(IC_{50} 100 µg/mL for palmitic acid). The activity demonstrated by the crude extract, solvent fractions, and the isolated compounds suggests that *Sargassum mcclurei* could be a useful functional food ingredient for the management of neurodegenerative disorders.

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QUALITY EVALUATION OF *CENTELLA ASIATICA* FROM VARIED GEOGRAPHICAL AREAS IN VIETNAM BY RP-HPLC/DAD AND CHEMOMETRICS

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Summary

Quality Evaluation of *Centella asiatica* from Varied Geographical Areas in Vietnam by RP-HPLC/DAD and Chemometrics

Quality of *Centella asiatica* samples collected from varied geographical areas in Vietnam were evaluated based on non-target peaks and asiaticoside content by a reversed phase HPLC/DAD method. The results indicated that there are differences in the chemical compositions between the samples collected in different regions of Vietnam. The content of asiaticoside, a major bioactive compound of *Centella asiatica*, ranged from 0.07% to 1.19%. The PCA-DA method on the non-target peaks showed a fairly good classification for the samples collected in Red River Delta, Northeast, South and North Central regions.

Keywords: *Centella asiatica, Asiaticoside, A reversed phase HPLC/DAD, Quality evaluation, Chemometrics*

1. Introduction

Centella asiatica (L.) (CA) is an important herb in traditional Vietnamese medicine. This plant is cooling, soporific, cardiotonic, nervine tonic, stomachic, improves appetite, antileprotic, antiseptic, tonic to nerves and memory. It is also used in diseases of skin, nerves and blood [1]. Various compounds, including triterpenoids, saponins, flavonoids, glycosides, alkaloids have been isolated from CA to date. The triterpenoid saponins including asiaticoside, madecassoside, and their aglycones, asiatic acid and madecassic acid are the most abundant pentacyclic triterpenoids in CA [1],[2],[3]. These chemical compounds exhibit numerous biological activities, including gastric ulcer healing, wound healing,

antitumor, memory enhancing, neuroprotective, immunomodulating, radioprotective, antitubercular and anti-inflammatory [1]. Numerous studies have been analyzed chemical constituents in CA by using different methods (HPLC/DAD, LC/MS, TLC-scanner) [4],[5],[6],[7]. CA was recognized by Vietnamese Pharmacopoeia (VP), Chinese Pharmacopoeia (CP) and Hong Kong Chinese Materia Medica Standards (HKCMMS) [8],[9],[10]. While CP and HKCMMS used asiaticoside and madecassoside as markers for controlling quality of CA [9],[10], no markers has been used for quantitative analysis in VP 2017 [8].

In order to provide more scientific basis for the quality of CA cultivated and grown in Vietnam, the whole plants of CA were collected in varied geographical areas of Vietnam for investigation the content of asiaticoside by using RP-HPLC/DAD method. The results of the present study contributed valuable evidences for geographical region selection and good medicinal material collection process in addition to suggest upgrade of the CA standardization in Vietnamese Pharmacopoeia in the future.

2. Experimental

2.1. Materials

A total of 41 samples of *Centella asiatica* (CA) were collected from 12 provinces in 4 different geographical regions: Red River Delta (**RRD**), Northeastern (**NE**), North Central (**NC**) and Southern (**S**). Whole plants of CA were collected from February 2021 to April 2021. These samples were cleaned, dried in the oven at (60°C) and grounded into powder before extraction. Detail collected samples were listed in Table 1. These samples were authenticated at the Department of Medicinal Material Resources and stored in the Department of Analytical Chemistry and Standardization, NIMM.

Table 1. The information of CA samples used in this study

No.	Collected location	Geographical region	No.	Collected location	Geographical region
M1 *a)*	Nam Cuong, Nam Truc, Nam Dinh	**RRD**	**M21***(b)*	Thai Dao, Lang Giang, Bac Giang	**NE**
M2*(a)*	Nam Cuong, Nam Truc, Nam Dinh	**RRD**	**M22***(a)*	Luc Hon, Binh Lieu, Quang Ninh	**NE**
M3*(a)*	Nam Truc, Nam Dinh	**RRD**	**M23***(a)*	Luc Hon, Binh Lieu, Quang Ninh	**NE**
M4*(a)*	Xuan Phu, Xuan Truong, Nam Dinh	**RRD**	**M24***(a)*	Hoang Quy, Hoang Hoa, Thanh Hoa	**NC**
M5*(a)*	Xuan Phu, Xuan Truong, Nam Dinh	**RRD**	**M25***(a)*	Lam Son, Bim Son, Thanh Hoa	**NC**
M6*(b)*	Xuan Phu, Xuân Truong, Nam Dinh	**RRD**	**M26***(a)*	Thach Long, Thach Thanh, Thanh Hoa	**NC**
M7*(a)*	Nam Tien, Nam Truc, Nam Dinh	**RRD**	**M27***(b)*	Thach Dong, Thach Thanh, Thanh Hoa	**NC**
M8*(a)*	Nam Tien, Nam Truc, Nam Dinh	**RRD**	**M28***(b)*	Sam Son, Thanh Hoa	**NC**
M9*(a)*	Nam Tien, Nam Truc, Nam Dinh	**RRD**	**M29***(a)*	Viet Tien, Thach Ha, Ha Tinh	**NC**
M10*(b)*	Yen Tien, Y Yen, Nam Dinh	**RRD**	**M30***(a)*	Viet Tien, Thach Ha, Ha Tinh	**NC**
M11*(b)*	Quynh Xa, Quynh Phu, Thai Binh	**RRD**	**M31***(a)*	Viet Tien, Thach Ha, Ha Tinh	**NC**
M12*(a)*	Quynh Xa, Quynh Phu, Thai Binh	**RRD**	**M32***(b)*	Cam Ha, Cam Xuyen, Ha Tinh	**NC**
M13*(a)*	Thai An, Thai Thuy, Thai Binh	**RRD**	**M33***(a)*	Dau Hoa, Minh Hoa, Quang Binh	**NC**
M14*(a)*	Phu Phuong, Ba Vi, Hanoi	**RRD**	**M34***(a)*	Thuan Loc, Hue	**NC**
M15*(a)*	Phu Chau, Ba Vi, Hanoi	**RRD**	**M35***(a)*	Quang Dien, Hue	**NC**
M16*(a)*	Van Con, Hoai Duc, Hanoi	**RRD**	**M36***(b)*	Tay Loc, Hue	**NC**
M17*(a)*	Hung An, Kim Dong, Hung Yen	**RRD**	**M37***(a)*	Tay Loc, Hue	**NC**
M18*(a)*	Hung An, Kim Dong, Hung Yen	**RRD**	**M38***(a)*	Tan Hoi Dong, Chau Thanh, Tien Giang	**S**
M19*(a)*	Huong Gian, Yen Dung, Bac Giang	**NE**	**M39***(a)*	Tan Hoi Dong, Chau Thanh, Tien Giang	**S**
M20*(a)*	Huong Gian, Yen Dung, Bac Giang	**NE**	**M40***(a)*	Binh Tan, Ho Chi Minh	**S**
			M41*(b)*	Da Phuoc, Binh Chanh, Ho Chi Minh	**S**

RRD- Red River Delta; NE- Northeastern; NC- North Central; S- Southern; a-training group; b-testing group.

2.2. Chemicals

Asiaticoside analytical standard was purchased from Chemfaces (CAS: 16830-15-2, Lot No: CFS202003) with the purity ≥ 98%. HPLC-grade acetonitrile solvent was purchased from Merck (Germany). All other solvents were of analytical grade.

2.3. Instrument and chromatographic conditions

The HPLC system (Shimadzu, Japan) used for the analysis was consisted of with a quaternary

pump, a degasser, an autosampler, an injector with a 200-µL loop. The HPLC system is equipped with a DAD (205 nm) and an Agilent C_{18} (250mm×4.6mm, 5µm) column. The flow rate is about 1.0 mL/min and the injection volume is 10 µL. The mobile phase consisted of 0.15% phosphoric acid (A) and acetonitrile (B): 0-15 min (21%B), 15-32 min (21-36%B), 32-50 min (36-40%B), 50-60 min (40-80%B).

2.4. Sample preparation

Weigh 0.5 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of methanol (80%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the supernatants and make up to the mark with methanol (80%). Filter through a 0.45-µm PTFE filter [10].

2.5. Standard solutions

Standard stock solution of asiaticoside was prepared by dissolving appropriate the amount of asiaticoside primary standard in methanol 80% to obtain concentration of 1000 µg/mL. They were then diluted to six concentrations for construction of calibration curve in the range of 6.87 - 687 µg/mL for asiaticoside. These solutions were stored at 4°C.

2.6. Method validation

The analytical method was validated for system suitability, specificity, calibration curve, accuracy, precision, detection limit (LOD) and quantitation limit (LOQ) following the current ICH guidelines [11]. The LOD and LOQ were determined based on the signal-to-noise ratio. The accuracy of the analytical method was assessed by spike recovery experiments at 3 levels (80%, 100%, 120%).

2.7. Calculation

Calculate the percentage of asiaticoside in the tested sample taken:

$$X\,(\%) = \frac{C \times V \times 10}{m \times (100 - a)}$$

C: the concentration of analyzed compound in the sample solution from the calibration curve equation (µg/mL); V: volume of the sample solution (mL); m: weight of tested sample taken to prepare the sample solution (mg); a: the moisture of powder (%).

2.8. Principal components analysis - Discriminant analysis (PCA-DA)

In this study, PCA and DA were used to differentiate *Centella asiatica* collected from different regions in Vietnam. The data of peak areas were used for PCA. Input data were in the form of a m×n matrix (with m and n being the number of samples and its peak areas corresponding to retention time, respectively). The construction of the classification and identification model according to the PCA-DA algorithm was performed by using the classification toolbox 4.2 (Matlab R2019a software).

3. Results and discussion

3.1. Validation of HPLC method

The CA sample used in validation of method was collected from Quynh Xa, Quynh Phu, Thai Binh (M11). The results of specification test were showed in Fig. 1.

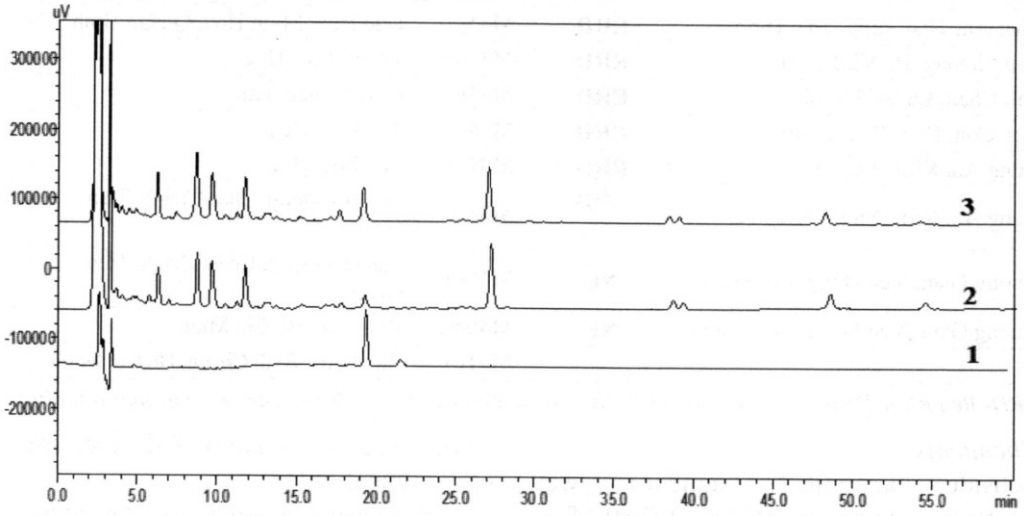

Fig. 1. The result of specification test
(1-asiaticoside, 2- CA sample, 3-CA sample spiked with asiaticoside)

The result of specification test (Fig. 1) showed that the retention time of asiaticoside peak (t_R = 19.56 min) in CA sample and CA sample spiked with asiaticoside was similar and peak area of asiaticoside in spiked sample solution was higher than that in sample without spike. The obtained chromatograms showed clear separation peaks, low background noise. Additionally, when comparing the UV spectrum of three points of analyzed peaks in sample, the peak purity of asiaticoside was 99.85%. The similarity of UV spectrum of asiaticoside peak in standard solution and sample solution (match ratio) was 0.9962. These demonstrated the specification of this method.

Different concentrations of asiaticoside standard were analyzed to validate the linearity criteria (Table 2). The result showed that in the range of determined concentrations of asiaticoside, there was a significant correlation between asiaticoside concentration and its corresponding peak area (Fig. 2)

Table 2. Concentration of asiaticoside and its corresponding peak area

Concentration (µg/ml)	Peak area (S, mAU*s)
687.5	2650331
550	2209540
412.5	1650789
275	1090870
137.5	542903
68.75	253096
34.38	141566
6.875	33711

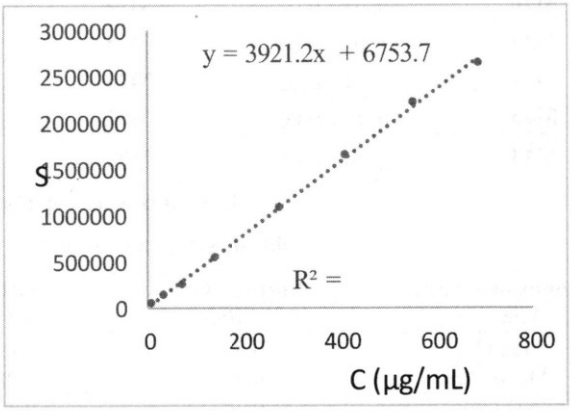

Fig. 2. Calibration curve of asiaticoside

The calibration curve equations were Y=3921.2X + 6753.7 (R^2 = 0.9991), where Y was the area of peak of analyte and X was its concentration (µg/mL); Linear correlation coefficient (R^2) values were above 0.999, therefore, it can be concluded that this developed method conforms the linearity.

The results of validation method (system suitability, precision, accuracy, limit of detection and limit of quantification) have been summarized in Table 3.

Table 3. The results of validation method

No	Test	Results
1	**System suitability** (n=6)	Retention time: Mean: 19.56 min; RSD = 0.02% Peak Area: Mean: 1098444; RSD = 0.87%
2	**Precision**	
	Intra-day (n=6)	Mean = 1.12%; RSD (%) = 1.41%
	Inter-day (n=6)	Mean = 1.13%; RSD (%) = 1.94%
3	**Accuracy (Recovery)**	95.2-102.6%
4	**LOD**	0.15 µg/mL
5	**LOQ**	0.45 µg/mL

RSD% of retention time, peak area and the intra- and inter-day precision were all under 2%. These indicated that the developed method was good precision and conforms system suitability criteria. The recovery values were obtained in the range of 95.2-102.6% (AOAC recovery criteria: 97-103% at the level of 1% and 95-105% at the level of 0.1%). In general, medicinal plants are complex samples, the matrix effect may also affect the recovery value (accuracy) of an analytical method; therefore, the recovery values (95.2-102.6%) indicated that the accuracy of the developed method were acceptable.

3.2. Determination of asiaticoside in CA samples by HPLC/DAD

The proposed method was applied to simultaneous determination of asiaticoside in 41 samples of CA collected from different regions in Vietnam. Each sample was analyzed in triplicate to determine the mean content (%). The results were summarized in Table 3.

Table 3. The content of asiaticoside in CA samples in Vietnam

Sample ID	The content of asiaticoside* (%)	Sample ID	The content of asiaticoside* (%)	Sample ID	The content of asiaticoside* (%)
M1	0.55 ± 0.03	**M15**	0.24 ± 0.02	**M29**	0.82 ± 0.02
M2	0.53 ± 0.02	**M16**	0.34 ± 0.03	**M30**	0.87 ± 0.02
M3	0.51 ± 0.03	**M17**	0.48 ± 0.05	**M31**	0.94 ± 0.04
M4	0.69 ± 0.04	**M18**	0.31 ± 0.03	**M32**	0.55 ± 0.05
M5	0.78 ± 0.05	**M19**	0.11 ± 0.01	**M33**	0.68 ± 0.04
M6	0.73 ± 0.05	**M20**	0.17 ± 0.02	**M34**	0.30 ± 0.02
M7	0.39 ± 0.03	**M21**	0.13 ± 0.01	**M35**	0.30 ± 0.03
M8	0.37 ± 0.03	**M22**	0.88 ± 0.03	**M36**	0.36 ± 0.04
M9	0.38 ± 0.04	**M23**	0.78 ± 0.03	**M37**	0.36 ± 0.02
M10	0.10 ± 0.02	**M24**	0.40 ± 0.03	**M38**	0.34 ± 0.03
M11	1.19 ± 0.04	**M25**	0.58 ± 0.04	**M39**	0.43 ± 0.02
M12	0.08 ± 0.00	**M26**	0.84 ± 0.04	**M40**	0.19 ± 0.01
M13	0.07 ± 0.00	**M27**	0.85 ± 0.05	**M41**	0.17 ± 0.03
M14	0.21 ± 0.03	**M28**	0.52 ± 0.03		

***The results were calculated on a dry - weight basis**

Table 4. Max, min, mean, SD and RSD of asiaticoside contents

Groups of samples	Northeastern	Southern	Red River Delta	North Central
Min (%)	0.109	0.172	0.072	0.300
Max (%)	0.883	0.435	1.196	0.926
Mean (%)	0.414	0.285	0.444	0.597
SD (%)	0.383	0.127	0.284	0.232
RSD (%)	92.5	44.5	64.0	38.8

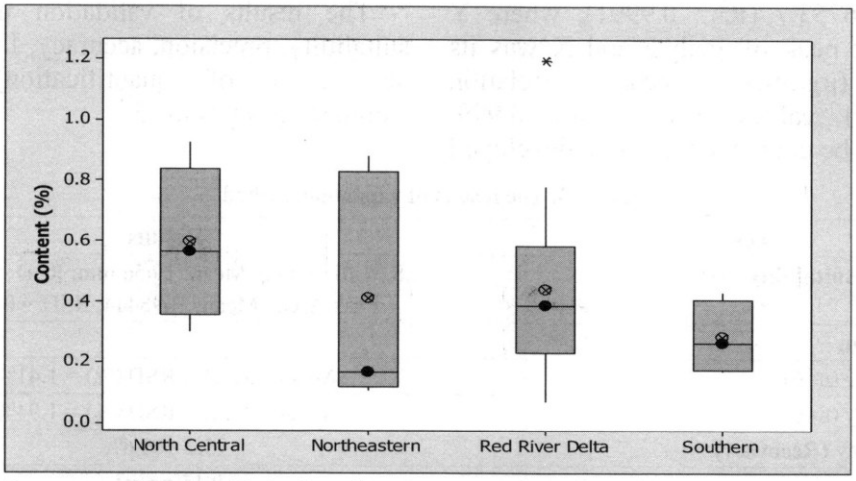

Fig. 3. Boxplot for the content of asiaticoside in *Centella asiatica* samples collected from 4 geographical regions in Vietnam

Based on the content of asiaticoside in CA samples collected from 4 geographical regions in Vietnam, the boxplot was constructed in the Fig. 4, in which the cross dots represent the mean values, and the black dots show the medians. The results showed that there was one sample (M11, collected from Thai Binh) outlier existed in the RRD region. The content of asiaticoside in this sample (1.19%) was much higher than other samples in the same group. The content of asiaticoside in the CA samples from NC region was considerable higher than that in other groups. Besides, the median of the asiaticoside content in CA samples collected from NE region was smallest among four groups, while the box of this group overlapped other groups. The difference between mean values of four groups was smaller than that between medians. The position of medians and mean values showed that the distribution of data was spread well and not

completely symmetrical. Moreover, it was very difficult to differentiate these group since their boxes overlapped each other.

3.3. Classification of CA by HPLC/DAD combined with PCA-DA analysis

To construct a model to classify CA samples, non-target peak areas were collected selectively according to the retention times. Ten peaks (**1-10**) were showed in Fig. 4.

The signal of qualitative peaks of 41 samples of CA was divided into 2 data groups: **Training group** (for building classification model) including 32 samples (15 samples from RRD region, 10 samples from NC region, 4 samples from NE region and 3 samples from S region); **Testing Group** (for testing the accuracy of the classification model) including 9 samples (3 samples from RRD region, 1 sample from NE region, 4 samples from NC region, 1 sample from S region). The PCA-DA results of training group showed that the most suitable number of PCs was 5 with the largest percentage of variance and the accuracy > 90%. The results showed in Fig. 5.

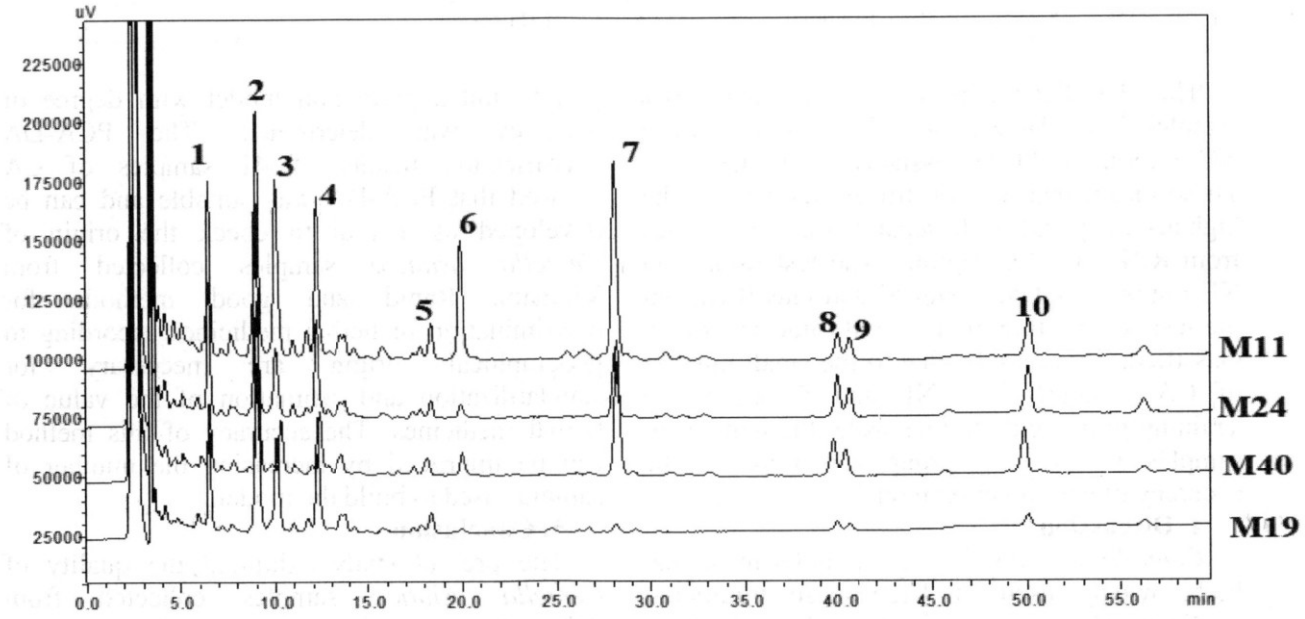

Fig. 4. HPLC chromatograms of CA samples collected from 4 geographical regions

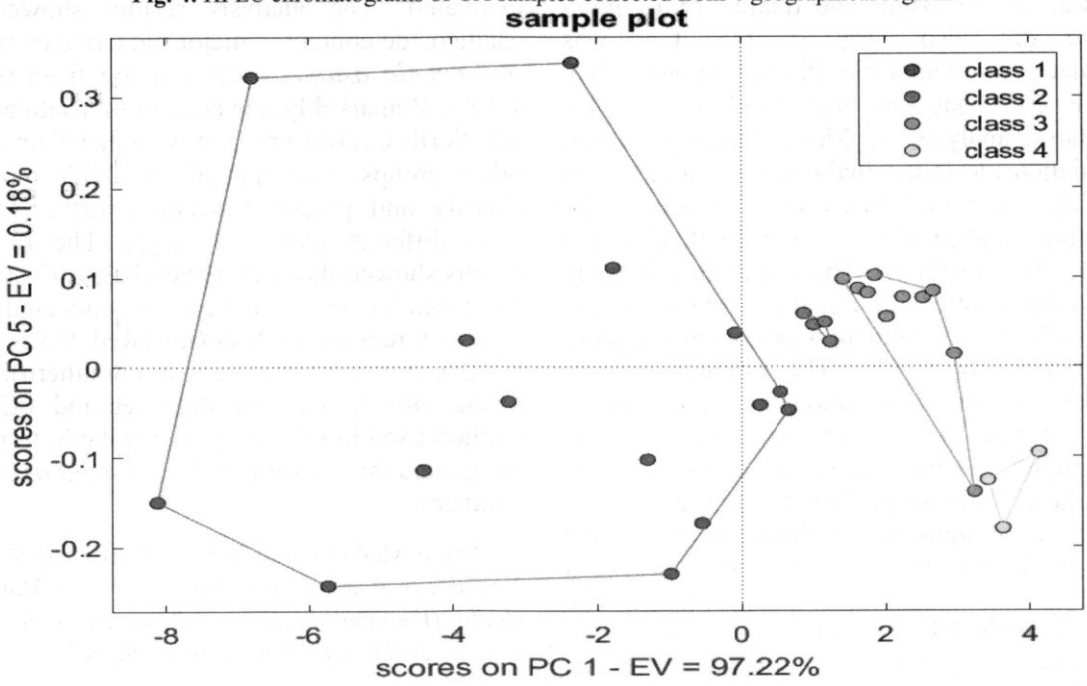

Fig. 5. The PCA-DA plot for Training group

The results of PCA-DA score plot and several trends were observed in Fig. 5. 15 samples from RRD region were discriminated clearly between samples from other regions. The samples from NC, NE and S regions were also classified. Given the good discrimination of the model, we used the PCA-DA model to predict the geographical origin of 9 samples of CA to check the accuracy of the classification model. The results showed in Table 5.

Table 5. The classification results of CA samples by PCA-DA

Group	9 test samples	Classification results (true/false)			
		RRD	NC	NE	S
RRD	3 samples	3 (true)			
NC	4 samples		3 (true)		
NE	1 sample			1 (true)	
S	1 sample		1 (false)		

The classification results showed that 100% samples from RRD region, 75% samples from NC region could be assigned and classified. These meant that the constructed model had the high accuracy and could separate the CA samples from RRD and NC regions. The test sample in NE region could be assigned and classified, but the test sample in S region could not be exactly classified. This may be due to the small number of CA samples from NE and S regions in Training group and the increasing the number of samples in Training group can improve the accuracy of classification model.

4. Discussion

Centella asiatica (CA) is a popular medicinal herb, widely used in Vietnamese traditional medicine. CA is grown from North to South in Vietnam, so the origin and quality of *Centella asiatica* are also very diverse. CA was recognized by Vietnamese Pharmacopoeia (VP), but VP 2017 has not been used markers for quantitative analysis [8]. Many studies published that asiaticoside is the main active ingredient in CA. This compound was used as a marker for controlling quality of CA in CP 2010, CP 2015 and HKCMMS [9],[10]. The results of this study showed that asiaticoside was the main component in CA collected in Vietnam with contents ranging from 0.07% to 1.19%. The obtained results provided useful information for the quality testing of CA in Vietnam and improving the monograph "*Herba Centellae asiaticae*" in Vietnamese Pharmacopoeia in the future.

The use of statistical methods to classify the origin of herbal medicinal samples has been carried out by many studies [12],[13],[14]. The PCA-DA method is a popular algorithm method which commonly used in sample classification. The PCA-DA analysis results are displayed in graphs and a prediction model with degree of accuracy was determined. The PCA-DA classification results of 41 samples of CA showed that PCA-DA was suitable and can be developed as a tool to check the origin of *Centella asiatica* samples collected from Vietnam. Rapid and good methods for discrimination of herbal medicines according to geographical origin are necessary for standardization and estimation of the value of herbal medicines. The accuracy of this method can be improved by increasing the number of samples used to build the model.

5. Conclusion

The present study exhibited the quality of *Centella asiatica* samples collected from different geographical areas in Vietnam were evaluated. The analysis results showed that asiaticoside content, a major bioactive compound in *Centella asiatica*, was ranging from 0.07 to 1.19%. Remarkably, the content of asiaticoside in the North Central group was higher than that in other groups. We applied PCA-DA model to classify and predict *Centella asiatica* samples from different places of origin. The PCA-DA results showed that a quite good classification for 41 *Centella asiatica* samples collected in 4 different regions in Vietnam (Red River Delta, North Central, Northeastern and Southern). These results proved that the data set and PCA-DA method used in this study was suitable to predict the geographical origin of the *Centella asiatica* in Vietnam.

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CYTOTOXIC AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS FROM THE FRUITS OF *ZANTHOXYLUM RHETSA* GROWN IN THANH HOA PROVINCE, VIETNAM

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Summary

Cytotoxic and Antimicrobial Activities of Essential Oils from the Fruits of *Zanthoxylum rhetsa* Grown in Thanh Hoa Province, Vietnam

The essential oils from the fresh and dried fruits of *Zanthoxylum rhetsa* grown in Thanh Hoa province were obtained by hydrodistillation and analyzed by gas chromatography (GC-FID) and gas chromatography/mass spectrometry (GC/MS). The major constituents of fresh fruit essential oil (FF) and dried fruit essential oil (DF) were sabinene, limonene, β-phellandrene, and α-pinene, but their contents in two essential oils were not the same, these values are 51.95%, 7.84%, 7.69%, 3.82% in FF and 32.88%, 20.95%, 9.20%, 4.22% in DF, respectively. In addition, α-phellandrene presents at high content in the DF (10.23%) but it is only the minor component in the FF (0.65%). The EOs were also tested for their cytotoxic and antimicrobial activities. The IC_{50} values revealed that these two essential oils exhibited a moderate activity against two tested cancer cell lines (MCF-7 and HeLa). For the antimicrobial activities, the FF showed its inhibitory effect on *E. coli* and *B. subtillis* (MIC values of 100 µg/mL), while the DF exhibited its potential antimicrobial activity against *E. coli, F. oxysporum* (MIC values of 100 µg/mL) and weak activity against *P. aeruginosa* (MIC value of 200 µg/mL).

Keywords: *Zanthoxylum rhetsa, Essential oil, Rutaceae, Thanh Hoa province, Vietnam.*

1. Introduction

Zanthoxylum rhetsa (Roxb.) DC, a flowering plant of the Rutaceae family, is found in India, Myanmar, Thailand, Laos and Vietnam. This is the medium-sized trees about 14 - 18 meter-height, straight body, thorny bark of trunk and