CYTOTOXIC ACTIVITY OF NEW PHENOLICS FROM *DIANELLA ENSIFOLIA (*L.) DC.

HOẠT TÍNH GÂY ĐỘC TẾ BÀO CỦA HỢP CHẤT PHENOLIC MỚI TỪ LOÀI XƯƠNG QUẠT (*DIANELLA ENSIFOLIA)*

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ABSTRACT

In our previous study, 10 compounds were isolated and structurally identified from the aerial part *of Dianella ensifolia*. Three of them (**1**, **2**, and **4**) were newly identified compounds and one (**3**) was obtained for the first time as natural product.With the aim to search for the cytotoxic agents from this plant, the present investigation was designed to evaluated cytotoxic activity of a phenolics (**1**-**4**) against four human cancer cell lines Hep3B, Hela, A549, and MCF-7. Of them, compound **4** (at 100μM) showed the most effects on A549 and MCF-7 cells, with the survival rates of 42.07% and 49.49%, respectively, while the positive control camptothecin (5μg/ml) resulting in the rates of 26.75% and 28.89%, respectively.

Keywords*: Dianella ensifolia*, *flavonoid, biflavan, cytotoxicity, A549*

TÓM TẮT

Từ nghiên cứu trước, 10 hợp chất đã được chúng tôi phân lập và xác định cấu trúc từ loài Xương quạt (*Dianella ensifolia*). Trong đó, ba hợp chất (**1**, **2** và **4**) đã được xác định là hợp chất mới và **3** lần đầu được tìm thấy trong tự nhiên. Tiến hành nghiên cứu hoạt tính gây độc tế bào của các hợp chất này (**1-4**) trên bốn dòng tế bào ung thư người Hep3B, Hela, A549 và MCF-7. Kết quả cho thấy, hợp chất **4** (tại nồng độ 100μM) có hoạt tính mạnh nhất trên 2 dòng tế bào A549 và MCF-7 với giá trị tế bào sống sót tương ứng là 42,07% và 49,49% trong khi chất đối chứng dương camptothecin (5μg/ml) có giá trị tương ứng 26,75% và 28,89%.

Từ khóa: *Dianella ensifolia, flavonoid, biflavan, gây độc tế bào, A549.*

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1. INTRODUCTION

A longstanding scientific research has shown that natural products, especially in terms of isolated metabolites from herbal plants, always play an important role throughout the anticancer drug discovery [1, 2]. Consequently, searching potential anticancer drugs from herbal plants gave strong attention to scientists.

Dianella ensifolia (the family Liliaceae) is an annual herbaceous plant native to tropical regions, especially in India, southern China, Japan, and Taiwan. The plant was renowned for the great role in folk medicine and pharmacological purposes, which can be mentioned to the treatment of kidney diseases and skin-related disorders like tinea [3, 4]. The phytochemical investigations on this species demonstrated that flavonoid is the predominant class of compound, which sets up a crucial role for various biological activities like antioxidant, antivirus, cytotoxicity, and tyrosinase inhibition [5-7].

D. ensifolia was found in Vietnam under the name as Xuong quat, which is mostly distributed in central provinces such as Thanh Hoa, Nghe An, and Lam Dong and it is used for abscess treatment [8]. In spite of the fact that, a large number of reports was published on *D. ensifolia* phytochemistry as well as pharmacological evaluation [7], there have been no chemical and biological activity study on this species growing in Viet Nam. To enrich the chemical diversity and afford scientific evidence for the traditional medicinal use of this plant, our previous chemical study on the aerial parts of *D. ensifolia* growing in Viet Nam led to the isolation and structural elucidation of 10 compounds [9-14]. By a combination of spectroscopic data (HR-ESIMS, 1D-nd 2D-NMR) with their comparison to those published in literatures, structures of 10 isolated compounds were clearly elucidated, including a tetralone7-acetyl-4*R*,8-dihydroxy-6-methyl-1-tetralone (**1**); three flavans2(S)-2',4'-dihydroxy-7-methoxyflavan (**2**), 2(*S*)-7,4'-dimethoxyflavan (**3**), and 5,7-dihydroxy-4'-methoxyflavan (**6**); a biflavan (5-hydroxy-7,4'-dimethoxy-(6,6''-methylen)-biflavan (**4**); an aromatic compound *β*-orcinolcarboxylate (**5**); a naphthalen glycosidedianellose(**7**); a biflavone amentoflavone (**8**); and two steroids stigmastenone (**9**), and *β*-sitosterol (**10**). Three new compounds (**1**, **2**, **4**), one compound (**3**) isolated from nature for the first time, and 6 known compounds (**5**–**10)** [5]. In the course of our ongoing search for anticancer agents from Vietnamese medicinal plants, we now report

the cytotoxic activity of new phenolics (**1**–**4**) against four human cancer cell lines Hep3B, Hela, A549 and MCF-7 [7, 12]. The cytotoxicity against several cancer cells of the other isolates were studied previously and reported elsewhere.

2. EXPERIMENTAL

2.1. General experimental procedures

The used characterization equipments and detailed experimental procedures are the same as described in our previous paper [5].

Four human cancer cell lines, including liver cancer cell (Hep3B), cervical cancer cell (Hela), lung cancer cell (A549) and breast cancer cell (MCF-7) were provided by Professor Jeong-Hyung Lee, Kangwon National University, Korea. The cancer cells were cultured according to *in vitro* procedure of Mosmann, 1983 [15].

2.2. Plant materials and isolation of compounds

Plant materials, extraction and isolation of all 10 compounds were describedin detailin our previous report [5]. With the aim to search for the cytotoxic agents from this plant, the present investigation was designed to evaluated cytotoxic activity of a phenolics (7-acetyl-4*R*,8-dihydroxy-6-methyl-1-tetralone (**1**); 2(S)-2',4'-dihydroxy-7-methoxyflavan (**2**); 2(*S*)-7,4'-dimethoxyflavan (**3**); (5-hydroxy-7,4'-dimethoxy-(6,6''-methylen)-biflavan (**4**)) against four human cancer cell lines Hep3B, Hela, A549, and MCF-7.

2.3. MTT colorimetric assay

The MTTcolorimetric method is based on measurement of the reduction of a yellow tetrazolium salt MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) [9]. This method was used to evaluate the cell survival effects when treating with the tested compounds (**1**–**4**) in four different human cancer cell lines Hep3B, Hela, A549 and MCF-7. According to the method, cells were cultured in either medium RPMI or DMEM in accordance to the guidance of ATCC at 37°C with the presence of 5% CO_2, 10% FBS (Fetal bovine serum), penicillin (100units/mL), and streptomycin (100µg/mL) for 46 h. Then, cells were seeded into a 96-multiwell culture plate with the volume of 200µl and a density of 2 x 10^5cells/well. After 24h, cells were treated with prepared samples at different concentrations in DMSO and growth for the remaining 72h of treatment. After that, 0.5mg/mL of MTT solution was added, incubated at 37°C and 5% CO_2 for 4h, and then the medium and MTT solution were removed. Isopropanol was added to all wells to dissolve the formazan crystals. Absorbance (OD) was measured on an ELISA Bio-Rad reader (Laboratories, USA) by using 570nm wavelength. The absorbance of the control sample (no compound treatment) corresponds to 100% cell survival at 48h. Camptothecin was used as a positive control.

Percentage of cell survival (%) was calculated by following equation:

$$\% \text{ cell survival} = [(OD_{sample} - OD_0) / (OD_{DMSO} - OD_0) \times 100] \pm SD$$

Where:

OD is the absorbance,

SD - standard deviation;

OD_0 - initial control at t = 0h.

2.4. Statistical analysis

Descriptive statistical calculations for the normality and homogeneity of variances were performed using the Microsoft Excel software. Results were expressed as mean ± standard deviation (SD) when each experiment was repeated in triplicate. The significant differences were assessed with $p < 0.05$.

3. RESULTS AND DISCUSSION

Cytotoxic effect *in vitro*

Compound **5**-**10** were reported for the cytotoxicity elsewhere in the literature, so they were not selected for cytotoxicity testing [7, 13]. The newly isolated compounds (**1**–**4**) from *D. ensifolia* were chosen for cytotoxic effect evaluation. Hep3B, Hela, A549 and MCF-7 human cancer cell lineswere selectedfor this test using the MTT method described above.Two concentrations (30 and 100µM) was used and cell survival (CS%) value for each compound was calculated.

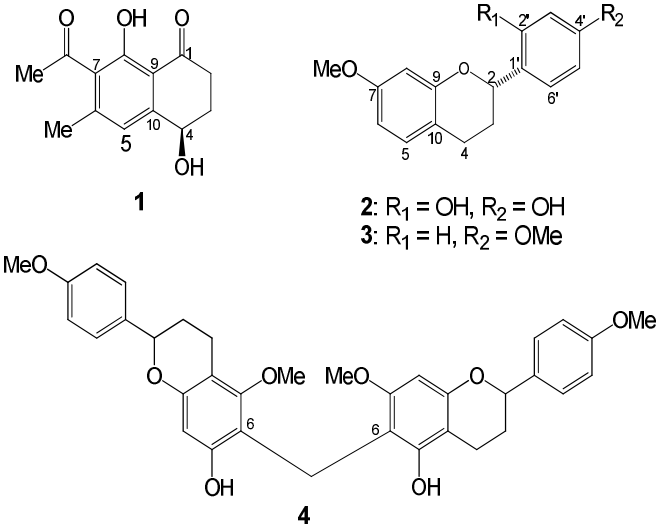

Figure 1. Structure of compounds **1**-**4** isolated from Dianella ensifolia

The results indicated that the biflavan was the most active component in the cytotoxic assay. Accordingly, compound **4** at 100µM moderately inhibited the proliferation of A549 and MCF-7 cells with respective viability of 42.07 ± 1.94% and 49.49 ± 2.14%. In comparison to the positive control, camptothecin, at 5µg/mL (14.37µM), resulted in the survival of 26.74 ± 2.16% for A549 cell and 28.89 ± 1.07% for MCF-7 cell. However, this compound failed to induce cytotoxicity to Hep3B and Hela cancer cells. Meanwhile, compound **1**-**3** weakly showed the effects in all tested cancer cells with the survival rate of more than 50%, regardless of concentration used (Table 1).

Table 1. Effect of tested compounds on cancer cell viability

Sample	Concentration μM	Cell survival (CS%)			
		Hep3B	Hela	A549	MCF-7
1	100	79.29 ±0.84	89.78 ±1.23	82.48 ±0.27	66.68 ±1.48
2		56.71 ±0.42	50.64 ±0.27	59.31 ±1.71	74.45 ±2.81
3		56.53 ±1.32	66.87 ±1.10	51.63 ±0.81	58.60 ±0.32
4		54.99 ±2.40	65.21 ±0.53	**42.07** ±1.94	**49.49** ±2.14
Camptothecin*	5μg/mL	37.65 ±1.21	18.61 ±0.56	26.74 ±2.16	28.89 ±1.07

*Camptothecin was used as positive control

4. CONCLUSIONS

Four new phenolics (**1**–**4**), isolated from Vietnamese *D. ensifolia* aerial parts were investigated for cytotoxic activity for the first time. Among tested compounds, the biflavan **4** (at 100 μM) showed moderate effect against A549 and MCF-7 cells with respective survival rates of 42.07 ± 1.94% and 49.49 ± 2.14%. Additionally, this is the first report of a biflavan exhibiting its cytotoxic effect against cancer cells. The notable effect of **4** suggested that biflavans might be important structures for cytotoxic activity study, which need more attention on the way of searching for anticancer drugs.

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