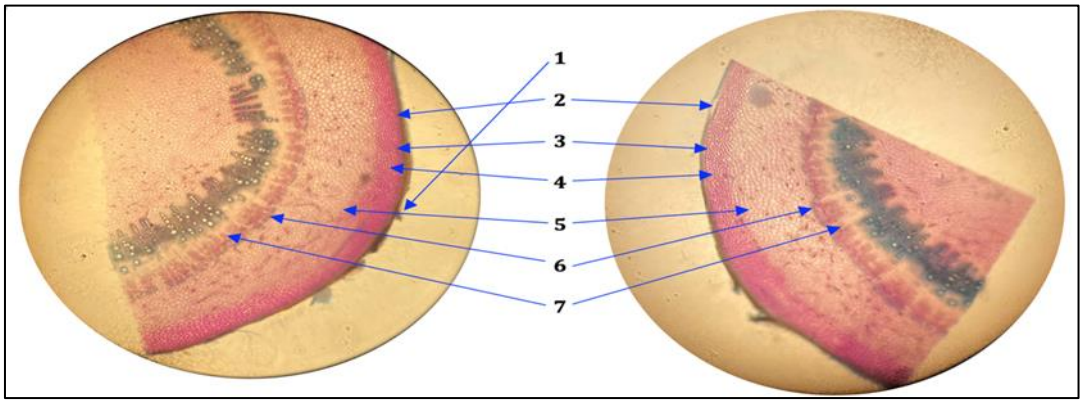
Overall, the leaf anatomy of *Ficus auriculata* exhibits the characteristic features of dicotyledonous plants, including a differentiated mesophyll system and a well-organized vascular tissue arrangement.

*3.2.3. Anatomical characteristics of the stem*

The transverse section of the stem of *Ficus auriculata* exhibits a well-developed secondary structure characteristic of dicotyledonous plants, as illustrated in Figure 3. The tissues are distinctly differentiated and arranged concentrically from the outermost region toward the center of the stem.

The outer surface is covered with epidermal hairs (1), which serve protective functions and contribute to minimizing water loss. Beneath this layer is the cork tissue (2), consisting of several layers of compactly arranged suberized cells that protect the stem against environmental stress and pathogen invasion. The cork cambium (3) is situated immediately below the cork and acts as a lateral meristem responsible for producing cork cells outward and secondary cortical tissues inward. The cortex (4) is composed mainly of chlorenchyma and cortical parenchyma cells. These cells are relatively large, thin-walled, and loosely arranged with evident intercellular spaces. In young stems, chlorenchyma cells may contain chloroplasts and participate in photosynthesis, whereas cortical parenchyma primarily functions in storage and short-distance transport. Laticifers containing milky latex are scattered throughout the cortical and vascular regions, representing a typical anatomical characteristic of species belonging to the family Moraceae [16]. Toward the inner region, the vascular tissues form a continuous ring. The secondary phloem (6) consists of living thin-walled cells responsible for the translocation of photosynthetic products. Adjacent to the phloem is the vascular cambium, which continuously generates secondary phloem outward and secondary xylem inward during secondary growth. The secondary xylem contains numerous vessel elements and lignified fibers involved in water conduction and mechanical support. Laticifers and xylem vessels (7) are clearly visible within the vascular region and contribute to

latex secretion and conduction functions. In the central part of the stem, the pith region contains large parenchymatous cells and distinct cavities or intercellular spaces (8), which are associated with storage and internal aeration.




1. Epidermal hair; 2. Cork; 3. Cork cambium; 4. Chlorenchyma and cortical parenchyma; 6. Secondary phloem; 7. Laticifer and xylem vessel.

(Observed at 200× magnification)

**Figure 3** Transverse section of the stem of *Ficus auriculata*

Overall, the stem anatomy of *Ficus auriculata* demonstrates the typical features of woody dicotyledonous plants, including extensive secondary growth, collateral vascular organization, and the presence of characteristic laticiferous structures commonly reported in members of the Moraceae family [15], [16].

### 3.3. Evaluation of Antioxidant Activity

The antioxidant activity of the ethanolic fruit extract obtained from *Ficus auriculata* was evaluated using the DPPH free radical scavenging assay. The results demonstrated a clear dose-dependent relationship between extract concentration and the percentage of DPPH radical scavenging activity, in which antioxidant activity increased progressively with increasing extract concentration (Figure 4).

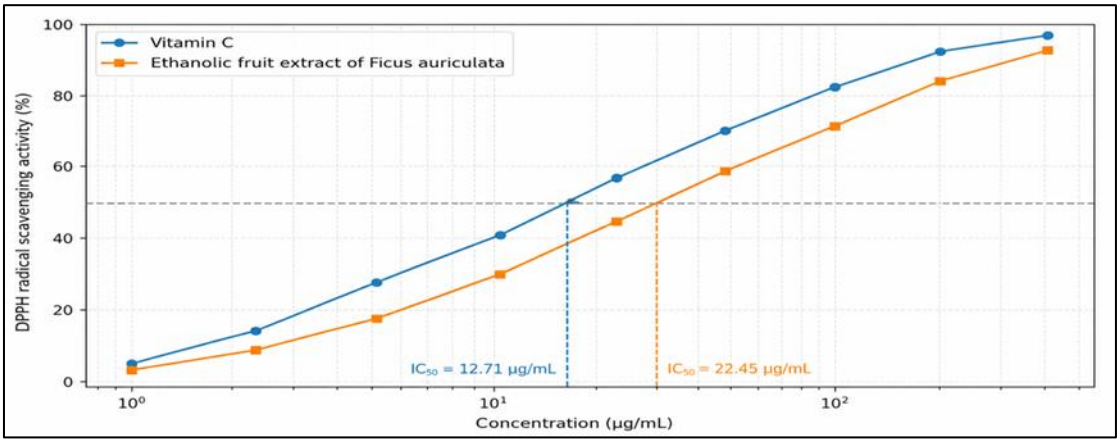



**Figure 4** Graph illustrating the relationship between extract concentration and DPPH radical scavenging (%) of *Ficus auriculata* leaf extract and Vitamin C

The present study demonstrated that the ethanolic fruit extract of *Ficus auriculata* exhibited strong antioxidant activity with an $IC_{50}$ value of 22.45 µg/mL in the DPPH free radical scavenging assay, whereas the reference antioxidant standard, vitamin C, showed a lower $IC_{50}$ value of approximately 12.71 µg/mL under comparable experimental conditions. According to the commonly accepted classification of antioxidant activity, extracts with $IC_{50}$ values below 50 µg/mL are considered to possess strong or very strong antioxidant capacity. Therefore, the ethanolic fruit extract of *Ficus auriculata* can be classified as a potent natural antioxidant source, although its activity remains lower than that of vitamin C.

The obtained results are consistent with previous studies reporting remarkable antioxidant potential in fruit extracts of *Ficus auriculata*, particularly in ethanolic and methanolic extracts rich in phenolic and flavonoid compounds. Shahinuzzaman et al. (2021) reported that optimized ethanolic fruit extracts exhibited an $IC_{50}$ value of 22.45 ± 0.18 µg/mL, which strongly correlated with high total phenolic content and total flavonoid content [3]. In addition, Basumatary et al. (2021) demonstrated that antioxidant activity varied depending on drying and extraction conditions, further supporting the importance of phytochemical composition in determining free radical scavenging capacity [4].

It should be noted that the DPPH assay primarily reflects the hydrogen-donating or direct radical scavenging ability of antioxidant compounds and may not fully represent all antioxidant mechanisms occurring in biological systems. Therefore, additional antioxidant assays such as ABTS, FRAP, and ORAC, together with comprehensive phytochemical profiling of flavonoids, phenolic acids, anthocyanins, and related metabolites, are necessary to better elucidate the antioxidant mechanisms and pharmaceutical potential of *Ficus auriculata* fruit extracts.

## 4. Conclusion

The present study clarified the morphological and anatomical characteristics of *Ficus auriculata*, including typical dicotyledonous structures and the presence of characteristic laticiferous tissues of the Moraceae family. In addition, the ethanolic fruit extract exhibited strong antioxidant activity in the DPPH assay with an $IC_{50}$ value of 22.45 µg/mL, indicating that the species is a promising natural source of antioxidant compounds for potential pharmaceutical and functional food applications.

## 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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