MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS, AND BIOACTIVE COMPOUND CONTENTS OF *STEMONA TUBEROSA* LOUR. COLLECTED IN VIETNAM

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Abstract: *Stemona tuberosa Lour. is one of the traditional medicines used for the treatment of cough, tuberculosis, antiseptics and external skin diseases. In Vietnam, Stemona tuberosa Lour is widely distributed from lowland to mountainous regions up to 1000 m altitude. To contribute to the database for study and use of this species, the information on morphology, anatomy, and bioactive compound content of alkaloids and tuberostemonines of 15 different provenances of Stemona tuberosa Lour. collected in different ecological regions of Vietnam is provided.*

Keywords: *Stemona tuberosa* Lour., *morphology, anatomy, alkaloid, tuberostemonine.*

1. Introduction

Stemona tuberosa Lour. belongs to the family Stemonaceae. Worldwide, *Stemona tuberosa* Lour. is distributed in India, Australia, Bangladesh, Cambodia, Laos, Malaysia, Myanmar, Philippines, Thailand and Vietnam [11]. In Vietnam, *Stemona tuberosa* Lour. has a wide distribution, including lowland, coastal, midland, and mountainous provinces (elevation below 1000 m). *Stemona tuberosa* Lour. is abundant in Cao Bang, Lang Son, Thai Nguyen, Tuyen Quang, Hoa Binh, Phu Tho, Bac Giang, and Thanh Hoa [1]. *Stemona tuberosa* Lour. is used to treat coughs, kill worms and insects [2], [7]. *Stemona tuberosa* Lour. also has antibacterial properties [6], that is applied to the treatment of tuberculosis, cough, skin antiseptic, and gynecological diseases [10,11]. *Stemona tuberosa* Lour. is considered as an essential medicine according to Circular No. 40/2013/TT - BYT dated January 1, 2014 of the Ministry of Health, promulgating the 6th list of essential drugs of traditional medicine and drugs from herbal ingredients. Currently, there are many companies wishing to expand the planting area of *Stemona tuberosa* Lour.. However, it is necessary to select good varieties with high yield and high total alkaloid content. In this study, we evaluated the morphological, anatomical characteristics, and bioactive compound content of 15 different provenances of *Stemona tuberosa* Lour. collected in some ecological regions in Vietnam.

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2. Materials and methods

2.1. Materials, time, and place of study

Plant materials: 15 different provenances of *Stemona tuberosa* Lour. were collected in some ecological regions in Vietnam based on the morphological characteristics described by Pham Hoang Ho (2000) [3] and Ragone [8].

Time to collect samples: from 6th to 20th of December 2020, collected all tubers and tuber buds of *Stemona tuberosa* Lour. varieties. Propagation of *Stemona tuberosa* Lour. and evaluation of growth, morphological and anatomical characteristics were conducted from January 2021 to October 2022.

Location of propagation and planting: North Central Research Centre for Medicinal Materials.

2.2. Methods

Collecting samples for morphological and anatomical analysis: Collection of *Stemona tuberosa* Lour. samples was done according to the method described by Tran Cong Khanh (1981) [4]. On each 3-4 years old plant, leaf samples (young, mature, old) were collected on each branch twice; 2-3 young to mature male and female flower clusters of each plant were collected; bundles in different sizes and colors on the same plant were collected for different sizes and colors.

Description of plant morphology: Based on the improved method of Nguyen Nghia Thin (2007), the descriptive parts include: srem, leaf, root, flower, fruit and seed [4]. Observation and description of morphological characteristics of vegetative and reproductive organs such as roots, stems, leaves, flowers, fruits were done along with photograph.

Anatomical analysis: Fresh anatomical specimens were prepared according to the method of R.M. Klein & D.T. Klein (1979) [5]. Observation and photograph of samples were conducted by using an optical microscope. Description of anatomical structure was done by the two-color iodized green alum staining method.

Analysis of bioactive compound content: Quantification of total alkaloids in medicinal herbs was carried out based on Vietnamese Pharmacopoeia. Total alkaloid content (X%) is calculated by the formula [1]

$$X\ (\%) = \frac{(10 - \mathrm{n})\mathrm{x}\ 3{,}75}{a}$$

n: 0.1 N sodium hydroxide solution used in ml.

a: Mass of medicinal powder to be quantified, with moisture removed, in grams.

3. Results and discussion

3.1. Sample collection of Stemona tuberosa Lour.

Fifteen samples of *Stemona tuberosa* Lour. varieties from 5 different ecological regions of Vietnam were collected in the present study. The results are shown in Table 1

Table 1. *General information on Stemona tuberosa* Lour. *samples collected in different ecological regions of Vietnam*

No.	Ecological regions	ID	Places
1	Northwest	BB8	Tan Thanh commune, Mai Chau district, Hoa Binh province
		BB14	Chieng Son commune, Moc Chau district, Son La province
		BB11	Ta Phin commune, Tua Chua district, Dien Bien town
		BB4	Ham Rong ward, Sa Pa town, Lao Cai province.
		BB09	Vien Son commune - Van Yen district - Yen Bai province
2	East Northern	BB13	Son Vi commune, Lam Thao district, Phu Tho province
		BB12	Quan Chu commune, Dai Tu district, Thái Nguyên province.
3	Red river delta	BB10	Minh Quang commune, Ba Vi district, Hanoi city.
		BB3	Ngu Hiep commune, Thanh Tri district, Hanoi city.
4	North Central	BB5	Thach Lam commune, Thach Thanh district, Thanh Hoa province.
		BB2	Hoi Xuan commune, Quan Hoa district, Thanh Hoa province.
		BB15	Ngoc Son commune, Ngoc Lac district, Thanh Hoa province.
		BB7	Minh Son commune, Do Luong district, Nghe An province.
		BB6	Son Loc commune, Bo Trach district, Quang Binh province.
5	South Central	BB1	Son Dung commune, Son Tay district, Quang Ngai province.

All the collected samples were examnined and their scientific name were identified as *Stemona tuberosa* Lour. (or *Stemona acuta* C.H. Wright), belonging to family Stemonaceae. All samples are being kept in the Specimen room of the Center of Medicinal Material Resources.

3.2. Morphological and anatomical characteristics

3.2.1. Stem characteristics

Stem morphological characteristics: Stem morphological characteristics of *Stemona tuberosa* Lour. are presented in Figure 1. Generally, stems are tendril, smooth cylindrical aerial stem with slight swelling at the node; the young stems are light green; it is darker green in mature stems.

Fig.1. *Stem morphological characteristics of Stemona tuberosa* Lour.

Stem diameter: The stem diameter of the 15 varieties of *Stemona tuberosa* Lour. are shown in Table 3.

Table 3. *The stem diameter of Stemona tuberosa* Lour.

No.	ID	Stem diameter (cm)	No.	ID	Stem diameter (cm)	No.	ID	Stem diameter (cm)
1	BB1	0,35	6	BB6	0,36	11	BB11	0,34
2	BB2	0,32	7	BB7	0,45	12	BB12	0,31
3	BB3	0,35	8	BB8	0,42	13	BB13	0,33
4	BB4	0,44	9	BB9	0,22	14	BB14	0,32
5	BB5	0,37	10	BB10	0,34	15	BB15	0,36

The stem diameter of *Stemona tuberosa* Lour. varies from 0,22 to 0,45 cm, in which BB9 sample collected in Van Yen - Yen Bai has the smallest stem diameter (0,22 cm). The rest of the varieties have stem diameter > 0,3 cm. For example, BB4 sample (Sapa City - Lao Cai) has a stem diameter of 0,44 cm; BB7 (Do Luong, Nghe An) stem diameter is 0,45 cm; BB8 (Mai Chau, Hoa Binh) has a stem diameter of 0,42 cm. These samples with a larger stem diameter reveals that in different geographical areas and soil types stem morphology have different adaptations to their living conditions.

Anatomy of the plant stem: The anatomical results of the stem are shown in Figure 2.

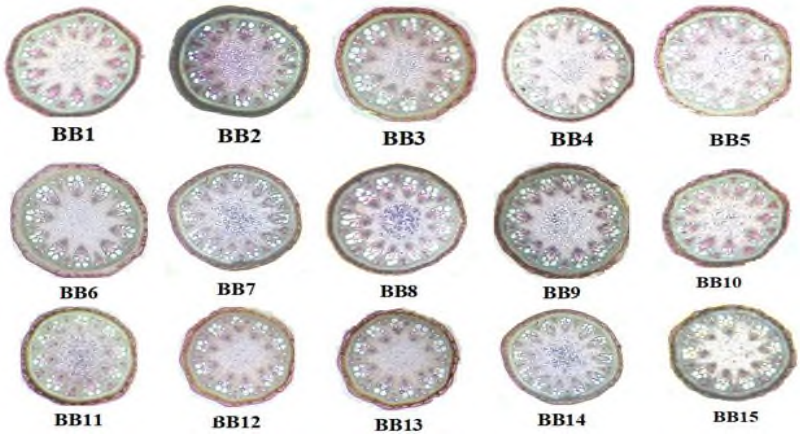


Fig.2. *Stem cross sections of Stemona tuberosa* Lour.

The results in Figure 2 show that there is almost no difference in terms of anatomy on cross-sections of 15 samples collected in 5 different ecological regions. However, there are differences in the number of vascular bundles in some samples. For example, in the BB1 and BB15 samples, the number of vascular bundles is 9; 10 in BB2, BB4, BB5, BB6, BB10, BB12, BB13 and BB14 samples; and 12 in BB7, BB8, BB9 and BB14 samples. This indicates that there is a change in the number of vascular bundles of the plant stem in response to different ecological and soil conditions.

3.2.2. Leaf characteristics

Morphological characteristics: Morphological characteristics of *Stemona tuberosa* Lour. leaves are shown in Figure 3 and 4.

Fig.3. *The leaf surface of Stemona tuberosa* Lour.

Fig.4. *The underside leaf of Stemona tuberosa* Lour.

The results in Figures 3 and Figure 4 show that the leaf morphology of 15 collected samples are not significantly different from each other. However, there are certain differences in leaf size. In BB9 sample, leaf size is relatively small compared to other samples. The leaf size parameters of 15 samples are presented in Table 4.

Table 4. *Leaf size of 15 samples of Stemona tuberosa* Lour.

No.	ID	Leaf width (cm)	Leaf length (cm)	Petiole lenght (cm)	No.	ID	Leaf width (cm)	Leaf length (cm)	Petiole lenght (cm)
1	BB1	8,0 ± 1,2	11,50 ± 1,3	5,2 ± 0,8	9	BB9	3,35 ± 0,5	5,2 ± 1,3	3,0 ± 0,6
2	BB2	8,70 ± 1,6	16,10 ± 1,9	5,7 ± 1,0	10	BB10	6,30 ± 1,0	14,50 ± 2,0	3,8 ± 1,0
3	BB3	8,2 ± 1,0	14,1 ± 0,9	5,0 ± 0,9	11	BB11	5,35 ± 0,6	13,60 ± 1,8	5,0 ± 0,6
4	BB4	6,8 ± 0,8	18,3 ± 2,4	6,3 ± 1,2	12	BB12	5,1 ± 0,7	13,40 ± 0,8	4,2 ± 0,9
5	BB5	6,40 ± 1,0	15,60 ± 1,0	4,4 ± 1,0	13	BB13	6,20 ± 0,8	14,1 ± 0,5	4,0 ± 0,9
6	BB6	6,15 ± 0,2	14,80 ± 0,8	4,3 ± 0,8	14	BB14	6,9 ± 0,9	13,9 ± 0,7	4,3 ± 0,8
7	BB7	8,70 ± 0,6	18,9 ± 1,0	3,8 ± 0,5	15	BB15	8,5 ± 1,0	14,7 ± 1,0	6,0 ± 1,0
8	BB8	8,4 ± 0,8	17,8 ± 0,8	5,6 ± 0,5					

The table 4 shows that the leaf length of *Stemona tuberosa* Lour. ranges from 5,2 to 18,9 cm, in which the BB9 sample (collected in Van Yen - Yen Bai) has the smallest leaf length, about 5,2cm. The other varieties have leaf length greater than 11 cm. The longest leaves are BB7, BB4 with lengths of 18,9 cm (Do Luong, Nghe An) and 18,3 cm (Sa Pa City, Lao Cai), respectively.

The leaf width of *Stemona tuberosa* Lour. ranges from 3,35 cm to 8,7 cm, in which the BB9 sample collected in Van Yen - Yen Bai has the lowest leaf width (3,35 cm). Some varieties have larger leaf width such as: BB4; BB5; BB6; BB7; BB8 and BB15 in which the BB7 and BB8 varieties have the largest leaf width of 8,7 cm.

The petiole length of 15 samples of *Stemona tuberosa* Lour. ranges from 3,0 to 6,0 cm, in which BB9 sample collected in Van Yen - Yen Bai had the lowest petiole length of 3,0 cm. The longest is the BB4 sample in Sa Pa - Lao Cai, with the petiole length of 6,3 cm. The remaining varieties have petiole length ranging from 3,8 to 6,0 cm.

Petiole anatomy: Petiole anatomy of *Stemona tuberosa* Lour. is presented in Figure 5.

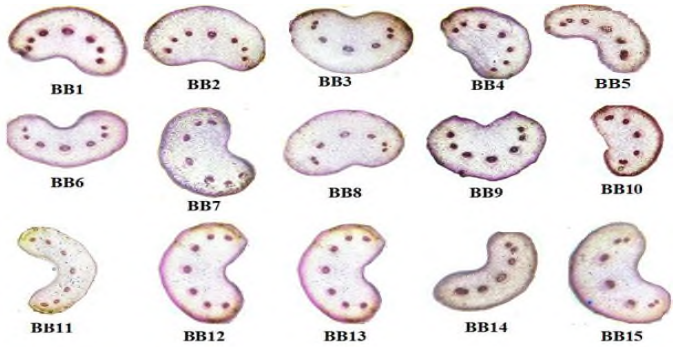


Fig.5. *Petiole anatomy of Stemon a tuberosa* Lour.

The Figure 5 shows the anatomical images of petiole of the 15 samples of *Stemona tuberosa* Lour.. There is not much difference in petiole anatomy of all collected samples. The only difference is found in BB5 and BB10 samples which have 6 and 5 vascular bundles, respectively; while other samples have 7 vascular bundles.. This result shows the adaptation of *Stemona tuberosa* Lour. to different soil and ecological conditions in their distribution area.

3.2.3. Flower characteristics

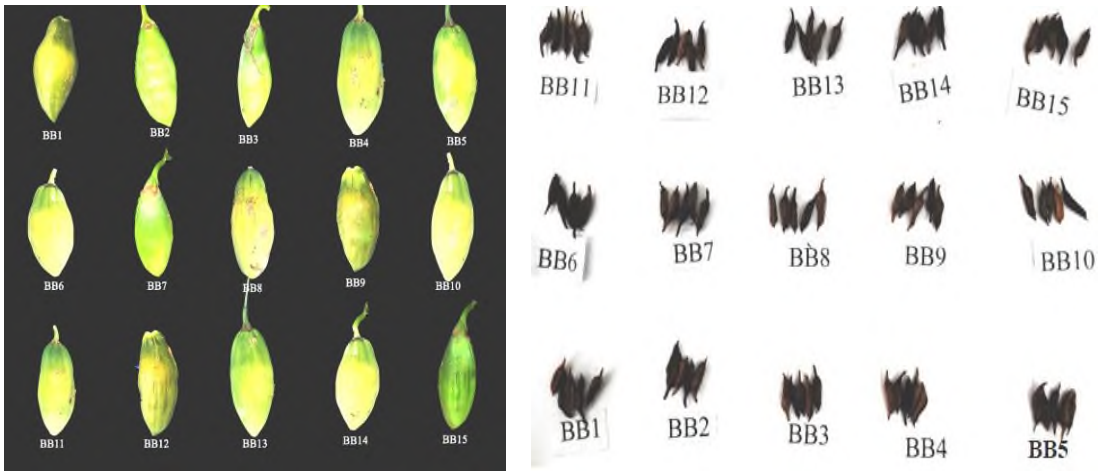
Morphological characteristics of flowers of 15 samples of *Stemona tuberosa* Lour are captured in Figure 6.

Fig.6. *Flower morphological characteristics of Stemona tuberosa* Lour.

The inflorescences of *Stemona tuberosa* Lour. arises from a leaf axil. The flower stalk is 4-6 cm length; each flower stalk has 2-6 yellow-green flowers with narrow bracts. Perianth consists of 4 similar, narow pieces, about 3-4 cm length, burgundy inner surface fades to the top. There is almost no significant difference in color, taste and flower growth style of 15 flower samples. On the petals, there are veins running vertically from the base of the flower to the tip.

3.2.4. Fruit and seed characteristics

Fruit and seed characteristics of *Stemona tuberosa* Lour. is shown in Figure 7a and Figure 7b.



a) **b)**

Fig.7. *Fruit (a) and seed (b) of Stemona tuberosa* Lour.

In general, the capsule is ovoid, the young fruit is green and it turns yellow from the stem to the top of the fruit when it is mature. When the fruit is ripe, the pods split on both sides to let the seeds spread. Fruit size, seeds, number of seeds per a fruit and weight of 1000 seeds are shown in Table 5.

Table 5. *Fruit and seed size of Stemona tuberosa* Lour.

No.	ID	Fruit		No. of seeds/fruit	Seed		Weight of 1000 seeds (g)
		Length (cm)	Width (cm)		Length (mm)	Width (mm)	
1	BB1	7,9 ± 0,3	3,3 ± 0,2	24 ± 2	2,05	0,4	82
2	BB2	8,5 ± 0,3	3,5 ± 0,3	28 ± 2	2,01	0,5	75
3	BB3	6,5 ± 0,5	3,0 ± 0,1	19 ± 3	1,90	0,4	84
4	BB4	8,2 ± 0,3	3,6 ± 0,3	24 ± 2	2,05	0,4	78
5	BB5	8,0 ± 0,4	3,0 ± 0,2	23 ± 2	2,00	0,5	68
6	BB6	6,5 ± 0,3	2,8 ± 0,3	15 ± 1	1,80	0,4	80
7	BB7	7,6 ± 0,6	3,5 ± 0,2	26 ± 3	1,90	0,4	83

8	BB8	7,3 ± 0,4	3,0 ± 0,4	28 ± 2	1,90	0,4	96
9	BB9	5,2 ± 0,4	2,6 ± 0,2	12 ± 2	1,70	0,4	68
10	BB10	6,8 ± 0,3	2,8 ± 0,3	15 ± 1	1,80	0,4	81
11	BB11	7,5 ± 0,6	3,7 ± 0,2	26 ± 3	1,90	0,4	85
12	BB12	7,8 ± 0,4	3,2 ± 0,4	28 ± 2	1,90	0,5	92
13	BB13	6,5 ± 0,3	2,6 ± 0,3	15 ± 1	1,80	0,4	79
14	BB14	7,6 ± 0,6	3,5 ± 0,2	26 ± 3	1,90	0,4	84
15	BB15	7,3 ± 0,4	3,0 ± 0,4	28 ± 2	1,90	0,5	92

The results of Table 5 show that the fruit length is from 5,2 to 8,5 cm; fruit width is from 2,6 to 3,7 cm. Each fruit contains from 12 to 28 seeds. When the seeds are ripe, they are dark brown in color, with wrinkles running along the seeds. Seed length is from 1,7 to 2,05 mm, seed diameter is from 0,4 to 0,5 mm. The weight of 1000 seeds ranges from 75 to 96 g, in which the BB9 sample (Van Yen, Yen Bai) has the smallest fruit size (5,2 x 2,6 mm), the larger fruit size samples are BB2 (8,5 x 3,5 mm); BB4 (8,2 x 3,6 mm), BB5 (8,0 x 3,0 mm). The number of seeds/fruit in BB9 is the lowest with 12 seeds/fruit; other samples such as BB3, BB6, BB10, BB13 have less than 20 seeds/fruit. The remaining samples have more than 20 seeds/fruit. Regarding grain size, the BB9 has the smallest seed size (1,7 x 0,4 mm). The average weight of 1000 seeds of is 78 g in BB5, BB9 samples and > 90 g in BB8, BB12 and BB15 varieties.

3.3. The bioactive compound content of Stemona tuberosa Lour. *seeds*

The content of total alkaloids and tuberostemonine were assessed in the seeds of 15 varieties of *Stemona tuberosa* Lour.. The results are shown in Table 6.

Table 6. *The content of total alkaloids and tuberostemonine in the seeds of 15 varieties of* *Stemona tuberosa* Lour.

No.	Sample	Content (%) of total alkaloids according to turberostemonin LG ($C_{12}H_{33}NO_4$) calculated by dry weight	Turberostemonin content (%) (calculated by dry weight)
1	BB1	0,56 ± 0,02	0,077 ± 0,001
2	BB2	0,69 ± 0,04	0,507 ± 0,004
3	BB3	0,60 ± 0,03	0,141 ± 0,003
4	BB4	0,56 ± 0,04	0,141 ± 0,003
5	BB5	0,67 ± 0,03	0,510 ± 0,003
6	BB6	0,67 ± 0,05	0,481 ± 0,005
7	BB7	0,56 ± 0,02	0,104 ± 0,003
8	BB8	0,72 ± 0,04	0,547 ± 0,002
9	BB9	0,39 ± 0,04	0,080 ± 0,002

10	BB10	0,51 ± 0,03	0,113 ± 0,003
11	BB11	0,67 ± 0,02	0,427 ± 0,001
12	BB12	0,68 ± 0,04	0,490 ± 0,001
13	BB13	0,47 ± 0,02	0,101 ± 0,003
14	BB14	0,67 ± 0,04	0,486 ± 0,004
15	BB15	0,73 ± 0,04	0,532 ± 0,005

The Table 6 shows that the total alkaloids of 15 seed samples ranges from 0,39 to 0,73%. There are 02 varieties having total alkaloid content of 0,5% which is lower than that specified in Vietnamese Pharmacopoeia V [1], namely BB9 (collected in Van Yen, Yen Bai) and BB13 (collected in Lam Thao, Phu Tho). There were 13 samples provided with satisfactory total alkaloid content compared to those specified in the Vietnamese Pharmacopoeia V (> 0,5%). There are 02 varieties with high total alkaloid content of more than 0,7%, which are BB08 varieties (collected in Mai Chau, Hoa Binh) and BB15 (collected in Ngoc Lac, Thanh Hoa).

The tuberostemonine content in seeds of 15 seed samples varies from 0,077 to 0,547%. There are 02 samples with tuberostemonine content < 0,1%, which are BB9 (Van Yen, Yen Bai) and BB1 (Son Tay, Quang Ngai). There are 05 samples with tuberostemonine content from 0,1 to 0,2%, which are BB3, BB4 (0,141%), BB7 (0,104%), BB10 (0,113%) and BB13 (0,101%). There are 08 samples with tuberostemonine content ranging from 0,4 - 0,5%, such as BB2, BB5, BB6, BB8, BB11, BB13, BB14, BB15. In which, there were 04 varieties with tuberostemonine content of more than 0,5%, namely BB2 (0,507%), BB5 (0,510%), BB8 (0,547%), and BB15 (0,532%).

4. Conclusions

Fifteen samples collected in 5 different ecological regions are identified as the species *Stemona tuberosa* Lour. (syn. *Stemona acuta* C.H.Wright), belonging to family Stemonaceae.

Morphology of stems, leaves, flowers, fruits and seeds of 15 samples of *Stemona tuberosa* Lour. is not significantly different from each other.

The size of stem and leaves of 15 collected samples shows no significant differences in 14 samples except for BB9 sample which has smaller sizes.

Anatomy of stem, petiole of 15 samples of *Stemona tuberosa* Lour. performs no significant differences.

Two seed samples containing total alkaloid content of < 0,5% are BB9 and BB13 which do not meet the standards of Vietnamese Pharmacopoeia V. There are 13 samples with total alkaloid content of > 0,5% which matches with the standards of Vietnamese Pharmacopoeia. 02 samples of BB15 and BB08 have total alkaloid content > 0,7%. There are 5 seed samples having tuberostemonine content of > 0,5%, namely BB2 (0,507%), BB5 (0,510%), BB8 (0,547%), and BB15 (0,532%).

References

[1] Do Huy Bich, Dang Quang Chung, *et al*. (2004), *Medicinal plants and medicinal animals in Vietnam*, Science and Technics Publishing House, 1st Episode, 118-122.

[2] Vo Van Chi (2012), *Dictionary of Vietnamese medicinal plants*, Medical Publishing House, 1st Episode, 82-84 .

[3] Pham Hoang Ho (1999), *An Illustrated Flora of Vietnam*, vol.1, Ho Chi Minh City: Tre Publishing House.

[4] Tran Cong Khanh (1981), *Practice of plant morphology and anatomy*, Hanoi: University and Professional High School Publishing House.

[5] Klein R.M. and Klein D.T. (1979), *Botanical study methods, Science and Technics* Publishing House (Translators: Nguyen Tien Ban, Nguyen Nhu Khanh).

[6] Luo, Hongli, et al. (2012), Isolation, classification and biosynthetic potential of endophytic actinomycetes from Stemona, *Wei Sheng wu xue bao = Acta Microbiologica Sinica*, 52(3): 389-395.

[7] MOH (2018), Vietnamese Pharmacopoeia V, Medical Publishing House, 2th Episode.

[8] Ragone, D. (2006), *Traditional Trees of of Pacific Islands: Their culture environment and use*, Edited by C.R. Elevitch. Permanent Agriculture Resources, Holualoa, Hawaii.

[9] Nguyen Nghia Thin (2007), *Plant research methods*, VNU Publishing House, Hanoi.

[10] Tsi ZH, Duyfjes BEE (2000), Stemonaceae. In: Wu ZY, Raven PH (eds) Flora of China, 24, Science Press, Beijing, 70-72.

[11] Wang, L., Wu, H., Liu, C. et al. (2022), A review of the botany, traditional uses, phytochemistry and pharmacology of Stemonae Radix. Phytochem Rev, 21, 835-862, https://doi.org/10.1007/s11101-021-09765-1.