

PERISTROPHE ROXBURGHIANA - A REVIEW

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Abstract:

Many synthetic pigments are now banned or limited in application to the foods because the evidence related to the ability to cause cancer and other adverse impacts on the health. Therefore, the study of natural colorants to replace the synthetic colors mentioned above is a challenge for many food scientists in Vietnam and around the world. Cam is abundant in rural area in the North of Vietnam. Currently, Cam leaves have been used as a popular pigment in Vietnam to replace synthetic colors in the traditional foods. Many studies have also shown that the dyes extracted from Cam leaves exhibited clear and beautiful appearance and durability during prolonged storage. Furthermore, the safety when using pigment of Cam leaves has been proven through the long history of the use and through the study of acute toxicity. Extracts of Cam leaves contain a large amount of pelargonidin, zizilan, carotenoids, β -sitosterol. These compounds are asserted to cure the tuberculosis, eliminates phlegm, acute bronchitis, antitoxic, anti-inflammatory, windpipe infection, hepatitis and diabetes. In order to reduce the cost of using these extracts on food application, many extraction techniques have been introduced to maximum the amount of pigments obtained to Cam leaves. Among of them, ultrasound and microwave are potential ones because of their lower cost and shortly consumed time.

Keywords: Peristrophe roxburghiana, Peristrophe baphica, Justicia bivalvis, Cam leaves, food colorant

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1. INTRODUCTION

Cam (*Peristrophe roxburghiana*) is an herbaceous perennial plant has various applications such as materials of medicines, food colorants and hedgerow trees (Do TX, Nguyen TPT, 2007). These plants have been located in many countries including Cambodia, China, India, Indonesia, Laos, Malaysia, Thailand and Vietnam. They have always been occupied in the forests above the sea altitude of 500-1000m (Hu J, Deng Y, Thomas FD, 2011). Botanical nomenclature for these plants has been changed over time such as *Justicia bivalvis* (1759), *Dicliptera bivalvis* (L) Juss. (1807), *Justicia tinctoria* Roxb (1820), *Peristrophe tinctoria* (Roxb) Ness (1832), *Peristrophe roxburghiana* (Schult.) Bremek (1955), *Peristrophe baphica* (Spreng) Bremek. (1957) (Do TX, Nguyen TPT, 2007; Shiva MP, 1998).

The serious adverse effects on health associated with abuse of the synthesis colorants

have been reported in recent years (Carmen S, 2008). Therefore, the natural pigments from plants have been chosen as a safety one replaced to the harmful synthesis colorants. Many studies have been shown that the Cam leaf extracts are highlighted in terms of health benefit (Tanavade SS, Naikwade N, Chougule DD, 2012; Trinh TT. et al, 2012; Wiart C. et al, 2004; Zhuang X, Lü J, Yang W, Yang M, 2003). For a long time, the Cam leaf extracts have been considered as the good colorant sources for food applications in Vietnam and some Asia countries (Li TSC, 2006). Therefore, this study focus on evaluation of the extraction processes, coloring durability in storage, health benefits, cultivation conditions, botanical characters of Cam in order to provide some preliminary estimation about Cam leaf extracts.

Botany

Cam (*Peristrophe roxburghiana*) belongs to *Peristrophe* genus, Acanthaceae family. It is an herbaceous plant which grows wildly in wet

areas. In Vietnam, Cam has been located in Muong Khuong, Lao Cai, Moc Chau, Son La and Thai Nguyen (Luu DC, 2005). Extracts from this plant have been used as food colorants in some tradition food in Vietnam such as taro-filled cake, glutinous rice dishes, banh tet (a traditional cake using in Lunar New Year) and dumplings.

Morphological characteristics of this plant were mentioned in the study conducted by Do and Nguyen (2007). *Peristrophe roxburghiana* is the herbaceous perennial shrub. It grows rapidly in the humid lowlands. Its height can be reach to 30-50 cm. The *Peristrophe roxburghiana* leaves have some basic characteristics such as opposite leaves, different shape from lanceolate to oval, 4-9cm long, 2-4 cm wide. Its leaves have been also presented sweet taste. The *Peristrophe* flowers are depicted as rose-purple color, foliaceous bracts, ovate shape, small fascicle, 2 cm long, 10-12 cm wide, base subcordate, apex acute, villose, peduncle 3-4 cm long, bracteoles 2, lanceolate, 6 mm long, 1.5 mm wide across the base, calyx 5-parted to the base, sepals lanceolate, 4-6 mm long, pilose, corolla bilabiate, tube slender, 1.8 cm long, limb 2-lipped, upper lip rounded, 1 cm in diameter, lower lip narrower, the apex shallowly 3-lobed, pilose without, statmens 2, exserted, filaments subglabrous, anthers 2-celled, connective narrow, thecae in a straight line, ovary ovoid, style filiform, stigma 2-lobed, the lobes

recurved, capsules clavate, stipitate, 1 cm long, pilose, acute. Its seeds are discoid, tuberculate and black (Do TX, Nguyen TPT, 2007; Hu J, Deng Y, Thomas FD, 2011; Hu SY, 2005).

Cam plants require some certain conditions for growing and accumulating pigments such as loam-rich, highly fertile, well drained, high moisture soil, low temperature and low light intensity. In the spring (from February to March), the health bud branches of Cam plants are picked up to prepare new season. The leaves in these branches are partially eliminated to limit the exceeded water evaporation. Then, the branches are cut into 15-20 cm long internodes with 2-3 bud eyes. After that, these internodes are planted with the distance of each about 30-40 cm. The amount of fertilizer is suggested to promote the growth and development of Cam plants was 8-10 tons per hectare. Phosphorous and potassium fertilizers with the amount of 100-150 kg and 50 kg respectively are added in order to optimize the growth and development of these plants. Then, watering and remaining the moisture of soils in first stage of 7-10 days in order to provide the best conditions for development of internodes. It is better to plant Cam beneath huge perennial fruit trees to reduce the light intensity for maximizing the pigment accumulation. After planting 3-4 months, Cam can be harvest for the first time. Cam can be harvested 2-3 times per year (Nguyen TPT. et al, 2009).

Tab. 1. Morphological characteristics of four types of Cam (*Peristrophe roxburghiana*) in Vietnam (Nguyen TPT. et al, 2009)

	Common names	Morphological characteristics of the leaves	Hue of extracts	Applications
1	Red Cam	Oval shaped leaves, tapered leaf base, dark green leaves with a lot of piloses, upper surfaces of leaves without white spots	Red	Chopsticks and glutinous rices
2	Purple Cam	Large egg-shaped leaves, rounded leaf base, light blue and thin leaves with a few of piloses, large white spot area along the veins of leaves	Purple	Glutinous rices
3	Magenta Cam	Oval leaves, rounded or tapered leaf bases, dark green and thick leaves with a few of piloses, a few of white spot along the veins of leaves	Purple	Glutinous rices
4	Yellow Cam	Oval leaves, tapered leaf bases, tapered leaf tip, both sides of leaves scattered with piloses, leaf blade usually wrinkled, especially leaf edges	Greenish yellow	Glutinous rices

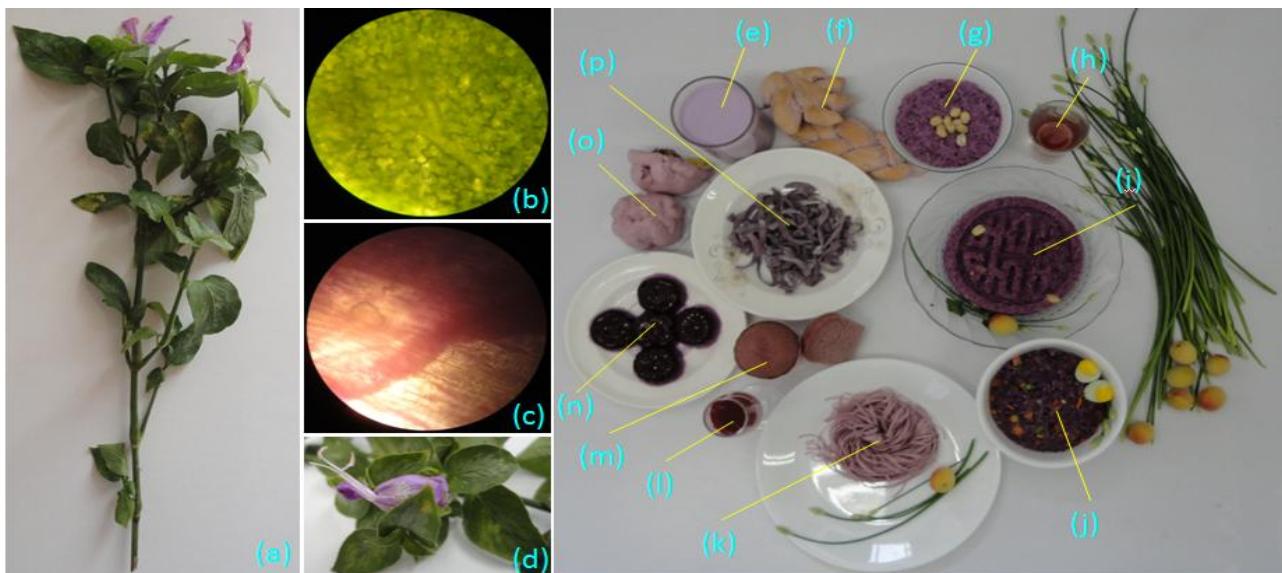


Fig. 1. Morphological of Cam plants and their applications: (a,d)-stems, leaves and flowers of Cam plants (b)-microscopic photographs of Cam leaves (x40 magnification), (c)- microscopic photographs of Cam petals (x40 magnification), (e)-purple soy milk, (f)-purple bread, (g)-purple sweetened porridge, (h)-purple liquor, (i)-purple glutinous rice, (j)-purple ten ingredients fried rice, (k)-fresh vermicelli, (l)-rice wine dying with red Cam pigments, (m)-purple sponge cake, (n)-purple agar jelly, (o)-purple dumpling and (p)-purple candied

Some applications and characters of Cam leaf extracts

Nowadays, food products are often consumed far from where they are produced. Approximately 75% of food products consumed in the developed countries have been processed into different forms. The results are the loss of the appearance of the food may be occurred during processing and transportation. Therefore, the use of food additives is necessary in order to (1) compensate the loss of color in the processing, (2) ensure uniformity of color, (3) intensify colors normally found in food, (4) protect other components (such as antioxidants), (5) obtain the best food appearance, (6) preserve characteristics associated with food, and (7) help as a visual characteristic of food quality⁷. At the present time, the use of chemical products in food production is negative impact on consumer health. Therefore, the use of natural products has been enhanced. Experts assert that this trend will continue to develop at least next 20 years⁷. Despite of long history in use for food in Vietnam, potential of pigments from Cam in replacement to synthesis colorants has been found in recent years due to the seriously adverse effects on health associated with abuse

of the synthesis pigments. In recent years, the study of pigments obtained to Cam plants has been received much attention of food scientists in Vietnam and China. For examples, Luu DC (2005) used water as a solvent to extract the pigments from Cam leaves. Luu also was applied these extracts to improve the appearances of glutinous rices, rice wines and pineapple core juices. Figure 1 showed some application of Cam leave extracts for food in Vietnam. Luu DC et al. (2006) reported that the amount of pigments was account for 12.72% in comparison with total volume of purple Cam leaves. In addition, Nguyen TTH (2009) who built the model to develop the coloring material sources from Cam plants in Moc Chau – Son La (Vietnam) also reported some primary research on chemical components and bioactives of these plant extracts. Besides, Do and Nguyen (2007) also distinguished ecological characteristics of Cam plants and La Dien plants (*Dicliptera chinensis* (L.) Juss.). Up to now, Nguyen et al. (2010) have first time announced the results of the peristrophe structure, a major pigment from the red Cam in Moc Chau, Son La and Thai Nguyen.

Jiang et al. (2011b) collected the red pigments from *Peristrophe roxburghiana* by ultrasonic

techniques combined with extraction solvent of ethanol found that the pigments from Cam plants were easily dispersed in water and ethanol. Cam leaf pigments are quite stable and exhibited the red-orange hue at pH 11. However, when the pH is greater than 12, the dye would turn into green yellow and lost their stability. Oxidative agents and ascorbic acid were significantly contributed in fading of the dye from the leaves of Cam. This pigment is quite stable to heat and light. Some food additives such as citric acid and potassium sorbate are very small effect on the stability of the Cam pigments. Metal ions which are abundantly presented in foods such as K^+ , Na^+ , Cu^{2+} , Mg^{2+} , Zn^{2+} and Ca^{2+} do not affect on the color stability of these pigments. In contrast, metal ions such as Fe^{3+} and Al^{3+} can be dimmed the reddish hue of these pigments.

Some studies have shown that the Cam leaf extract might be high in anti-microbial activity (Rajesh KV, Leena C, Sadhana K, 2008; Wiart C. et al., 2004). The activity against *Candida albicans* of methanolic plant extracts express as diameter of zones of antibacterial inhibition in descending order as follows: Leaves of *Knema glaucescens* Jack (16 mm) > roots of *Apama tomentosa* Engl. (13 mm) > whole plant of *Celosia argentea* Linn. (11mm) > whole plant of *Ancistrocladus tectorius* Merrill (10mm) ≈ bark of *Ancistrocladus tectorius* Merrill ≈ whole plant of *Polyalthia lateriflora* Kurz > whole plant of *Commelina communis* Linn. (9mm) ≈ leaves of *Solanum torvum* Sw. ≈ leaves of *Sonerila begoniifolia* Blume > leaves of *Cinnamomum malabatum* (Burm.f.) Blume (8mm) ≈ whole plant of *Eclipta prostrata* Linn. ≈ whole plant of *Euphorbia hirta* Linn. ≈ whole plant of *Peristrophe bivalvis* Merrill ≈ whole plant of *Piper stylosum* Miq. > roots of *Ancistrocladus tectorius* Merrill (7 mm) ≈ bark of *Apama corymbosa* Kuntze ≈ whole plant of *Apama tomentosa* Engl. ≈ leaves of *Ancistrocladus tectorius* Merrill ≈ whole plant of *Eleusine indica* Gaertn. ≈ bark and leaves of *Hedyotis congesta* Wall. & G. Don ≈ whole plant of *Hyptis suaveolens* Poit. ≈ whole plant of *Lycopodium cernuum* Linn. ≈ bark of

Nardostachys pallida Merr. ≈ whole plant of *Peperomia pellucida* H.B & K. ≈ whole plant of *Piper porphyrophyllum* N.E.Br ≈ leaves of *Trevesia burckii* Boerl (Rajesh KV, Leena C, Sadhana K, 2008). In addition, Sangeeta ST. et al. (2012) was published the minimum inhibitory concentrations (MICs) of both ethanolic Cam leaf and stem extract against *Candida albican* were 30-40 mg/ml. Four species of organisms related to food spolilage such as *Bacillus subtilis*, *Staphylococcus aureus* (positive Gram microorganisms), *Pseudomonas aeruginosa* and *Escherichia coli* (negative Gram microorganisms) were also treated with the ethanolic extracts of leaves and stems of *Peristrophe bivalvis*. The results were shown that all bacteria were also inhibited with low concentration of both Cam leaf and stem extracts. MICs values of leaf extract against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were repective in 30-40, 30-40, 20-30 and 20-30 and the stem extract were 50-60, 50-60, 30-40 and 20-30, respectively. Based on these data, it could be asserted that the leaf extracts from Cam could be used as natural preservatives for foods and medicines in treatment of diseases associated with intestinal disorders caused by harmful micro-organisms. Other application related to the Cam leaf extracts were recorded in some studies such as making salt by Apatani tribes in Arunachal Pradesh or treatment of tuberculosises, acute bronchitis and wrenchs (Srivastva RC, Singh RK, Apatani community, Mukherjee TK, 2010; Nguyen TTH, Pham HN, Tran VS, Trinh TT, 2010).

Major components, effects of Cam leaf extract on human health and evaluations of toxicity

The major component of the color aqueous extract (CAE) of Cam leaves was identified as peristropheine by spectral analysis (2D NMR spectra) (Trinh TT. et al., 2012). Some other substances were responsible for purple and red colors of Cam leaves also reported by Nguyen TPT. et al. (2009). These substances were illustrated in figure 2 as following:

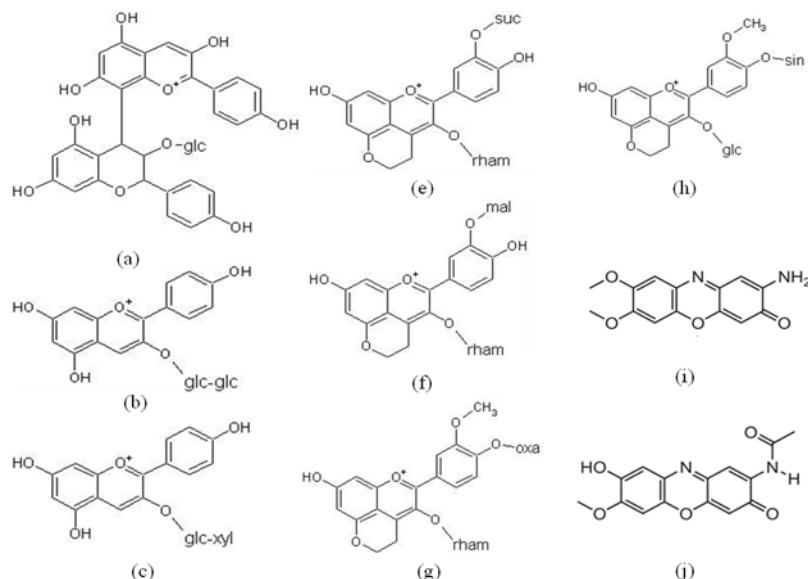


Fig. 2. Main component in Cam extracts including (a)-Afzelechin(4-8) pelargonidyl glucoside, (b)-Pelargonidine-3-O-gentiobiose and (c)-Pelargonidine-3-O-sambabiose in insoluble methanolic fraction of purple *P. bivalvis*, (d)-4'-sucxinoyl-3-rhamnozyl-(4H,5H) pyranocyanidin, (e)- 4'-maloyl-3-rhamnozyl-(4H, 5H) pyranocyanidin, (f)- 4'-oxaloyl-3-rhamnozyl-(4H, 5H) pyranopeonidin and (g)- 4'-sinpoyl-3-glucosyl-(4H, 5H) pyranopeonidin in soluble methanolic fraction of purple *P. bivalvis* and (h)- peristropheine in aqueous extracts of red *P. bivalvis* and (i, j)- perisbivalvine A in aqueous extracts of *P. bivalvis*

Figure 2 was shown that major components in the insoluble methanolic fraction of *P. bivalvis* purple belonged to pelargonidin group, but the soluble methanolic fraction was classified into the pyranocyanidin one. Pelargonidin is an anthocyanidin which presents as a natural colorant in strawberries and other fruits and flowers. Alzelechin (4-8) pelargonidyl glucoside has been also found in strawberries in which four purples pigment including alzelechin ($4\alpha \rightarrow 8$) pelargonidin 3-O- β -glucopyranose, epialzelechin ($4\alpha \rightarrow 8$) pelargonidin 3-O- β -glucopyranose, catechin ($4\alpha \rightarrow 8$) pelargonidin 3-O- β -glucopyranose and epicatechin ($4\alpha \rightarrow 8$) pelargonidin 3-O- β -glucopyranose and alzelechin ($4\alpha \rightarrow 8$) pelargonidin 3-rutinoside were detected (Fouad D, Vincenzo L, 2008). From these, the similarity between pigments in Cam leaves and strawberries was also indentified. Moreover, the soluble methanolic fraction of Cam leaves are also similar to components presented in *Prunus cerasus* L., blood orange, cherry and red wine (Dora BV, Francisco JLB, Josemaría AR, Isidro HG, 2011; Stéphanie G, Philippe S, Catherine MGR, 2009).

In recent years, the Zizilan compounds have been explored in the Cam leaf extracts. These were assigned to be able to treat diabetes, hepatitis B, anti-oxidative medicines, immune improvers or use in lotions or consider as a color agent in many application fields (Xie Y, Jiang X, Wen Y, 2003).

Hematochrome is generally term to show both β -carotene and their keto derivates distributed as the form of oily droplets in chloroplast (Oren A, 2005; Robert EL, 1999). These substances have been also occurred in Cam leaf extracts. Carotenoids and their derivates have been used as antioxidants to protect the retina and reduce risk of age-related macular degeneration based on their long-chain conjugated polyene structures (Carmen S, 2008; Yousry MAN, 2000). Two mechanisms of antioxidant actions of both substances can be distinguished including singlet oxygen quenching and reactions with radicals.

Eleven phytochemicals were isolated from *P. roxburghiana* including β -sitosterol, β -daucosterol, coumarin, 1-octadecanol, allantoin, lupeol, oleanolic acid, 3-acetyl oleanolic acid, cyanidin, cyanidin-3-O- β -D-glucoside and peristropheine estimated in anti-

inflammatory activity on lipopolysaccharide (LPS)-simulated RAW 264.7 cells and carrageenan-induced inflammation on BALB/c mice. The results were shown that eight of them exhibited ability of anti-inflammatory on normal cytokines TNF- α , IL-1 β , and IL-6⁴³. In addition, aliphatic components in *P. roxburghiana* were claimed to treatment of tuberculosis, eliminates phlegm, acute bronchitis, antitoxic, anti-inflammatory, windpipe infection, hepatitis and diabetes¹⁷. Moreover, *Peristrophe roxburghiana* have been traditionally used to remedy hypertension for many years ago in some local regions of China (Zhuang X, Lü J, Yang W, Yang M, 2003). Besides, lung heat cough, hacking of blood, blood ejection, infant fright wind, red swollen of throat, mouth and tongue sores, dribbling pain of urination, swelling welling abscess and sore and boil, scrofula, painful swelling from knocks and falls, bleeding due to external injury, poisonous snake bites have been cured by *Peristrophe roxburghiana* extracts (Jiaju Z, Guirong X, Xinjian Y, 2011). Cheng Z. et al. (2004) and Zhuang X. et al. (2003) claimed that *Peritrophe roxburghiana* extracts decreased Blood Pressure in Renal Hypertensive and Hyperlipidemic Rats due to higher the serum NO and lower angiotensin II in the thoracic aorta. The direct relaxant effect on smooth muscle of nitric oxide and reduces the vasoconstriction effects of low angiotensin II concentration are the reasons explained to this phenomenon (John S, Mazza G, Marc LM, 2002). Besides, Yang W. et al. (2002) was reported that *Peristrophe roxburghiana* extracts could be improved the hemorheological conditions and prevented the thrombosis forming in vasculars.

Tanavade SS. et al. (2012) were determined anticancer activity of ethanolic and aqueous extracts of leaves and stems of *Peristrophe bivalvis* *in vitro* by tryphan blue dye based on exclusion essay and brine shrimp lethality bioassay method. In this study, ethanolic extract was recognized more effectively than aqueous extract in anticancer activity. In addition, leaf extracts were higher in anticancer activity if compared to those of stems.

Furthermore, the CTC₅₀ and LC₅₀ values of ethanolic leaf extracts were 175.25±12.34 μ g/ml and 176.49±7.43 μ g/ml in EAC cell lines, respectively. These showed that the *Peristrophe bivalvis* got anticancer capacity. Besides, color aqueous extracts and peristrophine (a major bioactive component in Cam leaf extracts) are significantly declined the OCI cell number (Trinh TT. et al., 2012). In the study conducted by Qin SS. et al. (2010), the CCl₄-induced rat model of acute liver injury was treated by *Peristrophe roxburghiana* polysaccharides to observe the protective effects of these on rat livers. In this study, malondialdehyde levels were decreased, but the superoxide dismutase activity were increase that shown the polysaccharides extracted from *Peristrophe roxburghiana* had better protective effect on rat livers. Moreover, this study was also observed that polysaccharides from these plants inhibited harmful effects of aspartate aminotransferase and alanine aminotransferase on liver functions.

Techniques for extracting the colors from Cam leaves

There were many techniques to extract pigments from Cam leaves, but three common methods such as ultrasonic assisted extraction, microwave assisted extraction and solvent extraction would be introduced in this study. Jiang HZ. et al. (2011a) who used the ultrasound to extract natural colors from *Peristrophe roxburghiana* realized that the alcohol concentration of 70%, ultrasonic power of 300 W, extraction temperature of 70°C, extraction time of 45 minutes, the solid-liquid ratio of 1:35 have been chosen as the optimal conditions for extracting purple pigments from the Cam leaves. Meanwhile, Jiang HZ. et al. (2011b) were used ultrasound techniques to collect the red pigments from these plants with water as extraction solvent were recorded the optimal extracted conditions as follows: ultrasound power of 400W, the solid-to-liquid ratio of 1:35, extraction temperature of 65°C and the extraction time of 35 min. In another study, Nguyen TTH, Trinh TT (2010) was applied hot water extraction method at a temperature of 85°C-90°C in the extraction

duration of 60 minutes with two extraction stages at which one of 40 minutes and the other of 20 minutes were conducted for extracting the pigments from Cam leaves. According to the study of Luu DC (2005), pigments from red, yellow and purple Cam leaves could be extracted with the 40% alcohol extraction solvent. In another study, Luu DC. et al. (2006) also recognized that the extraction yield were the highest when the pigments were extracted from yellowish Cam (19.73%) by hot water. Besides, the haematochrome is one of the blood red pigment presented in variety of microbiology and plants such as *Haematococcus pluvialis*, *Trentepohlia* and *Euglena* as well as in the *red peppers* and *Peristrophe roxburghiana*. Niu G. et al. (2006); Droop MR (1954); Yi CK, Jiang HZ (2011) was also successfully extracted from *Peristrophe roxburghiana* by using microwave extraction technology. The best extraction conditions in this case were microwave power of 320W, extraction time of 75 sec and solid-to-liquid ratio of 1:20(w/v). Base on these data, the microwave technology were considered as the suitable method for extracting this pigment due to less time-consuming and high efficiency (Yi CK, Jiang HZ, 2011).

Analytical methods to determine the major component of Cam extracts

Headspace GC were used as the effective method for detecting the benzene series such as skellysolve B, benzene, methylbenzene and styrene from macro reticular resin in *Peristrophe roxburghiana* extracts (Ma H, Jiang ML, 2006).

According to Xie Y. et al. (2007), the zilanine could be use as insulin sensitizing agent improved the conditions of those related to disorders of livers and kidneys³⁸. This compound is one of pigment exhibited the purplish blue found into *Peristrophe roxburghiana* can be quantified by using many techniques such as Fluorescence Spectrophotometry with the parameters of pH, excitation wavelength, emission wavelength of 8, 590 and 625 nm, respectively. In addition, Micellar Eletrokinetic Capillary Chromatography could be used as the suitable

method to quantify this component in *Peristrophe baphica* extracts with capillary column of 50.2cm in long and 75µm in internal diameters, operation voltages of 25 KV, test wavelength of 585 nm, column temperature of 25°C, the mobile buffer of 20mmol/l boric acid and 20% acetonitrile (pH 8) with 10 mmol β-CD (Li J, Xie YC, Jiang XH, 2010). As far as we know, micellar electrokinetic capillary chromatography is a method of separation of neutral compounds using surfactant micelles that was developed in the early 90 by Shigeru Terabe to separate the mixture containing both ionic and neutral analyte (Gabriel H. et al, 2013). Another method could be established to evaluate the content of zilanine in the *Peristrophe baphica* extracts was RP-HPLC (Jiang XH, Xie YC, Huang YL, 2007).

2. CONCLUSIONS

Cam is not only useful for human health due to its bioactive substances but also beneficial for food industry because of its colorants. Moreover, this plant is easily to grow and does not require high nutrition. Therefore, this plant is suitable for agriculture economic development for ethnic minorities who have lived in the rural areas and alpine zones of Vietnam in which the soils are poor in nutrition due to erosion. In recent years, many techniques have been introduced to extract colorants from Cam leaves with the aims to reduce the costs of application related to Cam leaf extracts. As a result, commercialization of these colorants will become easier. As the results, the development of colorants from Cam leaves will be a suitable solution for reducing the poverty rate of ethnic minorities in Vietnam and other development countries.

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