Southeast-Asian J. of Sciences, Vol. 6, No. 1 (2018) pp. 73-79

ISOLATION OF HIPPADINE FROM THE BULBS OF CRINUM LATIFOLIUM L. AMARYLLIDACEAE

Nguyen Thi Tuyet Nhung[*], **Huynh Cong Tin**[*], **Phan Van Ho Nam**[†], and **Vo Thi Bach Hue**[†]

Faculty of Pharmacy,
Lac Hong University, Dong Nai, Vietnam
e-mail: tuyetnhung@lhu.edu.vn

[†]*Faculty of Pharmacy*
Ho Chi Minh University of Medicine and Pharmacy
Ho Chi Minh city , Vietnam
e-mail: vothibachhue@gmail.com

Abstract

The purpose of study is extraction and isolation of hippadine from the bulbs of Crinum latifolium for using as a reference standard for quality control of herbs and hippadine-containing preparations. Bulbs powder of Crinum latifolium was percolated with 70% ethanol, percolate obtained was evaporated at 65 ^0C to give a liquid extract. The extract was fractionated by distribution with n-hexane. The n-hexane extract was then chromatographed on silicagel 60 (0.04 - 0.063 mm). Hippadine was isolated, purified and elucidated by NMR, MS, IR and UV-Vis spectrum. The purity of hippadine was determined by HPLC-PDA. About 565,3 mg of hippadine was isolated and its purity was detemined as pure as 100%. Hippadine was isolated with the purity suitable for using as reference standard for quality control of herbs and hippadine-containing preparations.

1. Introduction

Amaryllidaceae is a widely spread family on the world, it contains about 90 genera and 1310 species (Benson L., 1970). The genus Crinum is an important

Key words: hippadine, Amaryllidaceae, Crinum latifolium, isolation.

representative of family Amaryllidaceae with broad geographical distribution throughout the tropics, subtropics and warm moderate regions (Mabberly DJ., 2008). And Crinum latifolium L. (Amaryllidaceae) has been used in Asian folk and traditional medicine in the treatment of various illnesses like rheumatism, fistula, tumors, earaches, rubefacient, tubercle and whitlow, it's also used in prostatitis adenoma, benign prostate enlargement (Ghosal S et al., 1985). In Vietnamese and Chinese traditional medicine, Crinum latifolium (CL) are used until nowadays because of its anti-inflammatory, anti-tumour and anti-microbial effects. These effects are mainly attributed to the presence of many different alkaloids in CL (Loi DT., 2000).

Today, a large number of functional foods, especially drugs from CL are circulating in the Vietnamese market such as Crila, CrilaOPC, Tadimax, etc. Therefore, the management and quality control of these products is essential, the study was carried out with the aim of isolating hippadine from the bulbs of Crinum latifolium L. as a reference standard for quality control of herbs and hippadine-containing preparations.

Materials and Methods

Plants materials.

The bulbs of CL were collected in Binh Dinh province, Vietnam, in March, 2016. The bulbs were identified and authenticated by Prof. Vo Thi Bach Hue, Analytical Department, Medicine and Pharmacy University of Ho Chi Minh city, Vietnam. After collection, the bulbs were cleaned and shade-dried in a cool place and then ground into a homogeneous powder.

Instrumentation.

The structure of the pure compound was characterized by spectral analysis. UV-Vis spectroscopy was performed on Thermo Scientific Evolution 300. Fourier-transform infrared spectroscopy (IR) was performed on Shimadzu IRTracer-100. Mass spectroscopy (MS) was performed on Shimadzu LCMS-8040. Nuclear magnetic resonance spectroscopy (NMR) was recorded on Bruker Avance 500 Mhz using CDCl3 solvent. The purity was determined by high performance liquid chromatography - photodiode array detector (HPLC - PDA) Agilent 1260.

Methods.

Bulbs powder of CL was percolated with 70% ethanol, percolate obtained was evaporated at 65 ^0C to give a liquid extract. The extract was fractionated by distribution with n-hexane.

Isolation was performed by conventional column chromatography for the separation of the pure compound from n-hexane crude extract. The column was prepared using silicagel (0.40 - 0.063 mm) by wet packing method. The column prepared for the separation and isolation of the phytoconstituents should be

properly grasped till the complete analysis. The sample was mixed uniformly and was introduced into the column using a long Pasteur pipette followed by addition of the eluent slowly from the top without disturbing the column. The eluent strength was changed from least polar solvent (n-hexane) system to polar solvent (methanol).

The pure compound was isolated, purified and elucidated by NMR, MS, IR and UV-Vis spectrum. The isolated compound was determined its purity by thin layer chromatography with three different solvent systems and by high performance liquid chromatography (HPLC).

Results and Discussion

Extract and Isolation.

Bulbs powder of CL (10 kg) was percolated with 70% ethanol. The ethanolic extract was heated to 65 ^{0}C to concentrate solution, give a 4200 g of concentrated ethanolic extract. Then, the extract was dissolved in 500 ml of HCl 1%. The obtained acidic ethanolic solution was filtered through paper and then was fractionated by distribution with n-hexane.

The n-hexane crude extract was chromatographed by conventional column chromatography. About 60,2 g of n-hexane extract was loaded into the column (85 × 7.5 cm) with 900 g of silica gel (40 - 63 μm) and mobile phases were n-hexane, chloroform, ethyl acetate and methanol.

After the implementation of column chromatography, 10 fractions were obtained, denoted by F1-F10. From the F3 fraction (n-hexane-chloroform (60:40)), an amorphous solid called CL-1 was achieved. CL-1 was crystallized in n-hexane, washed several times with cold n-hexane. Finally, we obtained 565,3mg of a colorless crystal CL-1.

Structure elucidation.

CL-1 was isolated as a white amorphous solid. It was a poor polarized compound, soluble in chloroform, slightly soluble in methanol and reacted with Dragendorff reagent.

UV-Vis spectrum in methanol for CL-1: $\lambda_{max}(nm)$ *at 248, 299 and 348 nm*

FT-IR spectrum for the CL-1: IR $\nu(cm^{-1})$ *1668,43 (C=O); 1618,28 (C=C); 1024,2 (- O-CH2-O-); and 929,69 - 632 (C=C).*

MS spectrum for the CL-1: It had the chemical formula C16H9NO3 as determined by ESI-MS (+) (286,0405 m/z $[M+Na]^+$*, calcd for* $[C16H9NO3Na]^+$ *).*

NMR spectrum for the CL-1:

^{1}H-NMR (125 MHz, $CDCl_3$): δ_H (ppm) 6,14 (2H; s; H-12); 6,87 (1H; d; J = 3,5 Hz; H-4); 7,44 (1H; t; J = 7,5 Hz; H-2); 7,59 (1H; s; H-11); 7,71 (1H; d; J = 7,5 Hz; H-3); 7,86 (1H; d; J = 7,5 Hz; H-1); 7,94 (1H; s; H-8); 8,01 (1H; d; J = 3,5 Hz; H-5).

Nature	CL-1
Color	White
Molecular formula	$C_{16}H_9NO_3$
Molecular weight	263
Mass	286,0405 $[M+Na]^+$
UV-Vis/ methanol λ_{max} (nm)	248, 299 and 348 nm
IR υ (cm^{-1})	1668,43 (C=O); 1618,28 (C=C); 1024,2 (- O-CH2-O-); 929,69 - 632 (C=C)

The ^{1}H-NMR spectrum displayed signals for five aromatic protons, two olefinic doublets, and a methylenedioxy group. Three of the five aromatic protons were determined to be in the same spin system due to the observed coupling constants from δ_H 7,86 (1H; d; J = 7,5 Hz), ?H 7,71 (1H; d; J = 7,5 Hz), and δ_H 7,44 (1H; t; J = 7,5 Hz). The doublet at δ_H 7,86 is most likely the result of unresolved meta coupling. The remaining two aromatic signals are both isolated singlets at δ_H 7,94 (1H; s), and δ_H 7,59 (1H; s). There was also a characteristic methylenedioxy resonance at δ_H 6,14 (2H, s). Lastly, the two olefinic protons were both coupled to one another with resonances at δ_H 6,87 (1H; d; J = 3,5 Hz) and δ_H 8,01 (1H; d; J = 3,5 Hz).
^{13}C-NMR (500 MHz, $CDCl_3$): δ_C (ppm) 101,7 (C-11); 102,3 (C-12); 108,0 (C-8); 110,6 (C-4); 116,6 (C-11a); 118,3 (C-1); 122,5 (C-3); 122,6 (C-11b); 123,5 (C-5); 123,9 (C-2); 128,4 (C-3a); 130,9 (C-11c); 131,6 (C-7a); 148,5 (C-10); 152,5 (C-9); 158,1 (C-7).

Comparison of the ^{1}H-NMR and ^{13}C-NMR literature data for hippadine (Thuy NHL., 2014) with the observed spectroscopic data confirmed the identity of CL-1 as the known compound hippadine. (Figure 1)

Determination of the purity

Thin layer chromatography was applied to test the purity of CL-1, with three different solvent systems: chloroform-n-hexane (1:4); toluene - acetone (3:1); n-hexane - acetone (7:3), detected by UV 365-254 and Dragendorff reagent, hippadine gave a single spot on the chromatograms.

The isolated compound - hippadine was determined its purity by HPLC-PDA with chromatographic conditions:

- HPLC system of Agilent 1260.
- Column: Eclipse RP C18 (100 x 4.6 mm; 3.5 μm).
- Mobile phase: Acetonitrile - acid H3PO4 pH 3 (46:54).
- Flow rate: 1 ml / min.
- Volume of injection: 10 μl.

Table 2. NMR spectral data of isolated compound CL-1 and Hippadine

Atom	CL-1		Hippadine	
	δC (*ppm*)	δH (*ppm*)	δC (*ppm*)	δH (*ppm*)
1	118,3	7,86 (1H; d; j=7,5 Hz)	118.4	7,91 (1H; d; j=8 Hz)
2	123,9	7,44 (1H; t; j=7,5 Hz)	124,0	7,47 (1H; t; j=8 Hz)
3	122,5	7,72 (1H; d; j=8 Hz)	122,6	7,74 (1H; d, j=8 Hz)
3a	128,4	-	128,4	-
4	110,8	6,87 (1H; s; j=3,5 Hz)	110,8	6,90 (1H; d; j=3,5 Hz)
5	123,5	8,01(1H; d; j=3,5 Hz)	123,5	8,04 (1H; d; j=3,5 Hz)
7	158,1	-	158,2	-
7a	131,6	-	131,7	-
8	108,0	7,94 (1H; s)	108,4	7,97 (1H; s)
9	152,5	-	152,6	-
10	148,5	-	148,6	-
11	101,7	7,60 (1H; s)	101,7	7,64 (1H; s)
11a	116,6	-	116,7	-
11b	122,6	-	122,6	-
11c	130,9	-	131,0	-
12	102,3	6,14 (2H; s)	102,3	6,16 (2H; s)

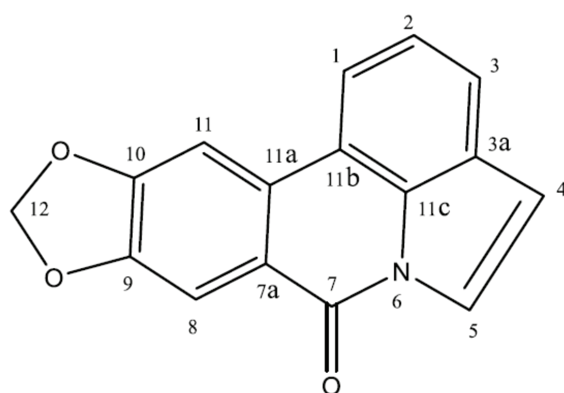

Figure 1. Structure of hippadine

- Column temperature: 30 ^{0}C.
- Wavelength of detector: 299 nm.
- Sample concentration: 10 μg / ml.

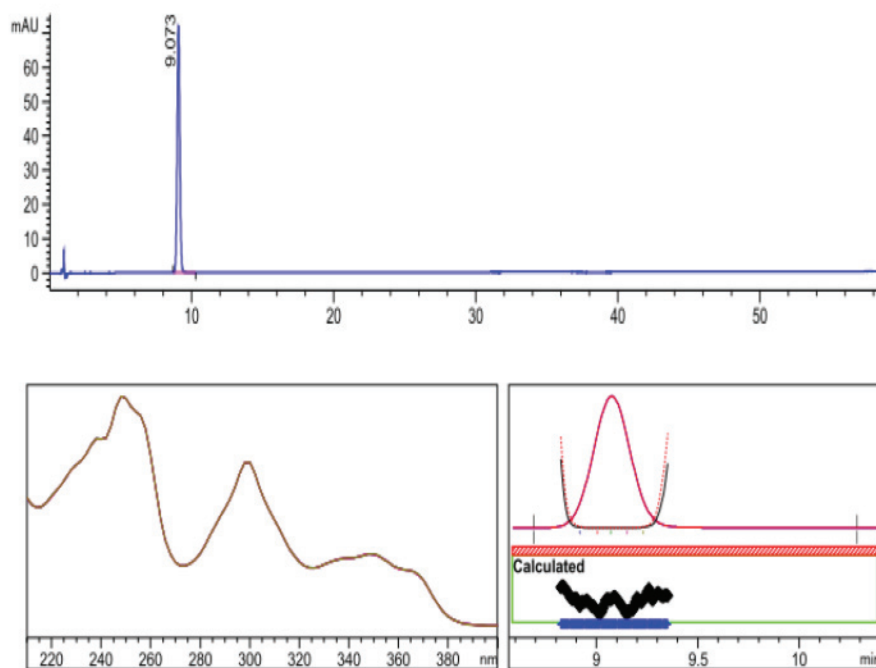

Figure 2. HPLC chromatogram of hippadine

The HPLC chromatogram of hippadine showed only one peak at 9,073 min (except the peak of solvent) with 100% purity. The result is shown in Figure 2.

Conclusions

In conclusion, 565,3 mg of hippadine was isolated and its purity was detemined as pure as 100%. Hippadine was isolated with the purity suitable for using as reference standard for quality control of herbs and hippadine-containing preparations.

Hippadine is a very weak base, a poor polarized alcaloid of the indole framework. In the acidic environment, the hippadine in the form of salt is easily return to the form of alglycon, therefore, hippadine was isolated from the n-hexane extract in the acidic environment.

References

[1] L. Benson, "Handbook of Plant Classification", 1970.

[2] S. Ghosal, K.S. Saini, and S. Razdan, *Crinum alkaloids: their chemistry and biology,* Phytochemistry, **24**(10) (1985), 2141-2156.

[3] D.T. Loi, "Traditional Plants of Vietnam" (in Vietnamese), Ha Noi Medicinal Publisher, 2000, 515-516.

[4] D.J. Mabberley, "The plant-book: a portable dictionary of the vascular plants", Cambridge University press, 2008.

[5] N. H. L. Thuy, "Research on chemical constituents, establish standard references and procedures for determination alkaloid and flavonoid of Crinum latifolium L., Amaryllidacceae" (Doctoral dissertation, Ho Chi Minh University of Medicine and Pharmacy), 2014.