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EVALUATION OF THE ROTUNDIN CONTENT OF *Stephania brachyandra* (Diels) GENE SOURCES IN THAI NGUYEN PROVINCE

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ABSTRACT:

The *Stephania brachyandra* plant, naturally found in Thai Nguyen, is scientifically known as *Stephania brachyandra,* which belongs to the genus Stephania Lour., family Menispermaceae, order Menispermaceae. This plant serves as a source of extracting L-tetrahydropalmatine (the active ingredient medicine known as rotundin) with the chemical formula $C_{21}H_{25}NO_4$, which is an alkaloid with sedative, neuroleptic, and and smooth muscle relaxant effects. The purpose of this study is to evaluate the rotundin content in the *Stephania brachyandra* species and analyze the correlation between the size of the species tubers and the content of rotudin in *Stephania brachyandra* naturally distributed in Thai Nguyen province. The analysis results show that the content of rotundin in 160 samples of naturally distributed *Stephania brachyandra* in Thai Nguyen province ranges from 90.4 to 981 mg/100 g. The analysis of the correlation coefficient shows a strong relationship between rotundin content and tuber diameter. This demonstrates diversity in active ingredient content among natural *Stephania brachyandra* plant samples, thereby confirming their significant potential as a medicinal resource.

Keywords: Extraction, L-tetrahydropalmatine, NMR, rotundin, *Stephania brachyandra* (Diels)

NGHIÊN CỨU ĐÁNH GIÁ HÀM LƯỢNG DƯỢC CHẤT ROTUNDIN CỦA CÂY BÌNH VÔI PHÂN BỐ TỰ NHIÊN TẠI TỈNH THÁI NGUYÊN

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TÓM TẮT

Loài cây Bình vôi phân bố tự nhiên chủ yếu ở tỉnh Thái Nguyên có tên khoa học là *Stephania brachyandra* (Diels), thuộc chi Stephania Lour., họ Tiết dê - Menispermaceae, bộ Menispermaceae. Loài cây này được sử dụng làm nguyên liệu để chiết xuất L-tetrahydropalmatine (hoạt chất thuốc có tên là rotundin) với công thức hóa học $C_{21}H_{25}NO_4$. Rotundin là một alkaloid có tác dụng an thần, gây ngủ, giảm đau và giảm co bóp cơ trơn. Mục đích của nghiên cứu này là đánh giá được hàm lượng dược chất rotundin của loài Bình vôi nhị ngắn và phân tích tương quan giữa kích thước củ Bình vôi và hàm lượng hoạt chất rotundin của củ Bình vôi nhị ngắn phân bố tự nhiên trên địa bàn tỉnh Thái Nguyên. Kết quả phân tích cho thấy hàm lượng rotundin của 160 mẫu Bình vôi phân bố tự nhiên tại tỉnh Thái Nguyên có sự biến động từ 90,4 - 981 mg/100 g. Phân tích hệ số tương quan giữa đường kính củ và hàm lượng dược liệu rotundin cho thấy hàm lượng rotundin có quan hệ rất chặt với đường kính củ Bình vôi. Điều này cho thấy sự đa dạng về hàm lượng hoạt chất trong các mẫu cây Bình vôi tự nhiên, từ đó khẳng định tiềm năng to lớn của nguồn dược liệu này.

Từ khóa: Chiết xuất, L-tetrahydropalmatine, NMR, rotundin, *Stephania brachyandra* (Diels)

I. INTRODUCTION

In recent years, along with advancements in medicine, sedatives and insomnia medications have become subjects of widespread research interest. Most sedatives currently on the market are derived from synthetic chemical processes and are imported. However, prolonged use of these medications often leads to side effects, prompting an increased focus on developing production processes for medications sourced from natural herbal remedies (Pham *et al.*, 2021; Vu *et al.*, 2016).

The tuberous root of *Stephania brachyandra* (Diels), commonly known as the *Stephania* plant and belonging to the Menispermaceae family, is a climbing plant widely distributed in China and Southeast Asian countries such as Vietnam. The stephania root contains various alkaloids, among which the compound rotundin (or L-tetrahydropalmatine) is present in large quantities. Traditional medicine has long utilized stephania root in decoctions or soaked in alcohol to treat insomnia, calm the nerves, relieve headaches, and alleviate stomach pains. Currently, rotundin is primarily employed for treating insomnia and inducing sedation, with notable advantages such as low toxicity, good drug tolerance, promotion of natural sleep, and enhancement of memory recovery (Chinh *et al.*, 2019; Hang *et al.*, 2014; Xieet *et al.*, 2015).

The supply of stephania root in our country is quite abundant. Therefore, it is a highly meaningful task to research and evaluate the rotundin content in Stephania plants naturally distributed in Thai Nguyen, achieve high extraction efficiency and industrial applicability, and make use of the rich domestic herbal resources available. This research not only helps to increase the economic value of the Stephanie root but also contributes to the development of the medicinal herb industry in Thai Nguyen province, providing practical benefits for patients through the supply of safer and more effective products.

II. MATERIALS AND METHODS

2.1. Materials

Fresh *Stephania brachyandra* (Diels) tubers were collected in October 2021 from 5 districts in Thai Nguyen province. The samples were scientifically identified based on morphological characteristics described in botanical and pharmacopoeial literature (Pham *et al.*, 2021). After harvesting, the raw materials were thoroughly washed to remove contamination, damaged parts were trimmed away, and the cleaned tubers were drained of water. They were then thinly sliced, dried, ground into a fine powder, and stored at room temperature in airtight plastic bags.

2.2. Methods

*2.2.1. Method for analyzing rotundin content in Stephania brachyandra (*Diels*)*

First, the *Stephania brachyandra* (Diels) were cleaned and peeled, then cut into appropriately sized pieces and soaked in an extraction solution containing water and inorganic buffers at an appropriate pH. The alkaloid active ingredients dissolving were extracted from the residue. The extraction process was repeated 2 - 3 times, and the residue is thoroughly pressed. The extract was allowed to undergo sedimentation, filtration, and subsequently neutralization at the suitable pH level in order to acquire a precipitate. The precipitate is filtered, dried. The crude product after drying contains approximately 27 - 32% rotundin (according to the Chinese Pharmacopoeia). The crude product is then purified into a pharmaceutical form containing 96 - 98% rotundin.

*2.2.2. Process of purifying rotundin in Stephania brachyandra (*Diels*)*

Stage I: The crude product was ground into small particles, dissolved in a buffer solution, and crystallized in an environment with a pH of 5. During this stage, most of the by-products

and impurities such as inorganic salts, cellulose, and starch are removed. However, this intermediate product (in powder form) remained dark in color, with an unstable melting point and a rotundin content of about 80%.

Stage II: In this stage, the semi-finished product was dissolved in a solvent mixture. The solvents selectively dissolved the alkaloids while leaving the inorganic salts and long-chain organic compounds such as cellulose, starch, and reducing sugars. To prevent the product from degrading and discoloring, only gentle heating was applied. The solution underwent hot filtration and followed by a gradual crystallization process at a low temperature over 10 - 12 hours. The crystals were filtered and repeatedly washed with the cooled solvent mixture until gained a rotundin content of up to 90%.

Stage III: Purification and decolorization. The former product with a 90% rotundin content was gradually dissolved in a solvent mixture and adjusted to the most suitable concentration. The reason was that a high concentration causes the crystallization process to occur too rapidly, resulting in an impure final product. Conversely, a low concentration results in a substantial loss and a low crystallization efficiency. Then, bleaching agents and antioxidants were added while stirring for 30 - 45 minutes. The mixture was allowed to crystallize. The product was formed as sulfate, nitrate, or chloride salts, depending on the preparation purpose. Next, vacuum filtration and re-crystallization were performed at low temperatures. The final product obtained consists of needle-shaped crystals, off-white in color. The purification process improved the rotundin content from 90% to 98.2%, with a purification efficiency of 88.2%. This stage played a crucial role, requiring the precise determination of the solvent dissolution ratio to achieve a high-quality final product with high purification efficiency.

2.3. Data analysis

The data resulting from this study were analyzed using SPSS and Microsoft Excel software.

III. RESULTS

3.1. Results of rotundin content research in *Stephania brachyandra* (Diels)

*3.1.1. Results of rotundin content research of Stephania brachyandra (*Diels*) in Vo Nhai district*

Table 1. Results of rotundin content research of *Stephania brachyandra* (Diels) in Vo Nhai district

No.	Sample code	Tuber diameter (cm)	Rotundin (mg/100 g)	Tuber diameter (cm)
1	11229753/DV.1	22.1	336	22.1
2	11229753/DV.2	24.2	348	24.2
3	11229753/DV.3	23.1	342	23.1
4	11229753/DV.4	21.3	287	21.3
5	11229753/DV.5	20.6	272	20.6
6	11229753/DV.6	19.9	258	19.9
7	11229753/DV.7	25.4	525	25.4
8	11229753/DV.41	16.4	210	16.4
9	11229753/DV.42	25.7	589	25.7
10	07236853/DV.25	12.1	105	12.1

No.	Sample code	Tuber diameter (cm)	Rotundin (mg/100 g)	Tuber diameter (cm)
11	07236850/DV.1	7.4	0	7.4
12	07236850/DV.2	12.6	109	12.6
13	07236850/DV.3	18.9	193	18.9
14	07236850/DV.4	17.2	142	17.2
15	07236850/DV.5	16.9	129	16.9
16	07236850/DV.6	14.2	125	14.2
17	07236850/DV.7	32.1	882	32.1
18	07236850/DV.8	7.6	0	7.6
19	07236850/DV.18	7.2	0	7.2
20	07236850/DV.19	7.6	0	7.6
21	07236850/DV.20	19.8	151	19.8
22	07236852/DV.1	17.3	131	17.3
23	07236852/DV.2	29.8	824	29.8
24	07236852/DV.3	11.9	94	11.9
25	07236852/DV.4	7.8	0	7.8
26	07236852/DV.5	12.2	101	12.2
27	07236852/DV.6	7.5	0	7.5
28	07236852/DV.7	8.1	0	8.1
29	07236852/DV.8	16.8	127	16.8
30	07236852/DV.9	20.1	168	20.1
31	07236852/DV.10	16.8	126	16.8
32	07236852/DV.11	28.6	827	28.6
33	07236852/DV.12	7.4	0	7.4
34	07236852/DV.13	19.2	141	19.2
35	07236852/DV.14	31.4	981	31.4
36	07236852/DV.15	32.5	981	32.5
37	07236852/DV.16	17.8	132	17.8
38	07236852/DV.17	33.1	981	33.1
39	07236852/DV.18	32.6	963	32.6
40	07236852/DV.19	30.2	913	30.2
	Average	**18.84**	**312.325**	

The tuber diameters range from 7.2 cm to 33.1 cm, with an average of 18.84 cm. Rotundin varied from 0 mg/100 g to 981 mg/100 g, with an average of 312.325 mg/100 g. The sample with the largest diameter (33.1 cm) had the highest rotundin content (981 mg/100 g), while the sample with the smallest diameter (7.2 cm) produced the lowest rotundin content (0 mg/100 g). Samples with the highest rotundin content all exhibited diameters over 31 cm, while samples with the lowest rotundin content (0 mg/100 g) showed diameters ranging from 7.4 cm to 8.1 cm. This indicates a relative relationship between tuber diameter and rotundin content: larger tubers generally contained more rotundin. The significant variation in rotundin content

among samples demonstrates the diversity in the quality of medicinal materials. Selecting larger samples might help optimize rotundin yield in the extraction process (Nong *et al.*, 2017; Fazekas *et al.*, 2008; Lahaye *et al.*, 2008).

*3.1.2. Results of rotundin content research of Stephania brachyandra (***Diels***) in Dinh Hoa district*

Table 2. Results of rotundin content research of *Stephania brachyandra* (Diels) in Dinh Hoa district

No.	Sample code	Tuber diameter (cm)	Rotundin (mg/100 g)	Tuber diameter (cm)
1	11229753/DV.8	18.7	213	18.7
2	11229753/DV.9	24.1	348	24.1
3	11229753/DV.10	28.9	542	28.9
4	11229753/DV.11	23.2	351	23.2
5	11229753/DV.12	27.6	551	27.6
6	11229753/DV.13	24.7	412	24.7
7	11229753/DV.14	19.6	237	19.6
8	11229753/DV.15	23.1	337	23.1
9	11229753/DV.16	24.9	399	24.9
10	11229753/DV.17	24.2	431	24.2
11	11229753/DV.18	18.4	272	18.4
12	11229753/DV.19	25.3	510	25.3
13	11229753/DV.20	24.6	381	24.6
14	11229753/DV.21	23.8	445	23.8
15	11229753/DV.22	26.4	581	26.4
16	07236851/DV.23	8.4	0	8.4
17	07236851/DV.24	29.6	730	29.6
18	07236851/DV.25	8.3	0	8.3
19	07236853/DV.1	9.1	0	9.1
20	07236853/DV.2	12.0	111	12.0
21	07236853/DV.3	7.8	0	7.8
22	07236853/DV.4	9.1	0	9.1
23	07236853/DV.5	12.0	116	12.0
24	07236853/DV.6	10.1	0	10.1
25	07236853/DV.7	13.7	121	13.7
26	07236853/DV.8	14.1	151	14.1
27	07236853/DV.9	7.5	0	7.5
28	07236853/DV.10	32.4	941	32.4
29	07236853/DV.11	12.1	100	12.1
30	07236853/DV.12	11.0	0	11.0
	Average	**18.49**	**276.000**	

An overview of the data reveals that there are 30 samples from the Dinh Hoa district. The tuber diameters ranged from 7.5 cm to 32.4 cm, with an average diameter of 18.49 cm. The rotundin content fluctuated from a minimum of 0 mg/100 g to a maximum of 941 mg/100 g, with an average of 276 mg/100 g. Many samples with diameters below 10 cm inducated a rotundin content of 0, while samples with larger diameters tended to have higher rotundin content. The sample produced the highest rotundin content of 941 mg/100 g showed a diameter of 32.4 cm. There was a positive relationship between tuber diameter and rotundin content; larger tubers tended to expressed higher rotundin content. It is important to thoroughly analyze samples with exceptionally small diameters and no rotundin content since they may not be completely mature or might include measurement inaccuracies. To increase rotundin content, it is essential to focus on developing tubers with larger diameters and further research the environmental or technical factors that affect tuber growth and rotundin content (Kang *et al.*, 2017).

*3.1.3. Results of rotundin content research in Stephania brachyandra (***Diels***) in Phu Luong district*

Table 3. Results of rotundin content research in *Stephania brachyandra* (Diels) in Phu Luong district

No.	Sample code	Tuber diameter (cm)	Rotundin (mg/100 g)	Tuber diameter (cm)
1	11229753/DV.23	17.8	219	17.8
2	11229753/DV.24	16.5	256	16.5
3	11229753/DV.25	22.3	350	22.3
4	11229753/DV.26	22.1	334	22.1
5	11229753/DV.27	18.1	272	18.1
6	11229753/DV.28	16.4	163	16.4
7	11229753/DV.29	22.8	357	22.8
8	11229753/DV.30	24.1	449	24.1
9	11229753/DV.31	22.4	391	22.4
10	11229753/DV.32	17.2	267	17.2
11	11229753/DV.33	18.1	284	18.1
12	11229753/DV.34	21.2	301	21.2
13	11229753/DV.35	18.1	279	18.1
14	11229753/DV.36	14.6	87.2	14.6
15	11229753/DV.37	24.7	471	24.7
16	11229753/DV.38	16.1	166	16.1
17	11229753/DV.39	18.9	301	18.9
18	11229753/DV.40	22.1	337	22.1
19	07236853/DV.22	14.5	124	14.5
20	07236853/DV.23	14.8	127	14.8
21	07236853/DV.24	16.2	133	16.2
22	07236850/DV.9	16.3	135	16.3
23	07236850/DV.10	17.2	139	17.2
24	07236850/DV.11	20.2	161	20.2
25	07236850/DV.12	14.1	122	14.1
26	07236850/DV.13	7.4	0	7.4
27	07236850/DV.14	7.8	0	7.8
28	07236850/DV.15	8.2	0	8.2
29	07236850/DV.16	8.8	0	8.8
30	07236850/DV.17	9.1	0	9.1
	Average	**16.94**	**207.507**	

An analysis of 30 samples from Phu Luong indicates tuber diameters ranging from 7.4 cm to 24.7 cm, averaging 16.94 cm, and rotundin content varying from 0 mg/100 g to 471 mg/100 g, averaging 207.507 mg/100 g with an average of 207.507 mg/100g. Samples with diameters below 10 cm typically have a rotundin content of 0. The sample with the highest rotundin content was 471 mg/100g, with a tuber diameter of 24.7 cm. A direct correlation was seen between the diameter of tubers and the concentration of rotundin, wherein bigger tubers tended to exhibit greater levels of rotundin. Samples with very small diameters and zero rotundin content needed to be carefully examined to determine the cause (Phong *et al.*, 2014). To enhance rotundin content, it is crucial to cultivate larger tubers and conduct further research into environmental and technical factors influencing growth. Overall, the data underscores that larger tubers tend to possess higher rotundin content, and maximizing rotundin content hinges on developing larger tubers.

*3.1.4. Results of rotundin content research in Stephania brachyandra (***Diels***) in Dai Tu district*

Table 4. Results of rotundin content research in *Stephania brachyandra* (Diels) in Dai Tu district

No.	Sample code	Tuber (cm)	Rotundin (mg/100 g)	Tuber diameter (cm)
1	11229753/DV.43	17.6	253	17.6
2	11229753/DV.44	17.2	90.4	17.2
3	11229753/DV.45	24.6	531	24.6
4	11229753/DV.46	26.8	612	26.8
5	11229753/DV.47	23.7	411	23.7
6	11229753/DV.48	25.1	476	25.1
7	11229753/DV.49	15.6	161	15.6
8	11229753/DV.50	22.2	403	22.2
9	11229753/DV.51	15.2	130	15.2
10	11229753/DV.52	17.3	178	17.3
11	11229753/DV.53	24.3	427	24.3
12	11229753/DV.54	14.8	122	14.8
13	11229753/DV.55	26.9	494	26.9
14	11229753/DV.56	18.4	283	18.4
15	11229753/DV.57	17.2	243	17.2
16	11229753/DV.58	25.6	456	25.6
17	11229753/DV.59	16.7	208	16.7
18	11229753/DV.60	17.8	271	17.8
19	11229753/DV.61	25.5	494	25.5
20	11229753/DV.62	16.7	220	16.7
21	11229753/DV.63	25.7	570	25.7
22	11229753/DV.64	15.6	158	15.6
23	11229753/DV.65	14.3	164	14.3
24	11229753/DV.66	13.2	107	13.2
25	11229753/DV.67	17.5	277	17.5
26	11229753/DV.68	17.1	242	17.1
27	07236851/DV.19	8.5	0	8.5
28	07236851/DV.20	7.9	0	7.9
29	07236851/DV.21	11.5	106	11.5
30	07236851/DV.22	7.7	0	7.7
	Average	**18.27**	**269.580**	

An overview of the data from 30 samples in Dai Tu shows that the tuber diameters range from 7.7 cm to 26.9 cm, with an average of 18.27 cm, and the rotundin content ranges were between 0 mg/100 g and 612 mg/100 g, with an average of 269.58 mg/100 g. Samples with diameters under 10 cm typically indicated no rotundin content suggesting possible underdevelopment or environmental influences. The sample with the greatest rotundin content was 612 mg/100 g, with a tuber diameter of 26.8 cm, illustrating a positive relationship between tuber diameter and rotundin content. Larger tubers generally produced higher rotundin content, suggesting that developing larger tubers could be an effective strategy to increase rotundin levels. Samples with extremely tiny sizes as well as without any rotundin warranted careful examination to determine the cause, which could be due to technical or environmental factors. To optimize rotundin content, efforts should focus on improving cultivation conditions to promote larger tuber development and conducting further research into environmental and technical factors impacting tuber quality (Scheunert *et al.*, 2014; Warwick *et al.*, 2010).

*3.1.5. Results of rotundin content research in Stephania brachyandra (***Diels***) in Dong Hy district*

Table 5. Results of rotundin content research in *Stephania brachyandra* (Diels) in Dong Hy district

No.	Sample code	Tuber diameter (cm)	Rotundin (mg/100g)	Tuber diameter (cm)
1	11229753/DV.69	19.1	313	19.1
2	11229753/DV.70	18.1	270	18.1
3	11229753/DV.71	20.1	322	20.1
4	07236851/DV.1	7.5	0	7.5
5	07236851/DV.2	10.3	109	10.3
6	07236851/DV.3	17.6	257	17.6
7	07236851/DV.4	28.9	768	28.9
8	07236851/DV.5	8.2	0	8.2
9	07236851/DV.6	12.8	104	12.8
10	07236851/DV.7	18.3	289	18.3
11	07236851/DV.8	9.6	0	9.6
12	07236851/DV.9	8.5	0	8.5
13	07236851/DV.10	9.4	0	9.4
14	07236851/DV.11	14.1	180	14.1
15	07236851/DV.12	8.5	0	8.5
16	07236851/DV.13	8.9	0	8.9
17	07236851/DV.14	10.7	0	10.7
18	07236851/DV.15	11.2	0	11.2
19	07236851/DV.16	11.7	110	11.7
20	07236851/DV.17	8.7	0	8.7
21	07236851/DV.18	9.6	0	9.6
22	07236853/DV.13	8.6	0	8.6
23	07236853/DV.14	7.9	0	7.9
24	07236853/DV.15	11.9	100	11.9
25	07236853/DV.16	7.6	0	7.6
26	07236853/DV.17	14.1	121	14.1
27	07236853/DV.18	14.3	126	14.3
28	07236853/DV.19	8.1	0	8.1
29	07236853/DV.20	7.8	0	7.8
30	07236853/DV.21	9.1	0	9.1
	Average	**12.04**	**102.300**	

An overview of the data from 30 samples in Dong Hy illustrates that the tuber diameters flutuated from 7.5 cm to 28.9 cm, with an average of 12.04 cm, and the rotundin content differed from 0 mg/100 g to 768 mg/100 g, with an average of 102.3 mg/100 g. Samples with diameters under 10 cm typically have no rotundin content, indicating poor development or unsuitable environmental conditions. The sample with the highest rotundin content is 768 mg/100 g, with a tuber diameter of 28.9 cm. Rotundin content was positively correlated with tuber diameter, with larger tubers typically exhibiting a higher rotundin content. This implies that the content of rotundin was significantly influenced by the extent of the tuber. Samples with very small diameters and zero rotundin content require meticulous examination to pinpoint the cause, which may stem from cultivation techniques or unsuitable environmental conditions. To enhance rotundin content, it is crucial to promote the development of larger tubers and conduct further research into environmental and technical factors influencing tuber growth and quality. Improving soil conditions, irrigation, and cultivation techniques could prove beneficial in increasing tuber size and quality.

3.2. Summary of rotundin content analysis results

Table 6. Summary of rotundin content analysis results

No.	Location (District)	Number of samples analyzed	Average tuber diameter (cm)	Average content (mg/100 g)
1	Vo Nhai	40	18.84	312.325
2	Dinh Hoa	30	18.49	276.000
3	Phu Luong	30	16.94	207.507
4	Dai Tu	30	18.27	269.580
5	Dong Hy	30	12.04	102.300
	Overall average		**16.915**	**233.542**

The average tuber diameter data across the province indicates that the exploitation of *Stephania brachyandra* (Diels) for rotundin extraction has led to a significant reduction in tuber size. Six to seven years ago, it was common to find tubers in the forest with diameters of 50 - 60 cm and weights of 70 - 80 kg. However, nowadays, only smaller tubers with diameters of 7 - 15 cm are predominant, and it is rare to find tubers with diameters over 20 cm. The rotundin content also varies by district, with the highest in Vo Nhai (312.325 mg/100 g) and the lowest in Dong Hy (102.300 mg/100 g). Since tuber diameter is closely related to age, this study focuses on the relationship between rotundin content and tuber diameter. The primary aim is to sustainably protect and develop *Stephania brachyandra* (Diels) resources, ensuring a long-term and high-quality supply for the pharmaceutical processing industry.

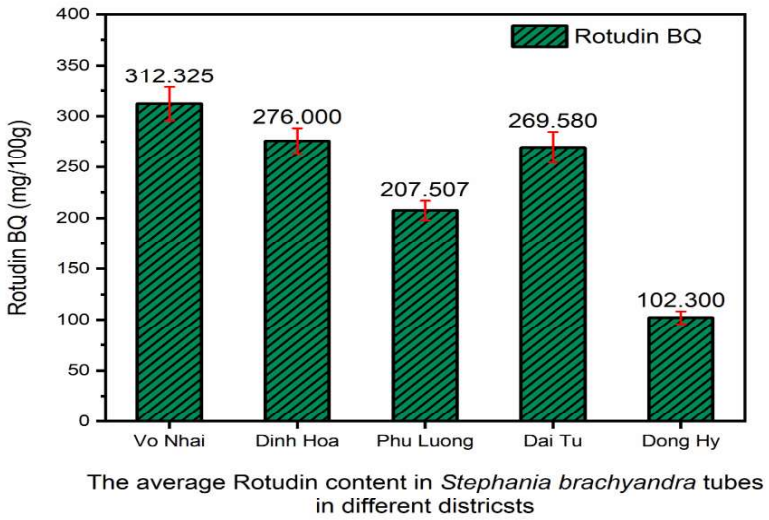

Figure 1. Rotundin content in *Stephania brachyandra* (Diels) in various districts

The average tuber diameter at various sampling locations exhibits significant variation. Vo Nhai district notably recorded the largest average tuber diameter, measuring 18.84 cm. Conversely, Dong Hy district had the smallest average tuber diameter, at only 12.04 cm. Overall, when aggregating data from different districts, the average tuber diameter for the entire province was 16.915 cm. This disparity may be due to a variety of factors such as soil conditions, climate, and cultivation techniques in each locality. These findings highlight the necessity of researching and applying appropriate agricultural practices to optimize tuber size in regions with smaller diameters, thereby improving production efficiency and the quality of agricultural products across the province.

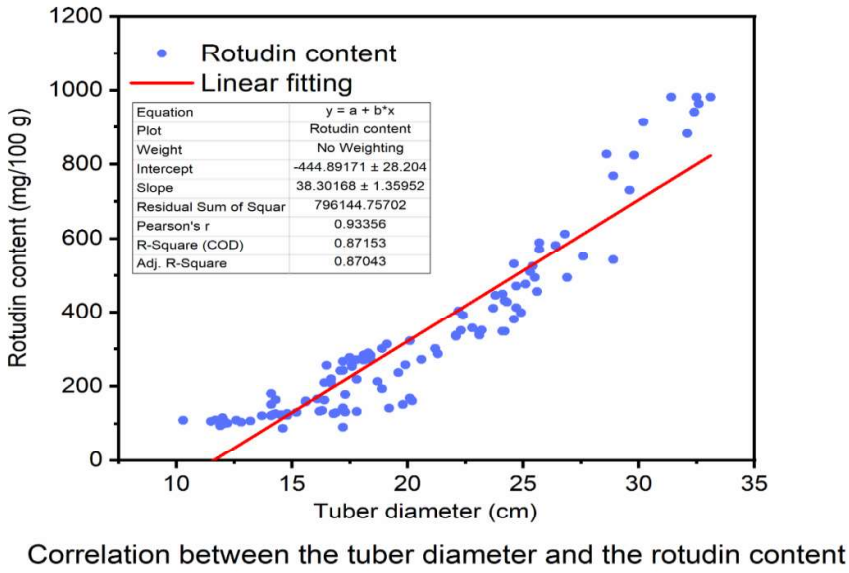

Figure 2. Correlation between rotundin content and tuber diameter of *Stephania brachyandra* (Diels)

The research results have determined a regression correlation between rotundin content and the diameter of *Stephania brachyandra* (Diels), with the equation

$$\text{Rotundin} = -444{,}89 + 38{,}302 \times \text{Tuber diameter} \quad (1)$$

The correlation coefficient R = 0.94 indicates a very high correlation, it was evident that there was a strong relationship between rotundin content and the tuber diameter of *Stephania brachyandra* (Diels). As mentioned earlier, the tuber's age was closely associated with its diameter. Hence, it could be inferred indirectly that rotundin content was influenced by the tuber's age. This will be further verified after the second round of rotundin content analysis, scheduled for October 2024. The upcoming analysis will focus on samples cultivated under the translocation conservation model and the original stock garden model, aiming to deepen our understanding of rotundin content dynamics in relation to tuber age and cultivation methods.

IV. CONCLUSION

The study analyzed the rotundin content of 160 naturally distributed *Stephania brachyandra* (Diels) samples in Thai Nguyen province. The results showed a wide variation in rotundin content, ranging from 90.4 to 981 mg/100 g. This fluctuation may be influenced by several factors, such as soil conditions, climate, and the age of the *Stephania brachyandra* (Diels). There was a high correlation between rotundin content and tuber diameter. A detailed analysis of these factors will enhance our understanding of the reasons behind these differences in rotundin content. Moreover, it will provide crucial insights for optimizing cultivation and harvesting methods to maximize rotundin content and ensure product quality.

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