Vietnam Journal of Chemistry, International Edition, **54**(4): 416-418, 2016
DOI: 10.15625/0866-7144.2016-00338

SCREENING FOR THE MAIN TRITERPENIC ACIDS IN *CENTELLA ASIATICA* SAMPLES FROM NORTH AND SOUTH OF VIETNAM

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Received 17 June 2016; Accepted for publication 12 August 2016

Abstract

The optimal conditions for extraction, hydrolyzation and quantitative determination of the total amounts of two main triterpene acids, asiatic acid, and madecassic acid in *Centella asiatica* by HPLC and column chromatography are described. Using this method the asiatic acid and madecassic acid content of three *Centella asiatica* samples collected in Sontay City, Namdinh province, and in Ho Chi Minh City have been screened. The result showed that the contents of both triterpenic acids are higher in Sontay sample and in each sample the content of madecassic acid is higher than that of asiatic acid.

Keywords. *Centella asiatica,* asiatic acid, madecassic acid, quantification.

1. INTRODUCTION

Centella asiatica (L.) Urban (syn. *Centella. coriacea* Nannfd., *Hydrocotyle asiatica* L.) (fam. Apiaceae) is a tropical medicinal plant, distributed in Southeast Asian countries, India, China, Sri Lanka, Madagascar and South Africa [1, 2]. Its leaves are edible. Vietnamese use the whole plant as vegetable, for making drinks, against fever [3]. In the folk medicine this plant has been used since long time ago for wound healing, memory enhancement effects, treatment of leprosy, as anti-inflammatory, antiulcer, hepatoprotective, antioxidant…[4, 5]. The main active principles in *Centella asiatica* are triterpenes, triterpene glycosides, flavonoids, sterols and essential oil [6].

In the searching the plant *Centella asiatica* with high amount of asiatic acid and madecassic acid for chemical transformations, we did the screening of *Centella asiatica* collected in Son Tay, Hanoi City, Nam Dinh province, and Ho Chi Minh City.

2. EXPERIMENTAL

2.1. Methods and equipment

Column chromatography used silica gel Merck 0.043-0.63 mm; TLC: silica gel G60 F254 precoated on the aluminum sheets. HPLC: Alliance series 2695, detector PDA 2996, Waters, USA. Mobile phase: Kanal A: H_2O+0.1 % formic acid. Kanal B: Acetonitril 1mL/min. gradient. Detector PDA, λ = 205 nm.

Analytical balance: Adam AAA 160 L (d = 0.0001). Establish of standard curve: 25 mg asiatic acid (Merck) was placed in a 25 mL standard flash, than fill with MeOH to have a stock solution with a concentration of 1 mg/mL. The solutions with a concentration of 0.05, 0.1, 0.3, 0.5 and 0.7 mg/mL were prepared from the stock solution. The prepared solutions will be given to the HPLC with the column Sunfire-C18Rp (4.6×150 mm), 5 μm. The results in table 1 are the averages of three measurements.

From the results in table 1 the equation of regression (y = ax+b) will be established. The estimated correlation coefficient is: R = 0.99989992 and the linear equation of regression is:

$$Y = 10570916.\ X_i - 60136$$

X_i: Concentration of component to be analyzed

Y: Peak area.

Similar procedure will be used for madecassic acid to estimate the standard curve.

Table 1: The HPLC date of asiatic acid for establishment of standard curve

No.	Concentration (mg/mL)	Peak area	Theoretical peak area	Retention time (min.)
1	0.05	455177	468410	19.7
2	0.1	969416	996956	19.7
3	0.3	3150939	3111139	19.7
4	0.5	5271350	5225322	19.7
5	0.7	7294451	7339505	19.7

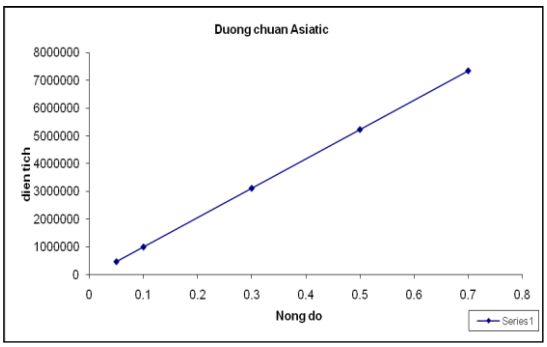


Fig. 1: The established standard curve for asiatic acid

2.2. Plant material

Centella asiatica samples were collected in Sontay, Hanoi City, and Namdinh province in April 2010, the sample from Ho Chi Minh City was collected in May 2010 and identified by Mr. Nguyen The Anh, Institute of Chemistry, VAST, 18 Hoang Quoc Viet, Hanoi.

2.3. Study the conditions for extraction, hydrolyzation and separation of asiatic acid and madecassic acid from *Centella asiatica*

The *Centella asiatica* sample (200 gram each) was dried at room temperature and extracted three times with EtOH/H_2O mixture 80:20 (each time 2 h) at 80^{o}C. After filtration and evaporation of the solvent, the residue was dissolved in 200 mL H_2O/MeOH 80:20 and the solution was extracted with *n*-hexane for the elimination of fats and essential oil. Then it was hydrolyzed with 20 % aqueous NaOH solution at 80 ^{o}C for 2 hours, let to room temperature, extracted with EtOAc. The aqueous layer was acidified with HCl to pH = 3-4 to give a residue. The residue was filtered, washed with H_2O and dried. This residue was given to the column chromatography and to the HPLC determination of the content of asiatic and madecassic acid.

2.4. Identification of asiatic and madecassic acid by TLC

5 mg of the residue were dissolved in 10 mL MeOH and evaluated on the TLC plates (elution solvent: CH_2Cl_2/MeOH 9:1). The developed TLC plates were sprayed with vanillin/H_2SO_4 reagent and heated (≈ 110 ^{o}C) until the spots were visualized.

2.5. Quantification by the HPLC method

Used the obtained standard curves for asiatic acid and madecassic acid.

2.6. Quantification by column chromatography (CC)

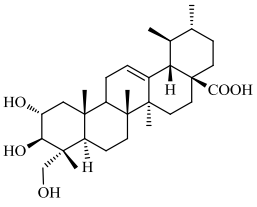
The residue obtained after hydrolyzation with NaOH was given to a column chromatography on SiO_2, eluted with a solvent mixture of CH_2Cl_2/MeOH 95:5 → 90:10 for separation of asiatic (**1**) and madecassic acid (**2**).

The optimal conditions for the extraction and hydrolyzation to obtain high amount of asiatic acid and madecassic acid was investigated. According to our study the best conditions for the extraction are the followings:

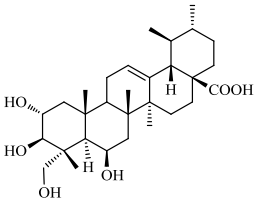
- Solvent for the extraction from *Centella asiatica*: EtOH/H_2O 80:20 (v/v)
- Temperature for the extraction: 80 ^{o}C
- Time of the extraction: 2 hours.

For the basic hydrolyzation:

- Concentration of the aqueous NaOH solution: 20% (w/w)
- Time: 2 hours
- Temperature: 80 ^{o}C, reflux
- Neutralization after hydrolyzation: with 5 % HCl solution.



1: Asiatic acid



2: Madecassic acid

3. RESULTS AND DISCUSSION

With the procedures described in the Experimental part the samples collected in Son Tay, Hanoi (RMST), Nam Dinh province (RMND) and Ho Chi Minh City (RMHCM) have been analyzed. The results of the CC method are given in table 2.

It was shown from the table 2, that in all three *Centella asiatica* samples the total content of madecassic acid is higher than that of the asiatic acid. The total content of asiatic acid and madecassic acid of *Centella asiatica* collected in Son Tay, Hanoi (RMST) is the highest, and in Ho Chi Minh (RMHCM) City is the lowest. The contents of asiatic acid and madecassic acid in RMHCM are similar. The hydrolyzed residues from three *Centella asiatica* samples were analyzed by the HPLC method. The total content in percent of asiatic acid and madecassic acid in the residue obtained after the hydrolyzation as well as calculated for the dry material (*Centella asiatica*) determined by HPLC are given in table 3.

Table 2: The total content of asiatic acid and madecassic acid in three *Centella asiatica* samples

Sample	Weight	Extract residue (g)	Residue after hydrolyzation (g)	Asiatic acid (g) (% to dry plant material)	Madecassic acid (g) (% to dry plant material)
RMST	200	30.12	4.25	1.02, (0.51)	1.54, (0.77)
RMND	200	29.25	4.02	0.92, (0.46)	1.58, (0.79)
RMHCM	200	25.0	1.67	0.43, (0.21)	0.49, (0.25)

Table 3: The total contents in percent of asiatic acid and madecassic acid in three *Centella asiatica* samples determind by HPLC method

Constituent	RMST 4.25 g residue		RMND 4.20 g residue		RMHCM 1.67 g residue	
	% Area	% in dry material	% Area	% in dry material	% Area	% in dry material
Asiatic acid	33.81	0.718	30.49	0.615	28.92	0.241
Madecassic acid	41.93	0.891	43.02	0.865	28.61	0.239

In comparison of the data in table 2 and 3 it can be concluded that the contents of asiatic acid and madecassic acid obtained by the CC method were lower than estimated by the HPLC method, namely about 71 % and 86 % of the HPLC method, respectively.

Acknowledgements. *This work has been supported financially by Vietnam National Foundation for Science and Technology Development (NAFOSTED) project code 104.01-2012.33.*

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