FLAVONOIDS AND THEIR CYTOTOXIC ACTIVITY FROM *TITHONIA DIVERSIFOLIA*

CÁC HỢP CHẤT FLAVONOID VÀ HOẠT TÍNH GÂY ĐỘC TẾ BÀO CỦA CHÚNG TỪ LOÀI CÚC QUỲ (*TITHONIA DIVERSIFOLIA)*

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ABSTRACT

Three flavones (**1**-**3**) were isolated from the aerial part of *Tithonia diversifolia (Hemsl.) A. Gray* growing in Hoa Binh province, Vietnam. Their structures were identified as hispidulin (**1**), nepetin (**2**) and luteotin (**3**) by analysis of these spectroscopic MS, NMR data and comparison with reported data. The compounds **1**-**3** showed cytotoxic activity against four human cancer (KB, Hep, Lu1 and MCF7) cell lines with IC_{50} values ranging from 16.13 ± 0.89μg/mL to 99.63±1.02μg/mL. Among them, compound **3** has shown the most potent effect on Hep and KB cell lines with IC_{50} 16.13 ± 0.89μg/mL and 24.45 ± 0.97μg/mL, respectively.

Keywords: *Tithonia diversifolia* (Hemsl.) A.Gray, *hispidulin, nepetin, luteotin,* cytotoxicity.

TÓM TẮT

Ba chất flavon (**1**-**3**) đã được phân lập từ phần trên mặt đất của cây Cúc quỳ (*Tithonia diversifolia* (Hemsl.) A. mọc ở tỉnh Hòa Bình, Việt Nam. Bằng cách phân tích dữ liệu phổ MS, NMR và so sánh với dữ liệu được công bố, cấu trúc của chúng được xác định là hispidulin (**1**), nepetin (**2**) và luteotin (**3**). Các hợp chất **1**-**3** có hoạt tính gây độc tế bào đối với bốn dòng tế bào ung thư người (KB, Hep, Lu1 và MCF7) với giá trị IC_{50} nằm trong khoảng từ 16,13 ± 0,89μg/mL đến 99,63 ± 1,02μg/mL. Trong đó, chất **3** có tác dụng mạnh nhất trên các dòng tế bào Hep và KB với IC_{50} lần lượt là 16,13 ± 0,89μg/mL và 24,45 ± 0,97μg/mL.

Từ khóa: *Cúc quỳ, hispidulin, nepetin, luteotin, gây độc tế bào*.

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1. INTRODUCTION

Tithonia diversifolia (Hemsl.) A. Gray (*T. diversifolia,* Compositae), is a well-known traditional medicinal plant [1]. *T. diversifolia* is used in folk medicine to treat muscle pain, infection, inflammation, diarrhea, fever, hepatitis, and malaria [2-4]. The phytochemical composition of this speciesmainly includes germacrane, eudesmane, and flavone analogues [5]. To date, most of the reports on cytotoxic activity have focused on tagitinins including prostate cancer, breast cancer, colon cancer and lung cancer cell lines. While flavones from this species have been only few publications [6, 7]. As part of our search for bioactive compounds from Vietnamese medicinal plants, this paper reports the isolation and structural elucidation of three flavones, hispidulin (**1**), nepetin (**2**) and luteotin (**3**) from the aerial part of *T. diversifolia* growing in Hoa Binh province, Viet Nam and their cytotoxic activity on KB, Hep, Lu1 and MCF7 cancer cell lines.

2. EXPERIMENTAL

2.1. Plant material

Plant materials were collected in Hoa Binh province, Viet Nam in June 2019. The species, *Tithonia diversifolia (Hemsl.) A. Gray* species, was identified by taxonomist Msc. Nghiem Duc Trong, Hanoi University of Pharmacy. A voucher specimen (No. ICH-TD/2019) was deposited at the Institute of Chemistry, VAST, Hanoi.

2.2. General experimental procedures

HR ESI MS spectrum was obtained on QStar Pulsar (Applied Biosystems).^{1}H NMR (500.13MHz), ^{13}C NMR (125.77MHz), HSQC, HMBC and NOESY spectra were recorded on a Bruker Avance 500 FT-NMR spectrometer at 25°C. The spectra were run as $CDCl_{3+}$ CD_3OD and CD_3OD (^{1}H δ 3.33; ^{13}C δ 49.0) for the internal standard. Coupling constants were reported in Hertz (Hz). Silica gel 60 F-254 (0.25mm, Merck); reversed phase RP_{18} F254S (0.25mm, Merck). CC: Silica gel 60 (230 - 400 mesh, Merck) for the first column, silica gel 60, 40 - 63μm (Merck) and Sephadex LH-20 for the following columns.

2.3. Extraction and isolation

The ground and dried aerial part of *T. diversifolia* (330g) were extracted three times with 80% aqueous EtOH, each ultra-soundedat 45°C for 15 minutes, then left overnight.

EtOH was evaporated *in vacuo,* and the aq. solution was partitioned with *n*-hexane followed by ethyl acetate (EtOAc) (three times each). The organic solvents were evaporated to yield corresponding *n*-hexane (30g), EtOAc (12g) and residue EtOH (10g) extracts, respectively. The EtOAc extract (TDE, 12g) was separated on silica gel using *n*-hexan-EtOAc (80:20→20:80) to afford 10 fractions (Fr1 → Fr10). The fraction (Fr8) was chromatographed on Sephadex LH-20 using MeOH to give 7 sub-fractions (Fr8.1 - Fr8.7). Fraction Fr8.7 (300mg) was further purified by CC on silica gel eluting with *n*-hexan-EtOAc (60:40) to provide compound **1** (28mg, 0.0243 %). Compound **2** (10mg), and **3** (8mg) were isolated from Fr9 (200mg) by chromatography on Sephadex LH-20 and then purified on silica gel eluting with *n*-hexan-EtOAc (60:40).

Hispidulin (4',5,7-trihydroxy-6-methoxyflavon, **1**): Light yellow crystals. HR ESI MS (+): *m/z* 301.0709 $[M+H]^+$ (calcd. for $C_{16}H_{13}O_6$, 301.0712). ^{1}H-, ^{13}C NMR (500/125MHz, $CDCl_3$+CD_3OD, *δ*ppm): See Table 1.

Nepetin (3',4',5,7-tetrahydroxy-6-methoxyflavon, **2**): Yellow amorphous powder. HR ESI MS (+): *m/z* 317.0696 $[M+H]^+$ (calcd. for $C_{16}H_{13}O_7$, 317.0661); ^{1}H-, ^{13}C NMR (500/125MHz, CD_3OD, $\delta_C$ppm): See Table 1.

Luteotin (3',4',5,7-tetrahydroxy flavon, **3**): Yellow amorphous powder. HR ESI MS (+): *m/z* 287.0568 $[M+H]^+$ (calcd. for $C_{15}H_{11}O_6$, 287.0556). ^{1}H-, ^{13}C NMR (500/125MHz, CD_3OD, *δ*ppm): See Table 1.

2.4. *In vitro* cytotoxic evaluation

The 3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay detects the reduction of MTT (Sigma) by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria andhence the measurement of cytotoxicity cell and viabilitys described in literature [8, 9]. Four human cancer cell lines including KB (mouth epidermal carcinoma cells), HepG2 (human liver hepatocellular carcinoma cells), Lu1 (human lung adenocarcinoma cells), and MCF7 (human breast cancer cells). Cells were maintained in Dulbecco's modified Eaglemedium, supplemented with 10% fetal bovine serum, L-glutamine (2mM), penicillin G (100UI/mL), streptomycin (100µg/mL), and amphotericine B (10 µg/mL). Stock solution oftest compounds were prepared in dimethylsulfoxide (DMSO)and diluted in distilled water (H_2O). Briefly, the cytotoxicity of the isolated compounds were carried outin 96-well microplates with 1×10^4 viable cells/mL and incubated at 37°C in air/CO_2 (95:5) with test compounds. After 72 hours incubation, 20µL of MTT were added per well. After 4 hours incubation, medium was removed, 100µL of DMSO were added per well and shaken for 5 to 10 minutes. Viable cells were estimated by optical density at 540nm with Epocher 2 (BioTek) microplates reader. The IC_{50} value wasdetermined as the concentration of compound that inhibits 50% cell growth compared to the control. Ellipticine wasused as a reference compound.

3. RESULTS AND DISCUSSION

Compounds **1**-**3** were isolated as yellow powder from ethyl acetate extract by repeated column chromatography on silica gel and Sephadex LH-20. The molecular formula of **1** was deduced as $C_{16}H_{12}O_6$ from the positive HR ESI MS data at *m/z* 301.0709 $[M+H]^+$ (calcd. for $C_{16}H_{13}O_6$, 301.0712). The ^{1}H-NMR spectrum of **1**, appeared a pair of two doublets at δ 7.83 (H- 2'/H-6') and 6.93 (H-3'/H-5') of four aromatic protons with *ortho* coupling constant (J = 9.0Hz). This data are agreement with two pairs of methine equivalents at *δ*129.43 (C-2'/C-6') and *δ* 117.01 (C-3'/C-5') in the ^{13}C-NMR spectrum. NMR spectral data suggested that ring B has two substituents at C-1' and C-4'. Two other aromatic methines groups resonated at *δ*H 6.60 (1H, s)/*δ*C 103.41 (C-3) and *δ*H 6.57 (1H, s)/*δ*C 95.29 (C-8). The signal appears at low field (δC 184.27) characteristic for the carbonyl group. In addition, the signal at *δ*C 60.94 connected with a single signal with integral strength at *δ*H 3.90 (3H, s) in the HSQC spectrum, is assigned to the methoxy group. From the above analyzed spectral data, the structure of **1** was determined to be 4',5,7-trihydroxy-6-methoxyflavone (hispidulin) [10]. Hispidulin is known to have remarkable anti-cancer and anti-viral activities [6, 11-12].

The molecular formula of **2** ($C_{16}H_{12}O_7$) was deduced from the positive HR ESI MS data at *m/z* 317.0696 $[M+H]^+$ (calcd. for $C_{16}H_{13}O_7$, 317.0661) with one hydroxy group more than compound **1**. The ^{13}C NMR and HSQC spectra of **2** indicated the presence of 16 carbons, including 1xOCH_3, 5xCH (95.2- 123.7), 10xCq including 1 carbonyl group at (*δ*C 184.22) (Table 1). The ^{1}H- and ^{13}C NMR data of **2** were similar to those of **1,** it was apparent that **2**contained an additional hydoxy group at *δ*C 147.01 (C-3') and the other remaining NMR data were similar to those of **1**. The ^{1}H-NMR spectrum of **2**, appeared two singlets at *δ*H 6.54 và 6.53 of H-8 and H-3, respectivety. Two doublets at δH 7.38 and 6.91 with *ortho* coupling constant (each d, J = 9.0Hz) correspond to two aromatic protons H-6' and H-5'. The remaining aromatic doublet signal at *δ*H 7.38 (d, J = 2.0Hz) is assigned to H-2'. In the HMBC, the signal at δH 3.90 (3H) has corelation with δC 132.85 (C-6) confirmed methoxy group connected with C-6. Combination of HR ESI MS, ^{1}H-, ^{13}C NMR, HSQC and HMBC data revealed that compound **2** was 3',4',5,7-tetrahydroxy-6-methoxyflavone (nepetin, eupafolin) [10]. This compound was also reported to have cytotoxic activity against the KB carcinoma cell line [13].

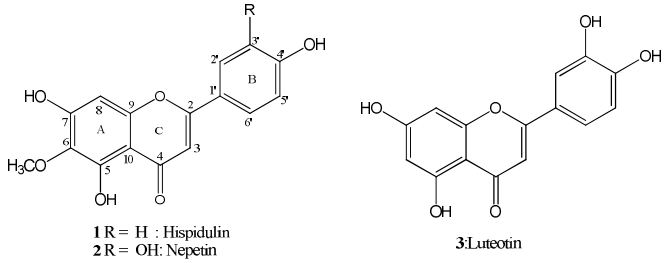

Figure 1. Chemical structures of compounds **1**-**3** isolated from Tithonia diversifolia

Compound **3** was obtained as yellow amorphous powder. Its HR-ESI-MS spectrum (positive ion) showed a *pseudo-molecular* peak at *m/z* 287.0568 $[M + H]^+$ (calcd. for $C_{15}H_{11}O_6$, 287.0556), indicating its molecular formula as $C_{15}H_{10}O_6$. The ^{1}H-, ^{13}C NMR and HSQC spectra proved that **3** was a flavone similar as **2**, except lacking of the methoxy group at ring A. The structure of **3** was confirmed as the luteolin according to the presence of two *meta* aromatic protons at δ_H 6.45 (H-8) and 6.23 (H-6) (each 1H, d, J = 2.2Hz); three ABX aromatic protons at δ_H 7.40 (1H, d, 9.0, H-6'), 6.92 (1H, d, 9.0, H5') and 7.38 (1H, d, 2.0, H-2') in ^{1}H NMR. The ^{13}C NMR and HSQC experiments supported the structure of **3** as 3',4',5,7-tetrahydroxy flavone (luteolin) [10, 15]. The complete ^{1}H- and ^{13}C NMR spectroscopic data of **1**-**3** and HMBC correlations of **2** are shown in Table 1.

Compounds **1**-**3** were evaluated for their cytotoxicity. KB, Hep, Lu1 and MCF7 human cancer cell lines were chosen for screening their inhibition effect using MTT method [8, 9]. Compounds **1**-**3** were initially screened at a fixed concentration of 100μg/mL and subsequently assayed at four concentrations (20.0, 4.0, 0.8 and 0.16μg/mL), and IC_{50} value for each compound was calculated. The cytotoxic effects of these compounds were estimated in terms of growth inhibition percentage and expressed as IC_{50} which is the concentration of compound which reduces the absorbance of treated cells by 50% with reference to the control (untreated cells). As the results shown in Table 2, compound **3** has shown the most moderate cytotoxic effect with IC_{50} values of 16.13 ± 0.89μg/mL and 24.45 ± 0.97μg/mL, meanwhile compound **2** exposed less cytotoxic activity than **3** with IC_{50} values of 43.76 ± 2.08μg/mL and 42.72 ± 0.55μg/mL on Hep and KB cell lines, respectively. All tested compounds **1**-**3** have shown weak effect on Lu1 and MCF7 cell lines with IC_{50} values from 67.28 ± 1.40μg/mL to 99.63 ± 1.02μg/mL, respectively (Table 2).

Table 1. ^{1}H- and ^{13}C NMR data of compounds **1**-**3**[a][125/500MHz, δppm, J in Hz]

C	1, $CDCl_3$ + CD_3OD		2, CD_3OD			3, CD_3OD	
	δ_C	δ_H (mult.)	δ_C	δ_H (mult.)	HMBC correlation (H/C)	δ_C	δ_H (mult.)
2	166.39		166.44			163.21	
3	103.41	6.60 (s)	103.44	6.53 (s)	C-2, C-4, C-10	103.89	6.55 (s)
4	184.27		184.22			183.87	
5	154.00		153.98			165.99	
6	132.87		132.85			100.11	6.23 (d, 2.2)
7	158.72		158.69			166.35	
8	95.29	6.57 (s)	95.24	6.54 (s)	C-7, C-9, C-6	94.99	6.45 (d, 2.2)
9	154.65		154.62			159.41	
10	105.77		105.76			105.32	
1'	123.28		123.72			123.72	
2'	129.43	7.83 (d, 9.0)	114.16	7.38 (d, 2.0)	C-3',	114.18	7.38 (d, 2.0)
3'	117.01	6.93 (d, 9.0)	147.01			147.03	
4'	162.73		150.47			150.97	
5'	117.01	6.93 (d, 9.0)	116.77	6.91 (d, 9.0)		116.78	6.92 (d, 9.0)
6'	129.43	7.83 (d, 9.0)	120.30	7.38 (d, 9.0		120.30	7.40 (d, 9.0)
6-OMe	60.94	3.90 (s)	60.94	3.90 (s)	C-6		

Table 2. Cytotoxic activity of compounds **1**-**3** and ellipticine[a]

Compound	IC_{50} (μg/mL)/cell line[b]			
	KB	Hep	Lu1	MCF7
1	82.77 ± 1.3	75.12 ± 1.24	99.58 ± 2.47	99.63 ± 1.02
2	42.72 ± 0.55	43.76 ± 2.08	67.28 ± 1.40	71.24 ± 1.82
3	24.45 ± 0.97	16.13 ± 0.89	88.13 ± 1.06	78.40 ± 2.26
Ellipticine[b]	0,45 ± 0,03	0,43 ± 0,03	0,39 ± 0,03	0,37 ± 0,03

[a] Ellipticine: positive control;

[b] Cell lines: KB (mouth epidermal carcinoma cells), HepG2 (human liver hepatocellular carcinoma cells), Lu1 (human lung adenocarcinoma cells), and MCF7 (human breast cancer cells).

4. CONCLUSIONS

In conclusions, three flavones, hispidulin (**1**), nepetin (**2**) and luteotin (**3**) were isolated and identified from the aerial part of *Tithonia diversifolia (Hemsl.) A. Gray*. The isolated compounds **1**-**3** showed cytotoxic activity against four human cancer cell lines (KB, Hep, Lu1 and MCF7) with IC_{50} values ranging from 16.13 ± 0.89μg/mL to 99.63 ± 1.02μg/mL. Among them, luteotin (**3**) has shown the most potent effect on Hep and KB cell lines with IC_{50} 16.13 ± 0.89μg/mL and 24.45 ± 0.97μg/mL, respectively.

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THÔNG TIN TÁC GIẢ

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