TAXONOMIC IDENTIFICATION OF AN ADDITIONAL SPECIES, *CURCUMA RANGJUED*, IN THE CENTRAL HIGHLANDS OF VIETNAM AND EVALUATION OF ITS INHIBITORY ACTIVITIES AGAINST CANCER CELL LINES

Phung Thi Kim Hue[1,8*], Nguyen Quoc Binh[2], Do Thi Thao[1,3], Nguyen Phuong Hanh[4], Nguyen Thi Duong[5], Tran Thi Quynh Ngan[8], Nguyen Le Bao Ngoc[8], Do Hoang Dung[8], Tran Van Loc[1,6], Le Nhat Minh [7], Le Tri Vien[1]

[1]*Institute of Health Research and Educational Development in Central Highlands - 73 Le Hong Phong Street, Dien Hong Ward, Pleiku City, Gia Lai Province, Vietnam*
[2]*Vietnam National Museum of Nature, Vietnam Academy of Science and Technology - 18 Hoang Quoc Viet Street, Cau Giay District, Hanoi, Vietnam*
[3]*Institute of Biotechnology, Vietnam Academy of Science and Technology - 18 Hoang Quoc Viet Street, Cau Giay District, Hanoi, Vietnam*
[4]*Institute of Ecology and Biological Resource, Vietnam Academy of Science and Technology - 18 Hoang Quoc Viet Street, Cau Giay District, Hanoi, Vietnam*
[5]*Thao Nguyen Duong Family Heirloom Traditional Medicine Clinic - Hamlet 7, Dak Ha Commune, Dak Glong District, Dak Nong Province, Vietnam*
[6]*Institute of Chemistry, Vietnam Academy of Science and Technology - 18 Hoang Quoc Viet Street, Cau Giay District, Hanoi, Vietnam*
[7]*Foreign Trade University - 91 Chua Lang Street, Dong Da District, Hanoi, Vietnam*
[8]*Hung Vuong High School for the Gifted - 48 Hung Vuong Street, Pleiku City, Gia Lai Province, Vietnam*

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ABSTRACT

Curcuma L. is a large genus in the Zingiberaceae, many species have been used as spices, medicines, dyes, some plants are grown as ornamentals. *Curcuma rangjued* has been grown for many years in the Central Highlands provinces of Vietnam. Traditional medicine products from *Curcuma rangjued* are used to treat common diseases, such as respiratory tract infections, bronchitis, asthma, stomachache, skin inflammation. However, the plant is only locally known as scorpion turmeric, based on the shape of the rhizome of this plant, without a scientific name. It is unlike any turmeric species found in China, Laos, Cambodia and Vietnam with the following characteristics: inflorescences growing between leaf sheaths; abaxially green, usually with red patches on along side of midvein, fertile bract pale green; coma bract pale red, white towards basal. Notably, the rhizome has an intensely bitter taste.

In this study, we identified and taxonomically described a new species of turmeric, *Curcuma rangjued*, contributing to the taxonomic diversity of Vietnam. Furthermore, pharmacological investigations revealed that the rhizome extract of *C. rangjued* exhibited significant inhibitory effects on the proliferation of four human cancer cell lines: A549 (lung carcinoma), MCF-7 (breast carcinoma), HT-29 (colorectal carcinoma), and SNU-1 (gastric carcinoma)."

Keywords: *Curcuma rangjued*, additional species, cancer, *nghệ bọ cạp*, Dak Nong.

1. INTRODUCTION

Curcuma L. as one of the largest genera in the family Zingiberaceae, is mainly distributed in South and Southeast Asia, extending to China, Australia and the South Pacific (Jana Leong-Škorničová et al. 2007). Tropical Asia and South Asia are the diversity hotspots of the genus. *Curcuma* has about 120 species worldwide (Leong-Škorničová, J., 2013), many new species of *Curcuma* have been described in recent years: *Curcuma bella* (Maknoi, K. Larsen & Sirirugsa, 2011), *C. arracanensis* (Vinita Gowda, W. John Kress, Thet Htun, 2012), *C. gulinqingensis* (Chen & Xia, 2013), *C. leonidii* (Škorničk. & Luru, 2013), *C. newmanii* and *C.*

*Corresponding author
Email: whitelily109@gmail.com **Phone:** (+84) 914730099 **Https:**//doi.org/10.52163/yhc.v65i13.1728

xanthella (Leong-Škorničová, J. & Trân, H.D., 2013), *C. corniculata* and *C. flammea* (Leong-Škorničková, J. et al. 2014), *C. arida* and *C. sahuynhensis* (Leong-Škorničová, J., 2015), *C. lampangensis* and *C. sabhasrii* (Sarayut Rakarcha et al., 2022), *C. borealis* and *C. retrocalcaria* (Piyaporn Saensouk et al., 2024). Vietnam is one of the countries in Southeast Asia with a high diversity of turmeric species, up to now, total of 40 are known, this is the second largest genus in the Zingiberaceae in Vietnam, after the *Alpinia* genus. A new species and a new record of *Curcuma* subgen. *Curcuma* (Zingiberaceae) from Northern Thailand. (Surapon Saensouk, et al., 2021). Many new species have been discovered in recent years: *Curcuma pambrosima* Skornick & N.S. Lý, *C. vitellina* Skornick & H.Đ. Trần (2010), *C. newmanii* Škorničk., *C. xanthella* Skornick., *C. leonidii* Škorničk., *Curcuma pygmaea* Skornick & Sida f. (2013), *C. cotuana* Luu, Škorničk. & H.Đ.Trần (2017), *C. sixsensesensis* D.D. Nguyen & T.A. Le (2022), *C. tuanii* H. T. Nguyen, D. D. Nguyen & N. A. Nguyen. (2023). Species in the genus *Curcuma* in Vietnam are distributed in all three subgen *Curcuma*: *Curcuma* subg. *Curcuma*; *Curcuma* subg. *Ecomata*; *Curcuma* subg. *Hitcheniopsis* (Leong-Škorničová, J., 2015). With the morphological characteristics of scorpion turmeric, the position of this species is in *Curcuma* subg. *Curcuma*.

Curcumin, a polyphenol extracted from *Curcuma longa* in 1815, has gained attention from scientists worldwide for its biological activities (e.g., antioxidant, anti-inflammatory, antimicrobial, antiviral), among which its anticancer potential has been the most described.

However, for *Curcuma rangjued*, the primary active ingredient is not curcumin, but rather D-limonene, β-pinene, caryophyllene - the compounds which possess anti-inflammatory and antioxidant properties (Gushiken et al., 2022). Therefore, this study aims to explore the anti-proliferative activity of *C. rangjued* on several cancer cell lines to determine its pharmacological properties.

2. MATERIALS AND METHODS

The studied *Curcuma rangjued* samples were collected from the Dak Ha commune, Dak Glong District, Dak Nong Province, Vietnam.

Cancer cell lines were provided by the laboratory of Professor Dr. John M. Pezzuto, Long Island University, USA and from the laboratory of Professor Dr. Chi-Ying Huang, National Yang Ming Chiao Tung University, Taiwan.

2.1. Species Identification

Specimens were sampled and processed using conventional methods guided by the Royal Botanic Gardens, Kew (Bridson & Forman, 1999). Detailed photographs and descriptions of taxonomically important characters of the newly recorded species were taken of fresh materials in the field using a digital camera. Taxonomic identification was done using morphological vegetative and reproductive characters following the aforementioned literature, especially Gusman & Gusman (2006).

2.2. Anti-proliferative Activity – MTT Assay

The applied method was first described in Journal of Immunological Methods by Mosmann (1983). Cancer cell lines, after achieving stable growth and seeded in a 96-well plate, were treated with 10 µL of the serially diluted test samples at different concentrations which was prepared previously. Wells only containing cancer cells without treatment (190 µL) + 1% DMSO (10 µL) were used as negative controls. Wells containing only the culture medium, without seeded cells and test extracts, were considered blank wells. The assay was replicated three times to ensure the statistical significance. Ellipticine at concentrations of 10, 2, 0.4, and 0.08 µg/mL was used as a reference control. The percentage of cell growth inhibition under the treatments was determined using the following formula:

$$\%\ \text{inhibition}\ (\%) = 100 - \frac{OD(tested\ sample) - OD(blank)}{OD(DMSO) - OD(blank)} x100$$

3. RESULTS

3.1. Taxonomic Identification of *Curcuma rangjued*

This new species has many characteristics similar to *Curcuma longa*, such as inflorescence shape, central inflorescence position, shape and size of calyx, corolla, labellum and staminodes. But different in the characteristics of the leaves: red patches along side of midvein, especially, rhizomes of 'scorpion turmeric' (in Vietnamese: *nghệ bọ cạp*) very bitter.

Rhizomatous herb, ca 0,6-0,8 m. Rhizome oval or ovoid, outside pale yellow, inside yellow, bitter, aromatic, small roots, slend. Leaf sheaths 19-30 cm long, glabrous, pale blue; petiole canaliculate, pale green, glabrous, 15-20 cm long; ligule thin, glabrous, entire, 1-1,5 mm long; blades elliptic, ca. 40-45 × 15-18 cm, glabrous on both sides, base cuneate, apex acute with uppermost tip hairy in two margin, adaxially green, usually with red patches on along side of midvein, 1,5-2 cm wide, then become faint, abaxially silver-blue. Inflorescence central, ca 12-15 x 4-6 cm; peduncle 12-20 cm long. Sheathing bract, lanceolate, pale green, size 10-12,5 × 1,8-2 cm, short point at apex, 1-1,5 mm long; fertile bracts below the inflorescence, occupies about 3/5-4/5 of the length of the inflorescence, connate to one another in lower 2/3, wide oval or nearly triangular (excluding the part attached to other bracts), glabrous, apex acute, ca. 2,8-3 × 2,5-2,8 cm, green above, green-white below, coma bracts oblong, ca. 8-9 × 2,6-3 cm, white-light green below, pale pink on over, slightly spread, no con-

tain flowers. Cincinni with 3 flowers at the base of the inflorescence, Each flower has 1 bracteoles, the flowers and bracteoles gradually become smaller. The bracteoles are thin, translucent white, ovoid like, ca. 2.5-3 × 1.6-2 cm. Calyx narrow bell-shaped, translucent white, 12-14 mm long, outer surface puberulent, with unilateral incision one side down ½ length, 3 unequal obtuse teeth at apex. Corolla translucent white, glabrous, villous at throat; floral tube 13-15 mm long, funnel-shaped middle, 8-10 mm long, funnel head diameter 5-7 mm, upper 3-lobes, ca. 13-14(-15) x 12-13(-14) mm; lateral lobes concave, glabrous, pure white, broadly oval; dorsal lobe longer and wider, triangular ovate, concave, glabrous, pure white, apex mucronate, mucro c. 2 mm long. Labellum wide obovate, 1,6-1,8 x 1,5-1,6 cm, yellow median band, two sides light yellow; apex emarginate, incised 3-4 mm. filament flat, pale green-yellow, 4-5 mm long, 3mm broad at base, 2mm broad at apex, anther almost perpendicular with filament, 3-4 mm, dehiscing along the entire length, sparsely puberulent, soft, anther spurs to 3 mm, without anther crest. Lateral staminodes 2, irregularly ovoid, 1,2-1,3 cm long, 7-9 mm large, pale yellow. Ovary wide oval, ca 2,5-3×1,5-2 mm, densely puberulent, white; style filiform, glabrous, white, stigma disc-form, ciliate. Epigynous glands pale yellow, 4-5 mm long. Fruit unknown (Figure 1).

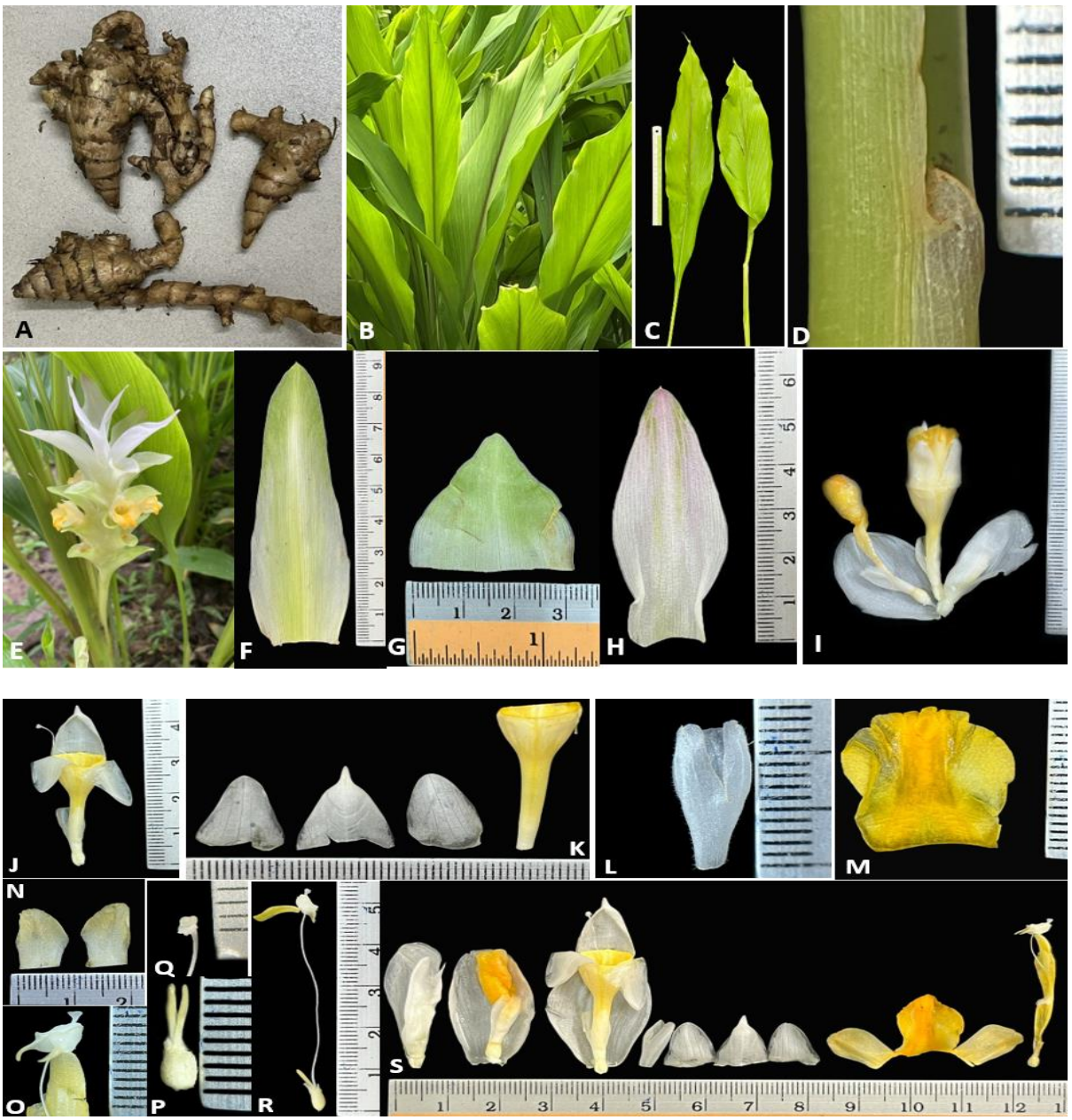

Figure 1: *Curcuma rangjued*.

A. Rhizome; B. Habit; C. Leaf ; D. Ligule; E. Inflorescence; F. Sheathing bract; G. Fertile bract; H. Coma bract; I. Cincinni with 3 flower; J. Calyx and corolla; K. Tube corolla and lobes corolla; L. Calyx; M. Labellum; N. Lateral staminodes; O. Filament and anthers; P. Ovary and epigynous glands; R. Ovary; epigynous glands, style, anther and stigma; S. Flower dissection (from left to right): Three flower in cincinni, bract and flower, calyx, three loles corolla,

staminoides and labellum, ovary-epigynous glands-corolla tube-filament-anthers-stigma.

Morphological analysis and in-depth comparison with specialized literature on the genus *Curcuma* revealed that scorpion turmeric shares similar characteristics with a previously documented *Curcuma* species, namely *Curcuma rangjued* Saensouk & Boonma (2021) (Surapon Saensouk et al., 2021), classified within the Zingiberaceae family. Therefore, it is proposed that *C. rangjued* should be included in the current catalogue of Vietnamese *Curcuma* species. This taxonomic placement has been validated by Dr. Nguyen Quoc Binh at the Vietnam National Museum of Nature.

3.2. Evaluating anti-proliferation activity on different cancer cell lines

The results of the antiproliferative activity tested on cancer cell lines from *C. rangjued* extracts are presented in Table 1.

Table 1. Cytotoxicity of the studied sample on cancer cell lines

Concentration (µg/mL)	Extract H							
	A549		MCF-7		HT-29		SNU-1	
	%Inhibition	SD	%Inhibition	SD	%Inhibition	SD	%Inhibition	SD
Extract H								
100	98.10	2.74	82.17	1.07	95.17	1.97	99.40	1.87
20	51.60	1.63	78.50	2.07	61.54	1.38	83.71	1.96
4	5.22	0.54	19.03	1.19	8.75	0.74	35.61	1.58
0.8	1.30	0.11	6.07	0.62	2.11	0.22	4.61	0.37
IC_{50}	24.01±0.97		11.36±0.64		18.05±0.86		6.85±0.29	
Extract E								
100	39.11	1.28	61.93	1.69	67.57	1.69	86.67	1.44
20	15.77	0.79	38.10	0.96	29.95	1.20	39.35	0.90
4	2.85	0.21	8.16	0.51	3.62	0.35	8.81	0.56
0.8	0.47	0.04	2.40	0.22	1.14	0.11	0.87	0.04
IC_{50}	>100		52.58±3.43		56.42±2.92		33.81±1.17	
Extract AC								
100	76.87	2.02	88.13	1.80	95.48	2.20	96.71	1.82
20	16.79	1.48	53.91	1.18	43.65	1.63	61.40	1.58
4	4.27	0.41	12.79	0.93	17.39	0.69	8.66	0.62
0.8	0.94	0.04	4.77	0.48	3.62	0.35	3.86	028
IC_{50}	63.50±2.97		21.82±0.96		26.01±1.49		18.16±0.91	
Ellipticine								
10	94.32	1.40	97.02	1.45	90.12	1.01	92.20	1.54
2	75.93	1.13	80.23	0.95	78.05	0.64	77.99	1.21
0.4	48.92	1.04	52.03	0.84	50.62	0.88	50.25	0.86
0.08	21.46	1.16	23.57	1.02	22.06	1.03	21.85	0.71
IC_{50}	0.46±0.03		0.32±0.02		0.34±0.02		0.35±0.03	

The fresh *C. rangjued* rhizomes were initially extracted using n-hexane, yielding Extract H at 1.05%. The remaining residue were then followed by ethanol extraction to obtain Extract E with a yield of 1.85%. The extracted *C. rangjued* rhizome residue was further extracted using Acetone, yielding Extract AC with a total efficiency of 2.7%. The results presented in Table 1 showed that Extract H exhibited inhibitory effects on four cancer cell lines, with the most potent effect against the SNU-1 gastric cancer cell line with an IC_{50} of 6.85 ± 0.29 µg/mL, followed by the MCF-7 breast cancer cell line with an IC_{50} of 11.36 ± 0.64 µg/mL, the HT-29 colorectal cancer cell line with an IC_{50} of 18.05 ± 0.86 µg/mL, and finally the A549 human lung adenocarcinoma cell line with an IC_{50} of 24.01 ± 0.97 µg/mL. This might be explained that Extract H, acquired from *C. rangjued* using a non-polar solvent n-hexane, contains active compounds such as D-limonene, β-pinene, and

caryophyllene, which are recognized as the primary agents to inhibit cancer cell growth.

Recent studies have shown that D-limonene has anti-tumor effects by inducing apoptosis in lung cancer cells (Yu et al., 2018), and β-pinene and limonene from *Piperrivinoides* Kunthessentialoilhaveanti-proliferative activity against oral squamous cell carcinoma cell lines (Machado et al., 2022). Moreover, β-pinene has been proved to inhibit tumor growth through cellular mechanisms such as apoptosis, cell cycle arrest, and necrosis (Machado and Da Fonseca et al., 2022). Caryophyllene has been reported to exhibit anti-tumor effects against colorectal cancer (Dahham et al., 2021). These findings highlight the necessity for further in-depth investigations of *Curcuma rangjued* as a promising candidate for cancer cell inhibition. This plant species is well-adapted to the Central Highlands area of Vietnam and can give an average yield of 15-20 tons of rhizomes per hectare after 18-20 months of cultivation. This indicates that the utilization of *C. rangjued* (or scorpion turmeric) at the Central Highlands provinces is a crucial strategic approach for developing a valuable medicinal source for cancer prevention.

5. CONCLUSION

Morphological observation and analysis which was conducted by experts from Vietnam National Museum of Nature identified the scorpion turmeric as *Curcuma rangjued*. This newly recognized species is an addition to the list of medicinal plant species in Vietnam.

Extraction of the rhizome using n-hexane yielded a crude extract (Extract H) containing biologically active compounds holding potential anti-cancer activities, including D-limonene, β-pinene, and caryophyllene. The Extract H demonstrated potent inhibitory activities against four cancer cell lines, including SNU-1, MCF-7, HT-29, and A549, with IC_{50} values ranging from 6.85 to 24.01 µg/mL. These findings open up new avenues for the development of new cancer treatments.

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