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Original Article

Morphological, Anatomical Characteristics, and Phytochemical Screening of the Leaves of *Goniothalamus flagellistylus* Tagane & V. S. Dang, Annonaceae

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Abstract: *Goniothalamus flagellistylus* Tagane & V. S. Dang (Annonaceae) is a new species of genus *Goniothalamus* discovered in 2015. Many species belonging to the genus *Goniothalamus*, which is widely used as traditional medicines for the treatment of miscarriage, fever, skin infections, and cancers. This study aims to investigate the morphology, microscopic characteristics, and chemical constituents of this plant in order to provide a valuable information for the taxonomical identification of this species in the genus *Goniothalamus*. Consequently, morphological characteristics: small tree, simple leaves, alternate, margin entire, flower simple cyme, bisexual, grows on the main stem and old branches, calyx and corolla are green in color, 6 petals regular. The anatomical characteristics of the leaf have been identified, including: main vein, leaf blade, and leaf stalk. Leaf powder has contented: covering trichomes, brachysclereids, parenchyma cells, stomata... The preliminary phytochemical screening revealed the presence of triterpenoids, proanthocyanidins, tannins, organic acids, polyuronic compounds, saponins, anthocyanidin glycosides, and anthraglycosides. The scientific name of plant was identified as *G. flagellistylus* Tagane & V. S. Dang by morphological characteristics and DNA sesquencing and this is the first reported for micromorphological features, leaf powder and phytochemical composition of this plant.

Keywords: Goniothalamus flagellistylus, Annonaceae, morphology, anatomy, phytochemical.

1. Introduction

The genus *Goniothalamus* is the largest genus in the Annonaceae family, comprising 160 species of shrubs or small trees that grow in tropical forests Asia [1].

The *Goniothalamus flagellistylus*, scientifically known as *G. flagellistylus* Tagane & V. S. Dang, is a newly discovered species within the genus *Goniothalamus*, identified in 2015. It was harvested in Khanh Hoa province. Currently, there have been no research studies

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conducted on the morphological characteristics, chemical compositions, or pharmacological effects of the *G. flagellistylus* species [2].

Many species within the genus *Goniothalamus* contained a variety of chemical constituents with diverse structures such as acetogenins, styryl-lactones, alkaloids, and flavonoids [3]. Acetogenins and styryl-lactones have been found to possess potent cytotoxic activity against various types of cancer cells, making the genus a potential source for the development of anticancer drugs. Additionally, some alkaloids and flavonoids exhibited a range of biological activities including antibacterial, antifungal, antimalarial, anti-inflammatory, and immunosuppressive effects [3-5].

In Malaysia, many species of the genus *Goniothalamus* have been widely used in traditional medicine to treat miscarriage, fever, and skin infections [6]. Indigenous people used them as a therapeutic treatment for cancer [6]. The seeds of *G. amuyon* Merr. was utilized to treat scabies, arthritis, and middle ear inflammation. Essential oil derived from the branches and roots of *G. macrophyllus* Hook.f. & Thomson exhibits strong antibacterial properties [7].

In Vietnam, several studies on the essential oil and chemistry composition from the leaves of this genus were carried out and its biological activities were reported such as: anti-microbial, anti-cancer, and cytotoxicity activities,… [8-11].

2. Material and Methods

2.1. Plant Material

The fresh leaves of *Goniothalamus flagellistylus* Tagane & V. S. Dang were collected in Ma Da forest, Dong Nai Nature and Culture Reserve, Vinh Cuu district, Dong Nai province, Vietnam in August 2023. Coordinates 11º22’55.5”N, 107º03’40.5”E, alt. 86 m.

The voucher specimen (GF042023.DN) was deposited at the Department of pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City.

2.2. Methods

2.2.1. Plant Morphological Characteristics Description

Plant morphology and botanical characteristics were described based on visual observation of fresh parts. Characteristics of fresh samples were compared with descriptions of *G. flagellistylus* published by Shuichiro Tagane and Dang Van Son [12].

Species identification using molecular markers based on DNA analysis of collected samples: Amplify a portion of the rbcL gene region and perform BLAST comparison against the NCBI gene bank, which were conducted by Phu Sa Genomics company.

2.2.2. Microscopic Characteristics of the Leaves of *G. Flagellistylus* Tagane & V. S. Dang

Leaves were microtomized, selected thin slices and stained with double Vert Iodine–Carmin staining method. The prepared slides were observed under an optical microscope at 4X, 10X, and 40X magnifications, in a distilled water, according to Appendix 12.18 of the Vietnam Pharmacopoeia V [13].

2.2.3. Leaf Powder and Epidermis Removal

The leaf powder was dried, ground, and sieved through a sieve size 32 to prepare a sample for investigating the powder and observed under an optical microscope at 10X and 40X magnifications.

Removal of the epidermal layer: To separate the epidermal layer, fresh leaves were used, and the lower epidermal layer was peeled off and observed under an optical microscope at 4X, 10X, and 40X magnifications.

2.2.4. Purity Test

The lost water of the dried sample was determined using the oven-drying method.

Total ash: The total ash of the dried herbal was determined according to the instructions in Appendix 9.8 of the Vietnam Pharmacopoeia V [13].

Extractives in the dried sample was conducted using the hot extraction method, according to Appendix 12.10 of the Vietnam Pharmacopoeia V [13].

2.2.5. Phytochemical Screening of *Goniothalamus Flagellistylus* Tagane & V. S. Dang

The plant samples (15-20 g) were extracted by using three different polarities of solvents: diethyl ether, ethanol, and water. Preliminary analysis of the chemical constituents was conducted using a modified Ciuley's procedure [14].

Briefly, the plant samples were firstly extracted with diethyl ether by soxhlet extraction method (50 mL × 3 times). The plant samples were eliminated the diethyl ether solvents, then extracted with 96% alcohol (50 mL × 3 times). The plant samples were finally extracted with water (50 mL) after removed ethanol solvents, by boiling on a water bath for 15 minutes in 3 times.

3. Results

3.1. Morphological Charactertistics of Goniothalamus Flagellistylus Tagane & V. S. Dang

Truck was a small tree, a 2-3 year old tree had an average height exceeding 10 meters and grew upright. The epicotyl and branches were green; when mature, they turned grey-green, with few rough patches.

Leaves: simple leaf, alternate, margin entire. Leaf blades were 26-33 cm long and 8.2-9.9 cm wide, oblong-elliptic, with a narrow base of leaf blades, acumen apex, and smooth surface. Penninerved, with the veins in a pinnate pattern, rominently raised on the lower surface and recessed on the upper surface, around 16-18 pairs of asymmetrical brochidodromous veins. Trucks and the adaxial leaf were typically dark green, while the abaxial leaf was lighter in color. The petiole was short, cylindrical, nearly about 1.0-1.4 cm: 1-2 cm in length and green. No stipule leaf (Figure 1).

Flower simple cyme, bixexual, grows on the main stem and old branches; petiole were long around 2.5 cm in lenght, green, and were covered with a fine layer of brown hairs. Calyx: The three sepals regular, each measuring 2 x 3 cm, were fused at the base by 0.1 cm, heart-shaped, green in color, and smooth on the surface. Corolla: 6 petals regular, greenish-yellow, divide into 2 rings: outer pentals has 3 choripetalous petals, oblong-elliptic, acumen apex, The outer surface had main veins, the main vein was clearly visible on the upper surface, sunken on the lower surface, and about 6-7 pairs of secondary veins, the bottom of the petal was convex, 0.2-0.3 cm in size, the outer surface was bulging. blue, concave inner surface forming a white, heart-shaped spoon.

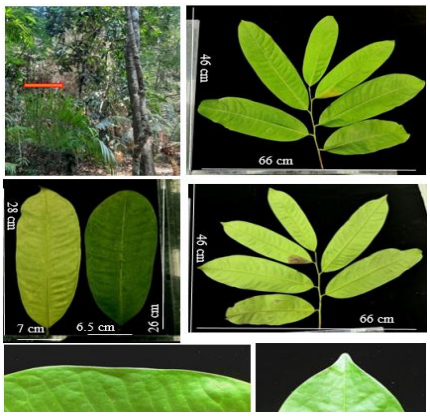

Figure 1. Morphological characteristics of *G. flagellistylus* Tagane & V. S. Dang.

The inner corolla consists of 3 choripetalous petals, shorter than the outer petals, measuring 2.5-2.6 cm in length. The blade was elongated prolate-shape, thick, with prominent raised primary veins on the outer surface; the inner surface was greenish-yellow with white streaks, slightly concave, and tapering to a sharp tip (Figure 2).

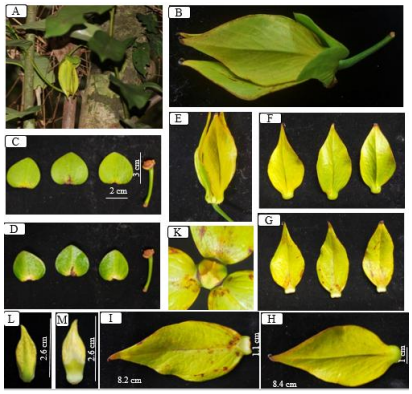

Figure 2. The perianth: (A, B): flower-bearing beanches; (C, D): calyx; (E, F, G, I, H): outer petals; (K, L, M): inner petals.

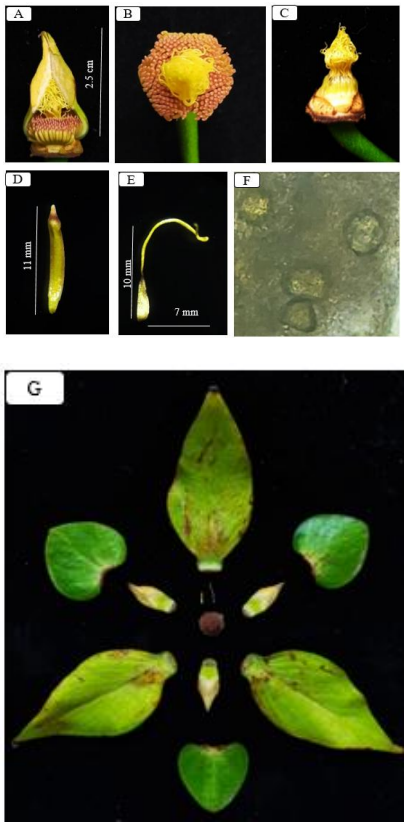

Figure 3. Structures of *G. flagellistylus* flower: (A): Androeceum and gynoeceum; (B): Stamen and pistil of the flower viewed from above; (C): receptacle; (D): stamen; (E): pistil; (F): pollen; (G): floral diagram.

The sequencing result (5'-3' direction), with a length of 525 bp, is presented as follows:

TTGACTTATTATACTCCTGAATATGA
AACCAAAGATACTGATATCTTGGCAGCA
TTCCGAGTAACCCCTCAACCCGGAGTTC
CGCCCGAGGAGGCAGGGGCTGCGGTAG
CTGCCGAATCTTCTACTGGTACATGGAC
AACTGTGTGGACCGTGGACTTACCAGTC
TTGATCGTTACAAAGGACGATGCTACCA
CATCGAGCCTGTTGCTGGAGAGGAAAAT
CAATATATTGCTTATGTAGCTTACCCTTT
AGATCTTTTTGAGGAGGGTTCTGTTACT
AATATGTTTACTTCCATTGTAGGTAATG
TGTTTGGGTTCAAAGCTCTACGAGCTCA
CGCCTAGAGGACTTGCGAATTCCTACTT
CTTATATCAAGACTTTCCAGGGCCCGCT
CACGGCATCCAAGTTGAGAGAGATAATT
GAACAAGTATGGCCGTCCTCTATTGGGT
GTACTATTAAACCCAAATTGGGGTTATC
CGCCAAGAACTACGGTAGGGCGGTTTA
GAATGTCTCCGCGGTGGA

The BLAST Nucleotide result on NCBI indicates that the sequence of the *Goniothalamus flagellistylus* chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, specimen-voucher: KYUM<JPN>:V1497, shows 100% similarity.

3.2. Microscopic Characteristics of the Leaves of Goniothalamus Flagellistylus Tagane & V. S. Dang

Midrib concaved on the upper surface in the middle, convex on the lower surface, and narrowed on both sides. Both upper and lower epidermis consist of a layer of uneven size, thickened, living rectangular cells, pinkish, thicker in the upper epidermis and thinner in the lower epidermis, the cuticle layer undulating along the epidermal cell walls. Covering trichomes on the protective layers were multicellular, few in number, scattered in the lower epidermis, with no trichomes on the upper surface. The upper lacunar collenchyma was thick, ranging from 8-10 layers in the upper epidermis, with flat, rectangular cells, and 5-12 layers of irregularly shaped polygonal cells in the lower collenchyma, varying in size. In the middle, there was a layer of flattened cells elongated on the lower surface. The sclerenchyma was evenly distributed around the midrib, ranging from 5-17 layers, irregular in size, polygonal in shape, with thick walls, narrow lumens, and no branching. The vascular system was arranged in a discontinuous ring, consisting of phloem and xylem, concentrated on the lower surface, with xylem on the inside and phloem on the outside. The phloem consisted of flattened cells, ranging from 12-14 layers, irregular in size, arranged disorderly, with cellulose walls, forming small bundles or rows just beneath the sclerenchyma layer. The xylem consisted of nearly round xylem vessels of varying sizes, stacked in rows, each row

containing 2-5 xylem vessels. The softwood tissue consists of polygonal cells, arranged alternating with xylem vessels. The pith soft tissue was parenchyma tissue consisting of nearly round or ellipsoidal cells, 2-3 layers thick, centrally depressed, forming a ring-like structure resembling two kidney beans.

Leaf blades have a thinkness equal to 1/5 of the thinkness of the midrib. The adaxial and abaxial epidermis is a single-layer tissue consisting of rectangular-shaped cells, adaxial epidermis has a prominent cuticle layer, thicker than that of the abaxial epidermis. Chlorenchyma comprises two layers of rectangular cells, cell walls contain cellulose, arranged tightly together and perpendicular to the upper epidermis adaxial epidermis. Aerenchyma consits of many layers of cells with different shapes and sizes, they are arranged haphazardly to form large defects.

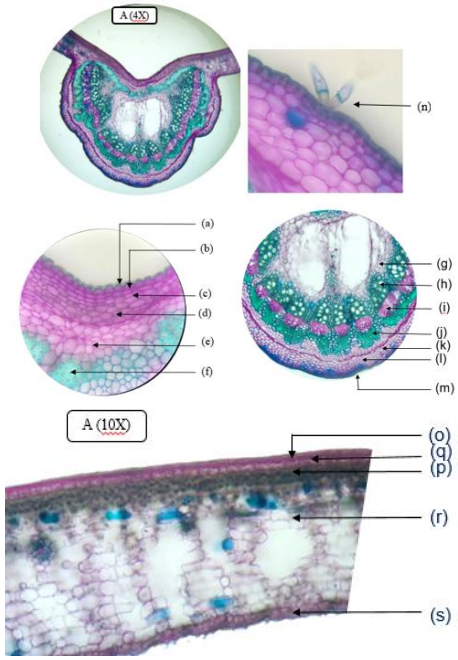

Figure 4: The cross-sectional anatomy of *G. flagellistylus* leaves under 4X, 10X, and 40X magnification: (A): midrib; (B): leaf blades; (a, o): cuticle; (b, m, p, s): epidermis; (c): collaenchyma angular; (d): calcium oxalate crystals; (e, l): parenchyma tissue; (f, g): rounded sclereid; (g): parenchyma; (h): xylem; (i): phloem; (n): covering trichomes; (q): chlorenchyma; (r): Aerenchyma

Petiole: The cross-section of the petiole is nearly circular, slightly concave on the upper surface. The epidermis consits of 1 layer of rectangular cells, uneven in size, with a moderately thin cuticle. Collaenchyma angular accounts for 1/3 of the microsurgery, comprising irregularly shaped polygonal cells of varying sizes, arranged straggerly. Sclerenchymatous rings are polygonal in shape, uneven in size, with thick cell walls and narrow lumens, forming large or individual clusters around the parenchyma and the collenchyma. The vascular system is arranged in discontinuous arc shapes, with xylem above and phloem below. Phloem consists of polygonal cells with curved cell walls, arranged in clusters. Metaxylem have a nearly circular polygonal shape, arranged haphazardly, surrounded by protoxylem. The cell walls are lignified or hardened into sclerenchymatous tissue. Parenchymatous pith comprises irregularly shaped cells of varying sizes.

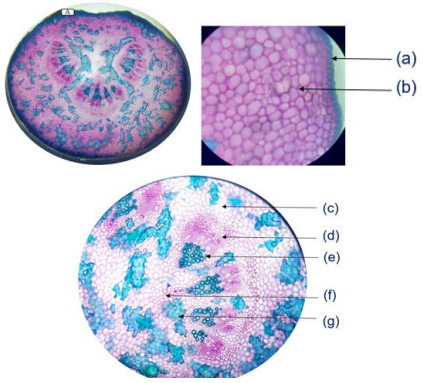

Figure 5. Microscopic characteristics of *G. flagellistylus* Tagane & V. S. Dang species' flower stalk under 4X, 10X, and 40X magnification: (A): Potiole;
(a): epidermis; (b): collaenchyma angular;
(c): parenchyma tissue; (d): phloem; (e): xylem;
(g): rounded sclereid.

3.3. Leaf Powder and Epidermis Characteristics of Goniothalamus Flagellistylus Tagane & V. S. Dang

The powder of *G. flagellistylus* leaves is green and has a distinctive, light, fragrant aroma. On microscopic inspection at 40X

magnification, covering trichomes, stomata, parenchymatous cells, rounded sclereid, branched sclereid, scalariform xylem vessel, point xylem vessel.

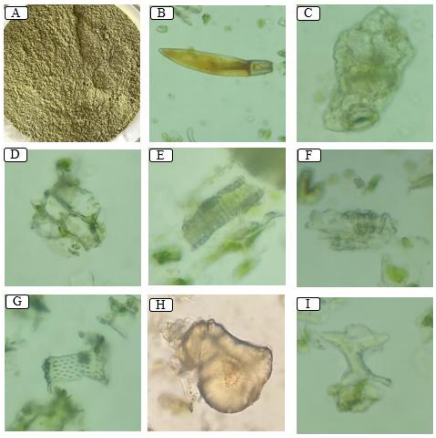

Figure 6. The leaves powder features of *G. flagellistylus* Tagane & V. S. Dang: (A): leaf powder; (B): covering trichomes; (C): epidermis and stoma; (D): parenchymatous cells; (E): fragments of scalariform xylem vessel; (F): spiral xylem vessel; (G): point xylem vessel; (H): rounded sclereid; (I): branched sclereid.

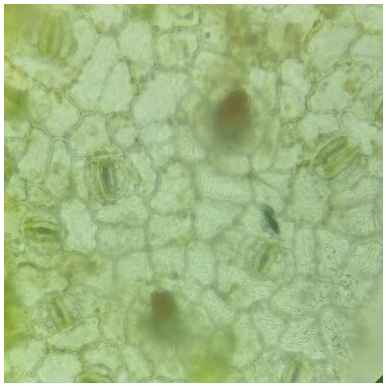

Figure 7. The epidermis with stoma.

Epidermis removal: the epidermis consists of disorganized, tightly packed cells, and cells with slightly undulating walls. Stomata were of the parallel-celled type.

3.4. Purity Test

Moisture content: The average moisture of the dry leaf powder was 9.05%, consistented with the limit for the purity test according to the Vietnamese Pharmacopoeia V.

Total ash: The results from three independent experiments of ash content showed that the average total ash of plant powder was 6.90% ± 0.12, and the average acid-insoluble ash of the herbal powder was 0.21% ± 0.08

Extractives from three different alcohol concentrations were highest in ethanol 50% as shown in Table 1, when compared to ethanol 96% and 70%.

Table 1. Extracting yields of leaf powder

Extracts	**Average ± SD (g)**
Ethanol 96%	19.55 ± 0.83
Ethanol 70%	24.26 ± 0.66
Ethanol 50%	27.18 ± 0.43

3.5. Phytochemical Screening of Goniothalamus Flagellistylus Tagane & V. S. Dang

The results of chemical compoud analytics of *G. flagellistylus* (Table 2).

The preliminary qualitative analysis revealed that *G. flagellistylus* contains free triterpenoids, proanthocyanidins, tannins, organic acids, polyuronic compounds, saponins, anthocyanidin glycosides, and anthraglycosides.

Table 2. Phytochemical screening tets of *G. flagellistylus* Tagane & V. S. Dang

Chemical constituents	The leaf extract of *G. flagellistylus*		
	Diethyl ether extract	Ethanol extract	Water extract
Fats	-	-	-
Essential oils	-	-	-
Free Triterpenoids	+++	-	-

Alkaloids	-	-	-
Coumarins	-	±	-
Antraglycosides	+	-	-
Flavonoids	-	-	-
Cardial glycosides	+	+	±
Anthocyanidins	-	-	-
Proanthocyanidins	+	-	+++
Tanins	-	+	+++
Hydrolysated tripenoids	-	-	-
Saponins	-	+	-
Polyuronides	-	-	+
Reducing compounds	-	++	++

Note: (-): Absent; (±): Possibly present; (+): Few; (++): Present; (+++): Many.

4. Conclusion

Based on the morphological characteristics and DNA barcoding, the plant samples collected in Ma Da rain forest, Dong Nai Nature and Culture Reserve, Vinh Cuu district, Dong Nai province showed the highest similarity to the plant collected in Hon Ba, Nha Trang. In this study, the floral formulae and floral diagram were reported to provide more accurate information for the species identification. In addition, the morphological characteristics of the leaves and phytochemical composition from the leaves of *G. flagellistylus* Tagane & V. S. Dang were reported for the first time. These findings provided preliminary data for the identification of the *G. flagellistylus* Tagane & V. S. Dang species.

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