Sharma R. P., Baruah J. N., Kulanthaivel P., Herz W. (1984), Flavonoid from *Millettia pulchra*. *Phytochemistry*, 23(2), 443-447. **7.** Yasuda T., Kano Y., Saito K., Ohsawa K. (1994), Urinary and biliary metabolites of daidzin and daidzein in rats. *Biological and Pharmaceutical Bulletin*, 17(10), 1369-1374. **8.** Buyinza D., Derese S., Ndakala A., Heydenreich M., Yenesew A., Koch A., Oriko R. (2021), A coumestan and a coumaronochromone from *Millettia lasiantha*. *Biochemical Systematics and Ecology*, 97, 104277. **9.** Na Z., Fan Q. F., Song Q. S., Hu H. B. (2017), Three new flavonoids from *Millettia pachyloba*. *Phytochemistry Letters*, 19, 215-219. **10.** Li X., Li J., Wang D., Wang W., Cui Z. (2009), Chromone and flavonoids from *Maackia amurensis*. *Asian Journal of Traditional Medicines*, 4(3), 98-103. **11.** Yoon J. S., Sung S. H., Park J. H., Kim Y. C. (2004), Flavonoids from *Spatholobus suberectus*. *Archives of Pharmacal Research*, 27(6), 589-592. **12.** Ngamga D., Yankep E., Tane P., Bezabih M., Ngadjui B. T., Fomum Z. T., Abegaz B. M., Isoflavonoids from seeds of *Millettia griffoniana* (Bail). *Zeitschrift für Naturforschung B*, 60(9), 973-977. **13.** Ding P., Qiu J. Y., Ying G., Dai L. (2014), Chemical Constituents of *Millettia speciosa*. *Chinese Herbal Medicines*, 6(4), 332-334. **14.** Calanasan, C. A., MacLeod J. K. (1998), A diterpenoid sulphate and flavonoids from *Wedelia asperrima*. *Phytochemistry*, 47(6), 1093-1099. **15.** Wang W., Li N., Wang J., Chen G., Huang R., Zhao W., Li J., Si Y. (2016), Bioactive benzofuran-chalcones as potential NQO1 inducers from *Millettia pulchra* (Benth) kurzvar-laxior (Dunn) Z. Wei. *Phytochemistry*, 131, 107-114. **16.** Ashok K. Singhal Nabin C.^Barua Ram P. Sharma Jogendra N. Baruahet (1983), A chalcone and an isoflavone from *Millettia pachycarpa* seeds. *Phytochemistry*, 22(4), 1005-1006. **17.** Pailee P., Mahidol C., Ruchirawat S., Prachyawarakorn V. (2019), Diverse flavonoids from the roots of *Millettia brandisiana*. *Phytochemistry*, 162, 157-164. **18.** Sritularaka B., Likhitwitayawuid K., Conrad J., Kraus W. (2002), Flavonoids from the roots of *Millettia erythrocalyx*. *Phytochemistry*, 61(8), 943-947. **19.** Ganapaty S., Pushpalatha V., Babu G. J., Naidu K. C., Waterman P. G. (1998), Flavonoids from *Millettia peguensis* Ali (Fabaceae). *Biochemical Systematics and Ecology*, 26, 125-126.

Journal of Medicinal Materials, 2022, Vol. 27, No. 4 (pp. 213 - 218)

INVESTIGATION OF CHEMICAL CONSTITUENTS OF *CARDIOSPERMUM HALICACABUM* L. AND THEIR BIOLOGICAL ACTIVITIES

Nguyen Thi Thuy Linh[1,2], Ba Thi Cham[1,2], Nguyen Thi Hoang Anh[1,2], Nguyen Phi Hung[1,3], Do Thi Thao[1,4], Nguyen Thi Loan[5], Le Thi Hong Nhung[5], Domenico V Delfino[6], Trinh Thi Thuy[1,2,]*

[1]*Graduate University of Science and Technology, Vietnam Academy Science and Technology;*

[2]*Institute of Chemistry, Vietnam Academy Science and Technology;*

[3]*Institute of Natural Products Chemistry, Vietnam Academy Science and Technology;*

[4]*Institute of Biotechnology, Vietnam Academy Science and Technology;*

[5]*Hanoi University of Industry, Vietnam;*

[6]*Department of Medicine and Surgery, University of Perugia, Perugia, Italy*

*Corresponding authors: thuy@ich.vast.vn

(Received May 09th, 2022)

Summary

Investigation of Chemical Constituents of *Cardiospermum halicacabum* L. and their Biological Activities

Four compounds were isolated from *n*-hexane and *n*-butanol extracts of *Cardiospermum halicacabum* growing in Vietnam by combined column chromatographic methods. Based on 1D-, 2D-NMR, ESI MS analyses, and comparisons with those published in work of literatures, the structures of these compounds were established as 3*β*-hydroxyglutin-5-ene (**1**), zerumbone (**2**), uridine (**3**), and daucosterol (**4**). It is intriguing to point out that, three out of them (**1-3**) were detected for the first time from the family Sapindaceae. Additionally, the cytotoxic activity of compound **3** was assessed against five human cancer cell lines (A549, T24, Huh-7, 8505, SNU-1) using the SRB method. Additionally, **3** was also evaluated for immunomodulatory capacity through stimulation of IL-2 expression in macrophages (RAW264.7). Compound **3** significantly stimulated IL-2 expression in RAW264.7 cells at a concentration of 100 µg/mL ($P<0.05$) while no affection was observed at all lower tested concentrations.

Keywords: *Cardiospermum halicacabum* L., *Uridine*, *Immunomodulatory effect, Cytotoxic activity.*

1. Introduction

Far from being a small family, Sapindaceae comprises about 144 genera and 1900 species, which are widely distributed in temperate to tropical regions [1]. Plants belonging to this family have been the topic of research in many phytochemical investigations and pharmacological activities due to their popular applications in traditionally medicinal treatments. One of the most studied species from the family, *Cardiospermum halicacabum* L. attracted the researcher's attention because of their containing structurally complex and biologically fascinating secondary metabolites such as alkaloids, carbohydrates, fatty acids, flavonoids, triterpenoids, and volatile esters [2],[3],[4].To now, many pharmacological investigations have been conducted on *C. halicacabum* extracts to

show the potential cytotoxic against various human cancer cells such as breast cancer (MCF-7), and Ehrlich Ascites carcinoma (EAC), and hepatocellular carcinoma (Hep-G2) and immunomodulatory effects [5],[6],[7]. These results encouraged scientists to find out phytocompounds, which are responsible for the anticancer and immunomodulating activities of *C. halicacabum* extracts. Taking all the above-mentioned factors into account, our present study aims to isolate and identify four different metabolites, together with its test for cytotoxic and immunomodulatory activities.

2. Materials and methods

2.1. Materials

Plant materials: The aerial parts of *C. halicacabum,* collected at Hoa Binh province, Vietnam in June 2021 were used as plant materials for this study. The scientific name of this plant was identified by taxonomist MSc. Nghiem DucTrong, Ha Noi University of Pharmacy. A voucher specimen (No. ICH-CH/2021) was stored at the Institute of Chemistry, VAST, Ha Noi.

Cells: The five carcinoma cell lines used in this study, including A549 (lung carcinoma), T24 (urinary bladder carcinoma), Huh-7 (hepatocarcinoma), 8505c (undifferentiated thyroid carcinoma), and SNU-1 (gastric carcinoma), were kindly provided by Prof. JM Pezzuto at Long Island University (US) and Prof. CY Huang at National Yang Ming Chiao Tung University (Taiwan). All media used for cell culture of solid cancer cell lines (A549, T24, Huh-7, 8505, and SNU-1), including serum and other reagents, were obtained from GIBCO Co. Ltd. (Grand Island, New York, USA).

2.2. General experimental procedures

The extraction, isolation, and structural elucidation of purified compounds were supported by: *Silica gel* 60 (230-400 mesh, Merck) and Sephadex LH-20 (for column chromatography); HR ESI MS was performed on an Agilent 6530 Accurate Mass Q-TOF instrument. Bruker Avance III NMR spectrometer, Switzerland (for ^{1}H NMR (500.13 MHz) and ^{13}C NMR (125.77 MHz)). Compounds were detected by thin layer chromatography and visualized by spraying with aqueous 10% H_2SO_4 and heating for 5 minutes.

2.3. Extraction and isolation

The above-ground parts of *C. halicacabum* were dried, powdered (10.2 kg), and then extracted with aqueous EtOH (15% H_2O) for three times, each time 24 hours. The combined ethanolic extract was evaporated in *vacuo* and then was partitioned successively with *n*-hexane, ethyl acetate (EtOAc), and *n*-butanol (three times for each) to yield the respective weights of 1.320 g, 720 g, and 540 g. To purify compounds, some eluated solvent systems were used like *n*-hexane-EtOAc (95:5→70:30), *n*-hexane-CH_2Cl_2 (5→30% CH_2Cl_2), EtOAc-MeOH (95:5→70:30), CH_2Cl_2-MeOH (90:10→75:25).

The *n*-hexane extract (1320 g) was chromatographed on *silica gel* column, eluting with gradient *n*-hexane-EtOAc (95:5→70:30) to afford 7 fractions (H1 - H7). Fr. H2 (250 g) was re-chromatographed on a *silica gel* column with an eluatedgradientsolvent system (*n*-hexane-CH_2Cl_2, 5 → 30% CH_2Cl_2) to afford 10 subfractions (H2.1-H2.10). Compound **2** (12 mg) was obtained by purification of subfraction H2.5 (170 mg) using *silica gel* column, *n*-hexane-CH_2Cl_2, 90:10. The separation of fraction H3 (320 g) was carried out on repeated *silica gel* columns with suitable evaluated solvent systems to furnish compound **1** (10 mg).

The *n*-BuOH extract (540 g) was given on a Sephadex LH-20 column, eluting with MeOH to furnish six fractions (B1 - B6). Fraction B3 (12.5 g) was reseparated on *silica gel* column, using EtOAc-MeOH (95:5→70:30) as evaluated solvent and then on Sephadex LH-20, MeOH to yield compound **3** (7 mg). The separation of fraction B5 (23.1 g) was carried out at first with a Sephadex LH-20 column, MeOH, and then with a *silica gel* column, CH_2Cl_2-MeOH (90:10→75:25), to yield compound **4** (15 mg).

3β-Hydroxyglutin-5-ene (**1**): White powder; ESI-MS: *m/z* = 427 $[M+H]^+$; ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR spectroscopic data (125 MHz, $CDCl_3$), see Table 1.

Zerumbone (**2**): Colorless crystal; ESI-MS: *m/z* = 218 $[M+H]^+$; ^{1}H (500 MHz, $CDCl_3$) and ^{13}C NMR spectroscopic data (125 MHz, $CDCl_3$), see Table 1.

Uridine (**3**): Colorless powder; HR ESI MS: *m/z* 279.0388 $[M+Cl]^-$ (calc. for $C_9H_{12}ClN_2O_6$, 279.0384), *m/z* 243.0620 $[M-H]^-$ (calc. for $C_9H_{11}N_2O_6$, 243.0617); ^{1}H- (500 MHz, CD_3OD) and ^{13}C-NMR spectroscopic data (125 MHz, CD_3OD), see Table 1.

Daucosterol (**4**): White powder. ^{1}H- (500 MHz, DMSO-d_6): δ_H 5.33 (1H, br s, H-6), 4.25 (d, *J*=8.0 Hz, glc H-1′), 4.60, 4.56, 4.16, 3.67 (glc H-2′- H-6′), 3.47 (br s, H-3), 2.95 (1H, t, *J*=8.0 Hz), 0.98 (3H, brs, CH_3-18), 0.92 (3H, d, *J*=6.5 Hz, CH_3-21), 0.85 (3H, t, *J*=8.5 Hz, CH_3-29), 0.82 (3H, d, *J*=7.0 Hz, CH_3-27), 0.79 (3H, d, *J*=6.7 Hz, CH_3-26), 0.67 (s, CH_3-19).

2.4. In vitro cytotoxic activity

Cancer cells were grown as monolayers in DMEM, supplemented with 2 mM of L-glutamine, 10 mM of HEPES, 1.0 mM of sodium

pyruvate, and 10% fetal bovine serum - FBS (GIBCO). Cells were sub-cultured every 3 days and maintained at 37°C in a humidified atmosphere containing 5% CO_2. Cytotoxicity of the isolated compound **3** was determined using methods from the American National Cancer Institute as previously described [8]. Assay sample, compound **3**, was initially dissolved in 100% DMSO and serially diluted to appropriate concentrations as mentioned below with culture medium immediately before the cytotoxicity assay. Cells were subsequently treated with 10 µL of compounds **3** or ellipticine as a positive control to reach final concentrations of 100 µg/mL, 20 µg/mL, 4 µg/mL, 0.8 µg/mL, and 0.16 µg/mL. The plates were then incubated for 48 h. The medium was discarded, and cells were fixed using 10% trichloroacetic acid. The fixed cells then were stained for 30 min by a staining solution sulforhodamine B (SRB). Protein-bound dye was dissolved in a 10 mMTris-base solution, and optical densities were measured at 510 nm using an ELISA plate reader (Biotek ExL800). The IC_{50} values were calculated using Table Curve 2Dv4 software. Ellipticine (Merck) was used as a positive control. The values reported for compound **3** are presented as averages of five determinations.

2.5. Evaluation of interleukin 2 expression

RAW 264.7 cells were seeded in a 96-well plate (2.5×10^5 cells/well). After 24h of incubation in 37°C incubator, tested compounds (10 µL) were added into the cells at different concentrations as 100 µg/mL, 20.0 µg/mL, 4.0 µg/mL, and 0.80 µg/mL. The wells of negative controls were set in presence of cells (190 µL) and DMSO 1% (10 µL). Then, lipopolysaccharide (LPS) (1 µL/mL) was added to the wells and continuously incubated for 24h. Finally, cell culture supernatants were taken under consideration to quantify of interleukin 2 by using IL-2 mouse ELISA Kit as instruction of the kit manufacturer (R&D systems, US).

3. Results and Discussion

From *n*-hexane and *n*-butanol extracts of the aerial parts of *C. halicacabum,* four compounds were isolated using chromatographic methods. Their chemical structures were elucidated including two terpenoids (**1** and **2**), one nucleoside (**3**), and one steroid glucoside (**4**) (Fig.1).

Compound **1** was obtained as a white powder from *n*-hexane extract of *C. halicacabum* aerial parts. Based on the ^{13}C-NMR spectroscopic data and combination with the ESI-MS data *m/z* 427 $[M+H]^+$, molecular formula of **1** was identified as $C_{30}H_{50}O$. ^{1}H- and ^{13}C-NMR spectra of **1** indicated the typical characters of a glutinane-type triterpenoid. The ^{13}C-NMR spectrum of **1** showed the signals of 30 carbons, whereas, from ^{1}H-NMR spectrum, eight methyl groups of singlet signals were clearly detected at δ_H1.04 (H-23), 1.14 (H-24), 0.85 (H-25), 1.09 (H-26), 1.00 (H-27), 1.16 (H-28), 0.95 (H-29), and 0.99 (H-30). The presence of a double bond at C-5/C-6 was predicted by ^{13}C chemical shifts at δ_C141.7 and 122.1, which was then agreed by signal of olefinic proton at δ_H 5.63 (dd, J = 4.0, 2.0 Hz, H-6). In addition, one oxymethine proton at δ_H 3.46 (br d, J = 12.0 Hz, H-3) suggested the presence of hydroxy group at C-3 position (δ_C76.4). As compared to spectroscopic data in the literature, compound **1** was determined to be 3β-hydroxyglutin-5-ene [9]. To now, many triterpenoids have been found from Sapindaceae family [10], but this is the first time triterpene 3β-hydroxyglutin-5-ene (**1**) was detected from this family.

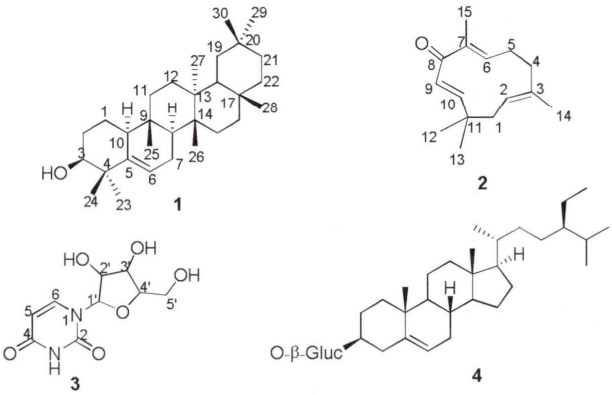

Fig. 1. Chemical structures of isolated compounds **1-4**

Compound **2**, a colorless crystal, was also isolated from *n*-hexane extract of the investigated plant. A combination of ESI-MS *m/z* at 218 $[M+H]^+$ and NMR spectral data determined the molecular formula of **2** was $C_{15}H_{22}O$, suggesting a sesquiterpene skeleton. From fifteen carbon signals in ^{13}C-NMR spectrum, one carbonyl carbon was underlined at δ_C 204.3 (C-8). Whereas, the ^{1}H-NMR spectra of **2** showed two proton signals at δ_H 5.98 (d, J = 16.5 Hz, H-9) and 5.86 (d, J = 16.5 Hz, H-10) corresponding to a *trans*-double bond at C-9/C-10; four tertiary methyl groups at δ_H 1.54 (s, H-12), 1.80 (s, H-13), 1.07 (s, H-14), and 1.20 (s, H-15); two trisubstituted double bonds at δ_H 5.25 (dd, J = 12.0, 3.0 Hz, H-2) at C-2/C-3 and δ_H6.02 (dd, J = 8.5, 1.0 Hz, H-6) at C-6/C-7. Depend on the above-mentioned spectroscopic analysis, structure of **2** was determined to be zerumbone, whose spectroscopic data were in good agreement with the reported literature previously

isolated from *Zingiber zerumbet* [11]. Zerumbone (**2**), a popular monocyclic sesquiterpene in medicinal plants. It has been widely investigated for both *in vitro* and *in vivo* pharmacological activities such as anti-inflammatory, analgesic, immunomodulatory, and anticancer [12],[13].

Table 1. NMR spectroscopic data of compounds **1**, **2**, and **3**

C	1, $CDCl_3$		2, $CDCl_3$		C	3, CD_3OD	
	δ_C	δ_H (mult.)	δ_C	δ_H (mult.)		δ_C	δ_H (mult.)
1	18.2	-	42.4	2.35 (m) 1.90 (d, 12.0)	1	-	-
2	27.8	-	125.0	5.25 (dd, 12.0, 3.0)	2	152.6	-
3	76.4	3.46 (br d, 12.0)	136.3	-	3	-	-
4	40.8	-	39.5	2.23 (m)	4	166.3	-
5	141.7	-	24.4	2.46 (m) 2.23 (m)	5	102.7	5.71 (d, 8.3)
6	122.1	5.63 (dd, 4.0, 2.0)	148.8	6.02 (dd, 8.5, 1.0)	6	142.7	8.02 (d, 8.3)
7	23.7	-	138.0	-	1'	90.8	5.92 (d, 4.5)
8	47.5	-	204.3	-	2'	75.7	4.20 (dd, 5.0, 10.0)
9	34.9	-	127.2	5.98 (d, 16.5)	3'	71.3	4.18 (dd, 5.0, 10.0)
10	49.7	-	160.7	5.86 (d, 16.5)	4'	86.4	4.00 (m)
11	33.2	-	37.9	-	5'	62.3	3.86 (dd, 3.0, 12.5) 3.75 (dd, 3.5, 12.5)
12	30.4	-	15.2	1.54 (s)		-	-
13	37.9	-	11.8	1.80 (s)		-	-
14	39.3	-	29.4	1.07 (s)		-	-
15	34.6	-	24.4	1.20 (s)		-	-
16	35.1	-	-	-		-	-
17	30.1	-	-	-		-	-
18	43.1	-	-	-		-	-
19	36.1	-	-	-		-	-
20	28.3	-	-	-		-	-
21	32.1	-	-	-		-	-
22	39.0	-	-	-		-	-
23	29.07	1.04 (s)	-	-		-	-
24	25.5	1.14 (s)	-	-		-	-
25	16.2	0.85 (s)	-	-		-	-
26	18.4	1.09 (s)	-	-		-	-
27	19.6	1.00 (s)	-	-		-	-
28	32.4	1.16 (s)	-	-		-	-
29	34.5	0.95 (s)	-	-		-	-
30	32.1	0.99 (s)	-	-		-	-

Compound **3** was furnished as colorless powder from *n*-butanol extract. Molecular formula of **3** was determined as $C_9H_{12}N_2O_6$ basing on the combination of HR-ESI-MS (negative ion) data *m/z* 243.0620 $[M-H]^-$ (calc. for $C_9H_{11}N_2O_6$, 243.0617), *m/z* 279.0388 $[M+Cl]^-$ (calc. for $C_9H_{12}ClN_2O_6$, 279.0384) and the NMR spectroscopic data. For that, the ^1H NMR spectrum showed the presence of an AB system at δ_H 5.71 (d, J = 8.3 Hz, H-5) and 8.02 (d, J = 8.3 Hz, H-6) and the anomeric proton signal at δ_H 5.92 (d, J = 4.5 Hz, H-1') demonstrated the presence of a sugar unit in **3**. Additionally, the ^{13}C-NMR spectra indicated two carbonyl groups at δ_C 166.3 (C-4) and 152.6 (C-2) and the signals of 5 carbons for sugar unit ranged from δ_C 90.8 to 62.3, indicating the presence of a ribose ring. These observations suggested a structure of nucleotide glycoside uridin for **3**, whose spectroscopic data was accordant with those of the published data [14]. Uridine (**3**) was first represented as a nucleoside in the chemical components of *C. halicacabum*.

Compound **4** was found as white powder from *n*-butanol extract. The ^{13}C-NMR spectrum of **4** indicated the presence of 35 carbon atoms, of which 29 were assigned to the aglycon moiety, while the remaining 6 were attributed to the sugar moiety (δ_C 61.1-76.9 and 100.7). From ^1H-NMR spectrum, typical proton signals at δ_H 5.33 (br s, H-6), 3.47 (br s, H-3), 0.79 (d, J=6.7 Hz, CH_3-26), 0.82 (d, J=7.0 Hz, CH_3-27), 0.85 (t, J=8.5 Hz, CH_3-29), 0.92 (d, J=6.5 Hz, CH_3-21), 0.67 (s, CH_3-19), 0.98 (brs, CH_3-18) supported us to find out structure of aglycon as sitosterol. The protons of β-glucose were observed at δ_H 3.67-4.60 and

4.25 (d, *J*=8.0 Hz, glc H-1′). Based on the above data and comparison with those reported data, compound **4** was identified as sitosterol-3-*O*-*β*-glucopyranoside (daucosterol) [15],[16].

In our continuing search for potential cytotoxic agents from medicinal plants [17],[18], we herein report the cytotoxic activities of isolated compounds from *C. halicacabum* species. Except for **3**, compounds **1**, **2**, and **4** have been investigated for cytotoxic activities against different cancer cell lines such as colon carcinoma (SW480), and prostate cancer cells [12],[19]. Hence, in this report, uridine (**3**) was chosen for cytotoxic test against five human cancer cells, including lung carcinoma (A549), urinebladder carcinoma (T24), hepatocarcinoma (Huh-7), undifferentiated thyroid carcinoma (8505), gastric carcinoma (SNU-1). The results indicated that **3** showed no cytotoxic activity against five tested cancer cells, regardless of concentration.

According to recently published reports, many nucleosides have been found to serve as a potential source of immunomodulatory effects [20]. Therefore, nucleoside **3** was chosen for immunomodulatory activity assay through IL-2 expression. Because IL-2 autocrinally stimulates T cell proliferation, this cytokine plays a crucial role in the immune system [21]. Hence, IL-2 expression is an important factor for a proper immune response.

The results showed that, compound **3** had significantly stimulated on IL-2 cytokine secretion in RAW264.7 cells at a concentration of 100 µg/mL ($P<0.05$ compared with the negative control), while at lower concentrations (20.0, 4.0 and 0.8 µg/mL), **3** exhibited unsignificant effect as compared to the control ($P > 0.05$) (Table 3).

Table 2. Cytotoxic activity of uridine (**3**) and ellipticine[b] by using SRB method

Tested samples	Concentration (µg/mL)	% Inhibition				
		A549[a]	T24[a]	Huh-7[a]	8505[a]	SNU-1[a]
Uridine (3)	100	17.26	18.83	11.99	22.07	19.74
Ellipticine[b]	2	71.99	71.89	78.52	82.38	80.33

[a]Cell lines: A549 (lung carcinoma), T24 (urinebladdercarcinoma), Huh-7 (hepatocarcinoma), 8505 (undifferentiated thyroid carcinoma) and SNU-1 (gastric carcinoma).
[b]Ellipticine: positive control.

Table 3. Effects of uridine (**3**) on IL-2 expression and control LPS[a]

Concentration (µg/mL)	Fold times change in IL-2 content compared to negative control
100	1.42* ± 0.18
20	0.97 ± 0.27
4	0.97 ± 0.09
0.8	0.94 ± 0.14
LPS[a]	4.16*** ± 0.05
Negative control	1 ± 0.01

[a]LPS:Lipopolysaccharide; *P<0.05 and ***P<0.001 compared to negative control.

4. Conclusion

In conclusion, our present study reported four compounds isolated from aerial parts of *C. halicacabum* collected in Hoa Binh, Vietnam. These isolates were 3*β*-hydroxyglutin-5-en (**1**), zerumbone (**2**), uridine (**3**), and daucosterol (**4**). Although, nucleosides are popular compounds, but this is the first time this group of compounds was found in *C. halicacabum* chemical components via detection of uridine (**3**). Our studies presented for the first time indirect evidence of immunomodulation through IL-2 expression of compound **3**. This compound significantly stimulated IL-2 expression at concentration of 100 µg/mL as compared to the negative control ($P<0.05$).

Acknowledgements: *The authors wish to thank the National Foundation for Science and Technology Development of Vietnam (NAFOSTED) for financial support (code 40/2021/TN 48.G06-0006) and Nguyen Thi Thuy Linh acknowledges financial support by the Ph.D. Scholarship Programme of Vingroup Innovation Foundation (VINIF), code VINIF.2021.TS.071.*

Rererences

1. Acevedo R. P., Welzen P. C., Adema F., Ham R. W. J. M. (2010), Sapindaceae, *Flowering Plants. Eudicots,* Springer246 (1789) 357-407. **2.** Deepan T., Alekhya V., Saravanakumar P., Dhanaraju M. D. (2012), Phytochemical and anti-microbial studies on the leaves extracts of *Cardiospermum halicacabum* Linn., *Advances in Biological Research*, 6(1), 14-18. **3.** Rao V., Chandra P. N. K., Shanta K. S. M. (2006), Pharmacological investigation of *Cardiospermum halicacabum* (Linn) in different animal models of diarrhea, *Indian Journal of Pharmacology,* 38(5), 346-349. **4.** Manju S.S., Mohammad A. (2019), A review of *Cardiospermum halicacabum* (Sapindaceae), *World Journal of Pharmacy and Pharmaceutical Sciences*, 8(5), 410-420. **5**. Aishwarya V., Abdulla S. S., Dheeba B., Renuka R. (2014), *In vitro*antioxidant and anticancer activity of *Cardiospermum halicacabum* L. against EAC cell

line, *International Journal* of *Pharmacy* and *Pharmaceutical Sciences*, 6(8) 263-268. **6**. Sagadevan P., Suresh S. N., Rathishkumar S., Gayathri S., Eswari D. V. (2013), Anticancer activity of methanolic leaf extract of *Andrographis paniculata* (Nees) and *Cardiospermum halicacabum* (Linn.) against human breast cell cancer cell line (MCF-7), *International Journal of Pharmaceutical and Life Sciences*, 4(9), 2983-2986. **7**. Jacob B., Natarajan S., Pattiyappan S., Sivaraman R., Suresh M. S. (2015), Study on anti-carcinogenic and apoptotic properties of methanolic stem extract of *Cardiospermum halicacabumin* MCF7 cell line, *Journal of Scientific and Innovative Research*, 4(1), 43-48. **8**. Ba Thi Cham, Nguyen Thi Thuy Linh, Nguyen Thi Hoang Anh, Nguyen Phi Hung, Do Thi Thao, Nguyen Quyet Tien, Nguyen Thanh Tam, Tran Duc Dai, Dang Ngoc Quang, Nguyen Thi Thu Hoa, Tran Khac Vu, Le Thi Hong Nhung, Domenico Delfino, Trinh Thi Thuy (2021), Cytotoxic and immunosuppressive activity of methyl caffeate isolated from *Balanophora laxiflora, Journal of Science and Technology,* 57(6), 114-118. **9**. Vu Kim Thu, Le Thi Kieu Anh, Dang Ngoc Quang, Nguyen Van Thang, Hoang Le Tuan Anh, Nguyen Xuan Nhiem, Dan Thi ThuyHang, Chau Van Minh, Phan Van Kiem (2015), Triterpenes from the leaves of *Glochidiono bliquum*, *Vietnam Journal of Science, Technology,* 53(2e), 103-106. **10.** Yu L., Tang X., Chen L., Wang M., Jian J., Cao S., Qiu F. (2012), Oleanane-type triterpenoid saponins from *Xanthoceras sorbifolia* Bunge, *Fitoterapia*, 83(8), 1636-1642. **11**. Dai J. R., Cardellina J. H., Mahon J. B., Boyd M. R. (1997), Zerumbone, an HIV-Inhibitory and cytotoxic sesquiterpene of *Zingiber aromaticum* and *Z. zerumbet*, *Natural Product Letters*, 10(2), 115-118. **12**. Sadhu S. K., Khatun A., Ohtsuki T., Ishibashi M. (2007), First isolation of sesquiterpenes and flavonoids from *Zingiber spectabile* and identification of zerumbone as the major cell growth inhibitory component, *Natural Product Research,* 21(14), 1242-1247. **13**. Somchit M. N., Mak J. H., Bustamam A. A., Zuraini A., Arifah A. K., Adam Y., Zakaria Z. A. (2012), Zerumbone isolated from *Zingiber zerumbet* inhibits inflammation and pain in rats, *Journal* of *Medicinal Plants Research*, 6(2) 177-180. **14.** Yalcin F. N., Ersoz T., Akbay P., Calis I., (2003), Phenolic, megastigmane, nucleotide, acetophenon and monoterpene glycosides from *Phlomis samia* and *P. carica, Turkish Journal of Chemistry,* 27(2003), 703-711. **15.** Voutquenne L., Lavaud C., Massiot G., Sevenet T., Hadi H. A. (1999), Cytotoxic polyisoprenes and glycosides of long-chain fatty alcohols from *Dimocarpus fumatus*, *Phytochemistry,* 50(1), 63-69. **16.** Ferrar L., Schettino O., Montesano D., (1996). Triterpenoids from *Cardiospermum halicacabum*, *Phytotherapy* Research, 10 (1), 192-194. **17**. Nguyen Thi Thuy Linh, Ba Thi Cham, Nguyen Thi Hoang Anh, Nguyen Thanh Tam, Dao Duc Thien, Tran Duc Quan, Tran Van Sung, Sabrina Adorisio, Domenico Delfino, Trinh Thi Thuy (2019), Ecdysteroids from *Vitex leptobotrys* inhibit proliferation of acute myeloid leukemia cells, *Vietnam Journal of Science and Technology*, 13-16. **18**. Trinh Thi Thuy, Ba Thi Cham, Nguyen Thi Thuy Linh, Phan Thi Ngoc Bich, Sabrina Adorisio, Domenico Delfino (2022), Phenolic acid analogues from *Balanophora laxiflora* inhibit proliferation of *in vitro* acute myeloid leukemia cells, *Records of Natural Products*, 16(3), 253-258. **19**. Zingue S., Gbaweng Y. A. J., Michel T., Ndinteh D. T., Rutz J., Auberon F., Blaheta R. A. (2019), Bioguided identification of daucosterol, a compound that contributes to the cytotoxicity effects of *Crateva adansonii* DC. (Capparaceae) to prostate cancer cells, *Journal of Ethnopharmacology,* 112251. doi:10.1016/j.jep.2019.112251. **20**. Conta A. C., Stelzner D. J. (2007), Immunomodulatory effect of the purine nucleoside inosine following spinal cord contusion injury in rat, *Spinal Cord*, 46(1), 39-44. **21.** Zimmermann-Klemd A. M., Reinhardt J. K., Morath A., Schamel W. W., Steinberger P., Leitner J., Gründemann C. (2020), Immunosuppressive activity of *Artemisia argyi* extract and isolated compounds, *Frontiers in Pharmacology,*11, 402. doi:10.3389/fphar.2020.00402.

Journal of Medicinal Materials, 2022, Vol. 27, No. 4 (pp. 218 - 222)

CALCIUM OXALATE CRYSTALLIZING INHIBITION OF EXTRACTS FROM SEVERAL *PANDANUS* SPECIES IN VIETNAM

Do Hoang Giang[1], Truong Thi Lien[1], Dang Viet Hau[1], Vu Duc Nam[1], Nguyen Thi Thu Thuy[2], Nguyen Tien Dat[1,*]

[1]Center for Research and Technology Transfer, Vietnam Academy of Science and Technology;
[2]Institute of Tropical Medicine, Vietnam - Russia Tropical Center, Vietnam
*Corresponding author: ntdat@ctctt.vast.vn
(Received June 20th, 2022)

Summary

Calcium Oxalate Crystallizing Inhibition of Extracts from Several *Pandanus* Species in Vietnam

Plants from the genus *Pandanus* were used as traditional medicines for urolithiasis treatment. Evaluation of calcium oxalate crystallizing inhibition of 14 extracts from 4 *Pandanus* species revealed that the extracts from *P. nanofrutex* and *P. amaryllifolius* exhibited stronger effects than those of *P. odoratissimus* and *P. tectorius*. The chemical quantitation and correlation analysis results indicated that phenolics and flavonoids significantly affect the calcium oxalate crystallizing inhibitory effect. These showed promising results for further investigation of antiurolithiatic compounds from *Pandanus* species.

Keywords: *Pandanus, Phenolic, Flavonoid, Alkaloid, Calcium oxalate.*

1. Introduction

Pandanus is one of the most common genera in the family of Pandanaceae which includes about 600 species. Among them, 17 species have been found in Vietnam, such as *Pandanus tectorius* Parkinson ex Du Roi, *Pandanus amaryllifolius* Roxb., *Pandanus odoratissimus* Linn., *Pandanus tonkinensis* B. Stone, *Pandanus nanofrutex* B. Stone, *Pandanus humillis* Lour… [1],[2]. Plants of the genus may vary in size with