organomercurials, *The Journal of Organic Chemistry*, 58, 2081-2085. 11. Shen C. C., Chang Y. S., Ho L. K. (1993), Nuclear-magnetic-resonance studies of 5, 7-dihydroxyflavonoids, *Phytochemistry*, 34(3), 843-845. 12. Lima C. S., Mottin M., de Assis L. R., Mesquita N. C. M. R., Sousa B. K. P., Coimbra L. D., Santos K. B., Zorn K. M., Guido R. V. C., Ekins S., Marques R. E., Proença-Modena J. L., Oliva G., Andrade C. H., Regasini L. O. (2021), Flavonoids from *Pterogyne nitens* as Zika virus NS2B-NS3 protease inhibitors, *Bioorganic Chemistry*, 109, 104719.

Journal of Medicinal Materials, 2023, Vol. 28, No. 3 (pp. 145 - 150)

CHEMICAL CONSTITUENTS FROM THE TUBER ROOT OF *STEMONA TUBEROSA*

Nguyen Thi Hong Anh[1], Dang Viet Hau[1], Nguyen Thi Thu Trang[1], Nguyen Van Tai[1,*], Pham Huu Nhat[2], Nguyen Thi Thuy Loan[2]

[1]*National Institute of Medicinal Materials (NIMM), Hanoi, Vietnam;*

[2]*Edupharm International Joint Stock Company, Hanoi, Vietnam*

*Corresponding author: nguyenvantai1111@gmail.com

(Received March 24th, 2023)

Summary

Chemical Constituents from the Tuber Root of *Stemona tuberosa*

Phytochemical investigation of the tuber root of *Stemona tuberosa* Lour resulted in the isolation of five compounds. They are composed of two alkaloids, a phenolic acid, an anthraquinone, and another unidentified compound. The structures of these compounds were confirmed using spectroscopic methods such as ESI-MS and NMR, and by comparing them with published spectroscopic data. The isolated compounds were identified as tuberostemonine O (**1**), neotuberostemonine (**2**), *p*-hydroxybenzoic acid (**3**), hydroxymethylfurfural (**4**), and physcion (**5**). Among them, compounds **1**, **3**, and **5** were isolated for the first time from *S. tuberosa* collected in Vietnam.

Keywords: *Stemona tuberosa, Alkaloid, Tuberostemonine O.*

1. Introduction

Stemona tuberosa Lour (Stemonaceae) is known as a traditional medicine in China and certain regions of South Asia [1]. In Vietnam, this plant, named as Bach Bo, which is a hairless, perennial herb that can reach lengths of 4-10 m [2]. The tuberous root of *S. tuberosa* (Radix Stemonae) has long been used in traditional medicine for its antitussive properties, lung moisturizing, and cough-suppressing effects, as well as its ability to combat parasites [3]. Phytochemical investigations on *S. tuberosa* have revealed the presence of various components including stilbenoids, tocopherols, and alkaloids (predominantly stemonine, stemine, tuberostemonine, oxytuberostemonine, isotuberostemonine, etc.). Alkaloids and stilbenoids have been recognized for their antitussive and antibacterial activities, while dehydrotocopherol derivatives exhibit antioxidant properties [4]. Recent attention has been drawn to the extracts from *S. tuberosa* due to their diverse biological functions, particularly their potential as anti-cancer agents [3]. Despite the wide distribution and significant utilization of *S. tuberosa* in Vietnam, there have been limited studies on its chemical composition [5],[6],[7],[8],[9]. Therefore, further investigations on the chemical constituents of this important medicinal plant in Vietnam are warranted. The present study aims to investigate the composition of tuber roots of *S. tuberosa*, and this article describes the isolation and structural elucidation of five compounds from the dichloromethane and ethyl acetate extracts of *S. tuberosa*.

2. Materials and methods

2.1. Plant material

The tuber roots of *Stemona tuberosa* Lour were collected in Phu Tho province in January 2022, and were taxonomically identified by MSc. Phan Van Truong (Department of Medicinal Material Resources, NIMM).

2.2. General experimental procedures

NMR spectra were recorded on Bruker AM 500 and 600 FT-NMR spectrometers (Karlsruhe, Germany) with TMS as an internal standard, *J* in Hz at 294 K. MS data were analyzed with an LC-MS/MS (Shimadzu, Japan). *Silica gel* (Merck, 0.040 - 0.063 mm particle size) and RP-C_{18} (Merck, 30 - 50 µm particle size) were used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* 60F_{254} (Merck, *silica gel*, 0.25 mm) and RP-18F_{254} (Merck, 0.25 mm) plates. Spots were detected under UV 254 and 366 nm or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating or Dragendorff's reagent.

2.3. Extraction and isolation

The tuber roots of *S. tuberosa* (5.0 kg) were extracted with 70% ethanol by refluxing for 3 hours. After three repetitions, the combined extracts were combined and concentrated under reduced pressure at 50°C, resulting in a residue. The residue was then sequentially extracted with *n*-hexane, dichloromethane, and ethyl acetate, followed by

evaporation to obtain the corresponding fractions: *n*-hexane (18.6 g), dichloromethane (25.1 g), and ethyl acetate (30.5 g).

The dichloromethane fraction (23.1 g) was subjected to chromatography on *silica gel* using a gradient of *n*-hexane - acetone (100:1 to 0:1, v/v) and divided into 10 subfractions (D1-D10). Subfraction D5 (1.65 g) was further chromatographed on *silica gel* CC using *n*-hexane - ethyl acetate (15:1 - 2:3, v/v) as the eluent, resulting in the isolation of compound **1** (15 mg). Subfraction D8 (2.21 g) was purified using *silica gel* CC with a gradient of *n*-hexane - ethyl acetate (100:1 to 0:1, v/v), yielding 5 smaller fractions (D8.1-D8.5). Subfraction D8.3 (0.1 g) was further separated using Sephadex LH-20 (MeOH), leading to the isolation of compound **2** (8 mg).

The ethyl acetate fraction (25.5 g) was chromatographed on *silica gel* using a gradient of dichloromethane - methanol (100:1 to 0:1, v/v), resulting in 12 subfractions (E1-E12). Subfraction E3 (4.0 g) was chromatographed on *silica gel* CC using dichloromethane - methanol (30:1 - 2:3, v/v) as the eluent, yielding compound **3** (10 mg). Subfraction E5 (2.18 g) was separated using RP-C_{18} gel CC with a gradient of methanol - water (1:3, 2:3, 1:1, v/v), resulting in the isolation of compound **4** (15 mg). Subfraction E6 (2.32 g) was purified using *silica gel* CC with a gradient of dichloromethane - methanol (5:1 - 2:3, v/v), yielding 4 smaller subfractions (E6.1 - E6.4). Subfraction E6.2 (0.12 g) was further separated using RP-C_{18} gel CC, eluting with methanol - water (1:2, v/v), leading to the isolation of compound **5** (10 mg).

Compound **1**: Yellow amorphous powder, $[\alpha]^{25}_D$ +11.0 (c, 0.1, $CHCl_3$), ESI-MS: *m/z* 376.2 $[M+H]^+$, ^{1}H-NMR (600 MHz, acetone-d_6), ^{13}C-NMR (150 MHz, acetone-d_6) and ^{1}H-NMR (600 MHz, $CDCl_3$), ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Compound **2**: Yellow powder, $[\alpha]^{25}_D$ +83 (c, 0.1, $CDCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Compound **3**: White powder, ESI-MS: *m/z* 136.7 $[M-H]^-$. ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H: 7.8 (2H, d, J = 7.5 Hz, H-2, H-6), 6.8 (2H, d, J = 7.5 Hz, H-3, H-5). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: 121.4 (C-1), 131.5 (C-2, C-6), 115.1 (C-3, C-5), 161.6 (C-4), 167.1 (C-7).

Compound **4**: Yellow oil, ESI-MS: *m/z* 127.3 $[M+H]^+$. ^{1}H-NMR (600 MHz, acetone-d_6) δ_H: 9.56 (1H, s, H-1), 7.36 (1H, d, J = 3.5 Hz, H-3), 6.57 (1H, d, J = 3.5 Hz, H-4), 4.64 (2H, s, H-6). ^{13}C-NMR (150 MHz, acetone-d_6) δ_C: 178.3 (C-1), 153.2 (C-2), 124.1 (C-3), 110.3 (C-4), 162.7 (C-5), 57.3 (C-6).

Compound **5**: Yellow amorphous powder, ESI-MS: *m/z* 283.5 $[M-H]^-$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 6.68 (1H, d, J = 2.4 Hz, H-2), 7.36 (1H, d, J = 2.4 Hz, H-4), 7.62 (1H, d, J = 2.4 Hz, H-5), 7.07 (1H, dd, J = 0.6; 2.4 Hz, H-7), 2.45 (3H, s, CH_3), 3.93 (3H, s, OCH_3), 12.09 (1H, s, OH-8), 12.29 (1H, s, OH-1). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 165.2 (C-1), 108.2 (C-2), 166.6 (C-3), 106.8 (C-4), 121.3 (C-5), 148.5 (C-6), 124.5 (C-7), 162.5 (C-8), 190.8 (C-9), 182.0 (C-10), 133.2 (C-11), 113.7 (C-12), 110.3 (C-13), 135.3 (C-14), 22.2 (CH_3), 56.1 (OCH_3).

3. Results and discussion

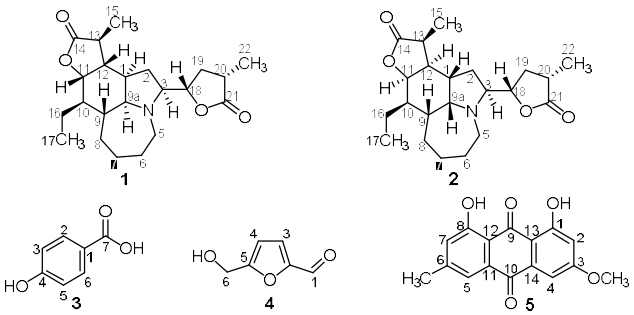

Fig. 1. Chemical structures of compounds **1-5**

Compound **1** was obtained as a light yellow amorphous powder and positive to Dragendorff's reagent. The molecular formula $C_{22}H_{33}NO_4$ of **1** was derived from the ESI-MS (*m/z* = 376.2 $[M+H]^+$) data. The ^{1}H-NMR spectrum of **1** (Table 1) exhibited signals for three methyl groups at δ_H 1.29 (3H, d, J = 7.8 Hz, H-15), 0.95 (3H, t, J = 7.2 Hz, H-17), and 1.19 (3H, d, J = 7.2 Hz, H-22). Two geminal protons appeared at δ_H 2.30 (1H, m, H-5α) and 3.31 (1H, dt, J = 13.2, 3.6 Hz, H-5β) and two protons of methine groups in the γ-lactone ring were observed at δ_H 4.36 (1H, m, H-18) and 4.38 (1H, m, H-11). Based on the combination of ^{13}C-NMR and HSQC spectra (Table 1), **1** was found to contain 22 carbons, including two carbonyl carbons at δ_C 179.8 (C-14) and 179.4 (C-21), two oxygenated carbons at δ_C 83.2 (C-11) and 82.5 (C-18), two *N*-methine carbons at δ_C 67.6 (C-3) and 70.6 (C-9a), an *N*-methylene carbon at δ_C 53.2 (C-5), three methyl carbons at δ_C 17.9 (C-15), 12.2 (C-17), and 15.1 (C-22), six methylene carbons at δ_C 30.3 (C-2), 31.2 (C-6), 32.1 (C-7), 24.2 (C-8), 24.2 (C-16), and 34.7 (C-19), and nine methine carbons. The spectroscopic data support the structural resemblance of **1** to tuberostemonine, a signature alkaloid commonly found in species within the *Stemona* genus [1].

The HMBC correlations of H_3-15 to C-12/C-13/C-14, H-11, H-12, H-13 to C-14, and H2-6′ to C-4′/C-5′ (Fig. 1) suggested the presence of an α-methyl-γ-lactone ring in the structure of **1**. The presence of an ethyl cyclohexane ring was verified by the HMBC correlations from H-10 to C-9/C-9a/C-11, H-9a to C-1/C-12, H-11 to C-1, C-16 and H3-17 to C-16/C-10. Furthermore, the HMBC correlations from H-9a to C-1/C-3/C-5/C-8/C-12, H-8 to C-9a/C-10, H-1 to C-2/C-9a/C-12, and H-5 to C-3/C-7/C-9a led not only to the construction of a pyrrolidine ring involving C-1, C-2, C-3, C-9a, and N but also to the connection of this ring to the 5 azepan ring involving C-5, C-6, C-7, C-8, C-9, C-9a, and N. The HMBC correlations from H-5 to C-3/C-9a and from H-9a to C-3/C-5 indicated that the presence of two 2 heterocycles formed by the linkage of the nitrogen atom, C-5-C-9a, and C-3-C-5. On the basis of the molecular formula and the observed NMR data mentioned above, the remaining nitrogen atom should be a tertiary amine, and one more ring was needed to fulfill the degree of saturation. In addition, the HMBC correlations (Fig. 2.) observed between H-11, H-13, H-15 to C-14, H-22, H-20, H-18 and C-21; H-22 to C-19/ C-20/C-21 allow to determine another α-methyl-γ-lactone ring. This ring binds to C-3 through the HMBC interaction between H-18 and C-3/C-2.

The correlations of H-12 (δ_H 2.14) with H-18 (δ_H 4.36), H-9 (δ_H 2.01), H-15 (δ_H 1.29), H-9 (δ_H 2.01) with H-17 (δ_H 0.95) together with of H-13 (δ_H 2.30) with H-9a (δ_H 3.04), H-22 (δ_H 1.19), H-9a (δ_H 3.04) with H-1 (δ_H 2.00) were also confirmed by the NOESY spectrum. Besides, the chemical shift of proton in the methyl group (CH_3-17) at δ_H 0.95 revealed the β-orientation, which was the same as in cases of tuberostemonine-type alkaloid from *Stemona tuberosa* species [7]. The results demonstrated that protons H-9, H-12, H-15, H-17, H-18, were β-oriented and protons H-1, H-9a, H-13, H-22 were α-oriented.

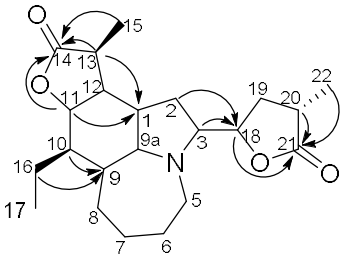

Fig. 2. Important HMBC (H→C) correlations of 1

Based on the analysis of 1D and 2D spectral data (measured in acetone-d_6 solvent) of compound **1** and comparison of the spectral data of compound **1** (measured in $CDCl_3$ solvent) with the spectral data of tuberostemonine O (measured in $CDCl_3$) [7], we have identified compound 1 as tuberosteomine O. Thus, compound **1** was determined as tuberostemonine O.

Table 1. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of compounds tuberostemonine O (**1**) and neotuberostemonine (**2**)

	Tuberosteomonine O	Comp 1					Neotuberostemoine	Comp 2	
Pos.	#δ_C (CDCl3)	a,bδ_C (CDCl3)	a,bδ_C (Aceton-d6)	a,cδ_H (Aceton-d6)	HMBC H → C	NOESY H → H	#δ_C (CDCl3)	a,bδ_C (CDCl3)	a,cδ_H (CDCl3)
1	37.0	36.9	37.9	2.04 (m)	C-2, C-9a, C-12		37.4	37.5	1.76 (m)
2	29.8	29.6	30.3	1.18 (m) 2.13 (m)	C-3, C-9a, C-18	H-9a	32.8	32.9	1.64 (m)
3	66.7	66.5	67.6	2.82 (dd, 7.2, 14.4)	C-18, C-5, C-19		66.6	66.7	3.33 (dd, 7.8, 15.6)
4						H-22			
5	52.4	52.3	53.2	3.31 (dt, 3.6, 3.2) 2.29 (m)	C-3, C-7, C-9a		50.0	50.1	3.08 (m) 2.95 (m)
6	30.6	30.4	31.2	1.49 (m) 1.64 (m)	C-8		29.8	29.9	1.68 (m) 1.68 (m)
7	31.5	31.3	32.1	1.18 (m) 1.98 (m)	C-9		23.2	23.2	1.48 (m) 1.62 (m)
8	23.7	23.5	24.2	1.43 (m) 1.51 (m)	C-9a, C-10,		28.9	29.0	1.93 (m)
9	39.3	39.2	40.3	2.01 (m)	C-3, C-8	H-12	36.3	36.4	1.83 (m)
9a	69.8	69.6	70.6	3.04 (dd, 7.2, 9.6)	C-1, C-3, C-5, C-8, C-12		66.2	66.3	3.19 (t, 4.2)
10	42.9	42.8	43.6	1.57 (m)	C-8, C-9, C-9a, C-11, C-17		34.8	35.0	1.73 (m)
11	83.4	83.1	83.2	4.38 (m)	C-1, C-14, C-16	H-19β	80.4	80.5	4.52 (t, 3.0)
12	45.7	45.5	46.0	2.14 (m)	C-1, C-9a, C-14	H-15, H-18	41.8	41.9	2.08 (m)
13	40.6	40.4	41.0	2.30 (m)	C-1, C-11, C-14, C-15		42.5	42.7	2.88 (t, 7.2)
14	180.4	180.1	179.8				179.3	179.5	
15	18.1	17.9	17.9	1.29 (d, 7.8)	C-12, C-13, C-14		10.2	10.3	1.23 (d, 7.2)

	Tuberosteomonine O	Comp 1					Neotuberostemoine	Comp 2	
Pos.	#δC (CDCl3)	a,bδC (CDCl3)	a,bδC (Aceton-d6)	a,cδH (Aceton-d6)	HMBC H → C	NOESY H → H	#δC (CDCl3)	a,bδC (CDCl3)	a,cδH (CDCl3)
16	24.2	24.1	24.2	1.52 (m) 1.44 (m)	C-9, C-11, C-17		21.1	21.1	1.37 (m) 1.69 (m)
17	12.1	11.9	12.2	0.95 (t, 7.8)	C-10, C-16		11.2	11.3	1.00 (t, 7.2)
18	82.1	81.9	82.5	4.36 (m)	C-2, C-3		78.9	79.0	4.39 (m)
19	34.5	34.3	34.7	252 (m) 1.59 (m)	C-3, C-18, C-20, C-21, C-22		34.5	34.7	2.37 (m)
20	35.1	34.9	35.3	2.68 (m)	C-19, C-21, C-22		34.9	34.9	2.61 (m)
21	179.6	179.3	179.4				179.1	179.3	
22	15.2	15.0	15.1	1.19 (d, 7.2)	C-19, C-21	H-2, H-3	14.8	14.9	1.26 (d, 7.2)

Compound 2 was purified as a yellow powder. It also has a characteristic yellow color on TLC with dragendoff reagent, so we predict it to be an alkaloid. The molecular formula $C_{22}H_{33}NO_4$ could be deduced from its NMR data. The ^{1}H-NMR and ^{13}C-NMR spectra of **2** were similar to those of **1**. The ^{1}H NMR spectrum of compound **2** has signals distributed in the high and medium fields, which are quite typical for an alkaloid from *S. tuberosa*. The ^{1}H-NMR spectrum of **2** included the signals of 3 methyl groups at δ_H 1.23 (3H, d, J = 7.8 Hz, H-15), 1.00 (3H, t, *J* = 7.2 Hz, H-17) and 1.03 (3H, d, J = 7.2 Hz, H-22). Two protons at the oxygenated carbons were shown at δ_H 4.36 (1H, m, H-18) and 4.38 (1H, m, H-11). The methylene protons (δ_H 1.48-3.45 ppm) were attributed to the 14 protons of two saturated rings (the lactone ring and the nitrogen-containing heterocyclic ring) - the two components of the alkaloids skeleton structure of *S. tuberosa*.

The ^{13}C-NMR and DEPT spectra showed 22 carbons including three methyls, seven methylenes, ten methines, and two carbonyls. The above characteristic data suggested that **2** is a tuberostemonine alkaloid. Three carbon signals at δ_C 10.3 (15-CH_3), 11.3 (17-CH_3), and 14.9 (22-CH_3) were assigned to three methyl groups. Seven methylene carbons at δ_C from 21.1 to 50.1 ppm. These methylene groups belong to two δ-lactone rings which are characterized for alkaloids from *S. tuberosa*. In addition, there were 2 carbonyl carbons at δ_C 179.5 (C-14) and 179.3 (C-21).

Compound 2 is an isomer of compound **1** so the configuration of compound **2** is determined based on the difference in chemical shift of the respective positions (H-1, H-9a, H-11, H). -18) compared with compound 1 (table 1), combined with reference comparison of polar rotation and NMR data. The ^{1}H and ^{13}C-NMR spectra of compound **2** also consisted of the corresponding data of the published neotuberostemonine [8]. Therefore compound **2** was neotuberostemonine. This compound is an tuberostemonine-type alkaloid that was first found in 1994 in *S. tuberosa* [9]. Neotuberostemonine had anti-inflammatory and anti-infective properties, and immunomodulatory effects [10].

Compound 3 was obtained as a white powder. The ESI-MS data exhibited an ion peak at *m/z* 136.7 $[M-H]^-$, indicating the molecular formula of **3** to be $C_7H_6O_3$. In the ^{1}H-NMR spectrum, **3** displayed two double signals at δ_H 7.8 (2H, d, *J* = 7.5 Hz, H-2, H-6) and δ_H 6.8 (2H, d, *J* = 7.0 Hz, H-3, H-5), corresponding to a para-disubstituted benzene ring. The ^{13}C-NMR spectrum confirmed the presence of a disubstituted benzene ring at the para positions (δ_C 121.4, 115.1, 131.5, 161.6) and a carbonyl group at δ_C 167.1. Based on the combined analysis of the ^{13}C-NMR and ^{1}H-NMR spectral data, along with literature references, **3** was identified as p-hydroxybenzoic acid [11]. This compound was naturally found in carrots (*Daucus carota*), oil palm (*Elaeis guineensis*), grapes (*Vitis vinifera*), east African satinwood (*Fagara macrophylla*), yellow leaf tree (*Xanthophyllum rubescens*), or can be chemically synthesized [12]. It exhibited antibacterial, antifungal, antialgal, antimutagenic, antisickling, and estrogenic activities [13].

Compound 4 was obtained as a yellow oil, and its molecular formula was determined to be $C_6H_6O_3$ based on the positive ion peak at *m/z* 127.3 $[M+H]^+$ in the ESI-MS. The ^{1}H-NMR spectrum revealed the presence of an aldehyde group at δ_H 9.56 (1H, s, H-1) and a hydroxymethylene group at δ_H 4.64 (2H, s, H-6). Two methine groups [δ_H 7.36 (1H, d, *J* = 3.5 Hz, H-3) and 6.57 (1H, d, *J* = 3.5 Hz, H-4)] were also observed. The ^{13}C-NMR spectrum displayed six carbon signals. Thus, the chemical structure of **4** was identified to contain a furan ring, an aldehyde group (CHO), and a methylene oxygen group (CH_2OH). Comparison of the data with the literature confirmed **4** as hydroxymethylfurfural [14]. It has been isolated from various species including *Laurencia undulata* [15], *Cornus officinalis*, and species of the genus *Schisandra* [16]. Moreover, hydroxymethylfurfural was also

found in honey, apple juice, orange juice, beer, wine, milk, cereals, and tomato products. Notably, hydroxymethylfurfural exhibited anti-cancer, antioxidant, anti-allergic, and anti-inflammatory activities [15].

Compound 5 was obtained as a yellow amorphous powder. The molecular formula of **5** was determined to be $C_{16}H_{12}O_5$ based on ESI-MS, which exhibited a pseudo-molecular ion peak at *m/z* 283.5 $[M-H]^-$. The ^{1}H-NMR spectrum indicated the presence of a methyl group at δ_H 2.45 (3H, s, CH_3) and a methoxy group at δ_H 3.93 (3H, s, OCH_3). Furthermore, it revealed the presence of four aromatic protons at δ_H 6.68, 7.07, 7.36, and 7.62, corresponding to H-2, H-7, H-4, and H-5, respectively. The ^{13}C-NMR spectrum exhibited 16 carbon signals, including two deshielded carbon signals at δ_C 190.8 and 182.0, assigned to two ketones. The remaining five signals at δ_C 148.5, 135.3, 133.2, 113.7, and 110.3 were attributed to aromatic carbons. Three signals at δ_C 166.6, 165.2, and 162.5 indicated carbon atoms on the aromatic nucleus bearing oxygen substituents. Additionally, signals corresponding to two methyl groups at δ_C 22.1 and 56.1 were observed. Based on the ^{1}H-NMR and ^{13}C-NMR spectral data, **5** was identified as 3-methyl-1,8-dihydroxy-6-methoxy-9,10-anthraquinone, also known as **physcion** [17].

According to published findings, a total of 10 compounds have been isolated from *S. tuberosa* in Vietnam. Among them, 7 compounds were alkaloids, namely neotuberostemonine, bisdehydrotuberostemonine [6], tuberostemonine, stanine, isotuberostemonine, isostemonine, and oxotuberostemonine [18],[19]. Additionally, 3 non-alkaloid compounds have been identified, including hydroxymethylfurfural, *trans*-4-hydroxycinnamate [20], and methyl ferulate [2]. This study contributes to demonstrate that the chemical composition of S. tuberosa in Vietnam has some chemical composition similar to *S. tuberosa* having studied in the world, that have not been found *S. tuberosa* in Vietnam. Currently in Vietnam, there is no published study on the content of compounds isolated from this study. In the world, also there is no research on the content of compound **1** in *S. tuberosa*. Compound **2** has a content of 0.5 - 3.2% in *S. tuberosa* [21]. In this study, we isolated 5 compounds from *S. tuberosa* collected in Son La, comprising 2 alkaloids and 3 non-alkaloids. Notably, tuberostemonine O (**1**), *p*-hydroxybenzoic acid (**3**), and physcion (**5**) were isolated for the first time from this plant in Vietnam.

4. Conclusion

Five compounds (**1-5**) were isolated from the tuber of *S. tuberosa* collected in Son La, Vietnam. Their chemical structures were identified as tuberostemonine O (**1**), neotuberostemonine (**2**), *p*-hydroxybenzoic acid (**3**), hydroxymethylfurfural (**4**), and physcion (**5**) through MS, 1D, and 2D-NMR spectral analysis, and comparison with published literature. Compounds **1**, **3**, and **5** were isolated for the first time from this plant in Vietnam. This finding contributes to the chemical composition database of this species in Vietnam and demonstrates the similarity in the chemical composition of *S. tuberosa* grown in Vietnam compared to this species worldwide, which is significant for its use in traditional medicine.

Acknowledgement: *This research was supported by the National Institute of Medicinal Materials (project: Study on the chemical composition of Stemona tuberosa).*

References

1. Xu Y., Xiong L., Y. Yan Y., Sun D., Duan Y., Li H., Chen L. (2022), Alkaloids from *Stemona tuberosa* and their anti-inflammatory activity, *Frontiers in Chemistry*, 10, 74. **2**. Nguyen T. M. P., Tran T. C., Dang N. Q., (2014), Anti-inflammatory activity of methyl ferulate isolated from *Stemona tuberosa* Lour., *Asian Pacific Journal of Tropical Medicine*, 7, S327-S331. **3**. Li Z., Sturm S., Stuppner H., Scharml E., Moser V. A, Siegl V. (2007), The dichloromethane fraction of *Stemona tuberosa* Lour. inhibits tumor cell growth and induces apoptosis of human medullary thyroid carcinoma cells, *Biologics*: *Targets and Therapy*, 1(4), 455-463. **4**. Kil Y. S., Park J., Han A. R., Wo H. A., Seo E. K. (2015), A new 9, 10-dihydrophenanthrene and cell proliferative 3, 4-δ-dehydrotocopherols from *Stemona tuberosa, Molecules*, 20(4), 5965-5974. **5**. Khamko V. A., Dang N. Q. and Pham H. D. (2013), Three new phenanthrenes, a new stilbenoid isolated from the roots of *Stemona tuberosa* Lour. and their cytotoxicity, *Natural Product Research*, 27(24), 2328-2332. **6**. Pham H. D, Phan V. K., Chau V. M. (2000), Alkaloids from the roots of *Stemona tuberosa* Lour., *Journal of chemistry*, 38(2), 64-67. **7**. Kil Y. S., Han A. R. and Seo E. K. (2014), Tuberostemonine O from the roots of *Stemona tuberosa, Bulletin of the Korean Chemical Society*, 35(6), 1891-1893. **8**. Chung H. S., Hon. P. M., Lin G., But P. P., Dong H. (2003), Antitussive activity of Stemona alkaloids from *Stemona tuberosa, Planta Medica*, 69(10), 914-920. **9**. Yang Y., Qin G. W. and Ren-Sheng X. (1994), Alkaloid from *Stemona tuberosa, Phytochemistry*, 37(4), 1201-1203. **10**. Jung J. E., Kil Y. S., Park H. R., Oh S., Kim H. K., Jeong M. G. (2014), Suppression of IL-2 production and proliferation of CD4+ T Cells by Tuberostemonine O, *Chemistry & Biodiversity*, 11(12), 1954-1962. **11**. Peungvicha P., Temsiririrkkul R., Prasain J. K., Tezuka Y., Kadota S., Thirawarapan S. S., Watanabe H. (1998), 4-Hydroxybenzoic acid: a hypoglycemic constituent of aqueous extract of *Pandanus odorus* root, *Journal of ethnopharmacology*, 62(1), 79-84. **12**. Khadem S., Marles R. J. (2010), Monocyclic phenolic acids; hydroxy-and polyhydroxybenzoic acids: occurrence and recent bioactivity studies, *Molecules*, 15(11), 7985-8005. **13**. Oksana S., Mahendra R., Shao H. (2012), Plant phenolic compounds for food, pharmaceutical and cosmetics production, *Journal of Medicinal Plants Research,* 6(13), 2526-2539. **14**. Serra-Cayuela A., Castellari M., Bosch-Fusté J. (2013), Identification of 5-hydroxymethyl-2-furfural (5-HMF) in Cava sparkling wines by LC-DAD-MS/MS and NMR spectrometry, *Food Chemistry*,

41(4), 3373-3380. **15.** Li Y. X., Li Y., Qian Z. J., Kim M. M., Kim S. K. (2009), *In vitro* antioxidant activity of 5-HMF isolated from marine red alga *Laurencia undulata* in free radical mediated oxidative systems, *Journal of Microbiology and Biotechnology*, 19(11), 1319-1327. **16.** Van Putten R. J., Van Der Waal J. C., De Jong E. (2013), Hydroxymethylfurfural, a versatile platform chemical made from renewable resources, *Chemical Reviews,* 113(3), 1499-1597. **17.** Danielsen K., Aksnes D. W., Francis G. W. (1992), NMR study of some anthraquinones from rhubarb, *Magnetic Resonance in Chemistry*, 30(4), 359-360. **18.** Pham T. K., Vu N. K., Nguyen X. D. (1991), A the main alkaloid in roots of *Stemona tuberosa* Lour. in Vietnam, *Pharmacology Journal*, 5, 4-5. **19**. Pfeifer S. (1968), Uber die Alkaloite aus Vietnamesischer *Stemona tuberosa, Die Pharmazie*, 23, 342-343. **20**. Phan T. H., Pham V. C., Bui T. L., Lam T. H. Y., Dang N. Q., Pham H. D. (2014), Chemical constituents of *stemona tuberosa* plant, *Journal of Science of Hnue*, 9(59), 37-42. **21**. Li S. L., Jiang R. W., Hon P. M., But P. P., Shaw P. C. (2007), Quality evaluation of Radix Stemonae through simultaneous quantification of bioactive alkaloids by high-performance liquid chromatography coupled with diode array and evaporative light scattering detectors, *Biomedical Chromatography*, 21, 1088-1094.

Journal of Medicinal Materials, 2023, Vol. 28, No. 3 (pp. 150 - 155)

CHEMICAL CONSTITUENTS FROM THE RHIZOMES OF *ADENIA CARDIOPHYLLA* (MAST.) ENGL.

Ma Chi Thanh*, Dong Thanh Hanh

Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam

*Corresponding author: mcthanh@ump.edu.vn

(Received April 24th, 2023)

Summary

Chemical Constituents from the Rhizomes of *Adenia cardiophylla* (Mast.) Engl.

Adenia cardiophylla is a species in the genus *Adenia* in the Passifloraceae family. The plant's rhizomes have a slightly sweet taste, and they have been used in ethnic medicine in An Giang province to treat lung inflammatory diseases. Five compounds were isolated and identified as 2,3-epoxydeca-4,6,8-triyn-1-ol (**1**), vanillic acid (**2**), demethoxypinoresinol (**3**), succinic acid (**4**), a mixture of deidaclin (**5a**) and tetraphyllin A (**5b**) from the rhizomes of *Adenia cardiophylla*. Their structures were determined by using spectroscopic methods (MS, 1D, and 2D NMR). In addition, the absolute configurations of deidaclin and tetraphylin were identified by comparing experimentally and calculated electronic circular dichroism (ECD) analysis methods.

Keywords: *Adenia cardiophylla*, Passifloraceae, *Cyanogenic glycoside, ECD calculations.*

1. Introduction

Adenia cardiophylla (Mast.) Engl. is a vascular plant in the Passifloraceae family. It is a canopy liana with tendrils up to 20 cm, primarily woody vines 25 m long, and rarely shrubs or trees. It is distributed in the Southeast region of Vietnam, such as Binh Duong, Dong Nai, Ba Ria-Vung Tau, An Giang, and Kien Giang provinces [1],[2],[3]. Several studies on the genus *Adenia* explored the main chemical components of the plants in this genus: toxic proteins [4], cyanogenic compounds [5],[6],[7], lectins [8],[9], polyacetylenes [10], and flavonoids [5]. In addition, species in the genus *Adenia* were reported to possess remarkable biological activities such as anti-oxidative [11], anti-hyperglycaemic [12], anti-spasmodic [13], anti-coagulation [14], anti-depressant [15], anti-anxiety [15], and cytotoxicity effects [9-10]. Until now, most publications on the chemical composition of species in the genus *Adenia* have been carried out on *A. digitata*, *A. gummifera, A. volkensii*, *A. cissampeloides*, *A. globosa*, *A. lobata* and *A. mannii* [4],[5],[6],[7],[8],[9],[10],[11],[12],[13],[14,[15]. To our knowledge, no research has been reported on the phytochemical and pharmacological effects of *Adenia cardiophylla*. Our present study on phytochemical constituents from the ethyl acetate fraction of the rhizomes of *A. cardiophylla* led to the isolation of five compounds. Their structures were identified by spectroscopic data and compared to those reported in the literature. Herein, experimental and calculated ECD spectra analysis confirmed the absolute configuration of compounds **5a**.

2. Experimental

2.1. Materials and Reagents

The rhizomes of *Adenia cardiophylla* (Mast.) Engl. were collected from Tinh Bien town, An Giang province, Vietnam, in November 2018. The voucher specimen (AC112018.AG) was deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City (UMP), Vietnam. The plant was identified by Dr. Vo Van Leo (Department of Pharmacognosy, UMP) and compared to the morphological characteristics as described in botanical references [11]. The solvents used for isolation and extraction were all in analytical reagent (AR) grade: ethanol (96%), *n*-hexane, ethyl acetate, chloroform, methanol, and *n*-butanol (Scharlau, Spain).

2.2. General experimental procedures

Silica gel (40-63 µm, Merck) and Sephadex LH-