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Kaempferia daklakensis N.H.Tuan & N.D.Trong (Zingiberaceae) - A new species from Daklak province, Central VietNam

Nguyen Hoang Tuan¹*, Nghiem Duc Trong¹, Nguyen Thanh Tung¹, Pham Ngoc Khanh^{1,2}

^{1*}Department of Pharmacognosy, Hanoi University of Pharmacy

² Insitute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

* Email: tuandl50@yahoo.com

Article Info	Abstract			
Received: 10-12-2019,	Through our survey on medicinal plant resources in Ea So nature reserve, Ea Kar			
Revised: 06-06-2019,	district, Daklak province, central Viet Nam, a new species of Kaempferia genus			
Accepted: 26-02-2020	from Vietnam, namely <i>Kaempferia daklakensis</i> N.H.Tuan & N.D.Trong, was			
Keywords: Kaempferia	discovered. A full description with color plates and notes on the variability in			
daklakensis, Zingiberaceae,	morphology, distribution, phenology, uses and relationship of this new species in			
Daklak province,	compare to other taxa were reported. This paper is also as an official announce for			
Viet Nam	this new Kaempferia species as new record for the flora of Vietnam.			

INTRODUCTION

Kaempferia Linnaeus is a genus of the second largest species members in the family Ginger (Zingiberaceae) (after Zingiber genus). Worldwide, it consists of 60 scientifically described species, which are distributed tropical regions from India to South East Asia (Sirirugsa, 1992). Pham Hoang Ho (1931-2017) in his the famous ethnobotanist of Vietnam, listed and described in detail 18 Kaempferia species, which are widely growing in the lowland and midland forests, relatively concentrated in dipterocarp forests especially in the Central Highlands, Vietnam (Ho, 2002). In previous papers, we described several Kaempferia species as new record of medicinal plant species for Viet Nam including K. parviflora Wall. ex Baker (Tuan and Cuong, 2016), K. marginata Carey ex Roscoe (Tuan and Duc, 2017), K. champasakensis Picheans. & Koonterm. (Tuan, Duc et al. 2018), K. laotica Gagnep (Tuan, Nam et al., 2018), K. attapeuensis Picheans. & Koonterm (Tuan, Hien et al. 2019) and K. daklakensis (in Vietnamese) (Tuấn and Trọng, 2017). The species Kaempferia daklakensis NH Tuan & N.D.Trong (locally name: Up dat, which means "ground digging") which was found during a field trip in Dak Lak province, Central Highland, Vietnam,

possesses characteristics completely distinguished from those of previously described *Kaempferia* species. In order to announce this new *Kaempferia* species as new record for the flora of Vietnam, in this paper, we reported all our results in morphological study of this new *Kaempferia daklakensis* in detail in English.

MATERIALS AND METHODS

The plant *K. daklakensis* was collected in September 2015 in Daklak province, Vietnam. Botanical identification was performed by Department of Pharmacognosy, Hanoi University of Pharmacy and a voucher specimen, HNIP/18153/16, is deposited at Department of Pharmacognosy, Hanoi University of Pharmacy.

Morphological characteristics of the plant was studied and analyzed by the Leica EZ4 stereoscope (Leica Microsystem, Heerbrugg, Switzerland). Descriptions are based on living materials and follow to the published documents (Sirirugsa 1992). The whole plant was dried in oven at 55 °C for 72h and then powdered by electric grinder for microscopic and physicochemical examinations. Identification of sample scientific name: Comparative morphological method was applied to identify the species (Leong-Skornickova and Newman, 2015; Larsen and Saksuwan, 2006) (Larsen, Saksuwan, 2006; Wu & Raven, 2000; Picheansoonthon and Koonterm, 2009; Sirirugsa, 1992); collected samples were analyzed in morphological characteristics the compared with those of taxonomic key and description in the documents (Tap, 2006; Thin, 2007) and to the authenticated plant specimens stored at Paris Natural History Museum (online).

RESULTS

Description of the new species

Scientific name: Kaempferia daklakensis N.H.Tuan et. N.D.Trong

Type: VIETNAM, Daklak Province, Ea Kar district, Ea So Nature Reserve, 12°59'17.4"N 108°39'54.2"E, at altitude 210 m above sea level, on 16th September 2015.

Vietnamese common name: Up dat (Up đất)

Family: Zingiberaceae

Description

This species is a perennial rhizomatous herb to 0.5 m tall. *Rhizome* short, rough surface, brown, cross-section nearly spherical, approximately 8-10 mm in diameter, fragrant, light yellow. *Root* tuberous bulb egg-shaped, externally brown, internally yellowish, and buried deeply in the ground. Root dissection is nearly circular, about 6-7 mm in diameter, divided into two concentric circular areas, externally whitish, internally milky white, fragrant.

Leaf single, usually 2 (rarely 3), grow near the ground; *lamina* elliptic-shaped, size 11-12 cm \times 8-9 cm, adaxially dark green, abaxially pale green, glabrous on both sides; *leaf base* nearly rounded; *apex* cuspidate; *leaf margin* entire, lightly curly; *leaf veins* parallel, curved towards the leaf apex, *midrib* dark green and bigger than veins; *leaf shealth* 4-4.2 \times 2-2.2 cm, gutter-shaped, thick, white in lower half, green in higher half, roll closely together to form a *pseudostem; ligule*

membranous, green; *petiole* 1-9 cm long, underside nearly greenish white, glabrous.

Inflorescence central, grow from the rhizome, between the two end-leaves, about 8-8.5 cm tall, coming up after leaves. Flowers not stalked, irregular, hermaphroditic, pattern 3. Bracts, bladeshape, $2.5-3 \times 0.2 - 0.3$ cm, point tip, lower part white, upper top light green. Bracteoles, $20-21 \times$ 1.0-1.5 mm in size, white at base, light green toward apex; *sepal* stick together into tube $3-3.2 \times$ 0.2-0.25 cm long, below white, above light green; corolla white, stick together at the bottom into a tube 6-6.2 cm long, upper irregular, strip-shape about 2.5-2.8 cm long, trilobed, the middle-plate opposite the lip 4 mm wide, 2 sided-strips about 0.2-0.3 cm wide. Stamens set up on the tube mount as two rounds. The outer ring consists of 2 double slits on both sides, transformed into two oval slices, 1.9 cm long, 1.3-1.4 cm wide, inside white, outside slightly violet, taper at base. The front stamens disappear. The inner ring consists of two front flanks and one fertile stamen, two delicately attached to one lip, inside white, outside purple, 1.9-2 cm long, 2.2-2.3 cm wide, bilobed. Fertile stamen standing opposite the lips, no filament, pollen blade-shape, white; connective along, 0.4-0.5 cm long, bending outward.

Ovary, elliptic-shape, 2-2.1 mm long, creamy white, trilocular, attached to a 3-cell gourd, 2 rows of locules at each cell, attached as ovary-center type; style 1, ca. 6-6.2 cm long, white, smooth, fibrous, stigma ca 1 mm wide, funnel-shaped with scattered short evelashes on the funnel mouth. Seeds, many seeds, nearly block-shaped, apex pointed, the edges and the bottom are obtuse, white opaque, size $3-3.1 \times 1.7-1.8$ mm, seed crosssection has two distinct layers, externally white, thin, internally much thicker, light brown (Fig.1). morphological characteristics The of Κ.

daklaknensis were compared to those of the known *Kaempferia* species including *K. champasakensis* and *K. galanga*. The results were shown in Tab. 1.

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Characteristics		Tested sample	K. champasakensis (Chayan and Supachai 2008)	K. galanga (Flora of China Editorial Committee 2000) (Chi 2003)
Plant form		Perennial plants	Perennial plants	Perennial plants
Rhizome, roots		 short, thick (lean) with rough, brown surface Tuberous roots grow in cluster 	 Rhizome slender, long Tuberous roots grow in cluster 	- Rhizome grow many tuberous roots close to each other
Leaf	Quantity	2 (3)	2 (3)	2 (3)
	Shape, size	 Oval to elliptic-shape 11-12 × 8 -9 cm 	 Egg to lanceolate shape 5,2-7,3 × 0,8-1,6 cm 	 Round or wide oval- shape 6-15 × 5-10 cm
	Leaf lamina	- <i>leaf base</i> nearly rounded; <i>apex</i> cuspidate; <i>leaf margin</i> entire, lightly curly; Green, glabrous	 <i>leaf base</i> slender to oval, <i>apex</i> pointed; <i>leaf margin</i> entire, lightly curly Green, glabrous 	<i>leaf base</i> slender, <i>apex</i> pointed; <i>leaf margin</i> entire, lightly curly - white or purplish leaf margin, with plenty of fine, long hair
	Leaf sheath	- $4-4.2 \times 2-2.2$ cm long, gutter- shaped, thick, white in lower half, green in higher half, closely arrange to form a pseudostem	- 1-2,3 cm long, glabrous	- 1-3 cm long - gutter – shaped, thick, glabrous
	Ligule	- Membranous, green	- Matt, unclear	- Thin membranous, white, brown margin, 1-2 mm high.
Inflorescence		- Buds, no stalk, c. 8-8.5 cm high	- No stalk, wrapped by leaf sheath	- No stalk, carrying many flowers, growing between the leaves
Bract		- Blade- shape, blade-shape, 2.5-3 × 0.2 - 0.3cm, acute apex, lower part white, upper top light green.	- 2 bracts, lanceolate to linear	- c. 2.5-4.5 × 0.8-1 cm in size, short and smaller from outside
Corolla		- White, tube-shaped	- Tube-shaped, c. 2.0-2.5 cm long, split one side, hairless, 3 apexes	- Tube-shaped, c. 3.0 - 3.5 cm long
Calyx		- White, lower part stick together into a tube 6 cm long, upper trilobed, thin irregular, blade- shaped, c. 2.5-2.8 cm long	- Lower part stick together into a tube 3.0- 4.2 cm long, white, hairless	 Lower part stick together into a tube 4-5 cm long Lobe 2.2-2.5 cm long, white
Stamen	Lip	 Size 1.9-2 × 2.2-2.3 cm, divided to 2 oval-shaped lobes Lower part white, outer part pale violett 	 Size 2-2.3 × 1.8-2 cm, divided to 2 egg-shaped lobes White colour 	 2.1-2.3 × 2.3-2.5 cm size, bilobed. White, lower part with large, violet spots

 Table 1: Comparison of morphological characteristics of K.daklakensis, K. champasakensis và K. galanga

	Staminode	 Petal-shaped, size 1.9-2 × 1.3-1.4 cm Lower part white, outer part 	- Egg-shaped, size 1.6- 1.8 × 1.2-1.7 cm	- Size 2-2.2 × 1.2-1.4 cm
		pale violett	- White	- White
	Fertile stamen	- Opposite the lip, without filament, white, strip-shaped anther. The connective extends into slabs of 0.4-0.5 cm long, bending outward	- Anther size 2-4 mm, eliptical apex	Opposite the lip, without filament, white, strip-shaped anther, white, 4-4.5 mm long, 2-3 mm wide
Pistil	Ovary	 Elliptic – shaped, white, 1.2 × 0.25 cm, glabrous Ovule 3, inferior trilocular ovary, micropyle 	 Oblong, 7.0 × 5.1 mm, glabrous Ovule 3, micropyle 	- inferior triocular ovary, each locule contain 2 rows of ovules, micropyle; milky white, flat, 2.5-3 mm long, 1-1.5 mm wide, scattered white, fine hairs
	Style	 Ca. 6-6.2 cm long, smooth, fibrous-shaped 	- Ca. 7 – 9 mm long, thin fibrous-shaped	
	Stigma	 1 mm wide, white, funnel- shaped, eyelashes on funnel mouth 	– Funnel-shaped	Funnel-shaped, eyelashes on funnel mouth
Seed		- Numerous, block-shaped, one apex, size 3-3.1 × 1.7-1.8 mm	- Numerous, near ecliptic- shaped, 2-3 × 1-2 mm size	-

Microscopic characteristics

Leaf microscopy

Observing the specimen under the microscope reveals the following characteristics (Fig. 2): The midrib was upper sided concave and under sided convex. The epidermis consists of oval-shaped cells (1). Soft tissue consists of polygonal cells that arrange messily to create intercellular spaces (2). Primary timber is irregular with xylem above (3) and phloem under (4); The xylem (wood) consists of small, irregular polygonal xylem tissue that untidily but aligned arrange. Fibrous cells untidily clutter (5).



Fig. 2 Microscopic investigation A. Transverse section of leaf passing through midrib; B. the sheath of K. daklakensis leaves. 1 - Epidermis, 2 – Soft tissues, 3 – xylem, 4 – Phloem, 5 – Fiber.

Leaf powder investigation

The fine powder of *K. daklakensis* was rinsed in glycerin and observed under microscope. It showed presence of soft tissue fragment with large,

polygonal cells (1), epidermal cells with stomatal pores or anomocytic stomata (2), spiral vessels (3), septate fibers (4), and polygonal calcium oxalate crystals (5) (Figure 3).



Fig. 3. Microscopic investigation of leaf powder of K. daklakensis

1 -Soft tissue, 2 -epidermal cells with stomatal pores or anomocytic stomata, 3 -spiral vessels, 4 -septate fibers, 5 -polygonal calcium oxalate crystals.

Rhizome microscopy

Observation under the microscope (Fig. 4) reveals following characteristics: Transvers section of the rhizome, circular, bifacial arrangement of cells. The upper region are suction root hairs with thin wall for absorption of water and nutrients from the soil (1). The cork layer consists of 4 to 6 layers of polygonal, thin walls, aligned in a sequence epidemic cells (2). The cortex region below the cork contains several calcium oxalate crystals. The soft tissue is divided into two regions: the outer layer consists of circular to oval-shaped distorted cells that arranged irregularly and created small defects (3); the inner layer is 2 to 5 layered oval-shaped cells. The inner smaller cells arrange radiated ranges and concentric rings (4). Endodermis is a layer of oval-shaped cells (5). Pericycle is a layer of polygonal or oval cells (9). Vascular bundles beneath pericycle consists of ca. 15 pairs of phloemxylem bundles arranging alternately on a ring. The phloem (libe) consists of small concentric clusters (7). The xylem (wooden logs) consists of 2-3 irregular, small vessels, concentric arrangement. The outer vessels are smaller than the inner vessels (6). The central region of the rhizome is divided into two regions: the outer is aligned polygonalshaped cells and the inner messily, round-shaped cells (Fig. 4).



Fig. 4. Transvers section of rhizome of K. daklakensis

Â. 1- Root hairs, 2- Cork, Cortex, 3 – Outer shell soft tissue, 4 – Inner shell soft tissue, 5 – Endodermic, 6 – Xylem, 7 – Phloem (Libe), , 9 - Medulary ray. B. 8 – Pith.

Rhizome powder investigation

The rhizome samples were dried, powdered and finely grounded to obtain yellowish brown, fragrant rhizomic powder. Observations on the microscope show the following characteristics (Fig. 5): Cork is pale brown, thick-walled cells (4); parenchyma are occupied by thin-walled cells containing simple starch grains or dark yellow oil droplets (3). Scalarform vessels (4). Starch grains numerous, egg- or oval-shaped, with hilum but unclear veins (2).



Fig. 5. *Microscopic investigation of rhizomic powder* 1 – Scalar-form vessels, 2 – Starch grains, 3 – Parenchymatous cells containing simple starch grains, 4 – cork

Discussion

morphological By comparing the characteristics of K. daklakensis with those described in the literatures (Sirirugsa 1992, Flora of China Editorial Committee 2000, Larsen and Saksuwan 2006, Chayan and Supachai 2008, Leong-Skornickova and Newman 2015) as well as comparing the plant specimens with authentic plant specimens bearing the number MNHN-P-MNHN-P-P01743496. P00672802. MNHN-P-P01743499. MNHN-P-P01743500 of the Herbarium Department, French National Museum of Natural history (Paris), the results show that the plant has full characteristics of Kaempferia genus and the most closely related to K. galanga and K. champasakensis. Compared to K. galanga, the two species share the same characteristics as plant type, number of leaves, flower size. However, by K. galanga, leaf underneath and ovary were occupied by fine hairs, while in the studied plant had not owned these characteristics. At another hand, K. galanga's lips were purple only in the root while the lobes, staminode, and the lips of the studied plant were white in the root and purple at the top. Compared to K. champasakensis, both species had the same plant form, flower size, and flower-parts' size. However, in K. champasakensis, the leaves were usually narrower, the stalks were shorter, and especially the flowers were pure white. In the studied Kaempferia plant, the leaves were larger and broader. Additionally, the lobes and the lips of the flowers were violet at the top. Other important characteristic for distinguishing this species from the two K. galanga and K. champasakensis species were the oval-shaped bulbous roots, oval-shaped leaves with broad blades, violet-colored flowers at the top and white in the root, the plant parts without hairs except the style with eyelashes. The microscopic investigation of the leaves, rhizome and the powder had also given more detailed results for the botanical identification.

Distribution: found in Ea So Nature Reserve, Ea Kar district, Daklak Province, Central Highlands, South Vietnam.

Ecology and phenology: growing well in sandy soil along streams or in lowland dry deciduous forest, altitude 100-300m above sea level. Flowering time: June to October.

Traditional usage: the leaves of *K. daklakensis*, local name Địa liền đăk lắc, Up đất, are used as salad by local ethnic people. The roots and rhizomes are used for treatment of stomach pain and for digestion stimulation. These all evidences indicated the studied species as the new plant. We named it as *Kaempferia daklakensis* N.H. Tuan & N.D. to dedicate to Daklak province, where this beautiful species was found. The research results have contributed to the flora of Vietnam and the world a new species of *Kaempferia daklaknensis* N.H.Tuan & N.D. This paper is also as an official announce for this new *Kaempferia daklakensis* species as new record for the flora of Vietnam.

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CONFLICT OF INTEREST STATEMENT We declare that we have no conflict of interest.

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