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# Micromorphology and phytochemical screening of *Piper betle* L. (Piperaceae)

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**ABSTRACT**

*Piper betle L., a common species in many countries, is used in many traditional medicines of countries and in many social, cultural, and religious ceremonies. P. betle has many traditional effects such as colds, bronchial asthma, cough, abdominal pain, rheumatism, bad breath, constipation, conjunctivitis, swollen gums, abscesses, and wound healing... Identifying P. betle species correctly is important for these activities, so this study aims to strengthen the criteria for accurate identification of P. betle species further. P. betle leaves were described microbiologically by staining sections, examining P. betle leaf powder and preliminary phytochemical composition of the leaves by modified Ciuley method. Microscopic characteristics of P. betle roots, stems, and leaves, microscopic characteristics of leaves include xylem-phloem in the leaf microstructure, a layer of palisade tissue present in the leaf anatomy, leaves powder (characterized by soft tissue fragments with colored masses, spiral veins), and preliminary chemical composition including essential oils, coumarins, flavonoids, organic acids, reducing agents and polyuronics. The study provides additional data on microscopic characteristics and chemical composition in the leaves of P. betle species.*

***Keyword:*** *Piper betle, leaves, micro-morphological*

## 1. INTRODUCTION

*Piper betle* L. (Piperaceae) is a well-known perennial vine from the *Piper* genus, native to central and eastern Peninsular Malaysia, and found in East Africa and tropical regions of Asia [1]. It is a commercial crop grown mainly in India, Bangladesh, Sri Lanka, Thailand, Taiwan, Malaysia, and several other Southeast Asian countries [2, 3]. *P. betle* is known as the 'green gold of India' because nearly 20 million people depend on the plant for their income from the production, transportation, handling, processing, and preparation of betel leaves. Leaves and areca nuts play a central role in Hindu culture as they are used in many social, cultural, and religious ceremonies [1]. Chewing is a common practice in many countries as it acts as a natural tonic and mouth freshener to prevent bad breath. The use of *P. betle* is found in many traditional medicinal systems, such as the Indian Ayurvedic and traditional Chinese medicine, and the folk medicinal systems of the West Indies and Latin America. Traditionally, *P. betle* has been used to treat a variety of ailments such as colds, bronchial asthma, coughs, stomachaches, and rheumatism, and other illnesses such as boils, bad breath, constipation, conjunctivitis, swollen gums, abscesses, injuries, and cuts, whether infectious or non-infectious [4]. The plant is also used for other purposes, such as in fish poisoning, as a fish bait, insecticide, or-namental, oil, perfume, and hallucinogen [5]. The pharmacological properties of medicinal plants are mainly due to a variety of bioactive phytochemicals of biomedical and pharmaceutical significance. The bioactivities of the plant include anti-inflammatory, analgesic activity, anti-anxiety activity, antioxidant activity, hepatoprotective properties, antihyperglycemic activity, etc. [5]. In Vietnam, studies mainly focused on the biological effects of P. betle on antibacterial, antifungal, and antioxidant effects [6]. The effects of *P. betle* are numerous and important, so correct identification of the species is necessary to exploit these effects. This study aims to strengthen further the criteria for accurately identifying of *P. betle* species.

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## 2. MATERIALS AND METHODS

### 2.1. Material

The materials in this study included *Piper betle* L. fresh medium-aged leaves from Van Xuan co., ltd. chloramine-T, chloral hydrate, acetic acid, Carmine-iodine green, $H_2SO_4$, Carr-Price's Reagents, KOH, HCl, Fehling's Reagents.

### 2.2. Method

#### *2.2.1. Procedures of micro-morphological study*

Micro-morphological characteristics of the samples were determined according to the guidelines of the Vietnamese Pharmacopoeia V [7] via the double staining method of iodine green carmine. The leaves, stems, and roots of *P. betle* 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 cross-sections were bleached with 5.0% (w/v) chloramine-T detergent and followed by 50% (v/v) chloral hydrate for 10 min. The 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 (ECLIPSE E200LED MV R) at 4×, 10×, and 40× magnifications.

Leaf powder preparation by chopping the medicinal herbs, drying at 60 - 70°C until dry, grinding, sieving through a 32 sieve (sieve hole diameter 0.1 mm), and observing the leaf powder features was also observed under the microscope with 10X and 40X magnification (ECLIPSE E200LED MV R).

#### *2.2.2. Phytochemical screening of P. betle*

10g dried powder samples were separately extracted by ultrasonication in 100 mL of chloroform, 96% ethanol, or distilled water for 30 min at 45°C. After that, these extracts were tested for phytochemical components, including carotenoids, fatty acids, essential oils, flavonoids, tannins, coumarins, alkaloids, triterpenoids, steroids (cardiac glycosides), saponins, amino acids, and carbohydrates by the Ciuley method [8]. These experiments were repeated independently in triplicate.

## 3. RESULTS

### 3.1. Micro-morphological characters

**Anatomical features of roots:** The cross-section of the roots is nearly circular. Cork consists of many layers, rectangle cells, organized in straight lines. Phelloderm consists of many layers, rectangle cells, cellulose walls, organized in straight lines. The cortical parenchyma is angular parenchyma, multi-layered, polygonal cells, cellulose walls, arrangement random. The endodermis is a single layer Casparian trip. The pericycle consists of 1 - 2 layers, polygonal cells, cellulose walls. The primary phloem is polygonal cells, cellulose walls, arrangement random. The secondary phloem consists of 3 - 4 layers, polygonal cells, cellulose walls, organized in straight lines. The secondary xylem vessels are polygonal cells, lignified cell walls, arrangement random; the parenchymatous secondary xylem is polygonal cells, lignified cell walls, organized in straight lines. The primary xylem vessels are polygonal cells, lignified cell walls, exarch. Ray consists of 4 - 5 lines, expanding towards the side of phloem's. The parenchymatous pith is angular parenchyma, multi-layered, lignified cells or cellulose walls, arranged randomly.

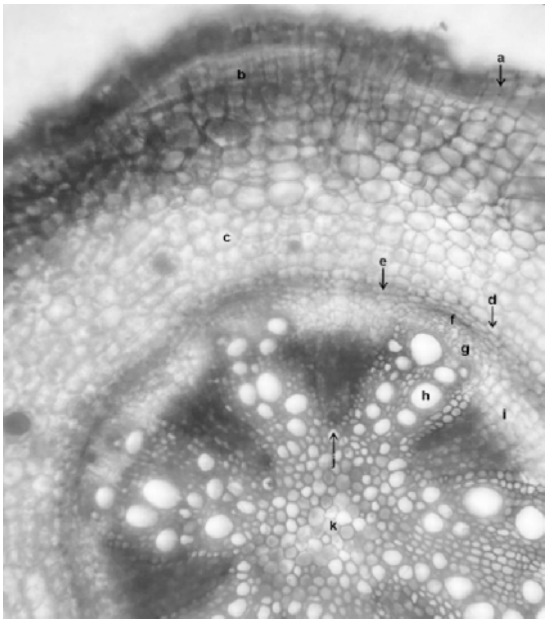


**Figure 1.** The features of cross-sectioned roots *a. Cork, b. Phelloderm, c. Cortical parenchyma, d. Endodermis with Casparian trip, e. Pericycle, f. Primary phloem, g. Secondary phloem, h. Secondary xylem, i. Ray, j. Primary xylem, k. Parenchymatous pith*

**Anatomical features of stems:** The cross-section of the stems is nearly circular. The epidermis consists of single layer, polygonal cells, cellulose walls; the cuticle layer is thin and flat. The trichomes single cell or multicellular cells, scattered across the epidermis. The lacunar collenchyma tissue is discontinuous, multi-layered, polygonal cells, cellulose walls, arrangement random. The cortical parenchyma is angular parenchyma tissue, 5 - 7 layers, polygonal or oval cells, cellulose walls, arrangement random. The endodermis is a single layer Casparian trip. The pericycle consists of 1 - 2 layers, polygonal cells, cellulose walls. The conductive tissue with two loops of phloem-xylem bundles, the phloems-xylem bundles are similar structure. The primary phloem is polygonal cells, cellulose walls, arrangement random. Each bundle of xylem has 4 - 6 vessels, the xylem vessels are polygonal cells, lignified cell walls; the parenchymatous xylem is polygonal cells, cellulose or lignified cell walls, arrangement random. The sclerenchyma tissue is arranged in a circle, below the outer phloems-xylem ring, polygonal cells, lignified cell walls, arrangement random. The parenchymatous pith is angular parenchyma tissue, multi-layered, cellulose walls, arrangement random. The secretory cavities consist of 7 - 8 cavities, in between the two rings of the phloem-xylem bundles and a secretory cavity at the center of the cross-section of the stems. The secretory cells are scattered across the cortical parenchyma and parenchymatous pith.

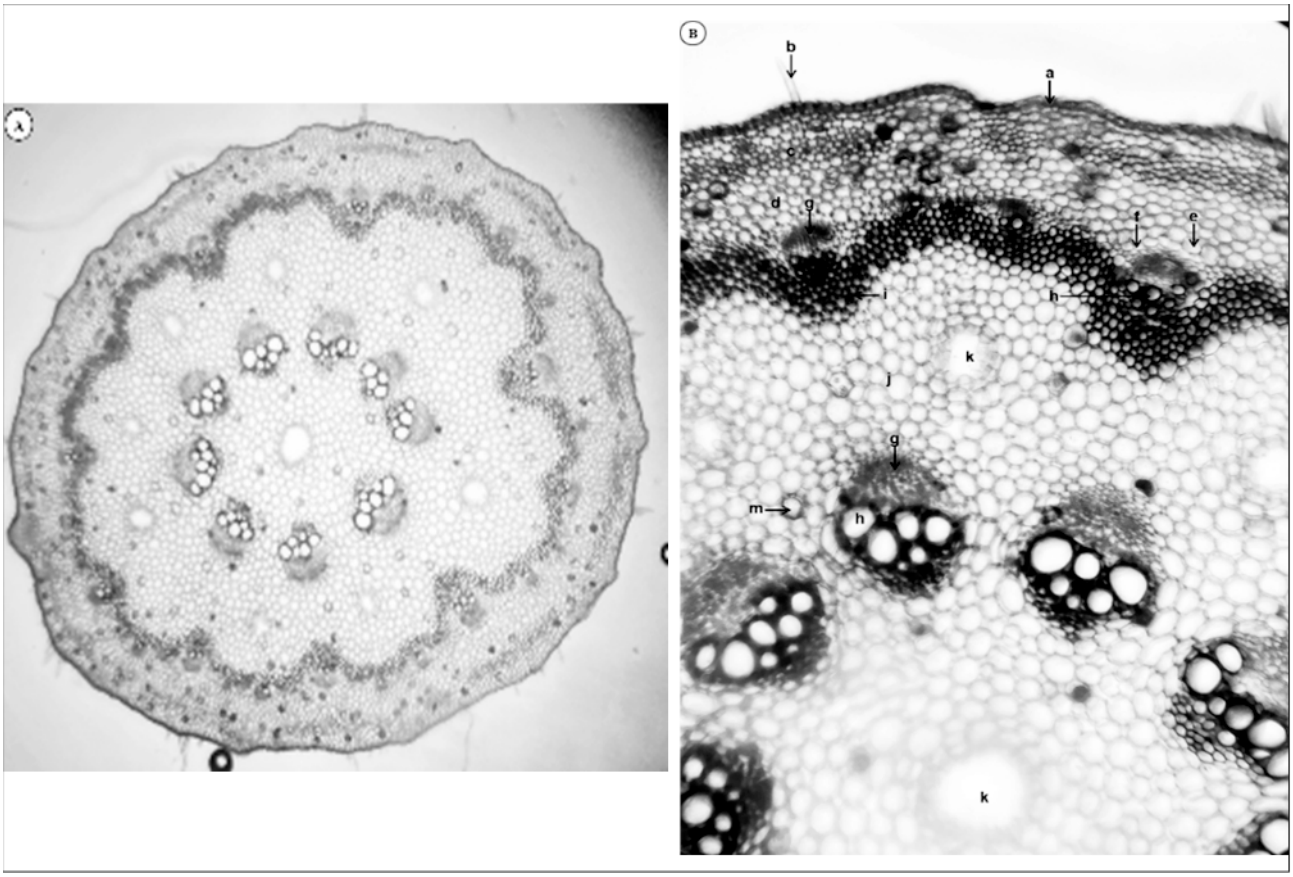


**Figure 2.** The features of cross-sectioned stems

*A. The features of cross-sectioned stems,*
*B. Partical of the features of cross-sectioned stems,*
*a. Epifermis, b. Trichomes, c. Collenchyma tissue,*
*d. Cortical parenchyma, e. Endodermis with Casparian trip,*
*f. Pericycle, g. Phloem, h. Xylem, i. Sclerenchyma tissue,*
*j. Parenchymatous pith, k. Secretory cavity, m. Secretory cell*

**Anatomical features of leaves:** The lower surface is more prominently curved than the upper surface. *Midrib region*: The upper and lower epidermis consists of a single layer, polygonal cells, cellulose walls; the cuticle layer is thin and flat. The trichomes multicellular cells are more concentrated on the lower epidermis. The upper lacunar collenchyma tissue has 3 - 4 layers and the lower lacunar collenchyma tissue 4 - 5 layers of polygonal cells, cellulose walls, arrangement random. The angular parenchyma tissue is polygonal cells, cellulose walls, arrangement random. The conductive tissue is a big phloem-xylem bundle with xylem on top and phloem underneath. The xylem vessels are circular polygonal cells, lignified cell walls, and arranged in rows of 3 - 4 vessels; the parenchymatous xylem is polygonal cells, lignified cell walls, arranged in 1 - 2 rows, alternating with rows of xylem vessels. The phloem are polygonal cells, cellulose walls. The secretory cavity is a cavity directly beneath the upper collenchyma tissue. The secretory cells are scattered across the parenchyma tissue. *Leaf blade region*: The upper and lower epidermis consists of a single layer, polygonal cells, cellulose walls; the cuticle layer is thin and flat; the stomata are more concentrated on the lower epidermis. The upper hypodermis has 2 layers and the lower hypodermis is single layer, polygonal cells, cellulose walls. The palisade mesophyll consists of 2 layers, rectangle cells. The spongy mesophyll region is thickness nearly equal to that of the palisade mesophyll region, cells have variable shapes.

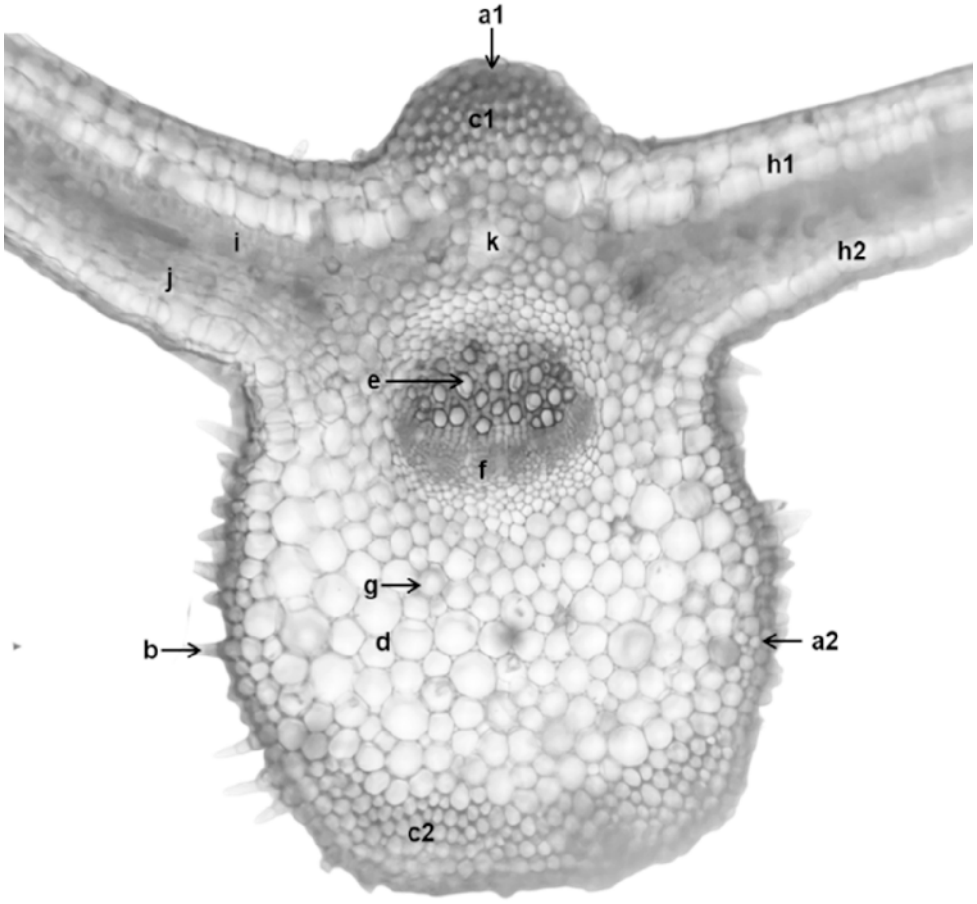


**Figure 3.** The features of cross-sectioned leaves

*a. Epidermis (1. Upper, 2. Lower), b. Trichomes, c. Collenchyma tissue (1. Upper, 2. Lower), d. Angular parenchyma tissue, e. Xylem, f. Phloem, g. Secretory cell, h. Hypodermis (1. Upper, 2. Lower), I. Palisade mesophyll, j. Spongy mesophyll, k. Secretory cavity*

**Leaf powder:** The leaf powder is yellowish brown and has a fragrant smell. Observed under a microscope, it consists of the following components: Translucent oil cells, a fragment of spiral xylem vessel, epidermis with stomata, and color masses.

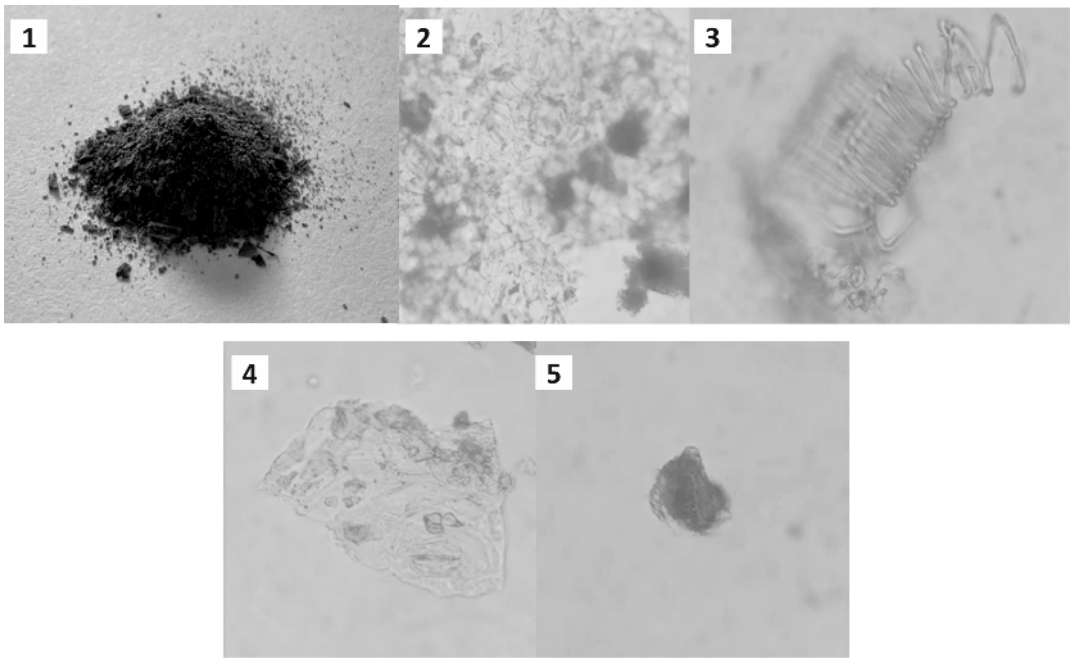


**Figure 4.** The features of leaf powder

*1. Leaf powder, 2. Translucent oil cells, 3. Fragment of spiral xylem vessel, 4. Epidermis with stomata, 5. Color masses*

### 3.2. Phytochemical screening

Phytochemical screening showed that the extract of *P. betle* had potential antibacterial properties. The screening results are shown in Table 1. It was concluded that the extracts had high concentrations of essential oils, antraglycosides, coumarins, flavonoids, organic acids, reducing agents, and polyuronides.

**Table 1.** Results of phytochemical screening of *P. betle*

| Phytocompound | Test | Result |
|---|---|---|
| Fatty acid | Stain test | - |
| Carotenoids | $H_2SO_4$ test/Carr-Price's test | - |
| Essential oils | Scent test | +++ |
| Triterpenoid | Liebermann Burchard test | - |
| Alkaloid | Valse-mayer's test, Bouchardat's test, Dragendoff's test | - |
| Coumarin | Luminescence in alkaline | ++ |
| Anthranoid | KOH test | ++ |
| Flavonoid | Shinoda (Cyanidin) test | + |
| Cardiac glycosides | Raymond's test, Xanthydrol's test | - |
| Anthocyanosid | KOH and HCl test | - |
| Proanthocyanidin | HCl and heat up test | - |

| Phytocompound | Test | Result |
|---|---|---|
| Tannin | Gelatin's test | - |
| Saponin | Foam test | - |
| Amino acids | $Na_2CO_3$ test | + |
| Carbonhydrates | Fehling's test | + |
| Polyuronides | Ethanol test | + |

*Notes: "+" and "-" indicate the presence and absence of the phytochemical constituents, respectively*

## 4. DISCUSSION

The anatomical characteristics of the stem, leaves, and petioles of *P. betle* have been described in detail, supplemented with clear illustrations. These findings provide critical foundational data that can be used to develop standardized testing methods for this medicinal plant. Such standards will play a crucial role in ensuring the quality and consistency of raw materials used in pharmaceutical applications. Additionally, the anatomical insights contribute to the identification and authentication of Piper betle, aiding in its classification and distinguishing it from related species [10].

Observations from Kuswandi PC (2023) on the transverse cross-section of the midrib of black betel leaves from four different locations revealed anatomical structures typical of the *P. betle* species. These include the presence of an epidermal layer, trichomes on the abaxial part of the leaf, several layers of hypodermis, visible vascular tissues, and secretion cells [11]. These findings are consistent with the present study, further validating the anatomical characteristics observed.

Moreover, anatomical data such as the arcuate arrangement of xylem and phloem in leaf microsurgery, the presence of a single layer of palisade tissue, and specific features of the medicinal powder - characterized by soft tissue fragments with colored masses and spiral veins - offer additional key markers for identification and quality assessment of *P. betle*.

Furthermore, the preliminary results of the phytochemical composition reveal the presence of essential oils, coumarins, flavonoids, organic acids, reducing agents, and polyuronides. These compounds are known to have various bioactive properties, supporting the traditional uses of *P. betle* in herbal medicine. The consistency of these findings with previous research [12] reinforces the reliability of the current study and highlights the plant's potential as a source of bioactive compounds. These results serve as a valuable reference for future pharmacological and clinical studies on *P. betle* in Vietnam, contributing to its broader application in medicine and health care.

## 5. CONCLUSION

The microscopic characteristics and preliminary phytochemical composition provided valuable insights for the identification of the species *P. betle*. Key micromorphological features include the arcuate arrangement of xylem and phloem in leaf microsurgery, the presence of a single layer of palisade tissue, and the unique traits of medicinal powder, characterized by soft tissue fragments with colored masses and spiral veins. Additionally, the preliminary phytochemical analysis revealed the presence of essential oils, coumarins, flavonoids, organic acids, reducing agents, and polyuronides. These findings serve as a basis for the accurate identification and further study of *P. betle.*

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# Đặc điểm vi học và sơ bộ hóa thực vật của Trầu không *Piper betle* L.(Piperaceae)

**Đỗ Thị Anh Thư, Trần Trung Trĩnh**

**TÓM TẮT**

*Trầu không là cây thuốc phổ biến được sử dụng nhiều trong y học cổ truyền của các quốc gia và được sử dụng trong nhiều nghi lễ xã hội, văn hóa và tôn giáo. P. betle có nhiều công dụng được sử dụng theo kinh nghiệm dân gian như cảm lạnh, hen phế quản, ho, đau bụng , thấp khớp, hôi miệng, táo bón, viêm kết mạc, sưng nướu răng, áp xe, làm lành vết thương… Việc xác định đúng loài P. betle là cần thiết để sử dụng các hoạt tính này, do đó nghiên cứu này nhằm mục đích củng cố thêm các tiêu chí để xác định chính xác nguồn nguyên liệu là P. betle. Lá P. betle được mô tả về vi học bằng phương pháp nhuộm các lát cắt, soi bột lá P. betle và sơ bộ thành phần hoá thực vật của lá P. betle bằng phương pháp Ciuley cải tiến. Đặc điểm vi học của rễ thân và lá P. betle, cấu tạo lá có gỗ-libe hình cung ở vi phẫu lá, 1 lớp mô giậu hiện diện trong giải phẫu lá, bột lá (đặc trưng là Mảnh mô mềm mang khối màu, mạch xoắn) và sơ bộ thành phần hóa học bao gồm: Tinh dầu, coumarin, flavonoid, acid hữu cơ, chất*

*khử và polyuronic. Nghiên cứu góp phần cung cấp thêm dữ liệu đặc điểm vi học và thành phần hóa học có trong lá của loài P. betle.*

***Từ khóa:*** *Piper betle, lá, vi học*

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