The Potential Use of a Food-Dyeing Plant *Peristrophe bivalvis* (L.) Merr. in Northern Vietnam

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Abstract. An investigation on the current usage status and experience in using *Peristrophe bivalvis* (L.) Merr. was conducted in the mountainous region of Northern Vietnam. Five varieties were collected and recorded as a source of foodstuff-dyeing and medicinal plants on indigenous knowledge of ethnic minorities in the study area, of which, three varieties were dyed purple, one red and one yellow-orange; with two varieties being used for the treatment of haemoptysis, dysentery, liver disease, kidney failure, and jaundice. Two extraction methods using organic solvents and aqueous solvent were applied to spectrophotometrically investigate the phytochemical contents of *Peristrophe bivalvis* varieties. Accordingly, the coloration components of *Peristrophe bivalvis* varieties were contributed by a complex compound of photosynthetic pigments, anthocyanins, flavonoids. Also, the total phenolics, total flavonoids and effect of extraction methods, dissolved solutions (water and methanol) on these contents were assessed. Besides, screening antioxidant capacity of *Peristrophe bivalvis* varieties was assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method which showed stronger radical inhibition of organic extractions than that of aqueous extractions. The results of determination of phytochemicals and antioxidant capacity support the knowledge and experience in using *Peristrophe bivalvis* varieties as natural dyeing production for food and pharmaceutical industry.

Introduction

The utilization of plant for food dyeing is no longer a novelty in the world. In Vietnam, using plants in processing and coloring food has been a long-standing experience favoured among the ethnic minorities - especially those residing in the Northern mountainous regions. Among food-dyeing plants, *Peristrophe bivalvis* (L.) Merr. is the most commonly used and widely grown to make foods more attractive-looking. The traditional foods dyed with *P. bivalvis* are very diverse and pregnant with various cultural values, which vary by each ethnic group and each region. However, the investigations revolved around *P. bivalvis* scattered and systematically deficient in Vietnam, especially on the local experience in dyeing food, cultivation modality, and economic value. Moreover, the indigenous knowledge in using such plant for common life is genuinely important. Article 8 (j) of the Convention on Biological Diversity precisely manifested that the link between biodiversity and cultural diversity plays a key role to maintain the in-situ conservation [1]. Therefore, the research and proposing solutions seek to preserve experience in using *P. bivalvis* in Northern Vietnam is essential.

According to Vietnam Plant Data Center, *Peristrophe bivalvis* (L.) Merr. [*Peristrophe roxburghiana* (Schult.) Bremek.] is essentially a herbaceous perennial plant which distributes widely in cool and humid climate areas. Based on the different colors of extracts and morphological characteristics, *P. bivalvis* appears in four varieties which are purple, purple-magenta, red, and yellow [2,3,4]. The identification of these varieties primarily bases on the difference between their phenotypes, such as the leaf shape and difference among colorants extracted from them. Apart from

decorating role in foodstuffs, *P. bivalvis* has many biological and pharmaceutical activities including the treatment of blood diseases, anti-hypertension, anti-hyperlipidemia, fungistatic and antibacterial properties [5-8]. Phaopongthai et al. [9] reported that *P. bivalvis* ethanolic extracts had the anti-snake venom activity against *Naja kaouthia* and *Trimeresurus albolabris* venoms. Another study highlighted the effectiveness of *P. bivalvis* ethanolic extracts on inhibition of bacteria consisting of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [10,11]. Thuy et al. showed the new application of *P. bivalvis* by evaluating the alkaloid components from purple and red varieties growing in Vietnam [12,13].

Although there were some scientific achievements on studying *Peristrophe bivalvis*, the comprehensive studies on *P. bivalvis* are quite limited. What is concerning the researcher is that most of the studies have only focused on a certain variety or an unclear variety which probably cause inaccurate analysis results. Therefore, it is really important to clarify the existence and phytochemical properties of *P. bivalvis* varieties in the northern Vietnam. This study is conducted to achieve the objectives including investigating the presence and current usage of *P. bivalvis* varieties for dyeing food-stuffs of ethnic minorities in the northern mountainous region of Vietnam, determining the pigment components, total phenolic content, total flavonoid content, and antioxidant capacity of various *P. bivalvis* varieties cultivated in Northern Vietnam.

Materials and methods

Plant materials

Plant samples were collected on some criteria as intact, fresh, and without signs of pests. After washing and draining in the field, samples were wrapped up by paper bags. After that, the samples including leaves and stems were dried at 35°C for 10 days in the oven and ground, thereafter kept at 4°C in the fridge until use.

The identification of *Peristrophe bivalvis* was referred from Vietnam Plant Data Center (<http://www.botanyvn.com>) and Plants Database Missouri Botanical Garden, United States (TROPICOS - <http://www.tropicos.org>). Also, the differentiation of varieties was implemented by testing the color of hot water decoction from each adequate samples in the field.

The plants' seedlings have been cultivated and vouchers have been deposited in Thai Nguyen University of Agriculture and Forestry, Vietnam. Details of the plants are shown in Table 1.

Field survey

An ethnobotanical investigation was conducted on the current usage status and experience in using *Peristrophe bivalvis* for foodstuff dyeing and other purposes among ethnic minorities in the northern mountainous region of Vietnam, including Lai Chau, Bac Kan and Thai Nguyen Provinces. The interviews were built on a questionnaire mainly carried out among 140 households of the ethnic minorities by the main author from the beginning of August 2015 to the end of September 2015.

Experimental survey

Plant extracts

The plant extractions are as described in Figure 1. Five different samples were used in the extraction process by using organic solvents and an aqueous solvent (distilled water). The extracts were named as the corresponding solvent and methods used.

Determination of Chlorophyll a, Chlorophyll b and total carotenoids

Photosynthetic pigment contents were determined according to Metzner, Rau, and Senger [14] and Fayez and Bazaid [15] with minor modification. The extraction method was unique to this experiment as follows: each dried ground sample (100 mg) was extracted in 2 mL of 85% (v/v) aqueous acetone. The mixture was vigorously shaken and centrifuged at 5000 rpm for 5 mins. The supernatant was collected and then diluted by the extracting solvent to a suitable concentration for spectrophotometric measurement. The absorbance was measured against a blank of the extracting solvent at three wavelengths of 452, 644 and 663 nm by a HACH DR/4000U spectrophotometer. Total photosynthetic pigments were calculated using the following equations:

$$\text{Chlorophyll } a \text{ (Chl } a) = 10.3 \times A_{663} - 0.918 \times A_{644}$$

$$\text{Chlorophyll } b \text{ (Chl } b) = 19.7 \times A_{644} - 3.87 \times A_{663}$$

$$\text{Total carotenoids} = 4.2 \times A_{452} - ((0.0264 \times \text{Chl } a) + (0.426 \times \text{Chl } b))$$

where A_{663} , A_{644} , and A_{452} are absorbance measured at 452, 644 and 663 nm respectively. The photosynthetic pigments were expressed as mg g^{-1} dry weight (DW).

Fig. 1 The extraction process of *Peristrophe bivalvis* samples

Determination of Total Anthocyanin Contents (TACs)

Quantification of total anthocyanins in *P. bivalvis* was evaluated with the pH differential method which integrated both methods of Giusti, Rodriguez-Saona, and Wrolstad [16] and Lee, Durst, and Wrolstad [17] with minor modifications. Each extract was diluted in turn by pH 1.0 buffer (potassium chloride, 0.025 M) and pH 4.5 buffer (sodium acetate, 0.4 M) to an appropriate concentration for spectrophotometric measurement. Subsequently, the absorbance of five *P. bivalvis* samples was measured at 520 nm and 700 nm by a HACH DR/4000U spectrophotometer. Anthocyanin content was expressed as mg cyanidin-3-glucoside equivalents in g dry weight (mg C3GE g^{-1} DW), as follows:

$$\text{TAC} = (A \times \text{MW} \times \text{DF} \times 10^3) : \varepsilon \times l$$

where $A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}$; MW = 449.2 g/mol = molecular weight of cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; l = pathlength in cm; $\varepsilon = 26900$ = molar extinction coefficient of cyd-3-glu in $\text{L} \times \text{mol}^{-1} \times \text{cm}^{-1}$; and 10^3 = factor for conversion from g to mg. TACs in the organic extraction was calculated by water-soluble anthocyanins plus methanol-soluble anthocyanins.

Determination of Total Phenolic Contents (TPCs)

Total phenolic content was calculated according to the Folin–Ciocalteu method described by Elzaawely et al. [18]. Concisely, 0.1 mL of extract was added in turn by 0.5 mL of 10% (v/v) Folin–Ciocalteu's reagent and 0.4 mL of 7.5% (w/v) Na_2CO_3 in water. After 2 to 3 mins, the mixture was shaken and incubated at room temperature for 30 mins. The absorbance was measured at 765 nm using a HACH DR/4000U spectrophotometer. TPCs were expressed as mg gallic acid equivalents in g dry weight (mg GAE g^{-1} DW). TPCs in the organic extraction was calculated by water-soluble phenolics plus methanol-soluble phenolics.

Determination of Total Flavonoid Contents (TFCs)

Total flavonoid content was spectrophotometrically determined according to the AlCl_3 method described by Djeridane et al. [19]. Briefly, 0.5 mL of extract was mixed with 0.5 mL of 2% AlCl_3 (w/v) in methanol. After a 15-minute incubation at room temperature, the absorbance was measured at 430 nm using a HACH DR/4000U spectrophotometer. TFCs were expressed as mg rutin equivalents in g dry weight (mg RE g^{-1} DW). TFCs in the organic extraction were calculated by water-soluble flavonoids plus methanol-soluble flavonoids.

Determination of antioxidant capacity by DPPH assays

The radical-scavenging assay described by Elzaawely, Xuan, and Tawata [20] was used to determine the antioxidant capacity of five *P. bivalvis* samples. A volume of 0.5 mL extract was mixed with 0.25 mL of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution and 0.5 mL of 0.1 M sodium acetate buffer (pH 5.5). After a slight shake, the mixture was incubated at room temperature for 30 mins without light exposure, followed by a measure at 517 nm using a HACH DR/4000U spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage based on the following formula in Son and Lewis [21]:

$$\% \text{ radical scavenging activity} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the control (DPPH solution without a sample, methanol instead) and A_{sample} is the absorbance of the *P. bivalvis* sample (DPPH solution plus extracts).

Statistical analysis

The analysis of data was conducted via ANOVA in the Minitab 16.0 software (Minitab Inc., State College, PA, USA). All experiments were carried out in triplicate and expressed as mean $\pm$ standard error (SE). Significant differences among the means were compared using Turkey's test method with a confidential level of 95%.

Results

Results of the ethnobotanical investigation in the northern mountainous region of Vietnam

Five villages in which two are in Lai Chau Province, one in Bac Kan Province and two in Thai Nguyen Province were selected as the fields for investigating the experience of using *P. bivalvis* for various purposes among local ethnic groups. Family members who are most accustomed to the use of *P. bivalvis* in each household are selected as respondents. As shown in Table 1, P1 and R varieties were commonly cultivated in Bac Kan and Thai Nguyen Province, while P3 and Y varieties could only be detected in Lai Chau Province, and P2 variety was sparsely cultivated in Thai Nguyen province. Most of the varieties were used by different cultural and ethnic groups for dyeing a number of traditional foods such as sticky rice and glutinous rice cakes serving in the special occasions.

Table 1. Samples of *Peristrophe bivalvis* collected in September 2015, and ethnobotanical results concerning the local use of these plants in the northern Vietnam

Variety	Voucher/ Abbreviation	Distribution location	Colors dyed	Local use
Purple <i>P. bivalvis</i>	P1	Thai Nguyen and Bac Kan province	Purple and black	Food dye and medicinal plant
Purple <i>P. bivalvis</i>	P2	Thai Nguyen province	Purple	Food dye
Purple <i>P. bivalvis</i>	P3	Lai Chau province	Purple	Food dye
Red <i>P. bivalvis</i>	R	Thai Nguyen and Bac Kan province	Red and scarlet	Food dye and medicinal plant
Yellow <i>P. bivalvis</i>	Y	Lai Chau province	Orange-yellow	Food dye (rarely)

The most commonly dyeing method used *P. bivalvis* was recorded as follows: the fresh stems and leaves were brought in boiling temperature with water for several minutes; the stems and leaves were then fished out of hot water decoction; after cooling, foodstuffs were soaked in that decoction to get the desired color. Most respondents said that one variety was used to dye a certain color; however, referring to the experience of Tay ethnic minority, P1 variety can generate both purple and black, and R variety can generate both red and scarlet. To get black color, an amount of straw ash was added into hot water decoction of P1 variety before soaking the foodstuff, whereas to get scarlet with R variety, vinegar or pineapple's leaves or *Elaeagnus latifolia* shoots were added into the decoction. Besides, P1 and R varieties were used as medicinal plants, which was introduced by two local healers in a village in Thai Nguyen Province. Accordingly, whole dried parts of P1 variety were mixed with some other herbs for the treatment of haemoptysis, dysentery, liver disease and kidney failure. In addition, the decoction of fresh leaves of P1 and R varieties was used to bath baby to cure jaundice. Nevertheless, the compounds and remedies remained covert because the healers believed that this would no longer be effective for treatment.

Determination of plant pigments

Total photosynthetic pigment contents

The differences of photosynthetic pigment contents are as illustrated in Table 2. While Chl *a* presented a minute amount and no significant difference between varieties, Chl *b* contents were significantly different among five *P. bivalvis* varieties. In particular, R variety exhibited the highest amount of Chl *b*, followed by P1 variety, P2 variety, P3 variety, and Y variety. Total carotenoids were only expressed by Y variety.

Table 2. Total chlorophyll a, chlorophyll b and carotenoids (mg g⁻¹ DW)

	P1	P2	P3	R	Y
Chlorophyll a	0.001±0.000	0.001±0.000	0.001±0.000	0.001±0.000	0.002±0.000
Chlorophyll b	0.584±0.012b	0.533±0.013c	0.446±0.023d	0.633±0.017a	0.222±0.002e
Carotenoids	0.000	0.000	0.000	0.000	0.025±0.001

Means that do not share a letter in the same row are significantly different ($P < 0.05$)

Total anthocyanin contents

Anthocyanins of *P. bivalvis* varieties are shown in Table 3. Three out of six extracts indicated the results of TACs which were MWH, HW, and W50 extracts. For MWH extract, all varieties performed TACs except P2 variety, in which R variety showed the highest concentration which was tailed by P3 variety, P1 variety, and Y variety. On the other hand, only P2 variety exposed TACs in

the HW extract. As for W50 extract, TACs were only presented by P1 and P2 varieties, of which TACs of P1 variety was higher than TACs of P2 variety.

Table 3. Total anthocyanin contents (mg C3GE g⁻¹ DW) of *P. bivalvis* samples

Method	TAC				
	P1	P2	P3	R	Y
MWH	0.078±0.001c	0.000	0.146±0.001b	0.244±0.003a	0.055±0.002d
HW	0.000	0.021±0.001	0.000	0.000	0.000
W50	0.038±0.001a	0.003±0.000b	0.000	0.000	0.000

Means that do not share a letter in the same row are significantly different ($P < 0.05$)

Determination of total phenolic contents

Effect of extraction methods used organic solvents on phenolic contents

There was a significant effect of extraction methods and dissolved solutions on phenolics of *P. bivalvis* varieties (Table 4). It is easy to see that the phenolics dissolved more strongly in water than in methanol. The highest mean of phenolics was obtained by MH extraction. Among five varieties, Y variety averaged the highest phenolic concentration, followed by P3 variety, R variety, P1 variety, and P2 variety.

Total phenolic contents

The total phenolics of *P. bivalvis* varieties are shown in Table 5. There were significant differences in TPCs among varieties in different extraction methods. The highest TPCs was observed in MH, MWH, and HW extracts. On average, Y variety experienced the highest TPCs in comparison with other varieties, of which Y variety presented the highest amount of TPCs in HW extract, whereas the lowest one was found in R variety in the same extract.

Determination of total flavonoid contents

Effect of extraction methods used organic solvents on flavonoid contents

The differences in flavonoid content between extracts used organic solvents are shown in Table 6. With the exception of P1 and P2 varieties in EWH and MH extract, most flavonoids of *P. bivalvis* samples dissolved more strongly in water than in methanol. In particular, P1 variety and P2 variety in EWH extract and P2 variety in MH extracts, all indicated the flavonoids concentration in methanolic solution higher than in water solution. The most prominent flavonoids content was showed in the MH extract. P3 variety contained the highest concentration of flavonoids, followed by P1 variety, R variety, P2 variety, and Y variety.

Total flavonoid contents

Total flavonoids of *P. bivalvis* varieties are described in Table 7. The TFCs were different among varieties in different extraction methods. The MH extract signified the highest TFC, in which the highest TFC was shown by P1 variety, the lowest one was shown in Y variety. On average, Y variety represented the lowest TFC, while P1, P2, P3, and R varieties showed the higher ones, respectively.

Table 4. Effect of organic extraction methods and dissolved solutions on total phenolic contents (mg GAE g⁻¹ DW) of *P. bivalvis* samples.

Method (M)	Dissolved solution (D)	TPC (P)					Mx/D mean	D mean	M mean
		P1	P2	P3	R	Y			
EWH	Water	14.04±0.07 ^O	12.87±0.02 ^P	17.05±0.04 ^L	16.30±0.04 ^M	18.75±0.06 ^J	15.80±0.56D		
	Methanol	1.06±0.04 ^X	0.83±0.05 ^Y	0.66±0.06 ^{YZA1}	0.78±0.03 ^{YZ}	0.17±0.03 ^{A4}	0.70±0.08G		
	Mx/P mean	7.55±2.90I	6.85±2.69m	8.86±3.67j	8.54±3.47k	9.46±4.16i			8.25±1.43D
MH	Water	22.32±0.08 ^E	17.71±0.03 ^K	24.43±0.05 ^B	23.91±0.03 ^C	25.34±0.05 ^A	22.74±0.72A		
	Methanol	1.72±0.01 ^W	1.85±0.03 ^W	2.10±0.04 ^{UV}	1.15±0.05 ^X	0.40±0.03 ^{A2A3}	1.44±0.16F		12.09±2.01A
	Mx/P mean	12.02±4.61d	9.78±3.55h	13.26±4.99a	12.53±5.09c	12.87±5.58b			
MWF	Water	16.85±0.03 ^L	11.43±0.02 ^Q	20.44±0.03 ^G	19.14±0.03 ^I	22.76±0.02 ^D	18.13±1.03C		9.3±1.72C
	Methanol	0.38±0.00 ^{A3A4}	0.61±0.01 ^{ZA1A2}	0.70±0.00 ^{YZA1}	0.52±0.01 ^{A1A2A3}	0.18±0.00 ^{A4}	0.48±0.05H		
	Mx/P mean	8.61±3.68k	6.02±2.42n	10.57±4.42g	9.83±4.16h	11.47±5.05e			
MWH	Water	20.51±0.05 ^G	15.61±0.03 ^N	19.87±0.03 ^H	20.32±0.01 ^G	21.98±0.05 ^F	19.66±0.57B		11.41±1.57B
	Methanol	1.89±0.02 ^{VW}	4.06±0.01 ^S	5.3±0.06 ^R	2.45±0.05 ^T	2.12±0.05 ^U	3.16±0.35E		
	Mx/P mean	11.20±4.16f	9.83±2.58h	12.58±3.26c	11.39±4.00e	12.05±4.44d			
DxP mean	Water	18.43±0.97b	14.40±0.73E	20.45±0.79B	19.92±0.82C	22.21±0.71A	19.08±0.49A		
	Methanol	1.26±0.18H	1.84±0.41G	2.19±0.57F	1.22±0.22H	0.72±0.25I	1.45±0.17B		
	P mean	9.85±1.85d	8.12±1.37e	11.32±1.96b	10.57±1.99c	11.46±2.27a			

Table 5. Total phenolic contents (mg GAE g-1 DW) of *P. bivalvis* samples in the different extraction methods

Method (M)	TPC (P)					M mean
	P1	P2	P3	R	Y	
EWH	15.11±0.11o	13.70±0.05p	17.71±0.03m	17.09±0.05n	18.92±0.04k	16.50±0.50C
MH	24.04±0.07e	19.56±0.03j	26.53±0.08b	25.06±0.08d	25.75±0.08c	24.19±0.66A
MWF	17.23±0.03n	12.04±0.03q	21.14±0.04h	19.66±0.03j	22.94±0.02f	18.60±1.01BC
MWH	22.40±0.06g	19.67±0.02j	25.17±0.08d	22.77±0.05f	24.10±0.01e	22.82±0.50A
HW	21.36±0.11h	25.00±0.06d	20.36±0.04i	11.34±0.06r	26.90±0.09a	20.99±1.44AB
W50	18.79±0.13k	15.21±0.08o	20.09±0.06i	18.13±0.04l	20.42±0.04i	18.53±0.50BC
P mean	19.82±0.75abc	17.53±1.06c	21.83±0.74ab	19.01±1.06bc	23.17±0.68a	

Means that do not share a letter in each subset are significantly different (*P*<0.05)

Table 6. Effect of organic extraction methods and dissolved solutions on total flavonoid contents (mg RE g⁻¹ DW) of *P. bivalvis* samples.

Method (M)	Dissolved solution (D)	TFC (F)					MxD mean	D mean	M mean
		P1	P2	P3	R	Y			
EWH	Water	4.37±0.03 ^{OP}	3.64±0.03 ^R	6.11±0.05 ^G	5.05±0.03 ^L	4.32±0.03 ^P	4.70±0.22D		
	Methanol	4.59±0.02 ^{MN}	5.03±0.03 ^L	4.47±0.02 ^{NOP}	4.54±0.02 ^{MNO}	1.58±0.02 ^U	4.04±0.33F		
	MxF mean	4.48±0.05 ^h	4.34±0.31 ⁱ	5.29±0.37 ^e	4.79±0.11 ^g	2.95±0.61 ^l			4.37±0.21 ^c
MH	Water	9.01±0.06 ^B	5.64±0.00 ^{HI}	9.38±0.06 ^A	7.54±0.06 ^{DE}	3.86±0.06 ^{OR}	7.09±0.56A		
	Methanol	8.19±0.04 ^C	7.75±0.04 ^D	7.32±0.08 ^F	5.52±0.04 ^{IJ}	1.61±0.04 ^U	6.08±0.64C		
	MxF mean	8.60±0.19 ^a	6.70±0.47 ^c	8.35±0.46 ^b	6.53±0.45 ^d	2.74±0.50 ^{mn}			6.58±0.43 ^A
MWF	Water	4.69±0.00 ^M	3.26±0.04 ^S	5.75±0.06 ^H	5.33±0.06 ^{JK}	3.99±0.04 ^Q	4.60±0.24E		
	Methanol	0.63±0.02 ^W	0.79±0.02 ^W	0.75±0.02 ^W	0.61±0.00 ^{WX}	0.40±0.00 ^X	0.64±0.04H		
	MxF mean	2.66±0.91 ⁿ	2.02±0.55 ^p	3.25±1.12 ^k	2.97±1.05 ⁱ	2.20±0.80 ^o			2.62±0.39 ^d
MWH	Water	7.49±0.06 ^{EF}	5.47±0.03 ^{IJ}	7.57±0.06 ^{DE}	6.31±0.03 ^G	4.54±0.03 ^{MNO}	6.28±0.31B		
	Methanol	3.01±0.02 ^T	4.65±0.04 ^{MN}	5.21±0.02 ^{KL}	1.77±0.03 ^U	1.12±0.02 ^V	3.15±0.42G		
	MxF mean	5.25±1.00 ^e	5.06±0.19 ^f	6.39±0.53 ^d	4.04±1.02 ^j	2.83±0.77 ^{lm}			4.71±0.39 ^B
DxF mean	Water	6.39±0.58 ^B	4.50±0.32 ^{DE}	7.20±0.43 ^A	6.06±0.30 ^C	4.18±0.08 ^F		5.67±0.22A	
	Methanol	4.11±0.83 ^F	4.55±0.75 ^b	4.44±0.71 ^E	3.11±0.60 ^G	1.18±0.15 ^H		3.48±0.33B	
	F mean	5.25±0.55 ^b	4.53±0.40 ^d	5.82±0.50 ^a	4.58±0.45 ^c	2.68±0.32 ^e			

Table 7. Total flavonoid contents (mg RE g⁻¹ DW) of *P. bivalvis* samples in the different extraction methods

Method (M)	TFC (F)					M mean
	P1	P2	P3	R	Y	
EWH	8.97±0.04 ^{jk}	8.68±0.06 ^k	10.58±0.06 ^g	9.59±0.01 ⁱ	5.89±0.04 ^o	8.74±0.42 ^B
MH	17.21±0.09 ^a	13.39±0.04 ^c	16.7±0.10 ^b	13.06±0.05 ^{de}	5.47±0.09 ^{pq}	13.17±1.12 ^A
MWF	5.32±0.02 ^q	4.04±0.03 ^s	6.5±0.08 ⁿ	5.94±0.06 ^o	4.39±0.04 ^r	5.24±0.25 ^C
MWH	10.5±0.07 ^g	10.11±0.03 ^h	12.78±0.04 ^{ef}	8.08±0.06 ^l	5.66±0.04 ^{op}	9.43±0.64 ^B
HW	13.32±0.07 ^{cd}	12.69±0.07 ^f	9.67±0.07 ⁱ	5.39±0.00 ^{pq}	8.27±0.07 ⁱ	9.87±0.78 ^B
W50	8.97±0.00 ^{jk}	6.58±0.07 ⁿ	9.25±0.07 ^j	7.71±0.00 ^m	8.06±0.07 ⁱ	8.11±0.26 ^B
F mean	10.72±0.91 ^a	9.25±0.79 ^a	10.91±0.77 ^a	8.29±0.62 ^{ab}	6.29±0.34 ^b	

Means that do not share a letter in each subset are significantly different (*P*<0.05)

Antioxidant capacity of *Peristrophe bivalvis* varieties

The above results pointed that the solubility of anthocyanins, phenolics, and flavonoids of *P. bivalvis* samples were stronger in water than in methanol, which resulted in only the samples dissolving in aqueous solution were selected to test DPPH scavenging activity. Table 8 shows the inhibition percentages of *P. bivalvis* varieties in different extracts at a concentration which was ten times diluted by methanol from the original concentration. It can be seen that the DPPH radical scavenging capacity of each variety varied in extraction methods and differed from others. The extractions used organic solvents exhibited DPPH scavenging activity were higher than those used aqueous solvent. Particularly, most of the *P. bivalvis* varieties performed DPPH scavenging activities higher than 50% in EWH, MH, and MWF extracts, except P2 variety which showed a 36.35% radical inhibition in MH extract. For extractions by water, only Y variety in HW extracts exhibited the inhibition percentage higher than 50%, meanwhile, the others showed the reverse.

Table 8. Inhibition percentages of aqueous extracts at a concentration, which were 10 times diluted by methanol from the original extracts.

Method (M)	Inhibition percentage (%)					M mean
	P1	P2	P3	R	Y	
EWH	67.22±0.09c	57.88±0.24hi	59.8±0.09f	58.79±0.28g	69.51±0.16b	62.64±1.28 _A
MH	60.73±0.09e	36.35±0.09p	62.41±0.09d	58.6±0.09gh	73.14±0.15a	58.24±3.22 _{AB}
MWF	69.85±0.09b	48.87±0.09k	69.39±0.18b	62.76±0.09d	69.48±0.00b	64.07±2.15 _A
MWH	43.57±0.19n	55.12±0.09j	47.04±0.16l	45.54±0.09m	59.06±0.09fg	50.07±1.6 _B
HW	12.97±0.17t	26.03±0.10r	47.17±0.10l	12.58±0.25t	57.54±0.10i	31.26±4.86 _C
W50	42.73±0.17o	23.16±0.26s	31.49±0.10q	31.4±0.17q	31.34±0.18q	32.02±1.67 _C
% mean	49.51±4.72ab	41.23±3.3b	52.88±3.03ab	44.95±4.35b	60.01±3.4a	

Means that do not share a letter in each subset are significantly different ($P < 0.05$)

Discussions

Indigenous knowledge and diversity of *Peristrophe bivalvis*

Indigenous knowledge has been seen as a treasure of accumulated knowledge and belief that are passed through many generations by transmitting the culture of the relationship between creatures including humans and their living environment [22]. The special relationship between indigenous knowledge and biodiversity are getting more and more attention recently [23, 24, 25], especially in the field of biodiversity conservation [1]. Vietnam, ranking 16th among most biodiversity-rich countries in the world, prioritizes the preservation of cultural diversity as well as of indigenous knowledge in using natural resources (for example, plants) [26].

The results of current usage and experience in using *P. bivalvis* varieties of ethnic minorities in the North of Vietnam has contributed more information to the indigenous knowledge and biodiversity in Vietnam. Besides the popular uses in coloring food and other traditional products, *P. bivalvis* used as a folk remedy plays a role as the premise for further research in the field of pharmaceutical extracts and natural dye large-scale production. Especially, P3 variety was detected as a new member of *P. bivalvis*. The variety that is used the same way to get purple but has different morphological characteristics and biochemical components compared to the other previously described varieties. Although the experiment conducted on biochemical components partly indicated differences, it is still necessary to be provided more evidence from genotypic analysis to affirm whether the P3 variety is in the taxonomic tree or not.

Phytochemical contents in *Peristrophe bivalvis* varieties

The colors in higher plants are basically assigned by pigments which are very diverse in structure and classification of which photosynthetic pigments and flavonoids are considered as the main groups contributing colorful countenance of plant. In particular, chlorophylls with the largest presence of Chl *a* and Chl *b* in plant cells are responsible for green color; while carotenoids are for red, orange or yellow which also play as accessory pigments in photosynthesis [27]. Meanwhile,

flavonoids, a group of secondary metabolites, have the widest color range from pale-yellow to blue, in which, anthocyanins, a class of flavonoids, take their color from the range of red, purple, or blue, depending on their pH [28] and are sensitive to temperature and light [29]. By spectral analysis, Thuy et al. [9] isolated the major component of the color aqueous extract of red *P. bivalvis* leaves which was so-called peristrophine. In this study, the color extract components of *P. bivalvis* varieties were also identified as photosynthetic pigments and anthocyanins.

As shown in Table 2, Chl *a* shows a negligible quantity which can be explained by using a dried sample for measurement. The degradation of chlorophylls was reviewed in many studies related to leaf senescence [30,31] and loss of moisture in leaves [32]. In the same situation, the degradation of carotenoids in *P. bivalvis* samples might be elucidated by the effect of drying and storage [33,34]. In summary, the photosynthetic pigments of *P. bivalvis* are certainly involved in the color formation of samples' extracts. Chlorophyll *b* occupied a positive amount in coloration of *P. bivalvis* varieties. The yellow-orange extract of Y variety is defined in part by carotenoids. Dried samples are not suitable to determine components of photosynthetic pigments in *P. bivalvis*.

Anthocyanins are water-soluble pigments and commonly extracted by acidified solvents like water, ethanol, alcohol or aqueous solvents [35,36]. By using spectrophotometric determination, the best result of anthocyanin contents was certified in MWH extract which most *P. bivalvis* varieties shared anthocyanin values, except P2 variety (Table 3). However, P2 variety presented a molar amount of anthocyanins in HW and W50 extract. Especially, the anthocyanins value of P2 variety treated by 100°C was higher than that by 50°C. It proved that the anthocyanins of P2 variety might be linked with some compounds that keep it more enduring in high temperature. Therefore, the unique absence in organic extracts and only presence in water extracts of anthocyanin in P2 variety may be explained by the effect of temperature and extraction solvent. In addition, only R variety presented methanol-soluble anthocyanins (3.70 mg C3GE g⁻¹ which was not shown in Table 3) in MWH extract, that is to say, methanol-soluble anthocyanins can be an indicator to identify and differentiate the R variety from other varieties.

Also, the differences in color can be clarified by referring to the flavonoid contents results (Table 6, Table 7). Accordingly, the average of water-soluble flavonoids was mostly higher than methanol-soluble flavonoids, meaning the water-soluble flavonoids as anthocyanins play an important role in coloration of *P. bivalvis*. Moreover, the significant differences in total flavonoid contents among five varieties can be used as an indicator [37] for *P. bivalvis* classification.

In summary, the coloration components of *Peristrophe bivalvis* varieties were contributed by a complex compound of photosynthetic pigments, anthocyanins, and flavonoids. Additionally, the determination of coloration components of *P. bivalvis* varieties was influenced by many factors such as drying condition, temperature, and type of extraction solvents.

Antioxidant capacity of *Peristrophe bivalvis* extracts

Antioxidants have a crucial role in neutralizing free radical species inducing DNA degradation, cell damage and physical and mental disorders. Thus, they are supposed to be capable of protecting human against infections and degenerative diseases due to the ability to prevent the deleterious consequences of oxidative stress [38]. Plants certainly contain a rich source of natural antioxidants. A plenty of previous studies reported flavonoids (flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols, anthocyanins and anthocyanidins) and phenolic compounds (derivatives of benzoic acid such as gallic acid, and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid) are the major groups of natural antioxidants which can help plant to cope with stresses and diseases [39-43]. Naturally, phenolic compounds produced in plants as secondary metabolites [44], and its concentrations not only heavily depend on species, but it is also affected by extraction methods and solvents. Several studies have proven that the antioxidant activity correlated with total phenolic compounds [45]. In this study, total phenolic contents of *P. bivalvis* samples (Y, P1, and P3) were found to follow the similar trend of DPPH radical scavenging activity. Therefore, this result confirmed that phenolic compounds contained in the extracts strongly influence antioxidant activity. Moreover, most of the organic extracts showed the antioxidant activity greater than 50%. This could simply be explained that the solvents, such as methanol, ethanol, acetone, ethyl acetate,

and their combinations are more productive in phenolics isolation from plant materials than water [38]. It is generally known that the antioxidant activity is not only affected by solvents alone, but it is also influenced by extraction time and temperature. However, the DPPH scavenging activity of *P. bivalvis* extracts was low in 100°C and 50°C water extractions. Obviously, high temperature probably causes phenolic oxidation, and consequently reduces its biological activity [41].

Conclusions

The indigenous knowledge in using *Peristrophe bivalvis* varieties of ethnic minorities plays an important role in researches on biodiversity and conservation of plant resource in Northern Vietnam in particular and in Vietnam in general. Apart from previous described *P. bivalvis* varieties, the discovery of P3 variety contributes to the diversity at the sub-species level or variety level of the species, which has received much attention recently. The results of phytochemicals determination and evaluation of antioxidant activity do not only support the classification of *Peristrophe bivalvis* but also can be informative in many fields, such as the production of natural dyes and pharmaceutical preparations from *Peristrophe bivalvis* through selecting the most appropriate extraction solvent.

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