INVESTIGATION OF BOTANICAL CHARACTERISTICS, DNA BARCODING AND CHEMICAL CONSTITUENTS OF *Stahlianthus campanulatus* KUNTZE IN DAK LAK PROVINCE, VIETNAM

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ABSTRACT

This study aimed to establish a scientific database on the biological characteristics and chemical constituents of *Stahlianthus campanulatus* Kuntze*,* a potential medicinal plant, collected from Chu Yang Sin National Park. The samples were identified to species level by DNA sequencing, along with detailed descriptions of morphology, microanatomy, and powdered crude drug characteristics, and preliminary analysis of chemical constituents. Sequencing of the *trnL–trnF* region yielded an 870 bps fragment, and Basic Local Alignment Search Tool (BLAST) showed similarity to *Stahlianthus campanulatus* Kuntze up to 99.84%. The rhizome powder is light brown, slightly pungent, and aromatic. The parenchyma composed fragments of polygonal thin-walled cells contain numerous ovoid and oval starch granules, along with scalariform vessels and fiber bundles. Chemical analysis indicated that the tubers of *S. campanulatus* contain several groups of compounds, including essential oils, free triterpenes, and coumarins, detected in ether and non-hydrolyzed alcohol extracts, existing in free form or as less-polar derivatives. The results contributed to the determination of species identity, morphological characteristics, and a preliminary profile of chemical constituents. These provide an important basis for more studies on the exploitation and conservation of this medicinal resource in the future.

Keywords: *Powdered crude drug, DNA barcoding, molecular identification, phytochemistry, S. campanulatus, microanatomy.*

1. INTRODUCTION

The Central Highlands is a such diverse ecosystems and notably rich medicinal plant resources region. According to Bui Van Thanh et al. (2020), there are more than 1,713 medicinal plant species recorded in this area, accounting for about 35.82% of the total native plant species nationwide. Simultaneously, the ethnic and cultural diversity of the Central Highlands has formed a valuable repository of traditional medical knowledge, with around 450 folk remedies and approximately 800 to 1,000 medicinal plant species commonly used in the daily life of local ethnic communities.

S. campanulatus Kuntze, also known by another scientific named *Kaempferia campanulata* (Kuntze) K. Schum., belonging to the genus Stahlianthus, Zingiberaceae family. It is a representative species which including biological value particular significance in traditional medicine. This species is primarily distributed in Thailand, Myanmar, China, India, Laos, Cambodia, and Vietnam (Mongkolsawat et al., 2018). In Vietnam, *S. campanulatus* is only sporadically distributed in several provinces such as Danang, Quangnam, Kontum, Dongnai, and Daklak (Pham, 1999). Notably, as recorded by Nguyen Tap et al. (2016), *S. campanulatus* is used by the Bana and Ede ethnic groups in Daklak in tonic remedies for postpartum women with considerable effectiveness.

Although *S. campanulatus* Kuntze has been reported to confer various health benefits, its widespread utilization remains limited and is mainly based on folk experience. The principal reasons are the lack of data on chemical composition and pharmacological activity, together with difficulties in propagation. Global research on *S. campanulatus* is still large limited, focusing mainly on propagation (Mongkolsawat et al., 2018). In Vietnam, the first study on this speciessamples collected in Laocai by Do et al. (2017) found that the roots of *S. campanulatus* contain a great deal of valuable essential oils. The main constituents of the oil are stahlianthusone (27.6%), α-copaene (16.7%), and camphor (14.7%). In addition, there are relatively high amounts of camphene (8.7%) and allo-aromadendren (6.0%). To date, no study has analyzed the chemical composition or evaluated the biological activity of *S. campanulatus* in the Central Highlands.

Furthermore, species identification based on

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traditional morphological methods is difficult and prone to confusion due to morphological similarities with other species in the family Zingiberaceae, affecting research and application.

This study focuses on species identification using molecular biology methods; detailed descriptions of morphology, microanatomy, and powdered crude drug; and a preliminary analysis of the chemical composition of *S. campanulatus* collected in Chu Yang Sin National Park. The results contribute to building a scientific database on species composition, morphological characteristics, and chemical constituents, and serve applied research directions, exploitation, and the sustainable development of this valuable medicinal resource in the Central Highlands.

2. MATERIALS AND METHODS

2.1. Materials

Fresh specimens of *S. campanulatus* Kuntze were collected in November 2024 from Chu Yang Sin National Park (12°29′25″N, 108°15′08″E). Rhizomes, roots, and leaves were used as materials for examinations of morphological and microanatomical characteristics. The rhizomes served as materials for a preliminary survey of chemical constituents and essential-oil composition. Young leaves were used as materials for DNA analysis and molecular identification.

2.2. Methods

2.2.1. Sample collection

Samples were recognized and collected to ensure representativeness and to comply with conservation regulations under the guidance of forest rangers. They were then provisionally identified in the field using morphological methods (following the description of Tap et al., 2016), with full records of GPS coordinates, habitat, and other relevant characteristics.

2.2.2 Molecular identification

Total DNA was extracted from fresh leaf material using the DNeasy Plant Mini Kit (Qiagen). DNA concentration was measured with a NanoDrop spectrophotometer, and DNA quality was checked by electrophoresis on a 1% agarose gel. PCR reactions were performed with the NEXpro™ Diagnostinnk Kit, including 10× e-Taq Buffer, 10 mM dNTP mix, e-Taq DNA Polymerase, sterile distilled water, primer pair, and template DNA. Reactions were run on a GeneAmp PCR System 2700 thermal cycler. The PCR mixture contained: 2× master mix – 7.5 µl, forward primer (10 pmol/µl) – 0.5 µl, reverse primer (10 pmol/µl) – 0.5 µl, template DNA (10 ng/µl) – 0.5 µl, and water – 6 µl, for a total volume of 15 µl. Thermal profile: 94 °C for 4 min; 35 cycles of (94 °C for 60 s, 54 °C for 40 s, 72 °C for 60 s); 72 °C for 10 min; hold at 4 °C. The primer pair specific to the *trnL* gene was designed with reference to Taberlet et al. (1991). Forward primer: *trnL*-F 5′-CGAAATCGGTAGACGCTACG-3′. Reverse primer: *trnL*-R 5′-ATTTGAACTGGTGACACGAG-3′.

PCR products were separated on agarose gel, purified with the Wizard SV Gel and PCR Clean-Up System (Promega), and sent for sequencing at Phu Sa Genomics Joint Stock Company (Cantho). The obtained sequences were compared with *trnL* sequences of other species in Stahlianthus and related genera deposited in NCBI using BLAST (Basic Local Alignment Search Tool), and a phylogenetic tree was constructed in MEGA11 (Tamura et al., 2021) with bootstrap values based on 1,000 replicates.

2.2.3. Morphological, microanatomical, and powdered-drug characteristics

Thin hand-cut sections of rhizome, root, tuberous root, leaf blade, and petiole were prepared with a razor, bleached with 50% Javel solution, then stained with Carmine–Iodine Green. Rhizomes were cut into 0.5–0.7 cm segments; roots 0.2–0.3 cm; the leaf blade was sectioned at the basal one-third including the midrib and lamina; and the petiole at the middle portion. Epidermal layers were separated, mounted in distilled water, and observed under a microscope at 10× and 40× to analyze microanatomical features following Hai (2022).

Rhizome powder of *S. campanulatus* was chopped, dried at 60–70 °C, finely ground, passed through a 32^{nd} mesh sieve, then examined under a light microscope (10X, 40X) and photographed for diagnostic features.

2.2.4. Preliminary phytochemical analysis

Raw materials comprised roots and rhizomes of Stahlianthus campanulatus, dried and milled to a coarse powder. Qualitative tests followed the modified Ciuley method (Hung, 2014). Twenty grams of powder were successively extracted with ethyl ether, 96% ethanol, and water. The 96% ethanol and aqueous extracts were hydrolyzed with 10% HCl. Compound classes were identified by characteristic reactions.

3. RESULTS AND DISCUSSION

3.1. Species identification

3.1.1. Morphological characteristics

S. campanulatus is a perennial herb, 30–50 cm tall (Fig. 1A). Leaves 3–5, arising in a cluster from the rhizome apex; petioles forming rounded, smooth sheaths that overlap to create a pseudostem; leaf blades elongate, more than 20 cm long and 3–4 cm wide, acute at both ends, glabrous, with many parallel secondary veins (Fig. 1B, 1C). Rhizomes subterranean, circular in cross-section, 0.5–1 cm in diameter, 4–5 cm long, dark yellow, bearing pointed scale leaves. Roots circular in cross-section, 0.1–0.3 cm in diameter and 6–8 cm long; the distal end bears fusiform or ovoid tubers 2–3.5 cm long and 1.5–1.7 cm in diameter, fleshy and light brown (Fig. 1D). The recorded morphological characteristics are entirely consistent with the description by Tap et al. (2016).

Figure 1. Morphological characteristics of S. campanulatus Kuntze: *(A, B) Whole-plant morphology collected from Chu Yang Sin National Park. (C) Leaf morphology. (D) Morphology of rhizome, roots, and tubers.*

3.1.2. Molecular identification results

According to the BLAST analysis, the *trnL* gene sequence of the studied sample showed 99.84% similarity to *S. campanulatus* (JQ409785.1) (Fig. 2).

Sequences producing significant alignments Download Select columns Show 10

select all 5 sequences selected — GenBank Graphics Distance tre of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Accession
Stahlianthus campanulatus voucher JLS 72446 iTNA-Leu (trnL) gene, partial sequence; trnL-trnF intergenic spacer, comple,...	Stahlianthus campanulatus	1151	1145	100%	0.0	JQ409785.1
Stahlianthus involucratus voucher JLS 71449 iTNA-Leu (trnL) gene, partial sequence; trnL-trnF intergenic spacer, compte sequence,...	Curcuma involucrata	1146	1145	100%	0.0	JQ409784.1
Curcuma sp. JLS 365 iTNA-Leu (trnL) gene, partial sequence trnL-trnF intergenic spacer, complete sequence; ,...	Curcuma newmani	1146	1145	100%	0.0	JQ409799.1
Curcuma plicata voucher Tran 80 ITNA-Leu (trnL) gene, partial sequence; trnL-trnF intergenic spacer, complete sequence,...	Curcuma plicata	1146	1145	100%	0.0	JQ409827.1
Curcuma reclinata voucher JLS 73467 ITNA-Leu (m (trnL) gene, partial sequence; trnL-trnF intergenic spacer, complete sequence,...	Curcuma reclinata	1146	1145	100%	0.0	JQ409810.1
Curcuma reclinata voucher JLS 73467 iTNA-Leu (trnL) gene, partial sequence; trnL-trnF intergenic spacer, complete sequence,...	Curcuma reclinata	1146	1145	100%	0.0	JQ409810.1

Figure 2. The result of BLAST with JQ409785.1

The phylogenetic tree constructed with 1,000 bootstrap replicates showed that the sample clustered in the same clade as *S. campanulatus* (bootstrap 66%) (Fig. 3). Combining the morphological analysis (Fig. 1) with sequence-based identification results, it can be concluded that the studied specimen is *S. campanulatus* Kuntze.

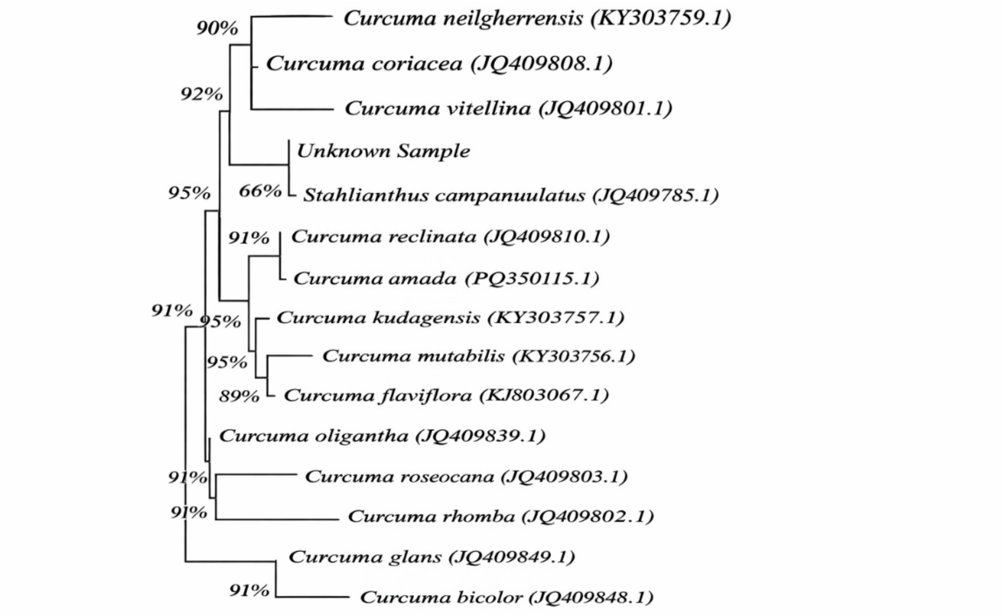

Figure 3. Phylogenetic tree showing the similarity of several species in the genera Stahlianthus and Curcuma based *on trnL–trnF* gene sequences (Unknown Sample is the studied specimen).

3.2. Microanatomical characteristics

Microanatomical analysis helps determine tissue and cell structures, thereby deepening understanding of plant morphology, anatomy, genetics, and variation. In this study, we examined cross sections of roots, rhizomes, storage tubers, and leaves.

3.2.1. Root microanatomy

Cross-sectional images of the root of *S. campanulatus* show the typical anatomy of a monocot root, with clearly differentiated tissue zones from the periphery toward the center. The outermost layer is the epidermis composed of compactly arranged cells with variably thickened walls, from which long root hairs arise, similar to the structure in *Curcuma sahuynhensis* (Van et al., 2022) (Fig. 4A). Immediately inside the epidermis lies the cortical parenchyma occupying most of the volume, consisting of large, thin-walled cells that form intercellular spaces and mainly store starch. The innermost layer of the cortex is the endodermis, a single row of regular cells with wall thickening on some faces. The root center is the stele, comprising many alternating xylem and phloem bundles, differentiated as protoxylem at the periphery and metaxylem centrally; xylem vessels have strongly lignified, dark bluish-purple walls. At the very center is the pith parenchyma with large, thin-walled cells functioning in storage (Fig. 4B and 4C).

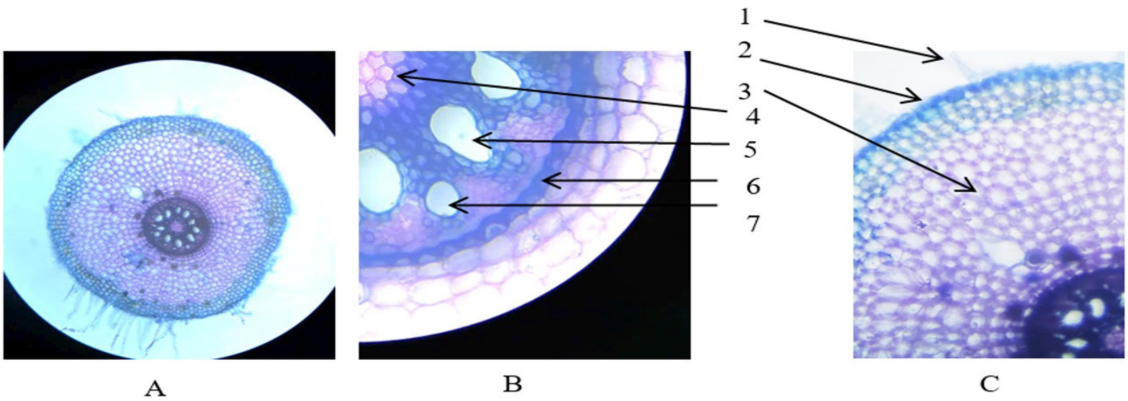

Figure 4. Root microanatomy of Stahlianthus campanulatus: *(A) Cross section of the root; (B, C) Partial views of the root region. 1. Root hair, 2. Epidermis, 3. Cortical parenchyma, 4. Pith parenchyma, 5. Metaxylem, 6. Endodermis, 7. Protoxylem.*

3.2.2. Rhizome microanatomy

Rhizome microanatomy shows that the rhizome of *S. campanulatus* has a circular cross-section and the typical anatomical structures of a subterranean monocot stem (Fig. 5). From the periphery toward the center, the rhizome comprises a cork (phellem) layer (1) with small polygonal cells. Internal to this are the cortical parenchyma (3) and an extensive pith parenchyma, both made of thin-walled parenchyma cells functioning in storage. Phloem–xylem bundles (2) are scattered throughout the parenchymatous ground tissue and are not arranged in distinct rings, a characteristic of monocot rhizomes. The number of vascular bundles increases with rhizome age (Nguyen et al., 2023). The endodermal layer (4) separates the cortex (a single layer of rectangular, thin-walled cells) from the stele containing numerous observable phloem–xylem bundles. Notably, the presence of secretory cells (6) dispersed in the cortical and pith parenchyma (5) is an important feature, indicating the potential accumulation of secondary metabolites with biological activity.

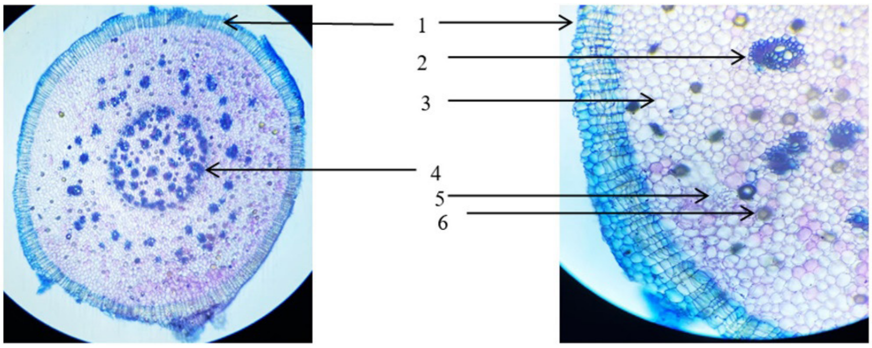

Figure 5. Rhizome microanatomy of *S. campanulatus* Kuntze: *1. Cork (phellem), 2. Phloem-xylem bundles, 3. Cortical parenchyma, 4. Endodermis, 5. Pith parenchyma, 6. Secretory cells.*

3.2.3. Microanatomy of the storage tuber

The microanatomical features of the storage tuber of *S. campanulatus* include an outermost single-layered epidermis bearing root hairs. Beneath the epidermis lies a broad cortical parenchyma composed of thin-walled cells functioning in storage (Fig. 6B). The endodermis clearly delimits the cortex and the stele. The stele has a polyarch structure, with numerous xylem and phloem bundles arranged alternately. The protoxylem is positioned exarch, whereas the metaxylem, with larger diameters, is concentrated toward the center. A pith parenchyma region develops at the center of the stele and also serves a storage role (Fig. 6A and 6C).

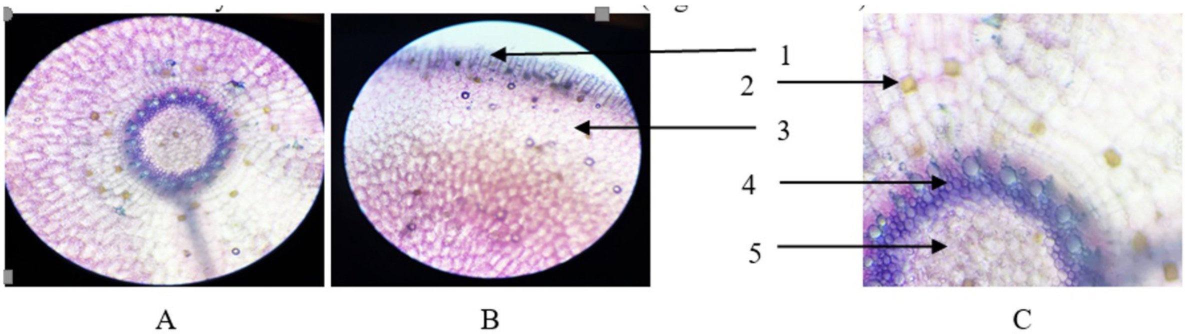

Figure 6. Microanatomy of the storage tuber: *(A) Partial microanatomy; (B) Cortex region: 1. Epidermis, 3. Cortical parenchyma; (C) Part of the cortex and stele: 2. Secretory cell, 4. Endodermis, 5. Pith parenchyma.*

3.2.4. Leaf microanatomy

Leaf microanatomy of *S. campanulatus* shows clear differentiation of the petiole, midrib, and lamina. Cross-sections of the petiole and midrib (Fig. 7A, 7B and 7D) consist of an outer epidermis, underlying collenchyma providing mechanical support, and parenchyma containing numerous air spaces (aerenchyma), similar to other Curcuma species (Anu et al., 2020). The air spaces are ovoid or irregularly elliptical, as in *Curcuma aurantiaca*. Phloem–xylem bundles (Fig. 7E and 7F) are distributed within the parenchyma, with xylem positioned above the phloem and surrounded by strengthening fibers. In the lamina (Fig. 7C), the mesophyll is homogeneous, not clearly differentiated into palisade and spongy tissues, and contains chloroplasts. Small vascular bundles are scattered throughout the mesophyll, and stomata occur on both epidermal surfaces, more abundant abaxially, representing the amphistomatic type. Stomata are surrounded by 4–6 irregular subsidiary cells, characteristic of the Zingiberaceae (Windarsih et al., 2022). Epidermal cells are irregular in shape with undulate walls, consistent with monocot features.

Overall, the leaf microanatomy reflects adaptation for photosynthesis and transpiration, with a well-developed vascular and supporting system and a stomatal apparatus optimized for gas exchange.

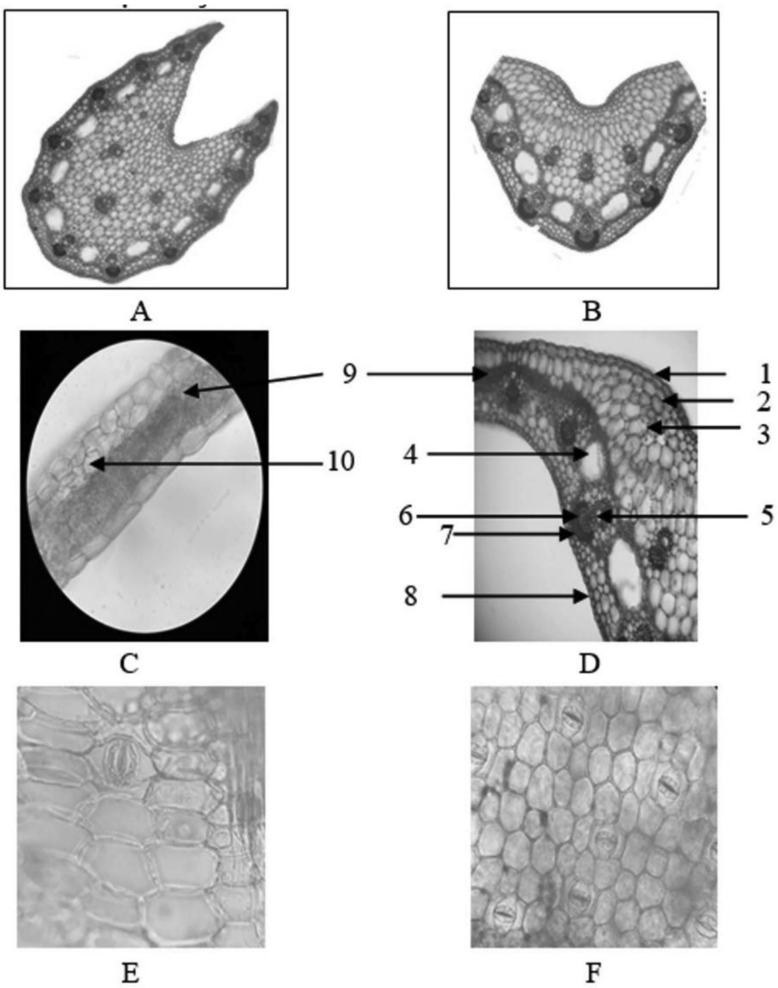

Figure 7. Leaf microanatomy of *S. campanulatus*: *(A) Petiole cross section; (B) Midrib cross section; (C) Partial view of the lamina; (D) Partial view of the midrib; (E) Stomata on the adaxial epidermis; (F) Stomata on the abaxial epidermis. (1) Upper epidermis; (2) Collenchyma; (3) Parenchyma; (4) Lacuna; (5) Xylem; (6) Phloem; (7) Fibers; (8) Lower epidermis; (9) Palisade mesophyll; (10) Lamina mesophyll.*

3.3. Powdered crude drug characteristics

The powdered crude drug is regarded as one of the important diagnostic features for the use of *S. campanulatus*. The rhizome powder is light brown, slightly pungent, and aromatic. Fragments of parenchyma with polygonal, thin-walled cells contain numerous ovoid or oval starch granules, together with scalariform vessels and fiber bundles (Fig. 8). These structures are basic features commonly recorded in Curcuma species (Ministry of Health, 2018).

In addition to the powdered material, histological components characteristic of this medicinal plant were also observed and recorded. Parenchymal fragments typically have thin walls and irregular shapes. Starch is prominent, occurring as single grains or aggregate granules of characteristic shapes and sizes, scattered individually or within parenchymal cell fragments. The abundance of starch confirms the storage function of the rhizome. Moreover, supportive structures such as fiber bundles are usually long, slender, and thick-walled were noted, indicating their mechanical support role in the rhizome. Fragments of scalariform vessels are also present, representing xylem elements with characteristic wall-thickening patterns and reflecting the function of water and nutrient transport in the plant salient traits of Zingiberaceae species (Bouzabata and Amel, 2015). The presence of resin (or other exudates) in the form of droplets or amorphous masses with darker staining is another noteworthy feature, consistent with the detection of secretory cells in the rhizome microanatomy.

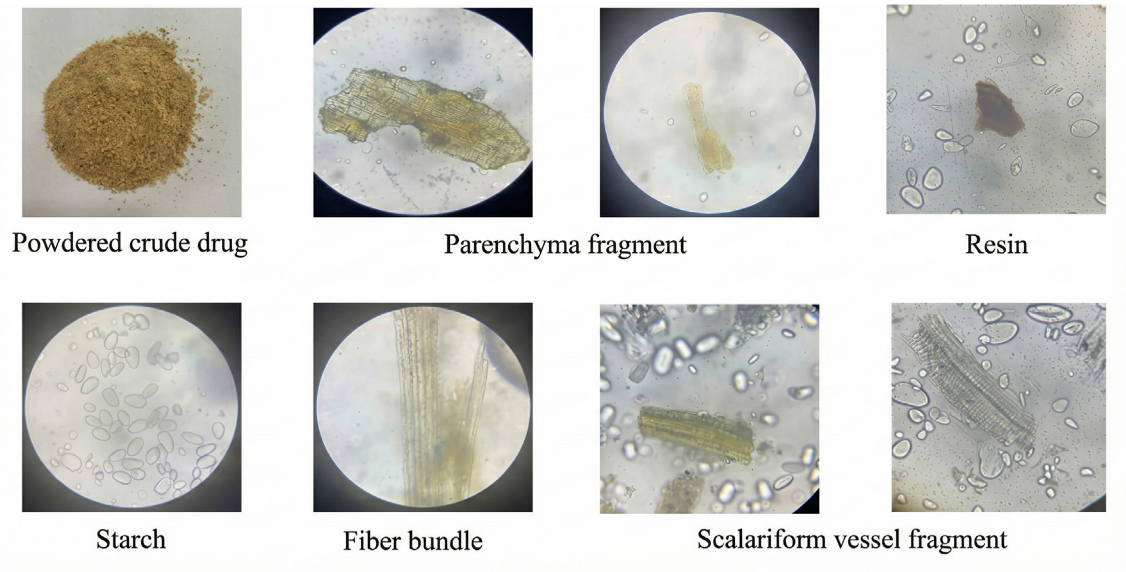

Figure 8. Rhizome powder of *S. campanulatus.*

3.4. Preliminary chemical composittion

Qualitative results (Table 1) showed that the tubers of *S. campanulatus* contain several major groups of compounds, including essential oils, free triterpenes, and coumarins, present in the ether and/or non-hydrolyzed ethanol extracts, indicating that they exist in free form or as less-polar derivatives. After hydrolysis, triterpenoids were confirmed in both the ethanol and aqueous extracts, pointing to the presence of triterpenoid glycosides. Reducing substances in the non-hydrolyzed ethanol and aqueous extracts demonstrate the sample's antioxidant capacity. Polyuronic compounds were also detected. Meanwhile, compound classes such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraglycosides, carotenoids, anthocyanosides, and organic acids were not detected or were below the detection limit of the qualitative method employed. The general saponin reaction was inconclusive, although hydrolyzed triterpenoids (possibly saponins) were confirmed.

Table 1. Compound classes in *S. campanulatus*

Compound class	Reagent/ test	Overall qualitative result*
Lipids (fats)	Spot the solution on paper	±
Carotenoid	H_2SO_4	–
Essential oils	Evaporate to dryness	+
Free triterpenes	Lierberman-Burchard	+
Alkaloid	General alkaloid reagents	–
Coumarin	Fluorescence in alkali	+
Anthraglycosid	10% KOH	–
Flavonoids	Mg/HCl	–
Cardiac glycosides	Lactone-ring test	–
	2-deoxy sugar test	–
Anthocyanosides	HCl	–
	KOH	–
Tannins	$FeCl_3$	–
Saponins	Lierberman	±
	Vigorous shaking of aqueous solution	
Organic acids	Na_2CO_3	–
Reducing substances	Fehling	+
Polyuronic compounds	Dilution with 90% ethanol	+

*Note: "+" = Present; "-"= Absentt; "±" = Possibly present in small amounts or with an inconclusive reaction; *Overall qualitative result is based on the combined outcomes of tests across the extracts.*

4. CONCLUSION

Our study accurately identified the plant specimen as *S. campanulatus*. A multidimensional analysis combining microanatomical characteristics and sequencing of the *trnL–trnF* region provided robust scientific evidence. Specifically, BLAST analysis showed sequence similarity of up to 99.84% to *S. campanulatus* (Kuntze), and the phylogenetic tree confirmed this relationship with high reliability, as evidenced by a bootstrap value based on 1,000 replicates. In addition, the study revealed a diversity of chemical constituents in *S. campanulatus*, affirming the species' medicinal potential. These results not only support accurate species identification but also open avenues for more in-depth research on natural compounds with biological activities potentially applicable in medicine and pharmaceuticals.

KHẢO SÁT ĐẶC ĐIỂM THỰC VẬT, MÃ VẠCH DNA VÀ THÀNH PHẦN HÓA HỌC CỦA CÂY TẢ LIỀN CHUÔNG (*Stahlianthus campanulatus* Kuntze) TẠI ĐẮK LẮK

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TÓM TẮT

Nghiên cứu này nhằm xây dựng cơ sở dữ liệu khoa học về đặc điểm sinh học và thành phần hóa học của Tà liền chuông (*Stahlianthus campanulatus* Kuntze), một loài dược liệu tiềm năng, thu thập tại Vườn Quốc Gia Chư Yang Sin, Đắk Lắk. Mẫu thu thập được định danh loài bằng phương pháp giải trình tự gen, mô tả chi tiết đặc điểm hình thái, vi phẫu, bột dược liệu, đồng thời phân tích sơ bộ thành phần hóa học. Kết quả giải trình tự đoạn gen *trnL-trnF* có độ dài 870 bp và dựa vào công cụ tìm kiếm trình tự gen (BLAST) cho thấy mức độ tương đồng với loài *Stahlianthus campanulatus* Kuntze lên đến 99,84%. Bột thân rễ có màu nâu nhạt, vị cay nhẹ, mùi thơm. Mảnh mô mềm tế bào đa giác vách mỏng chứa nhiều hạt tinh bột hình trứng và bầu dục, mạch vạch, bó sợi. Kết quả phân tích hóa học cho thấy, củ *S. campanulatus* chứa một số nhóm hợp chất hóa học gồm tinh dầu, triterpen tự do, và coumarin được tìm thấy trong dịch chiết ether và cồn không thủy phân tồn tại dưới dạng tự do hoặc các dẫn xuất kém phân cực. Kết quả nghiên cứu đã góp phần trong việc xác định thành phần loài, đặc điểm hình thái và sơ bộ thành phần hóa học. Đây là cơ sở quan trọng cho các nghiên cứu chuyên sâu hơn về khai thác và bảo tồn nguồn dược liệu này trong tương lai.

Từ khoá: *Bột dược liệu, DNA mã vạch, định danh phân tử, hóa thực vật, Tà liền chuông, vi phẫu.*

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