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# The First Agro-biological Characterization of Various Vine Tea (*Ampelopsis cantoniensis* (H&A) Pl.) Accessions Collected in Northern Vietnam

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## Abstract

Vine tea (*Ampelopsis cantoniensis* (H&A) Pl.) is a beneficial herbal tea with a high content of the natural antioxidant dihydromyricetin (DHM). Five accessions of vine tea were collected from different locations in Vietnam, *viz.* VT-CD01 (collected in Sapa), VT-CD02 (Lai Chau - 1), VT-CD03 (Lai Chau - 2), VT-CD04 (Son La), and VT-CD05 (Bac Giang), and were grown in Sapa, Lao Cai, Vietnam from 2017 to 2020 to evaluate their agro-biological characteristics and DHM contents. The leaf color, color of the young petiole, and leaflet shape of these accessions showed similar morphological characteristics to the snake wine vine. VT-CD01, VT-CD02, and VT-CD04 featured purple young leaves, whereas the leaves of VT-CD03 and VT-CD05 were green. VT-CD01 and VT-CD02 showed the best growth with the highest numbers of branches, high yields, and high DHM contents (35.02% in VT-CD01 and 35.25% in VT-CD02). The DHM in VT-CD03 (34.41%) and VT-CD04 (30.86%) also were high, whereas that in VT-CD05 (6.3%) was lower than the requirement of Vietnamese Pharmacopeia V (18%). The accessions VT-CD03, VT-DC04, and especially VT-CD01 and VT-CD02 should be cultivated and are recommended for the treatment of inflammatory-related diseases.

## Keywords

Agro-biological characteristics, Dihydromyricetin, gastric ulcer, *Ampelopsis cantoniensis*, Sapa

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## Introduction

Vine tea, called "Chè dây" or "Chè rừng" in Vietnamese, with the scientific names *Ampelopsis cantoniensis* (H&A) or *Nekemias cantoniensis,* is a healthy herbal tea with high contents of flavonoids, protein, potassium, calcium, and vitamins C, E, B1, and

B2. It is mainly distributed in China, India, Japan, Vietnam, Laos, Malaysia, and Indonesia (Wen *et al.*, 2014). Snake wine vine is an indigenous medicinal plant in Vietnam, and is distributed from the North to the South. It often grows in valley forests or alongside mountainside shrubs in Cao Bang, Lao Cai, Lang Son, Hai Phong, Quang Ninh, Ha Noi, Ninh Binh, and Quang Nam, among others. (Pham Thi Kim Thao *et al.*, 2017; Nguyen Ho Lam *et al.*, 2019). Vine tea is often recommended for its beneficial properties such as detoxification, reducing inflammatory-related diseases, clearing heat, acting as an anti-rheumatic, and strengthening tendons.

The leaves and stems of vine tea contain approximately 60 chemical components, including flavonoids, phenols, and other components. Approximately 30 of the flavonoids contain two types of sugar, glucose and rhamnose, both of which are substances that are quite safe for humans (Pham Thi Kim Thao *et al.*, 2017; Wu *et al.*, 2023). Among the flavones, dihydromyricetin (DHM, $C_{15}H_{12}O_8$) makes up the highest content and has significant anti-inflammatory properties (Carneiro *et al.*, 2021; Wu *et al.*, 2023). Previous studies have confirmed that the flavonoid component, especially DHM extracted from the snake wine vine, has a positive effect in treating stomach ulcers, preventing cancer, and enhancing the body's immune function (Nguyen *et al.*, 2022; Zeng *et al.*, 2023).

On the one hand, the market demand for vine tea has increased. More than half of the population in Vietnam is facing digestive system diseases and prefers treatment options with natural antioxidants and no toxicity, such as vine tea or herbal tea (Nguyen Ho Lam *et al.*, 2019; Nguyen *et al.*, 2022). In Vietnam, these raw medicinal materials have increased to hundreds of tons per year (Traphaco, 2012). On the other hand, wild resources in nature are gradually shrinking. The effectiveness of vine tea in disease treatment also differs depending on the varieties of plants used because of differences in the content of DHM or other flavonoids (Pham Thi Kim Thao *et al.*, 2017; Nguyen Ho Lam *et al.*, 2019). According to Vietnamese Pharmacopeia V (2018), only vine tea varieties that show a DHM content greater than 18% should be selected as therapeutic agents for inflammatory-related diseases. Therefore, the quantification of DHM content as well as characterization of agronomical traits of vine tea accessions collected in different regions in Vietnam is crucial to selecting good materials for medicinal food resources.

## Materials and Methods

### Materials and plant growing

Five vine tea accessions were collected for this study, namely VT-CD01 (collected in Sapa district, Lao Cai province, Vietnam), VT-CD02 (purple young stem, collected in Tan Uyen district, Lai Chau province, Vietnam; called Lai Chau - 1); VT-CD03 (green young stem, collected in Tan Uyen district, Lai Chau province, Vietnam, called Lai Chau - 2), VT-CD04 (collected in Thuan Chau district, Son La province, Vietnam), and VT-CD05 (collected in Luc Nam district, Bac Giang province, Vietnam) (**Figure 1**). They were propagated by stem cuttings and planted in Sapa, Lao Cai, Vietnam in 2017. The plantings were laid out as a randomized complete block design (RCB) with three replications. The experimental plot area was 20 $m^2$, excluding the protection band. The plants grew in shaded conditions with 30% sunlight. The applied planting processes followed Traphaco (2012).

### Data collection

The agronomical traits, namely growth duration, stem shape and type, stem color, pubescence, leaf length, leaf width, leaf color, leaflet characteristics, plant height, stem diameter, number of leaves per main stem, number of branches, chlorophyll content (recorded by SPAD-502, Minolta, Japan), and seed characteristics were measured in the second year. Individual yield was quantified by collecting 30 cm-long samples of all the young shoots, including leaves and stems, of five plants per treatment at the time of the 2$^{nd}$ harvest in each year from 2017 to 2020. In the third year, the dihydromyricetin (DHM) content was quantified according to Vietnamese Pharmacopoeia V (2018) in the laboratory of the Traphaco Company.

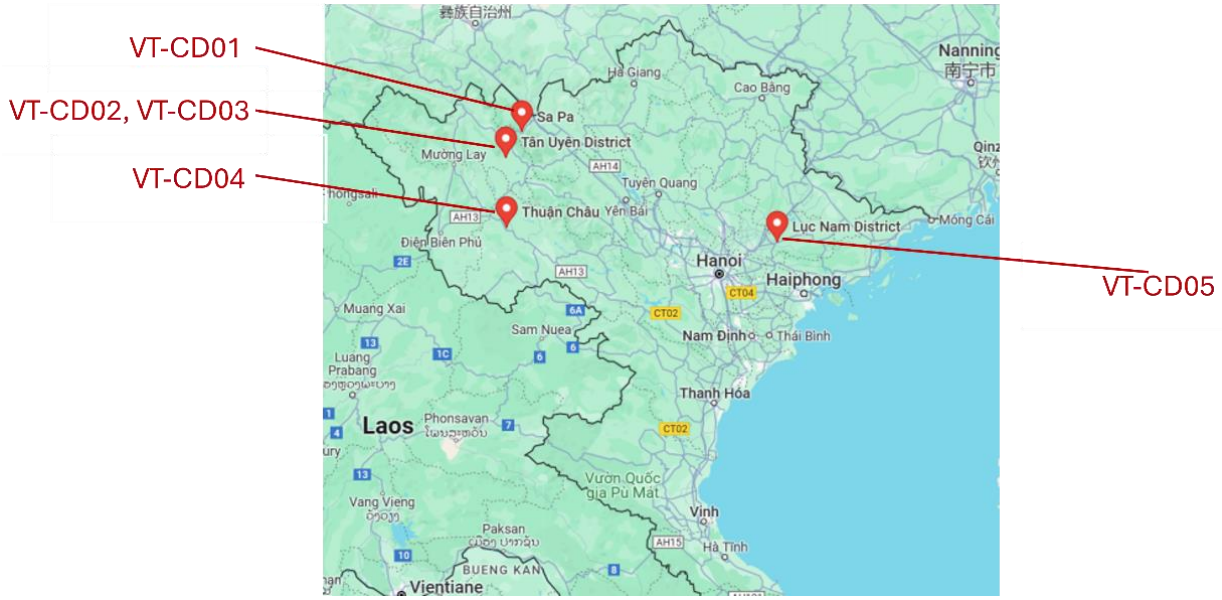


**Figure 1**. Geographical distribution of the five collected vine tea accessions in northern Vietnam

**Table 1**. Stem characteristics of the five vine tea accessions

| Genotype | Place of collection | Stem shape and type | Stem Color | | Tendril | Pubescence |
|---|---|---|---|---|---|---|
| | | | Young | Mature | | |
| VT-CD01 | Sa Pa, Lao Cai | branchlets are terete | purple | brown | yes, bifurcate, derived from shoot | sparsely pubescent when young |
| VT-CD02 | Lai Chau - 1 | branchlets are terete | purple | brown | yes, bifurcate, derived from shoot | sparsely pubescent when young |
| VT-CD03 | Lai Chau - 2 | branchlets are terete | green | brown | yes, bifurcate, derived from shoot | sparsely pubescent when young |
| VT-CD04 | Son La | branchlets are terete | green | brown | yes, bifurcate, derived from shoot | sparsely pubescent when young |
| VT-CD05 | Bac Giang | branchlets are terete | purple | brown | yes, bifurcate, derived from shoot | sparsely pubescent when young |

### Data analysis

One-way analysis of variance (ANOVA) was conducted by using IRRISTAT version 5.0. Multiple comparisons were performed using LSD method for least-square means at the significance level of 0.05.

## Results and Discussion

### Morphological characteristics of the five vine tea accessions

The results in **Table 1** show that all five accessions were common in terms of having stems that were cylindrical, solid with tassels, brown in maturity, and produced bifurcate tendrils. The accessions did, however, have various colors as young stems. The VT-CD01, VT-CD02, and VT-CD04 accessions were purple, whereas VT-CD03 (Lai Chau - 2) and VT-CD05 (Bac Giang) were green. The color of the young stems depended on the moisture in the stems and the color may change from green to purple in high moisture conditions. The obtained results in the current study were similar to the snake wine vine, namely Ra Zéh, grown in Tu commune, Dong Giang district, Quang Nam province (Nguyen Ho Lam *et al.*, 2019).

The morphology of vine tea leaves was characterized for each accession. The leaves of VT-CD04 were smaller than the other four.

**Table 2**. Leaf morphologies of the five vine tea accessions

| Genotype | Leaf type | Leaf | | Leaf blade color | | | Petiole color | | Leaflet | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Length (cm) | Width (cm) | Young | Mature (upper side) | Mature (lower side) | Young | Mature | Shape | Surface | Margin |
| VT-CD01 | pinnately compound (odd), having 2 basal 3-foliolate pinnae | 18.5 ± 0.32 | 17.5 ± 0.28 | purple and green | green | purple | purple | light purple | ovate | smooth | serrate |
| VT-CD02 | pinnately compound (odd), having 2 basal 3-foliolate pinnae | 18.2 ± 0.20 | 15.4 ± 0.10 | purple and green | green | green | purple | light purple | ovate-elliptic | smooth | serrate |
| VT-CD03 | pinnately compound (odd), having 2 basal 3-foliolate pinnae | 17.3 ± 0.19 | 16.5 ± 0.17 | light green | dark green | green | purple and green | purple and green | ovate | smooth | serrate |
| VT-CD04 | pinnately compound (odd), having 2 basal 3-foliolate pinnae | 13.6 ± 0.28 | 11.7 ± 0.18 | light green | green | light purple | light purple | light purple | ovate-elliptic | smooth | serrate |
| VT-CD05 | pinnately compound (odd), having 2 basal 3-foliolate pinnae | 17.6 ± 0.14 | 16.3 ± 0.06 | purple and green | dark green | green | purple and green | purple and green | ovate-elliptic | smooth | serrate |

**Table 3.** The morphological characteristics of the flowers and fruits of the five vine tea accessions

| Genotype | Flower color | Day to flower | Day to fruit | Fruit | Total fruit number (/plant) | Seed color | 1000-seed weight (g) |
|---|---|---|---|---|---|---|---|
| VT-CD01 | white | 188 | 269 | black ripened fruit, 3-4 seeded fleshy berries | 174.8 ± 8.0 | brown | 15.68 |
| VT-CD02 | white | 189 | 271 | black ripened fruit, 3-4 seeded fleshy berries | 167.0 ± 8.3 | brown | 15.46 |
| VT-CD03 | white | 197 | 278 | black ripened fruit, 3-4 seeded fleshy berries | 159.4 ± 6.8 | brown | 15.21 |
| VT-CD04 | white | 227 | 299 | black ripened fruit, 3-4 seeded fleshy berries | 140.0 ± 4.5 | brown | 15.77 |
| VT-CD05 | white | 194 | 274 | black ripened fruit, 3-4 seeded fleshy berries | 161.6 ± 5.4 | brown | 16.15 |

The leaflets of VT-CD01 and VT-CD03 were ovate while those of the other three accessions were ovate-elliptic. The colors of the young petioles of the VT-CD01 and VT-CD02 accessions were purple, whereas VT-CD03 and VT-CD05 were purple and green, and VT-CD04 was light purple. The leaf margins of VT-CD01, VT-CD02, VT-CD03, and VT-CD04 were serrated, while the leaf margin of VT- CD05 was a little thin serrate. All five accessions shared commonalities in having smooth upper leaf surfaces and serrate leaf margins. Most of the accessions had green mature leaves, while VT-CD05 featured very dark green mature leaves (**Table 2**). These results were similar to the descriptions of the vine tea accessions belonging to *Nekemias cantoniensis* (Hook. & Arn.) reported Wen *et al*. (2014), which were collected from Xichou Xian, Yunnan province, China.

The morphologies of the flowers, fruits, and seeds of the five accessions were similar. The flowers were white, the ripened fruits were black, and each fruit contained three to four seeds. These are the features of *Ampelopsis* genera (Wen, 2007; Wu *et al*., 2023). The seeds were brown, and the 1000-seed weight ranged from 15.21 to 16.15 g (**Table 3**). The five accessions only showed a difference in the growth duration from planting to flowering. VT-CD04 (227 days) had the longest duration, followed by VT-CD03 (197 days), VT-CD05 (194 days), VT-CD02 (189 days), and VT-CD01 (188 days).

## Growth and development characteristics of the five vine tea accessions

Snake wine vine is a plant with infinite growth and climbing stems, especially when it grows in shade or under a forest canopy (Nguyen Ho Lam *et al.*, 2019; Wu *et al.*, 2023). The plant heights of all the accessions increased gradually from the first year to the second year and were highest in the third year. However, the plant height depended on the accession and shoot harvesting. The research results in **Table 4** show that the plant height was highest in the third year of accession VT-CD01 (152.3cm), followed by VT-CD03 (143.5cm), VT-CD02 (141.2cm), and VT-CD04 (139.4cm), while VT-CD05 showed the lowest plant height (123.9cm).

The stem diameter and the number of branches/stems were determined by the stem/leaf ratio in the raw materials. These parameters may influence the quality of vine tea because it has been reported that the dihydromyricetin content is lower in stems compared to young leaves (Wu *et al.*, 2023). The stem diameter of the VT-CD02 accession was smaller than those of the other accessions in all three years. Specifically, in the first year, the stem diameter of VT-CD02 was only 7.02mm, while that of VT-CD05 reached 8.46mm. In the second year, the stem diameters increased rapidly, ranging from 22.5mm (VT-CD02) to 24.2mm (VT-CD03). In the third year, the highest stem diameter was obtained in VT-CD05 (38.2mm).

The number of branches and number of leaves per main stem showed the growth dynamics of the plants and these parameters may influence the quality of vine tea. The highest numbers of branches and leaves per plant were observed in the VT-CD02 accession in both the first and the third years but the values were not significantly different among accessions in the second year (**Table 5**). Of which, the number of branches reached 8.67 (in the first year), 13.33 (in the second year), and 20.67 branches stem$^{-1}$ (in the third year). In the third year, the number of branches in VT-CD05 (15.0 branches) and number of leaves per main stems (28.7 leaves) were lower than those of VT-CD02, VT-CD01, and VT-CD03 at $P < 0.05$ but were not significantly different from those in VT-CD04.

The lowest values were in the VT-CD05 accession in all three years. In addition to having the highest number of branches, VT-CD02 also showed the highest number of leaves per plant in all three years. In the third year, VT-CD02 had 43.2 leaves plant$^{-1}$ while VT-CD05 had significantly fewer leaves per plant (28.7) at a 95% confidence level.

## Leaf area index (LAI) and chlorophyll content of the five vine tea accessions

The five accessions showed differences in LAI in all three years, especially in the third year. In the first and second years, VT-CD04 and VT-CD01 had higher LAI than the other

three accessions. However, in the third year, the LAI in VT-CD04 (3.8) was much lower than those in VT-CD01 (4.8) and VT-CD02 (4.7). VT-CD03 and VT-CD05 always showed the lowest LAI in all three years (**Table 6**).

The chlorophyll content was quickly examined via SPAD due to their strong correlation (Ninh Thi Phip & Nguyen Thi Thanh Hai, 2016). SPAD increased over time and was influenced by the planting year (growth stage) and genotypes. There were no significant differences among the accessions in SPAD in the first and second years but significant differences were observed in the third year. SPAD in VT-CD03 (56.7) was not significantly different from VT-CD01 (53.5) but was higher than VT-CD02 (51.7), VT-CD05 (49.8), and VT-CD04 (47.8).

**Table 4**. Plant heights and stem diameters of the five vine tea accessions

| Accessions | Plant height (cm) | | | Stem diameter (mm) | | |
|---|---|---|---|---|---|---|
| | Year 1 | Year 2 | Year 3 | Year 1 | Year 2 | Year 3 |
| VT-CD01 | 100.0$^{ab}$ | 115.0$^{a}$ | 152.3$^{a}$ | 7.96$^{ab}$ | 23.8$^{a}$ | 35.2$^{b}$ |
| VT-CD02 | 99.3$^{ab}$ | 107.7$^{ab}$ | 141.2$^{ab}$ | 7.02$^{c}$ | 22.5$^{b}$ | 33.7$^{c}$ |
| VT-CD03 | 102.0$^{ab}$ | 110.7$^{ab}$ | 143.5$^{ab}$ | 7.98$^{ab}$ | 24.2$^{a}$ | 37.7$^{a}$ |
| VT-CD04 | 96.3$^{b}$ | 103.3$^{b}$ | 139.4$^{b}$ | 7.85$^{b}$ | 24.0$^{a}$ | 37.8$^{a}$ |
| VT-CD05 | 106.0$^{a}$ | 110.7$^{ab}$ | 123.9$^{c}$ | 8.46$^{a}$ | 23.8$^{a}$ | 38.2$^{a}$ |
| *$LSD_{0.05}$* | *8.28* | *8.27* | *12.57* | *0.58* | *0.75* | *0.69* |
| *CV%* | *4.4* | *4.1* | *4.7* | *3.8* | *2.9* | *2.0* |

*Note: Within columns, mean values followed by different letters are significantly different at P <0.05.*

**Table 5**. Growth dynamics of the number of branches per plant and the number of leaves/plant of the five vine tea accessions

| Accession | Total branch number | | | Number of leaves/main stem | | |
|---|---|---|---|---|---|---|
| | Year 1 | Year 2 | Year 3 | Year 1 | Year 2 | Year 3 |
| VT-CD01 | 7.00$^{b}$ | 13.00$^{a}$ | 18.00$^{b}$ | 9.3$^{c}$ | 23.6$^{ab}$ | 38.0$^{b}$ |
| VT-CD02 | 8.67$^{a}$ | 13.33$^{a}$ | 20.67$^{a}$ | 10.2$^{a}$ | 27.3$^{a}$ | 43.2$^{a}$ |
| VT-CD03 | 6.67$^{b}$ | 13.33$^{a}$ | 16.67$^{bc}$ | 9.6$^{b}$ | 18.1$^{b}$ | 34.0$^{b}$ |
| VT-CD04 | 6.00$^{bc}$ | 12.00$^{b}$ | 16.00$^{cd}$ | 9.7$^{b}$ | 23.6$^{ab}$ | 33.3$^{bc}$ |
| VT-CD05 | 5.00$^{c}$ | 9.33$^{c}$ | 15.00$^{d}$ | 9.5$^{bc}$ | 18.4$^{b}$ | 28.7$^{c}$ |
| *$LSD_{0.05}$* | *1.00* | *0.94* | *1.37* | *0.29* | *5.51* | *4.97* |
| *CV%* | *10.5* | *15.6* | *11.6* | *3.5* | *4.7* | *5.2* |

*Note: Within columns, mean values followed by different letters are significantly different at P <0.05.*

**Table 6**. SPAD index and LAI of the five vine tea accessions

| Accession | SPAD | | | LAI | | |
|---|---|---|---|---|---|---|
| | Year 1 | Year 2 | Year 3 | Year 1 | Year 2 | Year 3 |
| VT-CD01 | 29.7$^{a}$ | 42.1$^{a}$ | 53.5$^{ab}$ | 1.8 | 2.4 | 4.8 |
| VT-CD02 | 28.9$^{a}$ | 40.8$^{a}$ | 51.7$^{bc}$ | 1.7 | 2.2 | 4.7 |
| VT-CD03 | 29.5$^{a}$ | 45.3$^{a}$ | 56.7$^{a}$ | 1.6 | 2.2 | 3.3 |
| VT-CD04 | 27.4$^{a}$ | 41.7$^{a}$ | 47.8$^{c}$ | 1.8 | 2.4 | 3.8 |
| VT-CD05 | 31.8$^{a}$ | 42.5$^{a}$ | 49.8$^{bc}$ | 1.5 | 2.1 | 3.4 |
| *$LSD_{0.05}$* | *4.49* | *4.62* | *4.96* | | | |
| *CV%* | *7.7* | *5.5* | *4.7* | | | |

*Note: Within columns, mean values followed by different letters are significantly different at P <0.05*

**Table 7.** Individual yields and DHM of the five vine tea accessions

| Accession | Individual yield (g/plant) | | | DHM content in collected plants (%) | DHM content in plants cultivated in Sapa (%) |
|---|---|---|---|---|---|
| | Year 1 | Year 2 | Year 3 | Year 3 | Year 3 |
| VT-CD01 | 151.21 | 504.9 | 987.34[a] | 33.37 | 35.02 |
| VT-CD02 | 143.28 | 500.9 | 943.96[a] | 32.07 | 35.25 |
| VT-CD03 | 139.79 | 469.7 | 781.42[b] | 31.58 | 34.41 |
| VT-CD04 | 142.31 | 491.7 | 795.57[b] | 30.86 | 30.86 |
| VT-CD05 | 130.87 | 472.7 | 702.27[c] | 3.62 | 6.30 |
| *$LSD_{0.05}$* | | | *66.78* | | |
| *CV%* | | | *4.3* | | |

*Note: Within columns, mean values followed by different letters are significantly different at P <0.05.*


**Figure 2.** Stems and leaves of the four high-quality vine tea accessions

## Yield and dihydromyricetin contents in the five accessions

The yield of the vine tea plants increased over the experimental period and differed depending on the accession. VT-CD01 always produced the highest yield in all three years, with values of 151.21, 504.9, and 987.34 g $plant^{-1}$ in the first, second, and third years, respectively. The yields of VT-CD02 were also

as high as VT-CD01. The three other accessions showed much lower individual yields, especially in the third year with values of 795.57g in VT-CD04, 781.42g in VT-CD03, and 702.27g in VT-CD05 (**Table 7**). These accessions had approximately 2.7 tons per ha, whereas VT-CD01 had 3.2 tons per ha, and VT-CD02 had 3.0 tons per ha at the conclusion of the experiment.

Dihydromyricetin (DHM) is a natural antioxidant and the most important bioactive compound in vine tea. Extracted DHM from vine tea has significant anti-inflammatory properties and is recommended as a potential therapeutic agent for gastric ulcers and inflammatory-related diseases (Carneiro *et al.*, 2021; Nguyen *et al.*, 2022; Wu *et al.*, 2023). In the current study, we compared the DHM content in the stems and leaves of all the accessions between plants collected from natural conditions and those cultivated in Sapa in the experiment. The obtained results showed that the DHM contents (%) were higher in the plants grown in Sapa than in those collected from natural conditions. The highest DHM contents were observed in the VT-CD01 and VT-CD02 accessions at 33.37% and 32.07% (collecting) and 35.02% and 35.25% (in planted conditions), respectively (**Table 7**). The DHM contents in VT-CD03 and VT-CD04 were also higher than 30%. According to Vietnamese Pharmacopoeia V (2018), these four accessions passed the threshold requirement of 18% and can be recommended as good-quality accessions. Only VT-CD05 collected in Bac Giang province did not pass the threshold requirement because it showed very low concentrations in both the natural and cultivated sites, reaching only 3.62% (collected in nature) and 6.30% (grown in Sapa). According to the local people in Quang Nam province, the young stems with a purple or red color are believed to have a higher quality than the green ones (Nguyen Ho Lam *et al.*, 2019). This was not always true in our research. VT-CD05 had green leaves and stems and a low DHM content; however, VT-CD03 – collected in Lai Chau – also had green stems and leaves but a high DHM content. Therefore, analyzing the DHM in vine tea plants should be conducted to evaluate their quality. In the current study, four accessions, *viz.*, VT-CD01 collected in Sapa, VT-CD02 and VT-CD03 collected in Lai Chau, and VT-CD04 collected in Son La, are recommended as high-quality and can be used for medicinal purposes. The morphological characteristics of the stems and leaves are shown in **Figure 2**. These accessions also showed high resistance to diseases and pests such as beetles and mosquitos with slight infection rates (point 1, unpublished data).

## Conclusions

The five accessions analyzed in this experiment showed various features in terms of their agronomical characteristics. VT-CD03 and VT-CD05 had green leaves and stems, while the three other accessions, namely VT-CD01, VT-CD02, and VT-CD04, were marked with a purple color in the young stems and leaves. VT-CD01 and VT-CD02 showed high yields and DHM contents greater than 35%, followed by VT-CD03 and VT-CD04 with DHM contents greater than 30%. These four accessions, especially VT-CD01 and VT-CD02, are recommended for use in inflammation disease treatments. Only VT-CD05, which showed the lowest yield and DHM content, did not pass the requirement of Vietnam Pharmacopoeia V (2018), so it is not recommended for use as a medicinal food resource

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