BIODIVERSITAS
Volume 23, Number 11, November 2022
Pages: 5869-5883

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231141

Morphological, physicochemical, and phytochemical characterization of *Camellia dormoyana* (Pierre) Sealy from Vietnam

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Manuscript received: 31 August 2022. Revision accepted: 11 November 2022.

Abstract. *Luan DT, Chen TV, Nguyen DD, Truong QC, Thang KH, Nhi NTT, Giang VNL, Sang NX, Triet NT. 2022. Morphological, physicochemical, and phytochemical characterization of* Camellia dormoyana *(Pierre) Sealy from Vietnam. Biodiversitas 23: 5869-5883. Camellia dormoyana* (Pierre) Sealy has been used as a flavor, beverage, and folk medicine. Micromorphological features, physicochemical, and phytochemical aspects of *C. Dormoyana* have not been reported. Thus, further research is necessary to accurately verify the plants and determine their quality and safety for raw materials. This study aimed to characterize morphological, physicochemical, and phytochemical aspects of *C. dormoyana* from Vietnam. The results present that the species is easily distinguished macromorphologically by the superior ovary, 5-or 6-carpellate, glabrous, and styles glabrous, 5-or 6-parted, and uniformly fused. Micromorphologically, the structure of leaves and stems have similarities between the investigated species in the genus *Camellia*. Cork-wart, anisotricytic stoma, and stomatal clusters were found in the lower epidermis surface of leaves. The common presence of calcium oxalate crystals and sclereid cells was observed in the stem and the petiole. All epidermis fragments, parenchymal fragments, starch granules, calcium oxalate crystals, sclereid cells, spiral vessels, and red-brown oleoresin masses are standard components present in leaf powder. The physicochemical parameters of leaf powders all meet acceptable standards. The phytochemical compositions of the leaf extract contain lipids, carotenoids, essential oil, reducing compounds, amino acids, triterpenoids, saponins, tannins, and flavonoids.

Keywords: *Camellia dormoyana*, characterization, morphological features, pharmacognostical, phytochemical

INTRODUCTION

Tea is a popular product made from the leaves, buds, and flowers of the family Theaceae, such as *Camellia sinensis* (L.) Kuntze, *C. chrysantha* (Hu) Tuyama, *C. japonica* L., *C. reticulate* L., etc as well as non-Theaceae, such as *Dianthus chinensis* L., *Portulaca oleracea* L., *Corylus heterophylla* Fisch. ex Trautv., *Betula delavayi* Franch., etc (Zhang et al. 2014). Humans have used tea for thousands of years, and it is one of the most consumed beverages in Vietnam and every corner of the world. In Tea products, the genus *Camellia* is commonly known and widely used with various famous products, such as green tea (non-fermented tea), white tea, oolong tea, and black tea (fermented tea) (Meng et al. 2018).

Camellia L. is the largest genus in the family Theaceae and is widely distributed in East and South Asia (Lin et al. 2013). Up to now, there are 224 accepted species worldwide (Kew Science 2022), with more than 95 native species in Vietnam (Hoi et al. 2021). The flower *Camellia* is well attractive because of bearing colorful petals varying from white through yellow, pink to red. Among them, yellow flowering *Camellia* species occur only in South China and Vietnam with rich biodiversity that is proven by more than 45 taxa recorded, such as *C. chrysantha, C. impressivensis* H.T.Chang & S.Y.Liang, *C. kirinoi* Ninh, *C. megasepala* Hung T. Chang & Tran Ninh, *C. tuyenquangensis* V.D. Luong, Le & Ninh, *C. hamyenensis* M. Dealy, and *C. tienii* Ninh (Wei et al. 2017; Manh et al. 2019; Van Tuan et al. 2019; Van et al. 2020). Yellow flower *Camellia* species have long been used as a traditional medicine for the prevention and treatment of dysentery, hypertension, diarrhea, enteritis, hepatitis, jaundice, cirrhosis, sores, malignancies, and menstrual irregularities (He et al. 2018a, 2018b). With uncountable biological effects, yellow flower *Camellia* species are increasingly being exploited for uses. However, these *Camellia* species are difficult to distinguish based on only macro-morphological characters, because of variations in the size and shape of leaves, as well as the size and shape of reproductive parts even in an infrageneric population or a single individual (Zhao 2019), which leads to misidentifications. Erxu et al. (2009) suggested that morphological and anatomical characters, especially the

leaf part played an essential role in taxonomy and accurate species identification when there was not much information for flower variation or some species flower infrequently. A study on surveying and comparing the leaf epidermis characteristics of some *Camellia* species was reported by Ao et al. (2007), but this does not generalize to new species continuously being recorded. This suggests that combining micro-and macro-morphological methods for *Camellia* species identification has become a helpful method in overcoming limitations when comparing species whose macro-morphological is influenced by external factors.

Camellia dormoyana (Pierre) Sealy was described over a century and has not yet been rediscovered. The information from the original document does not have enough detail to differentiate it from the closely related species. We conducted numerous extensive investigations into the type locality of this species in southern Vietnam and verified it. Nevertheless, research on detailed descriptions of its micromorphological features has not been reported. Hence, it is essential to identify the micromorphological feature of *C. dormoyana*. Ultimately, the detailed and exact scientific proofs of the botanical features were derived from the study in which macro-and micro-morphological analyses were combined to precisely determine *C. dormoyana* (from Vietnam). Phytochemical constituents were also performed to identify specific components in *C. dormoyana* and standardization of the physicochemical parameters of medicinal herbs to assess the purity of raw materials. Therefore, the results of the study are valuable references for future research and development of *C. dormoyana*.

MATERIALS AND METHODS

Plant materials

Ten fresh samples were randomly collected and examined during our extensive fieldwork conducted in Dong Nai, Binh Phuoc, and Lam Dong Provinces, Vietnam. The morphological features of the samples were compared with: (1) the prior description of *Camellia dormoyana* (Pierre 1887), (2) the original drawing of *Camellia dormoyana* in 'Flore forestie`re de la Cochinchine, fasc. 8', and (3) specimens of Vietnamese *Camellias* deposited in herbaria Institude of Ecology ad Biological Resources, Vietnam National Museum of Nature, and Da Lat University as well as online digital images from the Muséum National d'Histoire Naturelle (https://science.mnhn.fr/), the Chinese Virtual Herbarium (http://www.cvh.ac.cn/), and JSTOR Global Plants (https://plants.jstor.org/).

Procedures

Morphological study

The samples were washed and processed immediately according to the following requirements for physicochemical, phytochemical screening, and micromorphological analyses.

Macromorphological characteristics

The collected plants were taxonomically identified by the morphological comparison method according to the guidance of the Vietnamese Pharmacopoeia V (Ministry of Health 2017) and some references. Such as Pierre (1887), Sealy (1958), and Quach et al. (2022) to identify the species name.

Micro-morphological characteristics

According to the guidance of Vietnamese Pharmacopoeia V (Ministry of Health 2017), free-hand sections (transverse about 10-20 μm) of the samples were stained with the iodine green-carmine double dye for morphological anatomy analyses. Thin-complete transverse sections were used for staining with 50% (v/v) chloral hydrate, 5.0% (w/v) chloramine-T, 1.0% (w/v) acetic acid, 0.3% (w/v) iodine green, and 1.0% (w/v) carmine reagents, respectively. These excess reagents, after every step, were completely removed using double-distilled water (ddw). The prepared slides in a glycerin-water mixture (1:1) were covered with a coverslip and observed under a light microscope at 4X, 10X, and 40X magnification (Carl Zeiss-PrimoStar, Germany).

The leaf epidermis analysis and leaf powder features were discovered by using the protocol of Vietnamese Pharmacopoeia V (Ministry of Health 2017). The leaf powder features were observed under a light microscope at 10X and 40X magnifications (Carl Zeiss-PrimoStar, Germany). The characterization of the organoleptic observations, including the shape, smell, color, and taste of the leaf powders was recorded (Amponsah et al. 2014).

Phytochemical study

Physicochemical evaluation

Camellia dormoyana leaves were air-dried until the raw sample could maintain a moisture content that fungi could not grow (about 12.0-13.0%). Then, the sample was pulverized into a powder to determine the moisture content, total ash value, and acid-insoluble ash value required by the Vietnamese Pharmacopoeia V for raw medicinal materials (Ministry of Health 2017).

Preliminary phytochemical screening evaluation

Phytochemical constituents include carbohydrates, essential oil, amino acids, fats, triterpenoids, tannins, flavonoids, alkaloids, coumarins, cardiac glycosides, saponins, and polyuronides were analyzed qualitatively using the Ciuley method with slight improvement (Van Chen et al. 2022b). The leaf samples were extracted using diethyl ether, then with ethanol, and finally with distilled water to obtain the respective extracts. The leaf extracts were then screened under the same conditions for the presence of phytochemical constituents.

Data analysis

Morphological data were analyzed and recorded followed by Pimple et al. (2012) and Van Chen et al. (2022a). The length and width sizes of the six sample parts were measured using a standard ruler. Micromorphologically, all values of cell size and cell layer

were measured and counted at 10X using a compound microscope (Carl Zeiss-PrimoStar, Germany) fitted with an eyepiece micrometer (Olympus, Japan). All values, including maximum and minimum values, were calculated and shown as mean values ± SD (Standard Deviation) by using Microsoft Excel 2016. The experiment of the physicochemical and phytochemical study was performed in triplicate. The results were analyzed (using Microsoft Excel 2016) and expressed as mean values ± SD (Standard Deviation) (Pimple et al. 2012).

RESULTS AND DISCUSSION

Morphological characters

History of nomeclature and typification of Camellia dormoyana

Camellia dormoyana was described more than a century ago by Pierre in 1887 (Zhao et al. 2016) based on specimens collected by himself a few years earlier in many localities in South Vietnam. This protologue consists of a colorless drawing clearly showing the overall habits, distinctive shapes of flowers and fruits (Figure 1). He classified it in the genus *Thea* L., and the species name 'dormoyana' was originally proposed by him. In 1958, a century later, Sealy changed the genus to *Camellia* L. (Zhao et al. 2017). During its taxonomic processing, this species, therefore, was recombined into *C. dormoyana*. He chose the specimen collected by Poilane as the type specimen for his description. This recombination is now accepted as the valid scientific name for this taxon. Thus, Poilane's specimen was accepted as the Holotype of the species. Its dried herbarium is deposited at Herbarium MNHN, Paris, P01903404 (Figure 2). The recorded locality was located in Dinh Quan area, Bien Hoa province, now known as Dinh Quan district, Dong Nai province, Southern Vietnam. One of Pierre's specimens also collected in the Bien Hoa area has been accepted as Syntype. After reviewing the original documents of Pierre (1887) and Sealy (1958), we concluded some typical characteristics of *C. dormoyana* are as follows: (1) inflorescence solitary, terminal with sessile pedicels covered by overlapping bracteoles; (2) medium-sized yellow flower 5.0-6.0 cm; (3) petals on 12 petals pale to dark yellow, waxy, brittle, thick with paler and semitranslucent margin; (4) Superior ovary, 5-or 6-carpellate, glabrous; (6) Styles glabrous, 5-or 6-parts, uniform fusion; (7) Mature fruit is irregular globose, capsule 5 to 6-locular, each with a longitudinal groove in the middle.

During the floristic investigation 2015-2021, in the score of Dong Nai Biosphere Reserve led by Nguyen Danh Duc, numerous specimens of a yellow *Camellia* species were collected with many characteristics that match the prior descriptions and the drawing of *C. dormoyana* mentioned above: similar in the size and shape of flower, tepals, perianth, as well as the size and shape of sexual organs. Independently, the team of the first author also collected similar specimens which were being on the anthesis, originally from this region and cultivated by a local guide (Figure 3).

Figure 1. The original drawing of *Camellia dormoyana* in 'Flore forestie`re de la Cochinchine, fasc. 8' (1887) (available from http://plantillustrations.org/species.php?id_species=1009434) and Pierre's specimen no P01903402, in MNHN, accessed from https://science.mnhn.fr/institution/mnhn/collection/p/item/p01903404?listIndex=3&listCount=15)

Figure 2. Holotype of *Camellia dormoyana* (Poilane's specimen no P01903404!, in MNHN, accessed from https://science.mnhn.fr/institution/mnhn/collection/p/item/p01903404?listIndex=3&listCount=15)

Unfortunately, all our specimens were collected without the fruits. Dong Nai Biosphere Reserve is composed of southern Cat Tien National Park and Dong Nai Nature Reserve. This area is the only remainder of the lowland evergreen forest in Dong Nai Province, which is suitable habitat for Camellias, and near Dinh Quan District, therefore, it is supposed as the outstanding type locality for *C. dormoyana*. Moreover, during floristic expeditions conducted sequentially by Truong Quang Cuong and Khuong Huu Thang, subpopulations of this yellow *Camellia* species were discovered, confirmed by digital photos of vegetative and reproductive parts of plants (Figure 4), extending the distributing range of this taxon in certainty outside Dong Nai Province.

Taxonomy

***Camellia dormoyana* (Pierre) Sealy**, Rev. Gen. *Camellia*: 45 (1958) ≡ *Thea dormoyana* Pierre ex Laness. in Pl. Util. Col. Franc. 296 (1887).

Holotype:-Vietnam, Indochinese, Bien Hoa Province, Dinh Quan, 3 December 1932, Poilane, E. 21598, (P01903404! Muséum national d'Histoire naturelle Paris, https://science.mnhn.fr/institution/mnhn/collection/p/item/p01903404? listIndex=3&listCount=15).

Syntype:-Vietnam, Indochinese, Bien Hoa Province, Chiao Phan, March 1873, L. Pierre 1332 (P01903402!, Muséum National d'Histoire Naturelle Paris, https://science.mnhn.fr/institution/mnhn/collection/p/item/p01903404? listIndex=3&listCount=15).

Epitype (designated here):- collected from the cultivated plant in Vietnam, Dong Nai Province, Tan Phu District, Tra Co Commune, 10 km from Dinh Quan Town, March 2021, Doan Thanh Luan, *NDD 493* (DLU), originally from Cat Tien National Park, Ta Lai Commune, Tan Phu District, Dong Nai Province (Figure 3).

Other field records:-Vietnam, Dong Nai Province, Vinh Cuu District, 100 m asl., 11^{0}23'52.2"N 107^{0}7'18.3"E, 4 December 2019, Nguyen Danh Duc *NDD 412*; Tan Phu District, Cat Tien National Park, 11°26'59.9"N 107°26'28.8"E, 100 m asl., 22 December 2020, Nguyen Danh Duc *NDD 490;* specimen *NDD 491* collected from a plant cultivated by Truong Quang Cuong in the botanic garden managed by Head Quater of Bidoup National Park, Da Nhim Commune, Lac Duong District, Lam Dong Province, originally from Lam Dong Province, Da Hoai District, Da Ton Commune, 200 m asl., 11°25'49.3"N 107°34'17.8"E, 9 May 2016, Madagui Forest, collector: Truong Quang Cuong; specimen NDD 492 collected from the botanic garden of Bu Gia Map NP, Binh Phước Province, original from Binh Phuoc Province, near Cambodian border, within Bu Gia Map National Park, 100 m asl., collector: Khuong Huu Thang.

Macromorphological features

Description:-shrub to small tree, 3-15 m tall. Young branches are smooth, pale green, and sparsely pubescent turning brown (or grey), and glabrous with age. Petioles 0.4-1cm long, glabrous; Lamina narrowly to broadly oblong-elliptic, 3.9-7.2 x 10.4-18 cm, apex acuminate, base subcordate to rounded, adaxially dark green and smooth, abaxially surfaces pale green, glabrous, brown glandular punctate, lateral venation pinnate, 6-9 pairs of veins, midrib and lateral veins clearly show in the abaxial surfaces, and margin denticulate. Flowers solitary, terminal, flower buds globose, flower (5-)5.5-6.0(-6.5) cm in diam., pedicels sessile, 0.1-0.3 cm long, smooth and glabrous covered completely by bracteoles. Bracteoles 6-7, persistent, light green, triangular, outside pubescent, inside glabrous, 1.5-3.0 x 1-2 mm. Sepals (5)6(-8), 0.8-1.6 x 0.9-1.8 cm, hemisphere, persistent, outside dark yellow to orange and pubescent, inside pale yellow and glabrous, arranged in 2 whorls forming a rather loose spiral, the outer whorl of 3(4) sepals and inner whorl of (2)3(4) sepals. Petals 12-22, almost round on the outer whorl near the base turning elliptic or obovate on inner whorls, dark yellow (sometimes pale yellow), waxy, brittle, thick turning paler and thinner on the margin, outside pubescent, inside glabrous, 1.7-2.8 x 1.4-2.4 cm. Stamens numerous, 1.5-2.0 cm long; filament glabrous, in tight circular formation 2.5-3 cm, outer filaments basally connate into a tube, filament tube 5-7 mm tall, 15-20 mm wide and adnate to the base of inner petals. Ovary superior, 5-or 6-carpellate, glabrous, each carpel not always bi-locular. Styles compound, glabrous, 5(6)-parted, proximally fused for two-thirds of its length, the remainder free, style 12-15 mm long, bright yellow, each end with an indistinct. Mature fruit unevenly globose, ca. 6 cm tall and 4-5 cm wide, sparse hair, capsule 5- or 6-locular, each locular having a ridge longitudinally, each locular with two seeds, pericarp 1-1.3 cm thick.

Taxonomic notes: C. dormoyana is classified in *Camellia* subg. *Camellia* Chang because the reduced pedicel is completely covered by bracteoles, and the

bracteoles are accompanied by closely followed ones and are indistinguishable from the sepals. These features differentiate *C. dormoyana* from the northern yellow flower of the sect. *Archecamellia* Sealy (*Camellia* subg. *Thea* (L.) Chang) has a pedicel and bracteoles that are clearly distinguished from the sepals. *C. dormoyana* belongs to a special sect. *Stereocarpus* because of the following typical features: persistent bracteoles, connate pattern, basally connate petals, androecium, and gynoecium smaller than the length of the petals.

In sect. *Stereocarpus*, *C. dormoyana* is similar to *C. quynhii* Luong, Quach & Hoang and *C. pubipetala* Y Wan & S.Z. Huang in general appearance as well as yellow and medium-sized flowers. The differences between *C. quynhii* with the former are lined out by Quach et al. (2022) (Table 1). The former differs from the third by some characters are as follows: (1) glabrous (vs. villous) young branch and petiole, (2) larger lamina 18-27.5 (-29.5) x 5-14 (vs. 10-17 × 5-8) mm, (3) terminal (vs. axillary or subterminal) flower, (4) filament glabrous (vs. pilose) with outer whorl basally connate into a shorter tube 0.5-0.7 mm (vs. 1-1.5) cm, (5) style 5(6)-parted (vs. 3(4)-parted), (6) capsular unevenly globose (vs. oblate), 5(6)-locular (vs. 3(4)-locular).

Table 1. Morphological comparison between *Camellia dormoyana* with *C. petelotii*, *C. euphlebia*, and *C. pubipetala*. The differences between the former with each remaining species are highlighted, characteristics of all three later cited from the taxonomic treatment of Ming and Bartholomew (2007) and available at http://www.efloras.org, accessed 25th August 2022

Characters	*C. dormoyana*	*C. petelotii*	*C. euphlebia*	*C. pubipetala*
Juvenile branches	Glabrous	Glabrous	Glabrous	Gray spreading villous
Leaf shape	Elliptic to broadly elliptic, base rounded leaf bases	Oblong-elliptic or oblong, base broadly-cuneate, rounded	Leaf blade elliptic to broadly elliptic	Elliptic-ovate, elliptic, or oblong-elliptic
Leaf size (cm)	18-27.5 (-29.5) x 5-14	9-23 × 3-7.5	(11-)14-20(-25) × (4.5-)5-8(-15)	10-17 × 5-8
Leaf apex	Sub-sharp or shortly acute	Shortly caudate	Abruptly shortly caudate to caudate and with a blunt tip	Acuminate to caudate-acuminate
Leaf based	Subcordate to rounded	Broadly cuneate to subrounded	Base obtuse to subrounded	Rounded to subtruncate
Leaf margins	Hairy, 20 far-spaced teeth	Serrulate	Serrulate	Serrulate
Lateral veins	7-11 pairs	8-10 pairs	11-13 pairs	6-8 pairs
Petiole	5-15(-20) mm long, glabrous	10-20 mm, glabrous	0.9-1.3 cm, glabrous	5-10 mm, yellowish brown villous to brown hirsute
Flower	Solitary	Solitary, 2-5 flowers clustered	Solitary or paired	Solitary
Flower position	Terminal	Axillary, subterminal	Axillary or subterminal	Axillary or subterminal
Bracteoles	Followed closely by and undifferentiated from sepals	Clearly on pedicel and differentiated from sepal	Clearly on pedicel and differentiated from sepal	Followed closely by and undifferentiated from sepals
Flower size	5-6(-6.5) cm diam.	5-6 cm	5.5-6.5 cm	5-6 cm
Sepals	Broadly ovate to ovate	Broadly ovate to ovate, 3-3.5 mm	Semiorbicular to broadly ovate, 4-5 × 5-7 mm	Broadly ovate to suborbicular, 1.3-1.5 cm
Sepal number	(5-)6 (-8)	5	5	5 or 6
Petals number	9-22	10-14	7-9	9-13
Petals shape	Waxy only the proximal (outer) 5-petaloid whorl distinctly inner irregularly obovate	Suborbicular, oblong-elliptic, broadly elliptic	Outer 2 or 3 petals sepaloid, suborbicular, 1-1.5 cm, inner petals broadly obovate to obovate-elliptic, 2.5-4 × 2-2.5 cm	Broadly obovate, obovate-elliptic to long obovate
Petal size (cm)	1.7-2.8 x 1.4-2.4	1.5-3.5 x 1.0-1.8		2-4 cm long
Filament	Glabrous, outer whorl basally connate into a 5-7 mm tube	Glabrous, outer whorl basally connate into a 7-10 mm tube	Glabrous, outer whorl basally connate for 1-1.5 cm	Pilose, outer whorl basally connate for 1-1.5 cm
Stamens	1.5-2 cm, lightly longer than styles (sub equally)	2-2.5 cm, stamens longer than styles (subequally)	2-3.5 cm (subequally)	2.5-3 cm
Style	12-15 mm long, 5(6)-parted, always uniformly fused, glabrous	15-20 mm, 3-parted, distinct	2-3.5 cm, 3-parted distinct	3(4)-parted, uniformly fused or free to ½ from the base, glabrous
Ovary	5(6)-carpellate, glabrous	3-locular, glabrous	Glabrous, 3-locular	3(4)-carpellate, pubescent
Capsule	Unevenly globose, 5(6)-locular with 5(6) longitudinal grooves	Oblate, apex retuse	Oblate, 3-locular	Oblate, 3(4)-locular with 3(4) longitudinal grooves

Camellia dormoyana is the most similar to two other species in sect. *Archecamellia*, *C. petelotii and C. euphlebia,* but differs in terminal flower (vs. axillary or subterminal) densely petals up to 22 (vs. petals 10-14 in *C. petelotii* and 7-9 in *C. euphlebia*), shorter stamen 1.5-2 cm (vs. 2-2.5 cm in *C. petelotii* and 2-3.5 cm in *C. euphlebia*), shorter style 12-15 mm long (vs. 15-20 mm in *C. petelotii* and 20-35 mm in *C. euphlebia*), style 5(6)-parted and permanently uniformly fused (vs. 3-parted, distinct), capsular unevenly globose (vs. oblate), 5(6)-locular (vs. 3(4)-locular).

Micromorphological features

Leaves

Midrib and vascular bundles

A transverse section of the leaf is comprised of a midrib and leaf blade (Figures 5A and 5C). The midrib has a concave upper surface, while the round convex lower surface (Figures 5A and 5B). The upper and lower epidermis are nearly round a rectangular layer, cellulose-impregnated walls, and uniform size (Figure 5a). The upper epidermal cells are twice as large as the lower epidermis cells, the cuticle and inner wall are also thicker (Figures 5a and 5b). The collenchymatous tissue is located below the upper and above the lower epidermis, consisting of 2-5 polygonal cell layers, irregular size, and messy arrangement (Figure 5c). The next layer is the parenchyma cells, consisting of many layers of polygonal cells, irregular in size (Figure 5d). The parenchyma tissues region is characterized by the presence of sclerenchymatous/stone tissues (Figure 5e) and sclereid cells which are very thick, branched, short, pointed, and concentrated mainly in the lower epidermis (Figure 5f). The pericycle, which is sclerenchymatous conjunctive tissue, includes 3-4 layers of polygonal cells, regular size, and forming a ring around the vascular bundle (Figure 5g). In the stele region, many bundles of conduction are arranged in an arc, xylem above and phloem below. Xylem vessels (Figure 5h) are developed with xylem rays (Figure 5i). The xylem tissues are nearly round polygonal cells, irregular in size, and arranged radially (Figure 5j), while the phloem is small, irregular polygonal cells arranged in clusters (Figure 5k). Moreover, the scattered spherical calcium oxalate crystals in xylem tissues and parenchyma tissue regions were also observed (Figure 5l).

Leaf blade

Both the upper and lower epidermis are made up of a rectangular cell layer, which has a very thick wall and is surrounded by a thick cutin layer (Figures 5C, 5a, and 5b). However, the upper epidermis cells are larger than the lower ones. Stomata with a sub-stomatal cavity are present only in the lower epidermis (Figures 5m). The leaf blade has an asymmetrical heterostructure. The palisade tissues in the upper layer are mainly columnar-shaped cells with a dense distribution that are arranged close together and perpendicular to the epidermis layer (Figure 5n), while the parenchyma cells in the lower layer contain polygonal cells with a relatively sparse distribution (Figure 5d). The mesophyll cells (spongy tissue) include 10-13 layers of heterogeneous polygonal cells with messy arrangements (Figure 5p). Extending from the upper epidermis to the lower epidermis, these cells are characterized by the presence of branched-pointed irregular sclereid cells (Figure 5f) and spherical calcium oxalate crystals (Figure 5g). The accessory bundle vessels surrounded by a ring of sclerenchymatous tissue are scattered (Figure 5q).

Epidermal cells and stomatal apparatus

Analysis of the leaf epidermis through microscopy showed that the upper and lower epidermis have irregular-sized cells, distinctly visible with curved anticlinal walls or undulating walls (Figures 6A, 6B, and 6C), containing starch granules (Figure 6a), chloroplasts (Figure 6b), and spherical calcium oxalate crystals (Figure 6c). However, stomata, guard cells (Figure 6d), subsidiary cells (Figure 6e), and cork-wart (Figure 6f) are observed only on the lower surface. The cells in cork warts seem to be suberized (Figure 6f). Furthermore, the type of stomata that appeared is anisotricytic (which has guard cells between three subsidiaries distinctly large), oval or nearly round, surrounded by 4-6 tangential elongated epidermis cells (Figure 6g). Additionally, under the field of view, there are also several stomatal clusters on the lower surface of the epidermis (Figure 6h). The trichomes (hairs) are not observed on both surfaces.

Leaf structure such as the pattern and size of epidermal cells, the layer of the epidermal cell, the thickness of palisade tissue and spongy tissue, and stomata characteristics were significant taxonomic tools for identifying species (Lu et al. 2012; Jiang et al. 2013; Qi et al. 2017). The leaves show a thick cuticle in *C. dormoyana*, similar to other *Camellia* species, such as *C. sinensis*, *C. assmica* subsp. *lasiocalyx*, etc. Rajanna and Ramakrishnan (2010) suggested that it was difficult to assort the thickness of the cuticle as an essential feature of taxonomics, therefore, it was necessary to combine many other classification features. However, a very thick cuticle observed in the leaves and stems of *C. dormoyana* and other *Camellia* species seems to have a bearing on its relationship to its inherent drought-tolerant. Moreover, various environments also affected the leaf structure (size of leaves, palisade tissue, and spongy tissue), photosynthesis, and metabolites in some *Camellia* species, such as *C. weiningensis*, *C. oleifera,* etc (Xu et al. 2022).

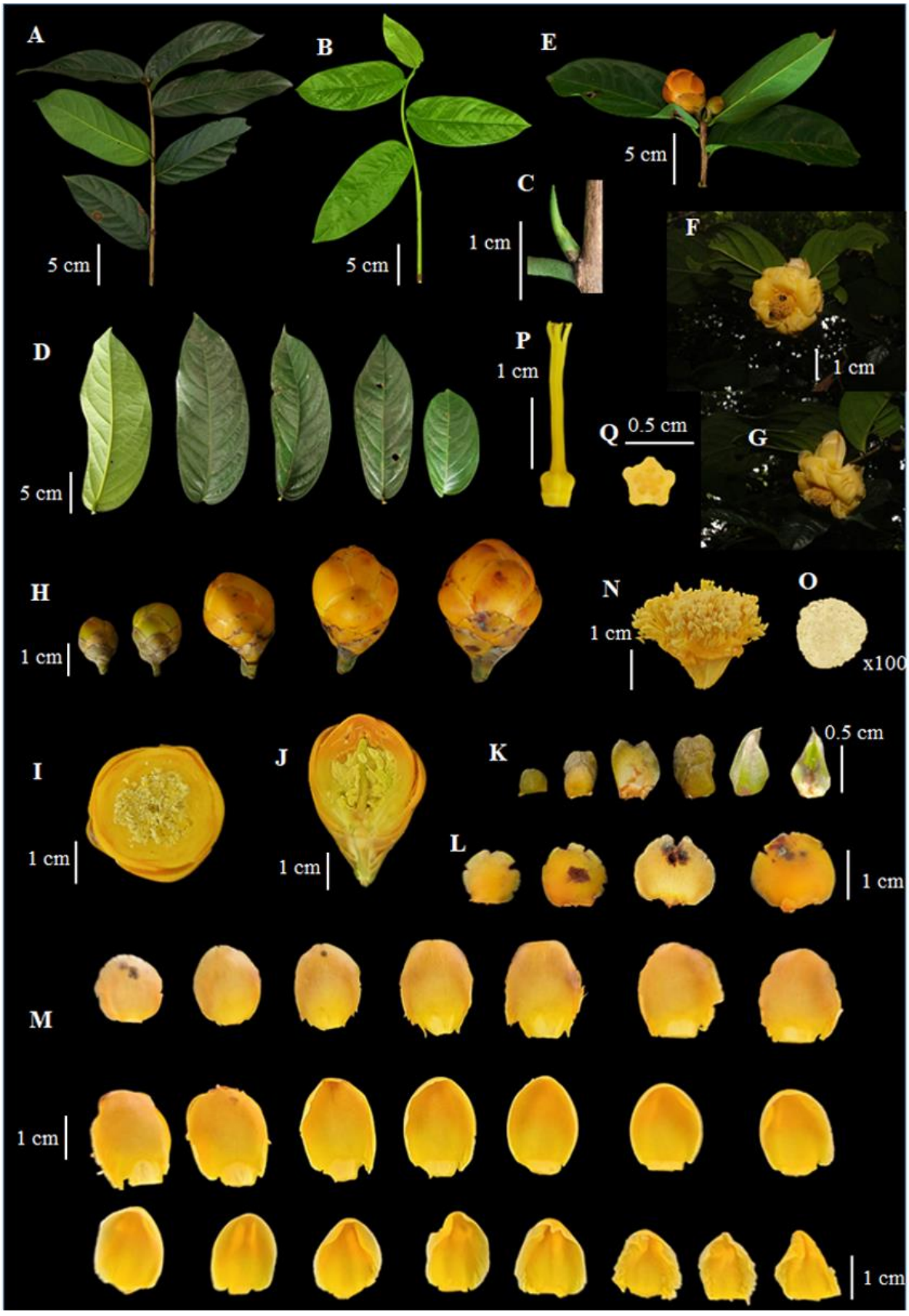

Figure 3. Morphological characteristics of *Camellia dormoyana* collected in Dong Nai Province: A. mature branch; B. young branch; C. lateral leaf bud; D. adult leaves abaxial and adaxial view; E. terminal inflorescence bearing flower buds; F. flower in front view; G. flower in side view; H. stages of flower buds: from left young to mature buds; I. flower buds in cross-section; J. flower bud in longitudinal section; K. bracteoles; L. sepals; M. tepals; N. stamen; O. pollen; P. pistil; Q. ovary in cross-section. Based on specimen *NDD 493*, photos by Doan Thanh Luan

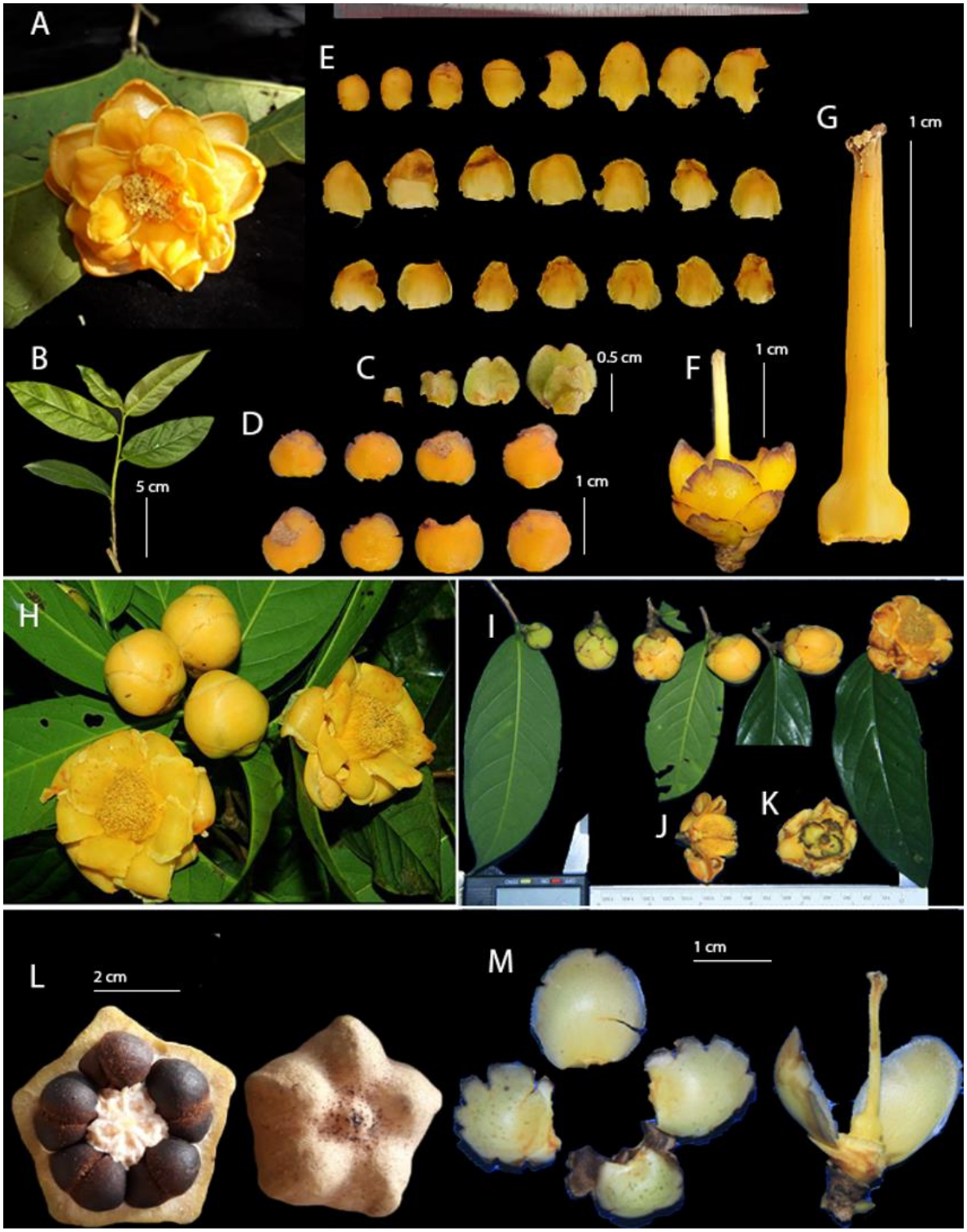

Figure 4. Morphological characteristics of *Camellia dormoyana*: A-G. from specimen NDD 491 collected in Lam Dong Province; H-M. from specimen NDD 492 collected in Binh Phuoc Province. A. flower in front view; B. young branch; C. bracteoles; D. calyx; E. tepals. F. flower removed petal and stamen, G. detail of pistil, H. inflorescences, I. stages of flower on branches: young bub, mature bub, and blooming flower; J. flower in cross-section showing stamen and pistil; L. mature fruit in cross section and back view; M. calyx and pistil. Photos A by Quang Cuong Truong, B-G by Doan Thanh Luan, H-M by Khuong Huu Thang

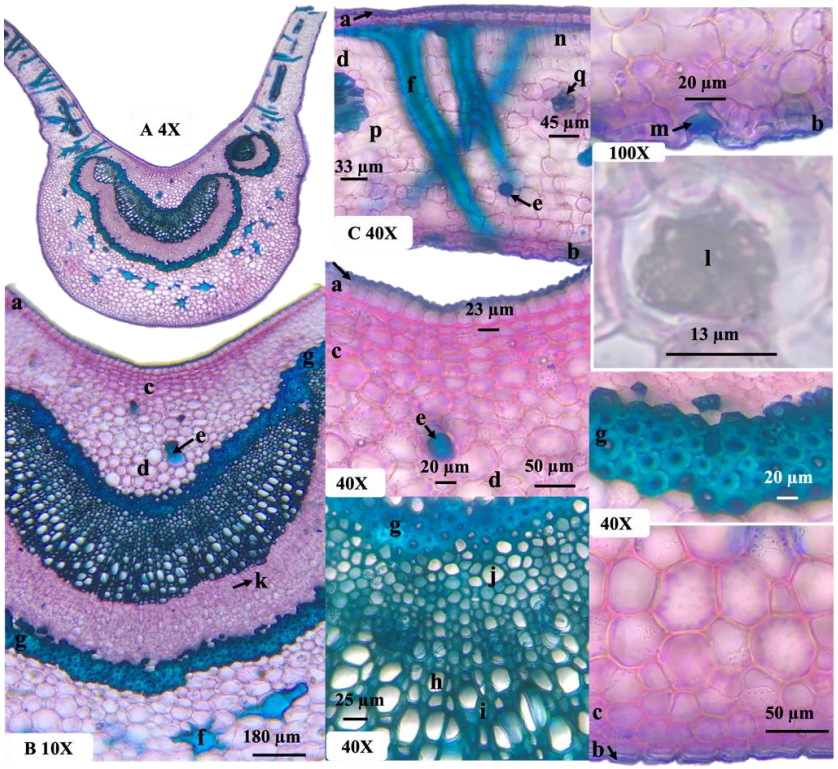

Figure 5. The features of cross-sectioned leaves of *Camellia dormoyana* (with magnifications 4X, 10X, 40X, and 100X): (A, B-4X) midrib, (C-40X) leaf blade with calcium oxalate crystals and stomata. (a) upper epidermis and (b) lower epidermis by covered thick-cutin; (c) collenchymatous tissues; (d) parenchymatous tissue; (e) sclerenchymatous/ stone tissues; (f) sclereid cells (often branched star-shaped); (g) pericycle with sclerenchymatous conjunctive tissues; (h) xylem; (i) xylem ray; (j) xylem tissues; (k) phloem; (l) spherical calcium oxalate crystals; (m) stomata with sub-stomatal cavity; (n) palisade tissues; (p) mesophyll (spongy tissue) with air cavity; (q) the accessory bundle vessels (100X)

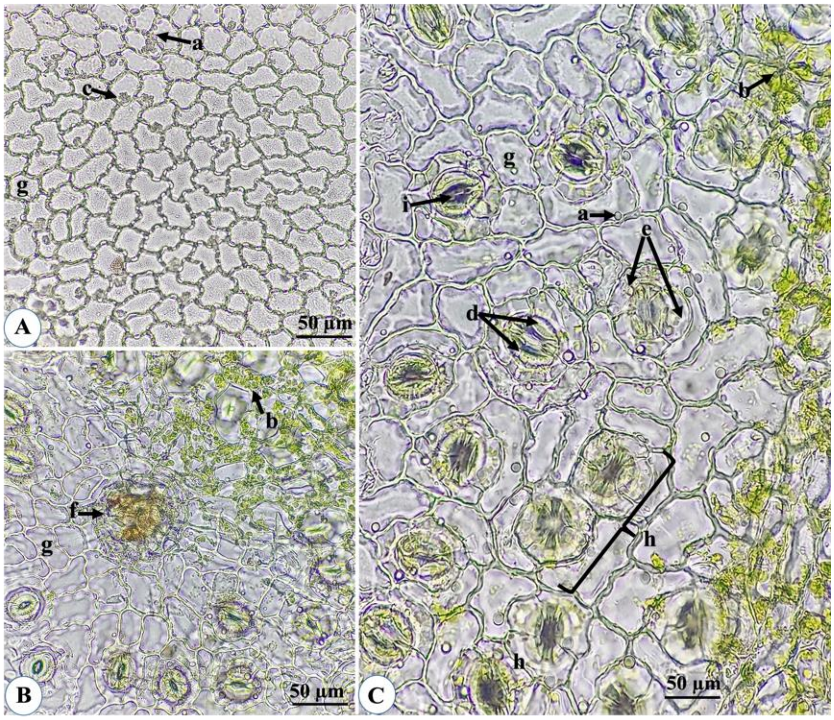

Figure 6. Epidermal cells and stomatal apparatus of *Camellia dormoyana* (with magnifications 40X): (A) upper surface view of the epidermis; (B, C) lower surface view of the epidermis with stomatal apparatus; (a) starch granules; (b) chloroplasts; (c) spherical calcium oxalate crystals; (d) guard cells; (e) subsidiary cells; (f) cork-wart; (g) epidermal cells; (h) stomatal clusters; (i) stomatal pore

Palisade tissue in *C. dormoyana* was often arranged adjacent to each other and formed a single-layered cuticle with a palisade tissue structure similar to *C. assamica*. Meanwhile, palisade tissue with 2 layers, such as *C. sinensis* and *C. assamica* subsp. *lasiocalyx* (Rajanna and Ramakrishnan 2010; Ekayanti et al. 2017) and 3-4 layers, such as *C. oleifera* (Hu et al. 2022). Thus, it is possible to rely on the number of layers of palisade tissue to classify *Camellia* species together.

Cork-warts were described as distinctive structures like circular black or brown spots on mature leaf blades and were made up of rows of radially arranged cells found on the epidermis of leaves (Qui et al. 2017). This type of structure has been reported on the leaves of *Camellia* (Zhao et al. 2016; Qi et al. 2017). Qi et al. (2017) suggested that cork-warts are responsible for air exchange and the adaptation of *Camellia* species to the more humid understory environment was correlated with cork-warts. Under the microscope, cork-warts were observed in the lower epidermal surface of *C. dormoyana* leaves. It was also found in several species of *Camellia*, such as *C. lanosituba, C. compressa, C. magniflora, C. hibisciflora, C. japonica* var. *japonica,* and *C. japonica* subsp. *rusticana* (Erxu et al. 2009).

Similar to other genera of Theaceae, trichomes on surfaces of *C. dormoyana* midrib were unicellular non-gland. Trichomes were found in both blade and midrib leaves *C. sinensis, C. oleifera, C. phellocapsa, C. jinshajiangica, C. villosa,* etc (Erxu et al. 2009; Ekayanti et al. 2017; Hu et al. 2022), while *C. dormoyana* was only found on the upper midrib surface. According to the Vietnamese Pharmacopoeia V's guidelines for quality control of medicinal herbs (Ministry of Health 2017), the type of leaf epidermal stomata is usually a relatively constant feature for a species. Therefore, the type and number of stomata proved to be significant for the taxonomic of the Theaceae. It has been demonstrated that leaf stomatal density is related to the rate of transpiration, gas exchange, and photosynthesis of leaves. The higher the stomatal density is, the more open the stomata are to absorb CO_2, and they are positively correlated with the stomatal conductivity, the rate of photosynthesis, and the rate of gas exchange. In other words, the number of stomata is inversely related to the rate of transpiration (Shiva et al. 2017; Van Chen et al. 2022b). This fact shows that the number of lower epidermal stomata is greater than that of the upper one of *C. dormoyana* leaves.

Stomatal clusters in the genus *Camellia* are generally with 2-4 stomata and arranged adjacent to subsidiary cells (Ao et al. 2007; Erxu et al. 2009). Stomata clusters were observed in many *Camellia* species such as *C. jinshajiangica, C. omeiensis, C. polyodonta, C. lanosituba, C. longigyna, C. lapidea, C. phelloderma, C. oligophlebia, C. uraku, C. edithae, C. paucipetala, C. henryana, C. tsingpienensis,* etc (Ao et al. 2007; Erxu et al. 2009). While many species without stomata clusters have also been recorded, such as *C. xifongensis, C. hongkongensis, C. brachygyna, C. hibisciflora, C. concina, C. glabsipetala, C. villosa,* etc (Erxu et al. 2009). In this study, *C.dormoyana* had stomatal clusters with two stomata. The feature of this stomatal cluster was easy to distinguish and is useful in classification.

Based on the number and arrangement of subsidiary cells, Ekeke et al. (2019) identified four types of stomata consisting anisocytic, anisotricytic, isotricytic, and tetracytic types. Ao et al. (2007) reported that the isotricytic type was predominant over the other three stomatal types. Isotricytic type has been recorded in *Camellia* species such as *C. krempfii, C. sasanqua, C. vietnamensis, C. luteoflora, C. edithae*, etc. In addition, anisocytic type has been identified only in *C. lienshanensis*. Furthermore, tetracytic type was found only in *C. longicarpa* and *C. assamica.* However, *C. paucipunctata* and *C. lancilimba* were identified as an anisotricytic stomatal type (Ao et al. 2007), which was also recorded in *C. dormoyana*. This feature is evidence that distinguishes *C. dormoyana* from other *Camellia* species.

Petiole

The cross-section of the petiole is nearly rounded and has a roughly leaf-like structure. The structure of the petiole is made up of the upper and lower epidermis, which are covered by a very thick cutin and sparse unicellular trichomes non-glandular, with the upper epidermis larger than the lower epidermis (Figures 7a and 7b), collenchymatous tissue of 15-20 layers (Figures 7c), parenchymatous tissues with polygonal intercellular spaces (Figure 7d), vascular bundles with vessel rays (Figures 7e, 7f, and 7g), sclerenchymatous tissues (Figure 7h), sclereid cells are often branched star-shaped, thick-walled, pointed (Figure 7l), and spherical calcium oxalate crystals (Figure 7m). However, the number of sclereid cells and spherical calcium oxalate crystals of the petiole is more than that of the leaf. Additionally, the leaf midrib surface was covered with thin unicellular trichomes non-glandular (Fig 7n), while the upper and lower epidermis of the leaf blade were absent trichomes.

Stem

The transverse section of the stem is nearly round in outline and includes two distinct areas with the cortex zone and the pith zone occupying about 1/5 and 4/5 of the section radius, respectively. The stem structure from outside to inside consists of the epidermis, the cortex region, and the pith region (Figures 8A-F). The epidermis is made up of a single layer of polygonal cells, irregular in size, and is covered by a thin layer of cutin (Figure 8a). Under the epidermis are 2-3 thick layers of angular, irregularly sized tissue cells, which are called collenchymatous tissues of the outer cortex (Figure 8b). The parenchymatous tissues of the inner cortex consist of 7-8 layers of nearly round polygonal cells, undulating walls, and the inner layer cells are flattened (Figure 8c). The sclerenchymatous pericycle consists of 2-4 layers of polygonal cells, regular size, arranged in continuous rings (Figure 8d), and sclereid cells (sclerenchyma or stone cells) scattered in the pericycle and parenchymatous cortex regions (Figure 8e). The next layer is the parenchymatous pericycle comprising a row of polygonal cells and arranged continuously (Figure 8f). The cambium zone includes

many layers of rectangular cells with undulating walls and gives rise to the secondary xylem and phloem. The primary phloem is a polygonal shape, cellulose wall, small in size, irregular, and arranged in clusters (Figure 8g). The secondary phloem consists of 2-4 layers of polygonal cells, and cellulose walls, which are arranged radially (Figure 8h). The secondary xylem is arranged continuously, with meta-xylem vessels polygonal or nearly round, uneven size distributed in the xylem parenchyma area (Figure 8i). The fibrous xylem parenchyma is small irregular polygonal or rectangular cells arranged in rows (Figure 8j). The narrow parenchyma rays (xylem rays) comprise 1-3 rows of oval or polygonal cells, impregnated with wood substance (Figure 8k). The primary xylem is evenly distributed, and each bundle includes 2-6 polygonal veins (Figure 8l). The primary xylem tissue is nearly round polygonal, cellulose-walled cells, varying in size, arranged in clusters evenly distributed in the parenchymatous pith region (Figure 8m). The pith region is composed of parenchymatous cells with oval or polygonal shapes, thin-walled, and triangular intercellular spaces present (Figure 8n). Calcium oxalate crystals are a spherical aggregate of individual crystals and are widely distributed in the primary phloem region and the pith region (Figure 8o). Additionally, the pith region also has the presence of scattered sieve plates (Figure 8p).

Calcium oxalate crystals (calciphytoliths) exhibit various sizes and shapes in most tissues and organs, such as roots, bark, stems, leaves, flowers, fruits, and seeds that have been observed to be a common ingredient in the plant kingdom and occur in more than 215 plant families (Nakata 2012; Paiva 2019). Plants form several specific crystals to adapt to various environments. This crystal appears to play an important role in proven functions, such as tissue calcium regulation and other minerals, protection from herbivory, pathogens, and heavy metal detoxification (Paiva 2019; Pérez-Aguilar and Cuéllar-Cruz 2022). In terms of the genus *Camellia*, micromorphological methods such as micro-anatomical and micro-powder under the microscope are increasingly used for identification because of their ability to detect unique components, such as tissue features, vascular bundles, starch granules, calcium oxalate crystals, etc. Moreover, as micro-mineral bodies, calcium oxalate crystals will be formed only as specific crystal types with morphologies characteristic of a particular species.

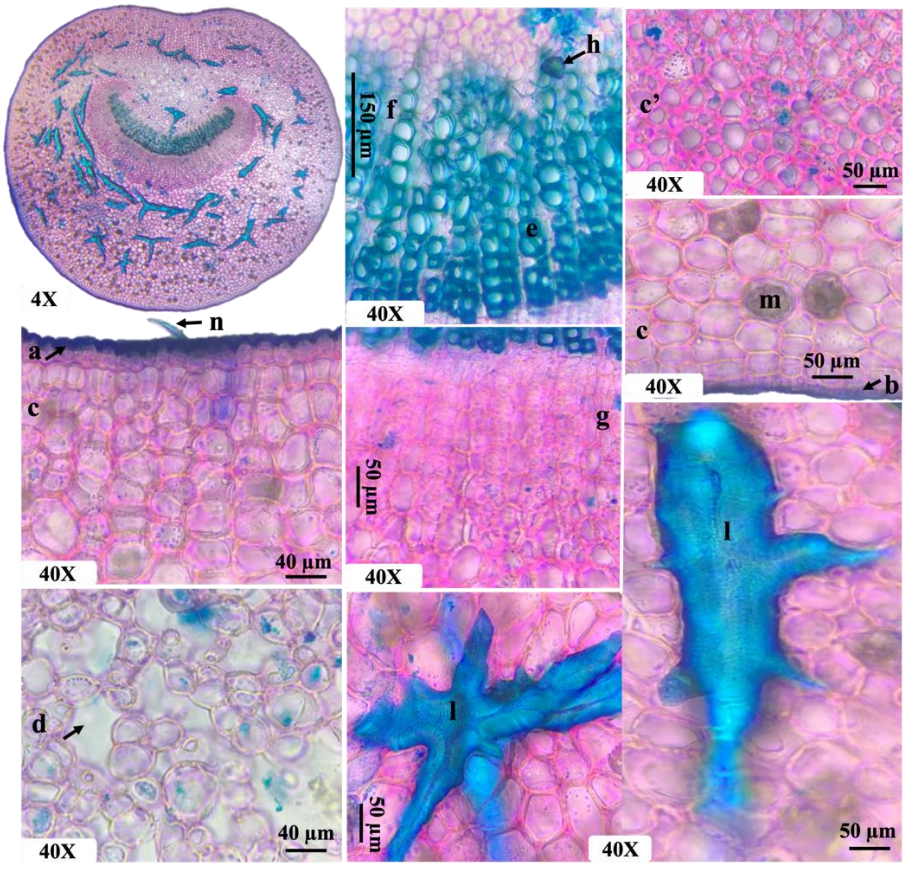

Figure 7. The features of the cross-sectioned petiole of *Camellia dormoyana* (with magnifications 4X, 40X): (a) the upper epidermis and (b) the lower epidermis by covered thick-cutin (arrow); (c) collenchymatous tissues and (c') thick-collenchymatous tissues; (d) parenchymatous tissues with polygonal intercellular spaces (arrow); (e) xylem; (f) parenchyma/ vessel rays; (g) phloem; (h) sclerenchymatous/ stone tissues (arrow); (l) sclereid cells (often branched star-shaped, thick-walled, pointed); (m) spherical calcium oxalate crystals; (n) unicellular trichomes non-glandular (arrow)

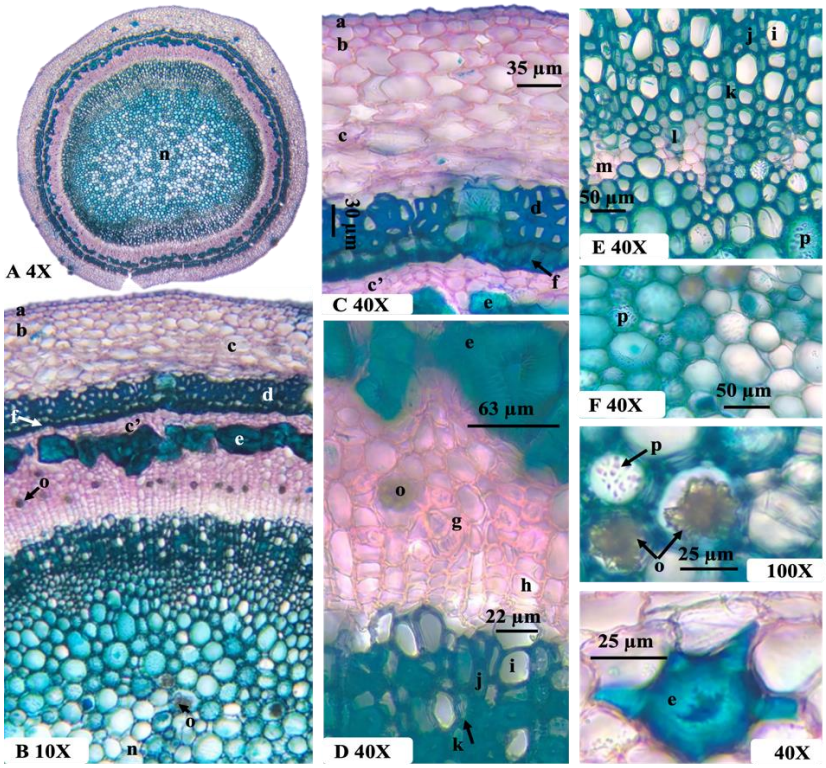

Figure 8. A-F. The features of the cross-sectioned stem of *Camellia dormoyana* (with magnifications 4X, 10X, 40X): (a) epidermis; (b) collenchymatous tissue of the outer cortex; (c) parenchymatous tissue of the inner cortex with intercellular spaces and (c') parenchymatous tissue; (d) sclerenchymatous pericycle; (e) sclereid cells (sclerenchyma or stone cells); (f) parenchymatous pericycle; (g) primary phloem; (h) secondary phloem; (i) secondary xylem; (j) fibrous xylem parenchyma; (k) parenchyma rays (xylem rays); (l) primary xylem; (m) primary xylem tissue; (n) parenchymatous pith with triangular intercellular spaces; (o) calcium oxalate crystals; (p) sieve plates

Herlina et al. (2019) reported that calcium oxalate crystals control morphology, which is regulated by the cells and genetics. This suggests that based on the presence of crystals, it is possible to separate individual species as well as identify different plant species (Zhang et al. 2014; Grechana et al. 2020). However, the morphological study of calcium oxalate crystals in plants of the Theaceae has been very limited. Further investigation is needed to sign whether this crystal is present in the leaves and stems of other yellow *Camellia* species. Zhang et al. (2014) identified five common types (including druses, styloids, raphides, prisms, and crystal sands) of calcium oxalate crystal morphologies of several other non-Theaceae plant species used as teas. These crystal types were found in some Theaceae plants, among them, the most common type was druses in the *Camellia*, while prisms, styloids, and raphides were absent in the one.

Spherical calcium oxalate crystals were found in the leaves of *C. dormoyana* in the current study, which was a component commonly found in the leaves of other *Camellia* species, such as *C. sinensis, C. synaptica, C. impressinervis, C. crassipes, C. reticulate, C. japonica, C. sasanqua, C. reticulata, C. costei*, etc. (Zhang et al. 2014, Ekayanti et al. 2017). Additionally, druses (a spherical aggregate of individual crystals) in *C. dormoyana* are mainly scattered in mesophyll cells, the upper epidermis surface of leaves and stems. However, in order to distinguish the *C. dormoyana* species from the others requires the presence of identifiable druses with its size. Thus, spherical calcium oxalate crystals in the leaves and stems would enable the identification of *C. dormoyana* with a significant degree of confidence.

The similarity of the stems structure of *C. dormoyana* with other *Camellia* species, such as *C. sinensis* and *C. chrysantha*, especially, from the outside to the inside of the stems are typical micromorphology features, including the epidermis, the cortex region, and the stele region. The common presence of spherical calcium oxalate crystals and sclereid cells observed in the stem was also observed in the petiole of *C. dormoyana*. However, round-polygonal stone cells were found in stems, while branched star-shaped, thick-walled, pointed sclereid cells were found in the petiole. The anatomical structure, the distribution of the spherical calcium oxalate crystals, and sclereid cells are considered three of the bases for determining this species.

Leaf powder

It is necessary to microscopically inspect the powdered material to accurately identify medicinal herbs (Nafiu et al. 2017). Moreover, microscopy, as a simple and fast tool, can be used to test the purity of raw material powders based on plant tissue characteristics (Osman et al. 2019). Leaf powder is moss green or chlorophyll green, fragrant, and bitter-acrid taste (Figure 9A). Microscopic examination of leaves powder revealed present the upper epidermis fragment (Figure 9B), the lower epidermis fragment with

stomata (Figure 9C), and the bundle of fibers is broken (Figure 9D). Additionally, the sclereid cell is large, branched, pointed, and irregular (often star-shaped) (Figure 9E) with sclereid fiber (Figure 9F). Spherical calcium oxalate crystals are scattered (Figure 9G), and fragments of thick spiral xylem vessel twisted circuit (Figure 9H), red-brown oleoresin masses (Figure 9I), scattered starch granules globose with an outer surface smooth (Figure 9J) which are also found in the observation field. Generally, fresh leaves and leaf powder have similar compositions; Moreover, the upper and lower epidermis can be identified through the stomata.

Physicochemical and Phytochemical characterization

Physicochemical parameters

The leaf powders' moisture content, total ash value, and acid-insoluble ash value were found to be 11.04 ± 0.17% (w/w), 9.96 ± 0.30% (w/w), and 0.31 ± 0.04% (w/w), respectively. Raw herbs are easily damaged by chemical changes or microbial attacks because of the high humidity of the crude drug and the relatively high temperature of the environment, which result in the low quality of raw materials. Salts of silicates, silica, carbonates, and phosphates are components present in total ash. A high total ash value is an indication of contamination, tampering, adulteration or mishandling of the material. Acid-insoluble ash indicates silica contamination, such as soil, sand, and gravel. Comparison of these ash values with the total ash values of the same sample will help distinguish contaminated materials and other components that are not present in the natural ash of the raw herbal material (Kaskoos and Ahamad 2014).

The total ash value in the current study revealed the presence of a high inorganic matter present in the herb material. Additionally, the acid-insoluble ash value (less than 1.0%) indicates the presence of a low level of the acid-insoluble inorganic component in that material. All pharmacognostic results were important identification and provided standardization parameters for the leaves of *C. dormoyana* to determine whether the sample has achieved purity for medicinal materials uses and for preparation of the monograph.

Phytochemical analysis

The phytochemical screening of the leaf extracts of *C. dormoyana* is presented in Table 2. The result reveals the presence of main phytochemical constituents, including lipids, carotenoids, essential oils, reducing compounds, amino acids, triterpenoids, saponins, tannins, and flavonoids. However, under preliminary analysis conditions, alkaloids, cardiac glycosides, coumarins, and polyuronides were not found in the *C. dormoyana* leaves.

In general, polyphenol compounds such as flavonoids and tannins present in the leaves of *C. dormoyana* are also commonly found in other yellow *Camellia* species, such as *C. chrysantha* (He et al. 2018c), *C. hakodae* (Tuyen et al. 2019). Phytochemical screening is known as an analytical method to determine the secondary metabolites' ability to react with certain specific reagents. In addition, the method uses solvents of different polarities to specifically extract secondary metabolites to achieve high performance.

Secondary metabolites in plants have been reported as substances with various biological effects. Previous studies have proved that flavonoids, tannins, and essential oils have different biological effects, including antioxidant, antibacterial, anti-inflammatory, anti-allergic, hypoglycemic, hypolipidemic, and other biological effects (Bouchelaghem et al. 2022; Gulcin et al. 2022; Xiao 2022). Therefore, these compounds have an important role in disease prevention and treatment, and since then, people have known to use the leaves and flowers to make tea with the name "Yellow Flower Tea" (Manh et al. 2019; Van et al. 2020) of *C. chrysantha* (Wei et al. 2017), *C. impressivensis*, *C. kirinoi*, *C. megasepala*, *C. tuyenquangensis*, *C. hamyenensis*, and *C. tienii*, etc (Van Tuan et al. 2019) as daily drink tea. Like other *Camellia* species, with proven biological effects, the leaves of *C. dormoyana* showed a promise to become raw material for tea products and medicinal herbs.

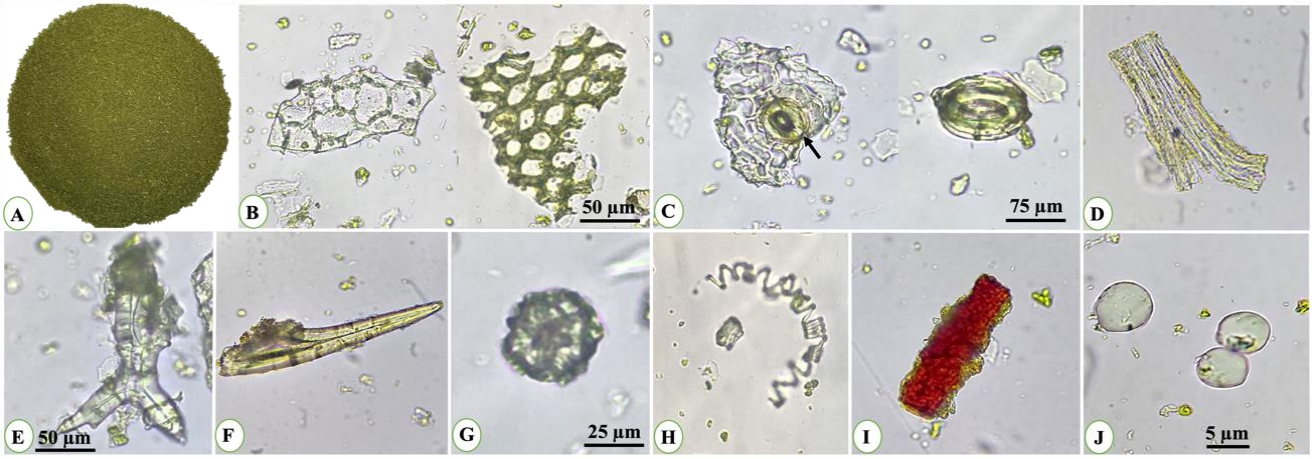

Figure 9. The features of *Camellia dormoyana* leaves powder (with magnifications 40X): (A) chlorophyll green leaf powder; (B) fragment of upper epidermal cells; (C) surface view lower epidermis with stomata (arrows); (D) vascular bundle (bundle of fibers); (E) sclereid cell (star-shaped); (F) thick sclereid fiber; (G) spherical calcium oxalate crystals; (H) thick spiral xylem vessel; (I) red-brown oleoresin masses; (J) starch granules

Table 2. Phytochemical contents of *Camellia dormoyana* leaves

Chemical constituents	The name of the test	The leaf extracts of *Camellia dormoyana*		
		Ether extract	Ethanol extract	Water extract
Lipids	Stain test	+	-	-
Reducing compounds	Molisch's test, Fehling's test	+	+	+
Carotenoids	H_2SO_4 test	+	-	-
Essential oil	Scent test	+	-	-
Triterpenoid	Salkowski test	+	+	+
Alkaloids	Wagner's test, Dragendoff's test, Murexide's test	-	-	-
Amino acids	Na_2CO_3 test	+	+	+
Cardiac glycosides	Raymond's test, Xanthydrol test	-	-	-
Saponins	Foam test	+	+	+
Coumarins	Lactone ring test	-	-	-
Flavonoids	Shinoda test	-	+	+
Tannins	Gelatin test, $FeCl_3$ test	-	+	+
Polyuronides	Ethanol 90% test	-	-	-

Note: "+" indicates the presence and "-" indicates the absence

The anatomical structure of *C. dormoyana* leaves and stems is similar to other *Camellia* species. This study provides for the first time a complete description of micromorphological features through micrographs, establishing their specific physicochemical parameters and chemical composition clarification by the preliminary phytochemical screening of *C. dormoyana*. Accordingly, these micromorphological similarities were drawn up as follows: the structure of leaves includes the epidermis covered with cutin, palisade tissue, spongy tissue, vascular bundle, sclereid cells, stomatal features, and the structure of stems includes the epidermis, the cortical region (outer and inner cortex), and the pith region with its vascular bundle system. However, the anatomical structure (1 layered palisade tissue, cork-wart, stomatal clusters, anisotricytic type, thin unicellular trichomes non-glandular), the distribution of the spherical calcium oxalate crystals, and sclereid cells are considered as the bases for determining this species. All epidermis fragments, parenchymal fragments, starch granules, spherical calcium oxalate crystals, sclereid cells, spiral xylem vessels, and red-brown oleoresin masses are common components present in leaf powder. In terms of phytoconstituents, the leaf extract contains lipids, carotenoids, essential oil, reducing compounds, amino acids, triterpenoids, saponins, tannins, and flavonoids. However, alkaloids, cardiac glycosides, coumarins, and polyuronides were absent in the *C. dormoyana* leaves. The physicochemical parameters all meet the allowable standards for medicinal materials. All of these provided additional insight into the guidelines for the identification of *C. dormoyana* powder and contributed effectively to the correct identification of this species.

In conclusion, *C. dormoyana* is easily distinguished macromorphologically by the superior ovary, 5-or 6-carpellate, glabrous, and styles glabrous, 5-or 6-parted, and uniformly fused. Micromorphologically, the structure of leaves and stems have similarities with other species within the genus *Camellia*. The leaf powder has a low moisture content, ranging from 10.87 to 11.21%. The total ash value varies from 9.66 to 10.26%. The percentage of acid-insoluble ash is present in small quantities, which varies from 0.27 to 0.35%. The leaves contain lipids, carotenoids, essential oils, reducing compounds, amino acids, triterpenoids, saponins, tannins, and flavonoids. Stomata, cork-warts, unicellular trichomes non-glandular, spherical calcium oxalate crystals, palisade tissue, spongy tissue, vascular bundle, and sclereid cells are prominent features of microscopic characterization. The highlights of the present study can be used to enrich new knowledge on macro-and micro-morphological features, physicochemical parameters, and chemical compositions that were previously unknown.

ACKNOWLEDGEMENTS

The authors would like to give the greatest gratitude to the Faculty of Pharmacy, Nguyen Tat Thanh University, Vietnam, for their facility support. We also thank Dr. Luong Van Dung (Director of the Center for Biodiversity and Climate Change; Department of Biology, Da Lat University, Vietnam) for his comments and advice, and Nguyen Huu Tri (Cat Tien National Park, Dong Nai Province, Vietnam) for providing research samples.

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