Microscopic characteristics and antioxidant activity of *Uvaria boniana*

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Abstract

Background and objectives: *Uvaria*, a genus within the Annonaceae family, encompasses around 150 species of flowering plants. *Uvaria boniana* is extensively found throughout Vietnam and is utilized in traditional medicine practices. The aim of this study was to determine the microscopic characteristics and evaluate the antioxidant activity of *Uvaria boniana*. **Materials and methods:** The stems and leaves of *Uvaria boniana* were collected in Huong Tra district, Thua Thien Hue province in October 2023. Micro-morphology of stems, leaves and powder properties were determined by the microscopic method. Antioxidant activity was assessed using the DPPH assay. **Results:** The microscopic characteristics of this species have been described. The methanol extract from the stem of *U. boniana* exhibited stronger antioxidant activity than the methanol extract from the leaf, with IC_{50} values of 45.19 ± 0.68 µg/mL and 70.94 ± 0.19 µg/mL, respectively. **Conclusion:** This is the first report on the microscopic characteristics and antioxidant activities of *Uvaria boniana*.

Keywords: *Uvaria boniana, anatomic structures, powder properties, microscopic characteristics,* antioxidant.

1. BACKGROUND

There are over 150 species of flowering plants in the *Uvaria* genus, which is part of the Annonaceae family. Predominantly, these species are either climbing shrubs or diminutive trees. They thrive in the moist, tropical climates found across Southeast Asia, tropical Africa, Northern Australia, Madagascar, and Indochina [1].

Uvaria is a large genus of the Annonaceae family, with 17 species found in Vietnam [2]. *Uvaria boniana*, as described by Fin. & Gagnep, is extensively found throughout Vietnam and is utilized in ethnomedicine [2]. The crushed leaves emit an aroma similar to that of cinnamon bark, and a decoction made from them can be ingested directly. Additionally, the fruits are employed in the treatment of intestinal ulcers [3]. The root's water decoction is specifically used for managing postpartum infections in women [4]. The number of studies related to this species is very limited. Thanh Tam Nguyen's research has isolated and determined the structures of five pure compounds, including: uvaridacol G, 4-methyl-4-[(2*Z*)-3'-phenylprop-2'-en1'-yl]cyclohex-2-en-1-one, 3,7-dimethoxy quercetin 4'-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside, *β*-sitosterol, and stigmasterol [5]. Son Ninh The reported on the chemical composition, anti-inflammatory and antibacterial activities of *U. boniana* essential oil [6]. As far as we know, there have been no studies on the microscopic characteristics and the antioxidant activity of this species. Therefore, the purpose of this study is to provide data on microscopic characteristics of the stem and leaf of this species and evaluate the antioxidant activities of *U. boniana*.

2. MATERIALS AND METHODS

2.1. Materials

The stems and leaves of *Uvaria boniana* were collected in Huong Tra district, Thua Thien Hue province in October 2023. The plant material was identified by Dr Anh Tuan Le (Mientrung Institute for Scientific Research, Vietnam National Museum of Nature, VAST, Vietnam). Voucher specimen has been deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy, Hue University, Vietnam. Some of pictures of the *Uvaria boniana* was displayed in figure 1.

Figure 1. The image of the *Uvaria boniana*

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2.2. Methods

2.2.1. Identification of microscopic characteristics

Micro-morphology: Fresh leaves and stems were sliced into thin sections using a razor blade. These sections were initially treated with a 5% sodium hypochlorite solution for around 30 minutes, followed by thorough rinsing in water. They were then submerged in 1% acetic acid for 3 to 5 minutes, and again rinsed well with water. The sections were then dyed with a 1% methylene blue solution for approximately 15 - 30 seconds and quickly rinsed with water. Sections were stained with 10% carmine red for approximately 30 min and washed several times with water. Subsequently, the stained sections were placed on a microscope slide, covered with a few drops of 10% glycerol for preservation, and a cover glass was applied. These sections were examined under a microscope (Eclipse E100, Nikon, Japan) and captured using a camera (Nikon D5100) [7].

Characteristics of the powder: Both the stems and leaves of the plant were dried and ground into a fine powder. This powder was sifted through a manual sieve with a mesh size of 0.125 mm to ensure its fineness. To prepare for microscopic examination, the powder was distributed on microscope slides, treated with several drops of 10% glycerol, and then covered with a cover glass. The observations were conducted using an optical microscope (Eclipse E100, Nikon, Japan), and images of the samples were captured using a camera (Nikon D5100) [7].

2.2.2. *Preparation of the extract*

The dried stems and leaves of *Uvaria boniana* were cut into small pieces (each, 10.0 g) and extracted with methanol (MeOH) (each, 100 mL x 3 times) at room temperature for three days. The residue was filtered, and solvents were then removed by an evaporator (Buchi, Switzerland) to yield the crude extracts.

2.2.3. *Evaluation of antioxidant activity*

The evaluation of antioxidant activity was conducted through the DPPH assay, with slight adjustments, measuring the absorbance at 517 nm. Quercetin served as the standard reference. The percentage of DPPH scavenging effect was calculated using the formula:

$$\text{DPPH scavenging effect} = [(A_{control} - A_{sample})/A_{control}] \times 100\%$$

where $A_{control}$ was the absorbance of DPPH solution and A_{sample} was the absorbance of DPPH solution in the presence of tested samples. The tests were carried out three times. The IC_{50} value is the concentration of 50% free radical neutralizer DPPH calculated in Microsoft Excel.

3. RESULTS

3.1. *Microscopic characteristics*

3.1.1. Anatomical characteristics of Uvaria boniana

Leaf midrib [Fig.2B]: The midrib, observed in cross-section, exhibited a slight concavity on the upper surface and convexity on the lower surface. Both the upper and lower epidermis (**B.2a**, **B.2b**) consisted of a layer of rectangular cells arranged adjacently. Trichomes were present on the upper epidermis (**B.1**). Under the epidermis, there were 2-3 layers of collenchyma (**B.3a**, **B.3b**) characterized by thick-walled, small and mostly round cells. The parenchyma (**B.4a**, **B.4b**) comprised numerous layers of polygonal cells that were different-sized, thin-walled, and arranged randomly. Within the parenchyma, some cells contained spherical oil droplets (**B.12**).

The vascular tissues were composed of multiple phloem-xylem bundles arranged in a central ring within the midrib. Xylem (**B.7**) catched the green color on the inside, the phloem (**B.6**) catched the red color on the outside, around the xylem forming a large arc. Surrounding the phloem were sclerenchyma cells (**B.5**), characterized by long, narrow cells with thickned walls. The pith region (**B.8**) consisted of parenchymatous cells interspersed with intercellular spaces, composed of polygonal cells and arranged randomly.

Leaf blade [Fig.2C]: Both the upper and lower epidermis (**C.9a**, **C.9b**) were comprised of a thin layer of rectangular or polygonal cells, with uniform sizing. Located close to the upper epidermis was the palisade parenchyma (**C.10**), which consisted of a layer of cylindrical cells with slightly thick walls. The palisade and spongy parenchyma (**C.11**) were clearly separated. The spongy parenchyma was made up of irregular cells, creating air-filled spaces within the leaf blade.

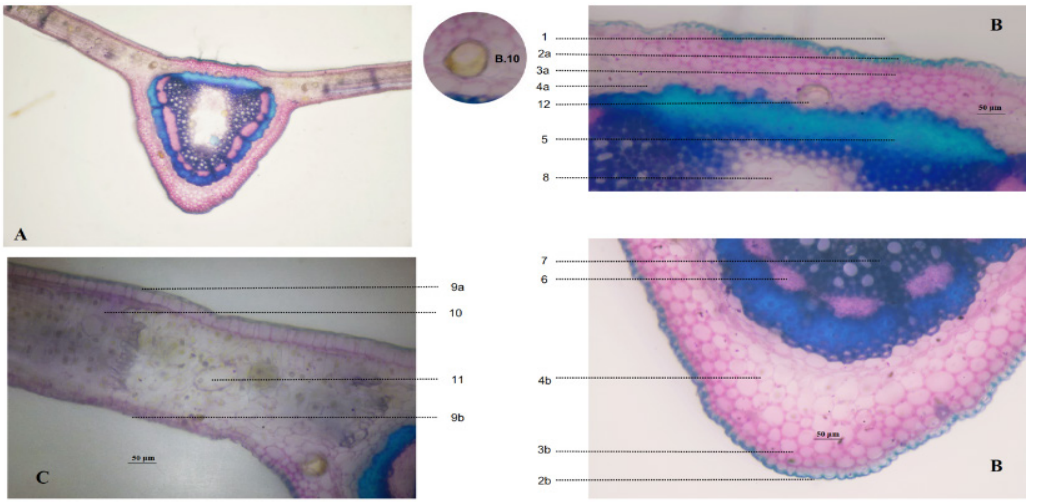

Figure 2. Microscopic characteristics of leaf cross-section of *Uvaria boniana*
***A**: Cross-section of the leaf (leaf midrib and leaf blade). **B**: Leaf midrib (1. Trichome, 2a. Upper epidermis, 2b. Lower epidermis, 3a. Upper collenchyma, 3b. Lower collenchyma, 4a. Upper parenchyma, 4b. Lower parenchyma, 5. Sclerenchyma, 6. Phloem, 7. Xylem, 8. Pith parenchyma, 12. Essential oil cell). **C**: Leaf blade (9a. Upper epidermis, 9b. Lower epidermis, 10. Palisade parenchyma, 11. Spongy parenchyma)*

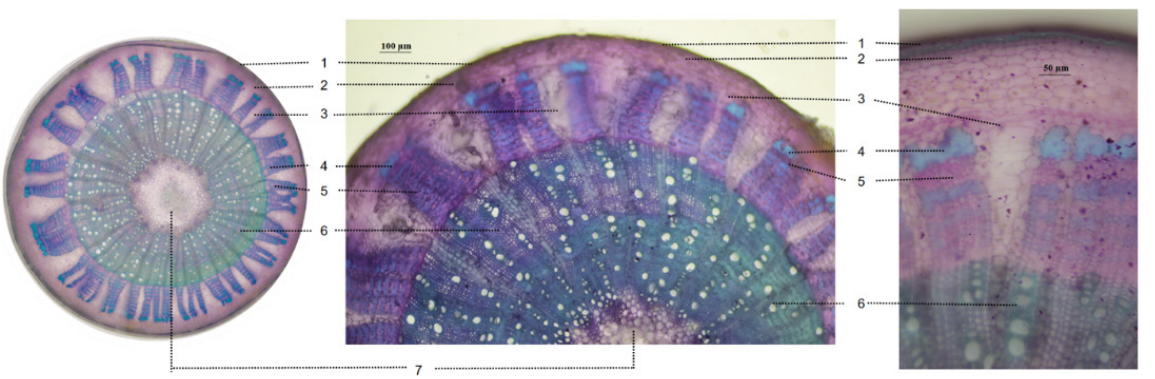

Figure 3. Microscopic characteristics of stem cross-section of *Uvaria boniana*
(1. Epidermis, 2. Collenchyma, 3. Cortical parenchyma, 4. Fiber, 5. Phloem, 6. Xylem, 7. Pith parenchyma)

Stems [Fig 3]: The transverse section of the stem of *U. boniana* (**Figure 3**) exhibited a circular shape, with distinct layers arranged from the outer to inner regions as follows: epidermis (**1**) consisted of 1 - 2 layers of rectangular cells arranged in a regular pattern. The cell membrane becomes corky and turns brown. Collenchyma (**2**) adjacent to the epidermis consisted of polygonal cells in 6-8 layers, with thickened walls at the corners, magenta-colored. Cortical parenchyma (**3**) comprised multiple layers of thin-walled polygonal cells, larger in size compared to collenchyma cells, and arranged randomly. Phloem cells (**5**) staining red, were interspersed between clusters of phloem fibers (**4**) that stain blue. Xylem (**6**) was made up of xylem vessels of varying sizes. Pith parenchyma (**7**) comprised numerous polygonal cells with thin walls, large in size, and arranged randomly within the stem's central region.

3.1.2. Powder features

Leaves powder [Fig.4]: A green powder had the characteristic of pleasant and aromatic odour. Powder features from the leaf were observed under a light microscope at 10X and 40X magnidications. The powder had several microsopic characteristics: fragment of epidermis (**1**), fragment of epidermis contained trichomes (**2**), fragment of epidermis contained stomata (**3**), bundle of fiber (**4**), fragment of vessel (**5**), essential oil cell (**6**), calcium oxalate crystals (**7**) and fragment of sclerenchyma (**8**).

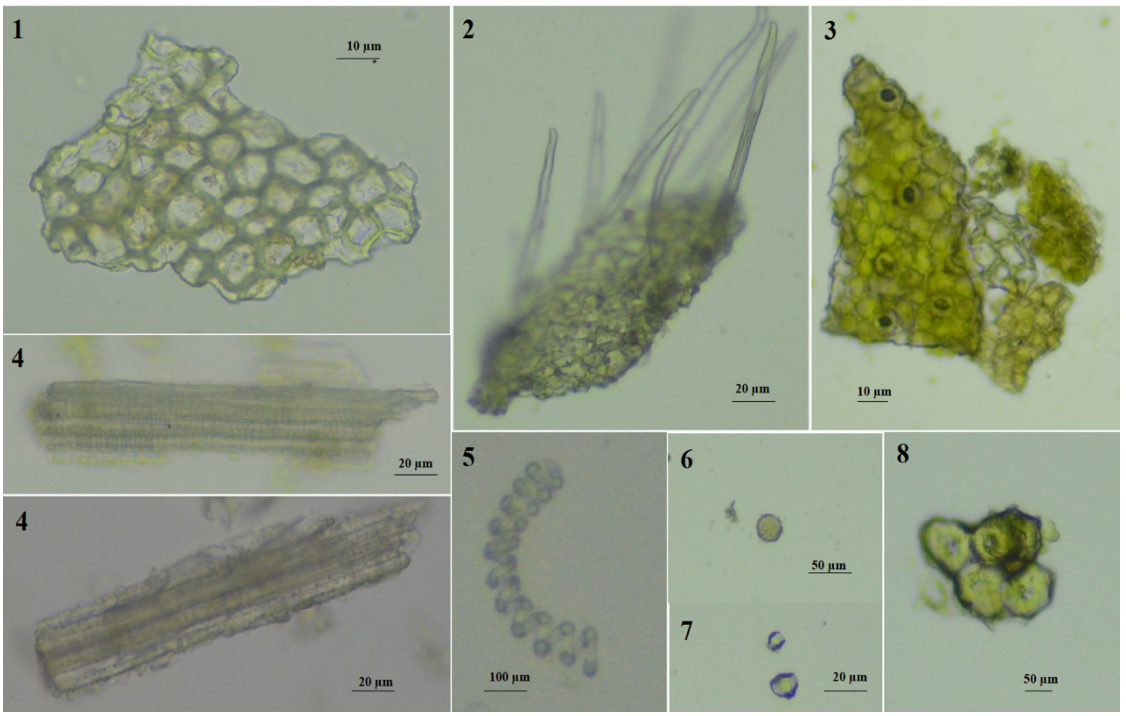

Figure 4. Microscopic features of the leaf of *Uvaria boniana*

Stems powder [Fig.5]: A brown-yellow powder had the characteristics of pleasant and aromatic oudour. Some microscopic features of the stem powder were observed under a light microscope at 10X and 40X magnifications, including: bundle of fiber (**1**), fragment of parenchyma (**2**), fragment of vessel (**3**), sclerenchyma tissue (**4**) and trichomes (**5**)

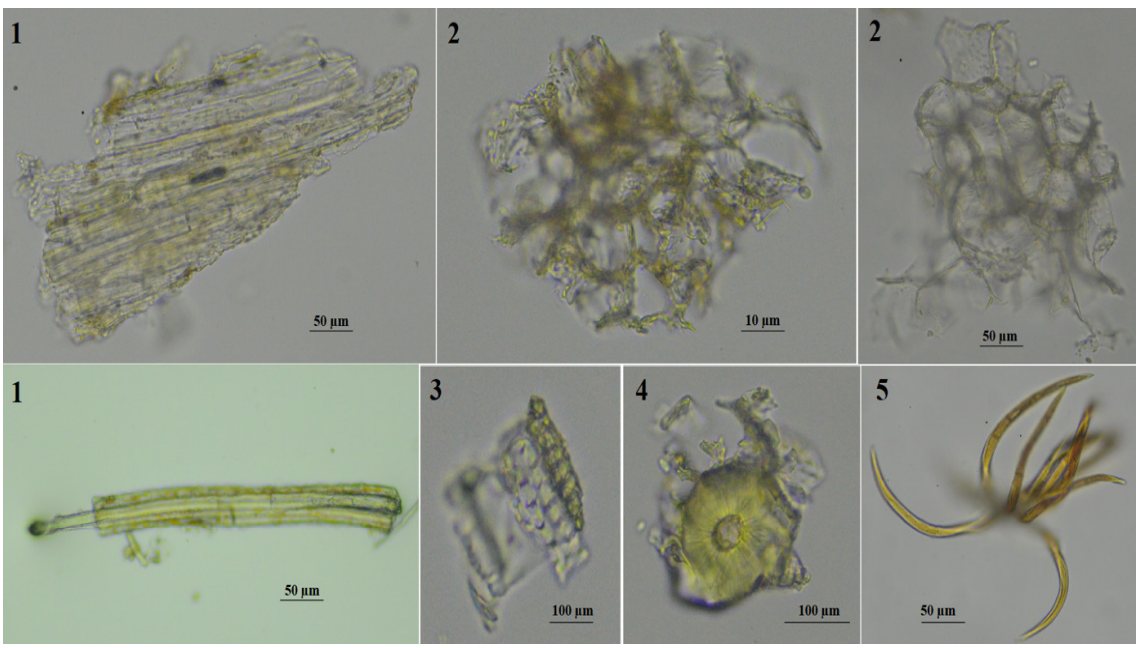

Figure 5. Microscopic features of the stem of *Uvaria boniana*

3.2. Evaluation of antioxidant activity

The scavenging efficiency of DPPH of the methanol extract from the leaf and the stem of *U. boniana* were showed in Table 1.

Table 1. DPPH free radical scavenging activity of *U. boniana*

No.	Sample	IC_{50} ± SD (μg/mL)
1	Leaf	70.94 ± 0.19
2	Stem	45.19 ± 0.68
	Quercetin	2.17 ± 0.06

As observed, the methanol extracts from *U. boniana* showed moderate *in vitro* DPPH radical scavenging activity. The extract from the stem displayed more potent antioxidant activity compared to that from the leaf, with IC_{50} values of 45.19 ± 0.68 μg/mL and 70.94 ± 0.19 μg/mL, respectively. This is the first report on the antioxidant activity of extracts from *U. boniana*.

4. DISCUSSION

Compared to the micro-anatomical descriptions of *Uvaria macrophylla*'s stem and leaf [8], this study reveals that the stem and leaf structures of both *U. boniana* and *U. macrophylla* share numerous similarities, suggesting these characteristics as typical of the *Uvaria* genus. Specifically, the leaves' vascular tissues are comprised of numerous phloem-xylem bundles arranged in a ring at the center of the midrib, with the phloem forming a large arc around the xylem, and sclerenchyma located outside the phloem in the midrib. Similarly, the stems are characterized by phloem interspersed among phloem fiber clusters. Despite these similarities, certain leaf structures can distinguish the two species: *U*. *macrophylla*'s xylem features three small, separate bundles, whereas *U*. *boniana*'s xylem does not form distinct bundles. The results of morphological characters of *U. boniana* have been described in specific detail as the scientific basis for the identification of this species.

Studies on the biological activities of *U. boniana* are very limited. Currently, only one study has been recorded on the bacterial and anti-inflammatory activities of *U. boniana* essential oil[6]. Besides, Nguyen Thanh Tam's research has reported on the antioxidant activities of some compounds isolated from *U. boniana* [9]. This study is the first report on the antioxidant activities of *U. boniana* extracts. However, this activity has been studied in many other *Uvaria* species such as *U. chamae* [10], *U. grandiflora* [11] and *U. rufa* [12]. Antioxidants are crucial in neutralizing free radicals in the body, which are unstable molecules that can cause oxidative stress, leading to cellular damage and various diseases. Preliminary studies on *U. chamae* or *U. grandiflora* extract suggest that they contain a rich blend of phytochemicals, including flavonoids and phenolic compounds, known for their effective antioxidant activities [10], [13], [14]. These compounds can scavenge free radicals, thereby protecting cells from oxidative damage. The antioxidant capacity of *U. boniana* extract could be attributed to its specific phytochemical composition, which interacts synergistically to enhance its free radical scavenging ability. This potential makes *U. boniana* an interesting subject for further research, aiming to explore its viability as a natural source of antioxidants for health applications, including the prevention of oxidative stress-related diseases. However, detailed scientific studies are required to fully understand its mechanisms and to validate its efficacy and safety for therapeutic uses.

5. CONCLUSION

The anatomical results derived from this study will be beneficial for the identification and standardization of *Uvaria boniana* stem and leaf materials, contributing to quality assurance and the preparation of a monograph on the plants. Additionally, the study has revealed that this plant exhibits antioxidant properties.

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