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(RESEARCH ARTICLE)

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Anatomical characterization and evaluation of antioxidant activity of *Ficus racemosa* L. in Thai Nguyen, Vietnam

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Abstract

Ficus racemosa L. (Moraceae) is a tropical plant widely distributed in Asia and traditionally used in herbal medicine. This study aimed to investigate the morphological and anatomical characteristics of *Ficus racemosa* and evaluate the antioxidant activity of its fruit extract collected in Thai Nguyen Province, Vietnam. Morphological observations showed that the plant is a medium-sized tree with alternate ovate to elliptic leaves and fruits growing in clusters on the trunk or large branches. Anatomical analysis of the root, stem, and leaf revealed typical dicotyledonous structures, including clearly differentiated phloem and xylem forming collateral vascular bundles. The stem also contained characteristic laticifers, while the leaf exhibited a dorsiventral structure with well-developed palisade and spongy mesophyll layers. The antioxidant activity of the ethanolic fruit extract was evaluated using the DPPH free radical scavenging assay. The results showed a concentration-dependent increase in DPPH inhibition, with an IC_{50} value of 14.02 µg/mL compared with 3.13 µg/mL for Vitamin C. These findings indicate that *Ficus racemosa* fruit possesses considerable antioxidant potential. The results provide a scientific basis for further phytochemical and pharmacological studies as well as potential applications of this species as a natural

Keywords: *Ficus racemosa*; Plant Anatomy; Antioxidant activity; DPPH assay; Thai Nguyen

1 Introduction

Ficus racemosa L., commonly known as cluster fig or country fig, belongs to the family Moraceae. It is a medium-sized tree widely distributed in tropical and subtropical regions of Asia, including Vietnam. The fruits of *Ficus racemosa* L. grow in clusters on the trunk or old branches, with a spherical to ovoid shape. When immature, the fruits are green and turn reddish-brown upon ripening. The fruit surface bears short hairs, and the flesh is spongy, containing numerous small seeds. In traditional medicine, the fruits of *Ficus racemosa* L. have been used to treat digestive disorders, diarrhea, sore throat, hemorrhoids, skin diseases, and to support the management of diabetes [1], [2]. Recent studies on the genus Ficus indicate that this group of plants is rich in chemical constituents, including flavonoids, phenolic compounds, tannins, triterpenoids, sterols, alkaloids, and saponins. These compounds possess notable biological activities such as antioxidant, anti-inflammatory, antimicrobial, and antihyperglycemic effects [3], [4]. In particular, *Ficus racemosa* has been reported to contain phenolic and flavonoid compounds with strong free radical scavenging capacity, contributing to cellular protection against oxidative stress [5]. Numerous in vitro and in vivo studies have demonstrated that extracts from the fruits of *Ficus racemosa* L. exhibit significant antioxidant activity, as evidenced by their ability to scavenge DPPH and ABTS free radicals and inhibit lipid peroxidation [6]. In addition, fruit extracts of *Ficus racemosa* L. have shown inhibitory activity against the enzyme α-glucosidase, thereby reducing postprandial glucose absorption and indicating potential applications in supporting diabetes treatment [7].

According to traditional Indian medicine (Ayurveda) and folk experience in Vietnam, the fruits of *Ficus racemosa* L. are used in fresh, dried, or concentrated extract forms to treat digestive disorders, infections, and to improve general health. However,

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scientific studies on the anatomical characteristics of the roots, stems, and leaves, as well as the antioxidant activity of *Ficus racemosa* fruit in Vietnam, remain very limited. Therefore, in this study, we investigated the morphological and anatomical characteristics of the roots, stems, and leaves, and evaluated the antioxidant activity of extracts from the fruits of *Ficus racemosa* L.. The results aim to provide a scientific basis for the exploitation, utilization, and development of products derived from *Ficus racemosa* fruit in the fields of medicine and functional foods.

2 Materials and methods

2.1 Materials

The material used for ethanol extract preparation was the fruit of *Ficus racemosa* L. The fruit samples were collected in Phan Đình Phùng Ward, Thái Nguyên Province, Vietnam. The plant specimen was identified by Assoc. Prof. Dr. Sỹ Danh Thường, Thai Nguyen University of Education, based on morphological botanical characteristics during the sampling process. The samples and voucher specimens were prepared and deposited at the Biological Museum, Faculty of Biology, Thai Nguyen University of Education, under the voucher number TNUE-FR2025-01.

2.2 Methods

2.2.1 Sample collection and preparation

Samples of the fruits (roots, stems, leaves, and fruits) of *Ficus racemosa* L. were collected under dry weather conditions. After collection, the samples were washed thoroughly with water and allowed to drain. They were then preserved in 90% ethanol and stored in the laboratory in a cool and well-ventilated place for further study and preparation of ethanol extracts, following the method described by Nguyễn Nghĩa Thìn (2008) [8].

2.2.2 Methods for morphological and anatomical studies

Morphology: The morphological characteristics of *Ficus racemosa* L. were observed, analyzed, and described based on plant taxonomic criteria according to the references by Trần Văn Ba and Hoàng Thị Sản (1998) [9], as well as Hoàng Thị Sản(2002) [10]. The description was carried out based on the typical characteristics of vegetative and reproductive organs to ensure accuracy in species identification.

Anatomy: Roots, stems, and leaves were processed to prepare temporary anatomical slides and then observed under a light microscope. The histological structures of these organs were recorded, analyzed, and described following the methods of Trần Văn Ba and Hoàng Thị Sản (1998) [9] and Kixeleva (1998) [11].

2.2.3 Microscopic imaging method

The anatomical slides were observed and photographed using a STE-1 CAM optical microscope (China) in combination with Motic microscopy software. Micrographs were captured at a magnification of 200X for the analysis and description of histological structural characteristics.

2.2.4 Preparation of ethanol extract

The fruits of *Ficus racemosa* L. were washed thoroughly after collection, drained, and dried at 50 °C for 48 hours until completely dry. The dried samples were then ground into a fine powder for extraction. The extraction process was carried out using a maceration method combined with ultrasonic treatment, with a ratio of 10 g of fruit powder in 30 mL of 90% ethanol. The extraction was performed for three days to obtain the extract solution. The extract was then filtered through Whatman No.1 filter paper (Merck, Germany). The filtrate was subsequently concentrated using a Rotavapor R100 rotary vacuum evaporator (Buchi, Switzerland) to obtain the ethanol crude extract. The extract was stored at 4°C for subsequent experiments [12].

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

The antioxidant activity of the fruit extract of *Ficus racemosa* L. was determined using the DPPH free radical scavenging assay following the procedure described by Tabart et al. (2009) [13]. Specifically, 100 µL of the extract at concentrations of 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 µg/mL was added to test tubes, followed by the addition of 2.9 mL of 0.1 mM DPPH solution in methanol. The mixture was thoroughly mixed and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis 1800 spectrophotometer (Shimadzu). The percentage of DPPH radical scavenging was calculated using the following equation: DPPH (%) = 100 × (Ac − As) / Ac where Ac is the absorbance of the control sample and As is the absorbance of the test sample. The antioxidant activity

of the extract was expressed as the EC_{50} value, which represents the concentration required to neutralize 50% of DPPH free radicals.

3 Results and discussion

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

Field surveys conducted in Thai Nguyen Province recorded the fig tree, *Ficus racemosa* L. (family Moraceae), as a medium-sized woody species reaching approximately 10–15 m in height. The trunk is generally straight and branches profusely, forming a broad canopy. The bark ranges from grey-brown to reddish-brown in color with a slightly rough surface, and exudes a white latex when injured. Leaves are arranged alternately; the leaf blade is ovate to elliptic-ovate with an entire margin, averaging 6–15 cm in length and 3.5–6 cm in width, while the petiole is approximately 1–5 cm long. The leaf surface is relatively smooth, with veins that are particularly prominent on the abaxial side. Fruits develop in clusters directly on the trunk or on large branches, forming a typical fig-type structure (syconium) that is nearly spherical to slightly pear-shaped, with a diameter of approximately 2–3 cm. Immature fruits are green and gradually turn orange-red to dark red when fully ripe.

The morphological characteristics observed in this study are generally consistent with previous descriptions of *Ficus racemosa*. According to Kumar et al. (2021)[14], this species possesses ovate to elliptic leaves of similar size and is characterized by the cauliflorous habit, in which fruits develop directly on the trunk. Likewise, Marcello Wiart (2021) [15] reported that the fruits of F. racemosa occur in clusters on the trunk and major branches, typically measuring around 2–3 cm in diameter at maturity. Recent reviews on the biological and medicinal properties of this species also describe comparable features of leaves, stems, and fruit morphology, indicating a strong agreement between the specimens observed in Thai Nguyen and previously published scientific descriptions (International Journal of Pharmaceutical Sciences Review and Research, 2024) [16].

3.2 Evaluation of Antioxidant Activity

3.2.1 Anatomical characteristics of the root

The transverse anatomical structure of the root of *Ficus racemosa* L. is described from the outermost to the innermost layers as shown in Figure 1.

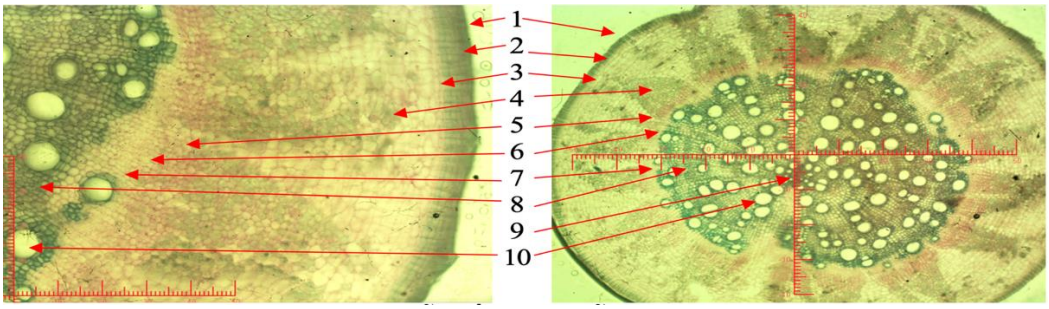

Figure 1 Transverse anatomical structure of the root of *Ficus racemosa* L.

1. Cork; 2. Cork cambium; 3. Chlorenchyma and cortical parenchyma; 4. Secretory cells; 5. Sclerenchyma ring; 6. Phloem; 7. Vascular cambium; 8. Xylem; 9. Pith; 10. Xylem vessel lumen. (Image observed at 200× magnification).

1. Cork: Consists of several layers of tightly arranged cells with suberized walls that stain blue and function as a protective tissue; 2. Cork cambium: Composed of living cells, small in size, with thin cellulose walls. The cells divide tangentially; the outer side forms cork cells, while the inner side produces cortical parenchyma; 3. Chlorenchyma and cortical parenchyma: Consist of multiple layers of relatively large cells arranged loosely, forming distinct intercellular spaces; 4. Secretory cells: Scattered within the cortical parenchyma, larger than surrounding cells and containing characteristic secretory substances; 5. Sclerenchyma ring (lignified sclerenchyma): Composed of cells with thick, lignified walls that stain with methylene blue; 6. Secondary phloem: Formed outside the vascular cambium, consisting of polygonal cells with thin walls, closely arranged and stained pink with carmine; differentiation occurs centripetally; 7. Vascular cambium (phloem–xylem cambium): Composed of living, slightly elongated rectangular cells dividing tangentially; the inner side produces secondary xylem while the outer side forms secondary phloem; 8. Secondary xylem: Differentiates centrifugally; xylem vessels vary in size and are arranged in relatively continuous masses; 9. Pith: Consists of large parenchyma cells with thin walls; 10. Xylem vessel lumen: Hollow cavities of conducting vessels in the

secondary xylem, varying in size, lightly stained, and responsible for transporting water and mineral salts from the roots to the stem.

Thus, in the secondary structure of the root of *Ficus racemosa*, the phloem and xylem are arranged in an open collateral vascular bundle, which is characteristic of roots undergoing secondary growth.

3.2.2 Anatomical characteristics of the leaf

The transverse anatomical structure of the leaf of *Ficus racemosa* L., from the outermost to the innermost layers, includes the parts labeled in Figure 2.

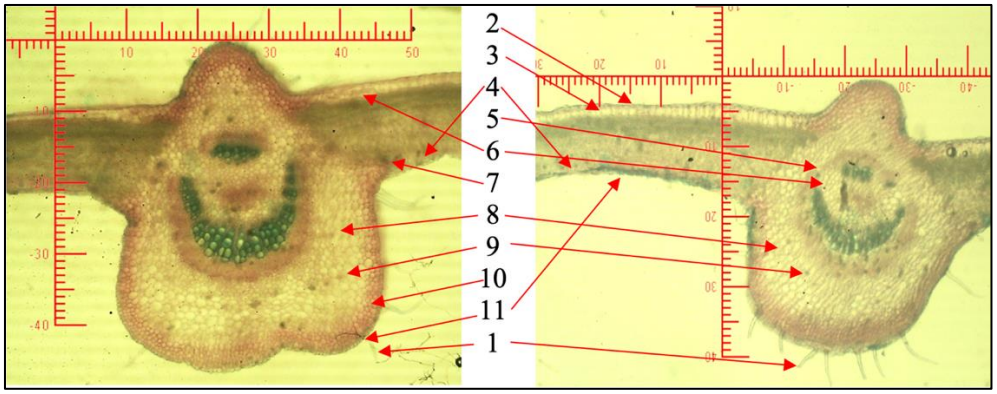

Figure 2 Transverse anatomical structure of the leaf of *Ficus racemosa* L.

1. Epidermal hairs; 2. Upper epidermis; 3. Palisade mesophyll; 4. Spongy mesophyll; 5. Sclerenchyma ring; 6. Phloem; 7. Xylem; 8. Ground parenchyma of the leaf vein; 9. Secretory cells; 10. Collenchyma; 11. Lower epidermis. (Image observed at 200× magnification).

Observation of the transverse section shows that the anatomical structure of the leaf of *Ficus racemosa* is clearly organized into characteristic tissue layers. From the outermost to the innermost layers: 1. Epidermal hairs: Distributed on the leaf surface, functioning in protection and reducing water loss; 2. Upper epidermis: Consists of a single layer of rectangular cells arranged closely together, usually lacking chloroplasts; the outer wall is covered with a thick cuticle that protects the leaf and reduces water loss; 3. Palisade mesophyll: Located just beneath the upper epidermis, consisting of 5–7 layers of elongated cylindrical cells arranged relatively closely with narrow intercellular spaces; the cells contain numerous chloroplasts and are mainly responsible for photosynthesis; 4. Spongy mesophyll: Distributed below the palisade tissue and adjacent to the lower epidermis; composed of 4–5 layers of polygonal cells loosely arranged, forming large intercellular spaces favorable for gas exchange; 5. Sclerenchyma ring: Consists of cells with thick, lignified walls that stain with methylene blue; these cells are dead at maturity and are aggregated into nearly continuous clusters forming a supporting ring around the vascular bundle; 6. Phloem: Composed of small, living polygonal cells arranged compactly and stained pink with carmine; located outside the xylem and forming a continuous arc; 7. Xylem: Located inside the phloem, consisting of several layers of cells of different sizes with lignified walls that stain with methylene blue. The xylem and phloem together form a closed collateral vascular bundle, typically arc-shaped in the leaf vein; 8. Ground parenchyma of the leaf vein: Consists of several layers of nearly round cells with thin walls that stain pink with carmine, functioning in storage and tissue connection; 9. Secretory cells: Scattered within the parenchyma, larger than surrounding cells and containing characteristic secretory substances; 10. Collenchyma: Consists of several layers of cells with unevenly thickened cellulose walls, round or polygonal in shape, functioning in mechanical support; 11. Lower epidermis: Composed of a single layer of rectangular cells arranged closely together, usually lacking chloroplasts; numerous stomata are present to facilitate gas exchange and transpiration.

Thus, the anatomical structure of the leaf of *Ficus racemosa* exhibits the characteristics of a dorsiventral leaf, with well-developed palisade mesophyll on the upper side and spongy mesophyll on the lower side. The vascular bundle is a closed collateral bundle, with the phloem located externally and the xylem internally, surrounded and supported by sclerenchyma and collenchyma tissues, contributing to enhanced mechanical support and physiological adaptation of the leaf.

3.2.3 Anatomical characteristics of the stem

The transverse anatomical structure of the stem of *Ficus racemosa* L. is described from the outermost to the innermost layers as shown in Figure 3.

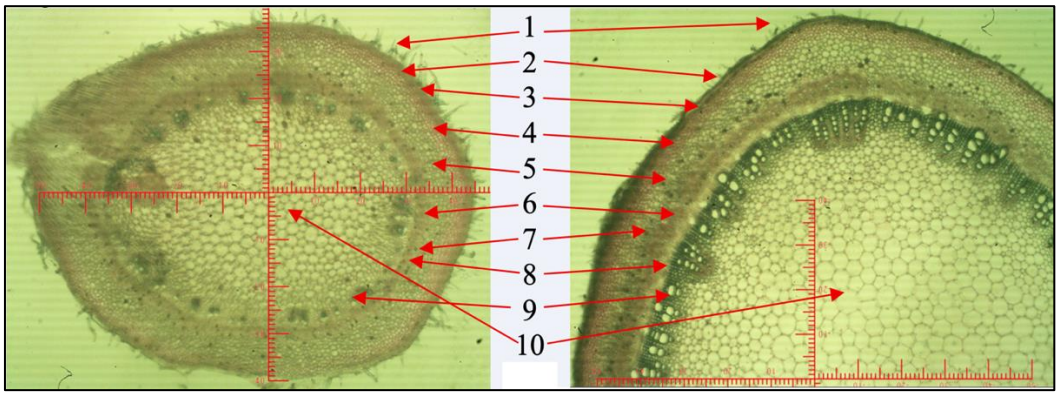

Figure 3 Transverse anatomical structure of the stem of *Ficus racemosa* L.

1. Epidermal hairs; 2. Cork; 3. Cork cambium; 4. Chlorenchyma and cortical parenchyma; 5. Sclerenchyma ring; 6. Secondary phloem; 7. Laticifer (latex tube); 8. Vascular cambium; 9. Secondary xylem; 10. Pith parenchyma. (Image observed at 200× magnification).

Observation of the transverse section shows that the stem structure is clearly differentiated into characteristic tissue layers. From the outermost to the innermost layers: 1. Epidermal hairs: Distributed on the surface of young stems, representing extensions of epidermal cells, functioning in mechanical protection, reducing water loss, and protecting the plant from adverse environmental effects; 2. Cork layer: Consists of dead, flattened cells (the radial diameter smaller than the tangential diameter) with suberized walls; the cells are usually empty and contain no protoplasm. The cells are tightly arranged without intercellular spaces. Cork is impermeable to water and gases and functions as a protective tissue; 3. Cork cambium: Composed of living cells, nearly quadrangular in shape and flattened in the radial direction, capable of dividing tangentially; the outer side forms cork while the inner side forms cortical parenchyma; 4. Chlorenchyma and cortical parenchyma: Consist of relatively large cells arranged loosely, forming distinct intercellular spaces. Chlorenchyma cells contain chlorophyll and are capable of photosynthesis in young stems; they also serve storage functions; 5. Sclerenchyma ring: Composed of cells with thick, lignified walls that stain with methylene blue; these cells are dead at maturity. The cells aggregate into nearly continuous clusters forming a mechanical supporting ring; 6. Secondary phloem: Formed outside the vascular cambium, consisting of living polygonal cells with thin walls, closely arranged and stained pink with carmine; differentiation occurs centripetally; 7. Laticifers (latex tubes): Larger than surrounding parenchyma cells, distributed in the phloem and parenchyma tissues, containing latex characteristic of the mulberry family; 8. Vascular cambium (phloem–xylem cambium): Consists of living, slightly elongated rectangular cells dividing tangentially; the inner side produces secondary xylem while the outer side forms secondary phloem; 9. Secondary xylem: Develops inside the vascular cambium forming a continuous ring of several layers of lignified cells that stain with methylene blue. The xylem includes vessels of different sizes (approximately 12 µm in diameter) interspersed with xylem parenchyma forming perivascular parenchyma; 10. Pith parenchyma: Located in the center of the stem, consisting of polygonal or nearly round cells with unequal sizes, functioning mainly in storage.

Thus, the secondary structure of the stem of *Ficus racemosa* shows a vascular system forming a continuous ring, with xylem located internally and phloem externally, arranged as open collateral vascular bundles, together with the presence of laticifers characteristic of this group of plants.

3.3 Evaluation of Antioxidant Activity

The antioxidant activity of the ethanol extract from the fruit of *Ficus racemosa* L. was determined using the DPPH method to evaluate its ability to neutralize free radicals under in vitro conditions. The results showed that the percentage of DPPH inhibition increased with increasing extract concentration, demonstrating a clear dose-dependent trend (Figure 4).

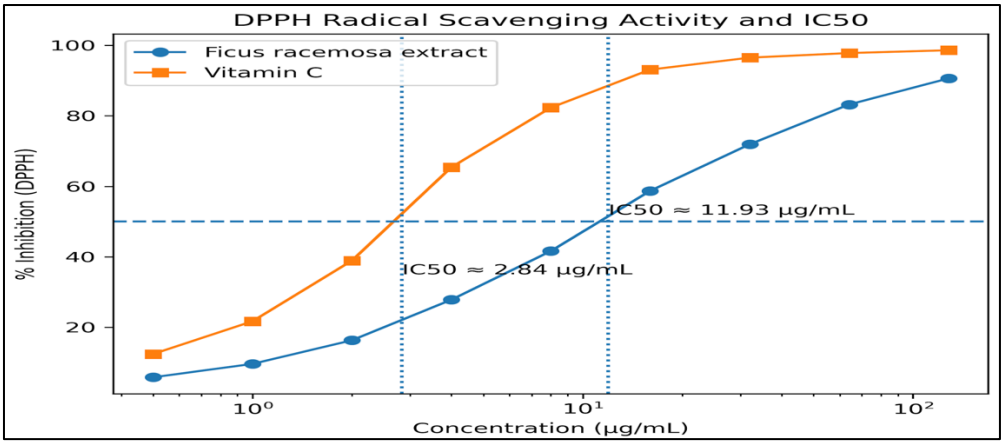

Figure 4 Relationship between extract concentration and DPPH radical scavenging activity (%) of the ethanolic fruit extract of *Ficus racemosa* L. compared with Vitamin C.

The IC_{50} value of the extract was 14.02 µg/mL, while that of the positive control (Vitamin C) was 3.13 µg/mL. According to the DPPH activity classification scale (IC_{50} < 50 µg/mL: strong or very strong; 50–100 µg/mL: moderate; 100–250 µg/mL: weak), the extract can be categorized as having high antioxidant activity. Although its activity was lower than that of Vitamin C, the results still indicate a considerable antioxidant potential of the studied material.

It should be noted that the DPPH assay mainly evaluates the ability of compounds to donate electrons or hydrogen atoms to neutralize stable free radicals, and therefore does not fully represent all antioxidant mechanisms occurring in biological systems. The observed activity may be associated with the presence of secondary metabolites such as polyphenols, flavonoids, anthocyanins, and phenolic acids in the extract. These compounds are capable of stabilizing free radicals and inhibiting lipid peroxidation processes. To further clarify the nature and extent of this activity, additional biochemical assays and more advanced experimental models are required.

4 Conclusion

The present study provides information on the anatomical characteristics and antioxidant activity of *Ficus racemosa* L. collected in Thai Nguyen, Vietnam. The anatomical investigation revealed that the root, stem, and leaf exhibit well-organized tissue systems typical of dicotyledonous plants undergoing secondary growth. The vascular tissues are clearly differentiated into phloem and xylem, forming collateral vascular bundles, while the presence of laticifers and supporting tissues such as sclerenchyma and collenchyma contributes to the structural stability and physiological functions of the plant organs.

In addition, the ethanolic extract of *Ficus racemosa* fruit showed notable antioxidant activity in the DPPH radical scavenging assay, with an IC_{50} value of 14.02 µg/mL, indicating strong free radical scavenging capacity. Although the activity was lower than that of the positive control (Vitamin C), the extract still demonstrated considerable antioxidant potential. These findings provide a scientific basis for further phytochemical and pharmacological studies, as well as for the potential utilization of F. racemosa as a natural source of antioxidant compounds.

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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