CYTOTOXIC ACTIVITY OF FRACTIONS AND COMPOUNDS FROM *PEPEROMIA PELLUCIDA*

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ABSTRACT

Peperomia pellucida is a widely distributed herb and commonly used in folk medicine for the treatment of various disorders. The present study reported the isolation, elucidation of compounds from the whole plant and their cytotoxic activities on Hep G2 (HB - 8065™, hepatocellular carcinoma), A549 (CCL-185™, pulmonary adenocarcinoma), and MCF-7 (HTB - 22™, breast cancer) cell lines. As a result, velutin exhibited the most significant cytotoxicity against Hep G2, A549, and MCF-7 cell lines with IC_{50} values of 34.00, 30.85, and 41.33 µg/mL, respectively.

Keywords: *Peperomia pellucida, Velutin, Flavonoid, Flavone, Cytotoxicity*

I. INTRODUCTION

Peperomia pellucida is a widely distributed herb in tropical and subtropical countries. Besides being a common vegetable, it is a remedy for treating eye, gastrointestinal and respiratory tract disorders in Brazilian, Cameroonian, Bolivia, and Vietnamese folk medicine. Previous studies on this plant indicated the presence of lignans, sesquiterpenes, and radionuclides with anti-infective, bone healing, anti-diabetic, analgesic, and anti-inflammatory activities [1]. Several publications on this *Peperomia* species reported the cytotoxic activity of solvent extract and chemical compounds. *P. pellucida* leaf extract possessed significant cytotoxic activity against the human breast adenocarcinoma (MCF-7) cell line with IC_{50} of 10.4 µg/mL [2]. Peperomin A isolated from *P. dindygulensis* exhibited apoptosis inhibition on human prostate cancer (DU145) cells [3].

In our previous investigation, two sesquiterpenes and one phenylpropanoid were isolated from the *n*-hexane fraction. Based on spectroscopic data and comparison with reported literature, they were elucidated to be pellucarotine (**1**), daucol (**2**), and dillapiole (**3**) [4]. In this study, we isolated one more compound from ethyl acetate fraction and measured the cytotoxic activity of all four compounds on HepG2, A549, and MCF7 cells.

II. MATERIALS AND METHOD

2.1. Plant materials

A collection of *P. pellucida* whole plant was made in Haiphong, Vietnam in February 2022 and identified by Professor Dam Duc Tien, Centre of Marine Pharmacognosy, Haiphong university of Medicine and Pharmacy. A voucher specimen (HPMUSP-2022-03) was deposited at the Herbarium, Centre of Marine Pharmacognosy, Haiphong University of Medicine and Pharmacy.

2.2. General experimental procedures

The ^{1}H, ^{13}C NMR, HSQC, HMBC spectra were measured using an AVANCE NEO 600 MHz spectrometer with tetramethylsilane (TMS) as an internal standard with the chemical shifts recorded in δ values (ppm). Silica gel (Merck, 40 – 63 µm particle size) and RP silica gel (Cosmosil 75 C18-PREP,

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40 µm particle size) were used for column chromatography. Thin layer chromatography was performed using Merck silica gel 60 F254 and RP C-18 F254 plates, and spots were detected under UV light (254 and 366 nm) and by spraying with 10% H_2SO_4 in ethanol (*v*/*v*), and then heating.

2.3. Extraction and isolation

The whole plant of *P. pellucida* (2.0 kg) was extracted with ethanol 3 times at room temperature for 3 days and filtered. The filtrate was combined and concentrated *in vacuo* to remove the solvent. The total ethanol extract (40.0 g) was suspended in a minimum volume of distilled water and then partitioned with *n*-hexane and EtOAc, successively. Each fraction was evaporated *in vacuo* to yield n-hexane (9.7 g), EtOAc (5.8 g), H_2O (13.5 g) fractions. Compounds **1** – **3** were isolated from the *n*-hexane soluble fraction of *P. pellucida* whole plant and described in our previous study [4]. EtOAc soluble fraction was subjected on a silica gel open column eluting with a solvent system of *n*:hexane: EtOAc (10:1) to obtain 22 sub-fractions. Sub-fraction EA14 were further chromatographed over RP-C18 silica gel open column using a solvent system of MeOH: H2O (2:1) to yield compound **4** (3.2 mg).

2.4. Cytotoxicity assay

Cytotoxic activity was performed based on the MTT (3-(4,5-dimethylthiazol-2 - yl)-2, 5 - diphenyltetrazolium) method first described by Tim Mosman [5]. This method evaluates cell viability through the ability to reduce transformation of MTT (yellow) to a formazan complex (purple) by the activity of mitochondria dehydrogenase enzyme. The formazan product was dissolved with DMSO and the optical density (OD) was measured at 540 nm.

Hep G2 (HB - 8065TM, hepatocellular carcinoma), A549 (CCL-185™, pulmonary adenocarcinoma), and MCF-7 (HTB - 22TM, breast cancer) cell lines were kept in liquid nitrogen, activated and maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal Bovine Serum). Cells are grown under standard conditions (5% CO2, 98% humidity, temperature 37°C, sterile). At a density of 3 × 106 cells/mL, cytotoxic test was conducted with the compounds at various concentrations. DMSO was used as the sample dissolving solvent, final concentration of DMSO was less than 0.1%. 10 µL test compounds were add to each well contained 190 µL cell culture broth in standard conditions and kept for 72 h. Subsequently, each well was continued to be incubated with 10 µl MTT (5 mg/ml) for 4 hours. After removing the supernatant, the formazan crystals were dissolved with 100 µl of 100% DMSO. Cell viability was measured by OD at 540 nm. Ellipticine (Sigma - Aldrich) was used as a positive control. Each dose of sample was repeated in triplicate. The concentration of sample that inhibits 50% of cell growth, the IC_{50} value, were measured. The results are presented as mean ± standard error of mean (SEM).

III. RESULTS AND DISCUSSION

3.1. Isolation and structural elucidation of compounds

Along with three compounds (**1** – **3**) reported in our previous study, one more compound (**4**) was isolated from the EtOAc soluble fraction.

Velutin (4',5-dihydroxy-3',7-dimethoxyflavone) (4) – Pale yellow amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 12.79 (s, 1H, 5-OH), 7.49 (dd, J = 8.4, 1.9 Hz, 1H, H-6'), 7.332 (d, J = 8.4 Hz, 1H, H-1'), 7.037 (d, J = 1.9 Hz, 1H, H-5'), 6.56 (s, 1H, H-3), 6.489 (d, J = 2.1 Hz,

1H, H-8), 6.373 (d, J = 2.1 Hz, 1H, H-6), 4.006 (s, 3H, 7-OCH_3), 3.886 (s, 3H, 3'-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 182.42 (C-2), 165.51 (C-7), 164.07 (C-2), 162.26 (C-5), 157.72 (C-8a), 149.27 (C-4'), 146.89 (C-3'), 123.45 (C-1'), 120.78 (C-6'), 115.01 (C-5'), 108.38 (C-2'), 105.59 (C-4a), 104.55 (C-3), 98.09 (C-6), 92.68 (C-8), 56.19 (3'-OCH_3), 55.81 (7-OCH_3).

Compound **4** was obtained as pale-yellow amorphous powder. Based on ^1H and ^{13}C NMR spectra data and comparison to published literature, its structure was determined to be velutin [6, 7]. Structures of isolated compounds from *P. pellucida* are shown in Fig. 1. Velutin was also found in other species, including *Ceanothus velutinus*, *Euterpe oleracea*, *Rhus retinorrhoea*, *Euterpe oleracea*, and *Drimys arfakensis* [6]. To the best of our knowledge, this is the first report of this flavone from the genus *Peperomia*.

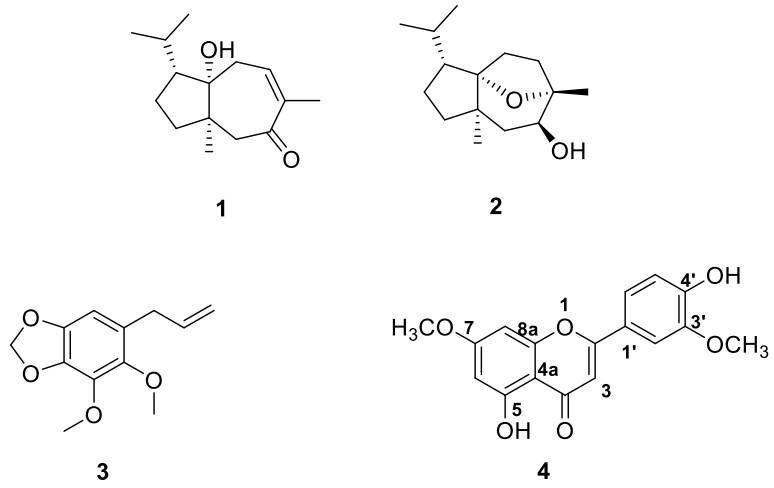

Fig. 1. ***The structures of 1−4 isolated from*** **P. pellucida.**

3.2. Cytotoxic activity of solvent fractions and isolated compounds

Table 1. ***Cytotoxic activity of solvent fractions and compounds (1 – 4) on tested cell lines***

Sample	IC_{50} (µg/mL)		
	HepG2	A549	MCF7
Total extract	143.20±3.40	127.52±2.05	129.82±3.87
n-Hexane Fr.	139.44±4.87	145.84±4.45	141.30±2.13
EtOAc Fr.	151.40±3.41	148.26±3.43	137.64±2.82
H2O Fr.	-	-	-
1	-	73.71±3.67	-
2	-	-	-
3	-	-	-
4	34.0±2.82	30.85±1.61	41.33±1.88
Ellipticine	0.43±0.02	0.43±0.02	0.44±0.03

(-): Not determined, more than 256 µg/mL with solvent fractions and 128 µg/mL with isolated compounds.

Total ethanol extract, *n*-hexane fraction, EtOAc fraction, H_2O fraction, and four compounds (**1** – **4**) were investigated for their cytotoxic activity against Hep G2, A549, and MCF-7 cell lines (Table 1). As results, compound **4** exhibited the most significant cytotoxicity against Hep G2, A549, and MCF-7 cell lines with IC_{50} values of 34.00, 30.85, and 41.33 µg/mL, respectively. Compound **1** inhibited cell growth of A549 with IC_{50} of 73.71 µg/mL. Meanwhile, all extracts show weak activity on all three cell lines with IC_{50} ranging from 127.52 – 151.40 µg/mL. Velutin (**4**) was reported for its anti-inflammatory, antibacterial, and anti-osteoarthritic [6]. At concentrations of 0.5-2µM, velutin inhibited osteoclast differentiation and downregulated HIF-1α via the NF-κB pathway. Moreover, velutin was reported to possess inhibition on human nasopharynx carcinoma (KB) cells with IC50 of 4.8 µM. Its strong effect may be due to the presence of hydroxyl groups at C-5 and C-4' and methoxyl groups at C-7 and C-3' [8]. Despite of various activities reported, the current investigation provided further data on bioactivity of velutin as well as other compounds and solvent fraction of *P. pellucida*.

IV. CONCLUSION

One flavonoid was isolated and determined to be velutin from the ethylacetat soluble fraction of *P. pellucida*. This compound exhibited the most significant inhibitory activity on Hep G2, A549, and MCF-7 cell lines with IC_{50} values of 34.00, 30.85, and 41.33 µg/mL, respectively.

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