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The cytotoxic activity of flavonoid glycosides and alkaloids from the male *Carica papaya* flowers

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Abstract

The cytotoxic activity of six flavonoid glycosides (kaempferol 3-*O*-*α*-*L*-rhamnopyranoside (**1**), kaempferol 3-*O*-*β*-*D*-glucopyranoside (**2**), kaempferol 3-*O*-*α*-*L*-arabinopyranoside (**3**), quercitrin (**4**), quercetin 3-*O*-*β*-*D*-galactopyranoside (**5**), myricitrin (**6**)), and two alkaloids (1-benzyl-5-(hydroxymethyl)-1*H*-pyrrole-2-carbaldehyde (**7**), indole-3-aldehyde (**8**)) from the male *Carica papaya* flowers are conducted for lung cancer cells (A549), liver cancer cells (Hep3B), and breast cancer cells (MCF-7). The results, flavonoid glycosides (**1**), (**3**), (**6**), an alkaloid (**7**) exhibited medium cytotoxic activity on these cancer cells with IC_{50} values from 26.72±0.76 to 64.37±3.42 µg/mL. Flavonoid glycosides (**2**), (**4**), and (**5**) showed weak cytotoxic activity on these cancer cells with IC_{50} values from 71.52±3.27 to 91.37±3.40 µg/mL. Alkaloid (**8**) did not display cytotoxic activity on these cancer cells. Ellipticine was a positive control.

Keywords: Flavonoid, alkaloid, A549 lung cancer cells, Hep3B liver cancer cells, MCF-7 breast cancer cells

1. Introduction

Carica papaya (Caricaceae) is one of the valuable nutraceutical fruit plants in Asia zone. It is native to tropical America and is commonly known as Papaya in English, Papita in Hindi, and Erandakarkati in Sanskrit. Papaya is widely planted in the delta provinces, along the rivers, and on alluvial soils in Vietnam.

Male *Carica papaya* flowers, one of the natural materials, have many pharmacology activities, including anticancer. Recently, local people in Quangnam - Danang have used male *Carica papaya* flowers to treat respiratory diseases such as pharyngitis, cough, bronchitis, hoarseness, or hearing loss in adults and children. In addition, male *Carica papaya* flowers have long been used to support the treatment of lung cancer, liver cancer, and breast cancer [1]. There have been many studies on the chemical constituents and biological activities of *Carica papaya* leaves, stems, and fruits [2-5]. However, only a few researches have been done on their flowers [6-10], especially the cytotoxic activity of compounds. The cytotoxic activity on cancer cells of A549, Hep3B, MCF-7 of lignans, and monoterpenoids isolated from the male *Carica papaya* flowers in Quangnam - Danang was announced [11].

Continuing previous research on the cytotoxic activity of lignans and monoterpenoids, the present study aimed to determine the cytotoxic activity of flavonoid glycosides, alkaloids from male *Carica papaya* flowers in Quangnam - Danang on lung cancer cells (A549), liver cancer cells (Hep3B), and breast cancer cells (MCF-7).

2. Materials and Methods

Plant materials

The male *Carica papaya* flowers were collected at Quangnam - Danang, Vietnam in December 2016. Its scientific name was identified by botanist Dr. Ngo Van Trai (Vietnam National Institute of Medicinal Materials), MSc. Nguyen The Anh, and MSc Ho Ngoc Anh (Institute of Chemistry). A voucher specimen No. DD001 was deposited at the Herbarium of the Institute of Chemistry, Vietnam Academy of Science and Technology.

Chemicals and equipment

NMR spectra were recorded on a Bruker 500 MHz spectrometer. ESI mass spectra were recorded on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 230-400 mesh, Merck) or RP-18 resins (150 µm, YMC), thin layer chromatography using a pre-coated silica gel 60 F254 (0.25 mm, Merck) and RP-18 F254S plates (0.25 mm, Merck).

Methanol (MeOH), *n*-hexane, methylene chloride (CH_2Cl_2), ethyl acetate ($CH_3COOC_2H_5$), acetone (CH_3COCH_3), *n*-butanol, distilled water (H_2O), sulforhodamine B (SRB), dimethyl sulfoxide (DMSO), trichloroacetic acid, acetic acid, tris(hydroxymethyl)aminomethane, ellipticine get analytical standards.

Tested cell lines: A549 (lung cancer), MCF-7 (breast cancer), and Hep3B (liver cancer) produced by J.M. Pezzuto, University of Hawaii, USA, and Jeanette Maier, University of Milan, Italy.

Extraction and isolation

The dried powdered male *Carica papaya* flowers (5 kg) were extracted three times with methanol using a sonicator to yield 300 g of dark solid residue. This extract was suspended in water and successively partitioned with *n*-hexane, methylene chloride (MC), ethyl acetate (EA), and *n*-butanol to obtain corresponding *n*-hexane (54 g), MC (52 g), EA (20 g), and *n*-butanol (70 g) residues.

The EA (20 g) was fractionated by silica gel CC, eluted with a stepwise gradient of CH_2Cl_2/MeOH (50/1, 25/1, 10/1, 5/1, and 1/1) to yield five fractions, EA1 (2.0 g), EA2 (2.4 g), EA3 (1.6 g), EA4 (6.2 g), and EA5 (4.4 g). Fraction **EA3** (1.6 g) was subjected to chromatography on a silica gel CC YMC and eluting with CH_3OH/H_2O (1.2/1, v/v) to afford two sub-fractions, EA3A (25 mg) and EA3B (37 mg). Sub-fraction EA3A (25 mg) was purified by Sephadex LH-20 column and eluted with MeOH/H_2O (1/1, v/v) to give compound (**4**) (11.0 mg). Compound (**2**) (9.2 mg) was purified by Sephadex LH-20 column using MeOH/H_2O (1/1, v/v) from sub-fraction EA3B (37 mg). Fraction **EA4** (6.2 g) was separated by silica gel CC YMC using MeOH/H_2O (1/1, v/v) to yield two sub-fractions, EA4A (300 mg) and EA4B (410 mg). Sub-fraction EA4A (300 mg) was also purified by silica gel CC and eluted with $CH_2Cl_2/CH_3OH/H_2O$ (3/1/0.2, v/v/v) to afford two sub-fractions, EA4A1 (58 mg) and EA4A2 (42 mg). Sub-fraction EA4A1 (58 mg) was further purified by Sephadex LH-20 column, eluted with MeOH/H_2O (1/1, v/v) to obtain compound (**1**) (9.0 mg). Compound (**5**) (8.1 mg) was also purified by Sephadex LH-20 column using MeOH/H_2O (1/1, v/v) from sub-fraction EA4A2 (42 mg). Sub-fraction EA4B (410 mg) was separated by silica gel CC, eluted with $CH_2Cl_2/CH_3OH/H_2O$ (3.5/1/0.15, v/v/v) to yield two sub-fractions, EA4B1 (62 mg) and EA4B2 (71 mg). Sub-fraction EA4B1 (62 mg) was further purified by the Sephadex LH-20 column and eluted with MeOH/H_2O (1/1, v/v) to give compound (**3**) (9.6 mg). Compound (**6**) (8.7 mg) was also purified by Sephadex LH-20 column using MeOH/H_2O (1/1, v/v) from sub-fraction EA4B2 (71 mg) [7].

The MC residue (52 g) was roughly separated on a silica gel CC, eluting with CH_2Cl_2/MeOH (0-100% volume of MeOH) to give 5 fractions MC1-MC5. Fraction **MC2** (4 g) was chromatographed on a silica gel CC, eluting with CH_2Cl_2/MeOH (20/1, v/v) to give 4 smaller fractions MC2A-MC2D. Compound (**7**) (5.0 mg) was isolated from fraction MC2A (1.2 g) using silica gel CC YMC and CH_3COCH_3/H_2O (2/1, v/v) as an eluent. Fraction **MC4** (3.5 g) was separated on a silica gel CC, eluting with CH_2Cl_2/MeOH (20/1, v/v) to give 3 fractions MC4A-MC4C. Fraction MC4A (0.6 g) was repeatedly chromatographed on a silica gel CC, eluting with $CH_2Cl_2/CH_3COOC_2H_5$ (4/1, v/v) to give 4 fractions MC4A1-MC4A4. Compound (**8**) (9.0 mg) was isolated from fraction MC4A1 using a silica gel CC and CH_2Cl_2/ CH_3COCH_3 (4/1, v/v) as an eluent.

Evaluation of the cytotoxic activity of compounds

The cytotoxic activity of the compounds was determined by the Monks method [12]. The test was carried out to determine the total cellular protein content based on the optical density (OD) measured when the protein composition of the cells was stained with sulforhodamine B (SRB). The calculated OD value is directly proportional to the amount of SRB attached to the protein molecule, so the more cells, the larger the OD value. The test is carried out under the following specific conditions: The reagent (10 μL) mixed in 10% DMSO (in sterile distilled water) was introduced into the wells of the 96-well plate to have a screening concentration of 100 μg/mL. The active reagent is determined IC_{50} using a concentration range of 100; 20; 4; 0.8 μg/mL. Each concentration of test sample is prepared in 3 wells. Trypsinizing experimental cells to leave cells and counting in the counting chamber to adjust the density ($3x10^4$ cell/mL) to suit the experiment. Add the appropriate number of cells (190 μL of medium) to the test wells and let them grow for 3-5 days. Another 96-well plate without reagent but with cancer cells (190 μL) was prepared in 3 columns for day 0 control. After 01 hour, the day 0 control plate cells were fixed with trichloroacetic acid-TCA. The day 0 plate was a separate experimental plate. The experimental procedure of the day 0 control plate was similar to the reagent test plate. After the growth phase in a CO_2 incubator, cells were fixed to the bottom of the well with TCA for 30 minutes and stained with SRB for 1 hour at 37°C. Discard the SRB, and the test wells were washed three times with 5% acetic acid and allowed to dry in air at room temperature. Finally, use 10 mM tris(hydroxymethyl)aminomethane solution to dissolve the bound SRB and stain the protein molecules, put on a plate shaker, shake gently for 10 minutes, and use the ELISA Plate Reader (Bio-Rad) to read the results of the color content of SRB dyes through the absorption spectrum at 515-540 nm. The percentage of cells that are inhibited (%) in the presence of reagents will be determined through the following formula:

$$\%\ Cell\ inhibited = 100\% - \frac{OD\ (regents) - OD\ (day\ 0)}{OD\ (negative\ control) - OD\ (day\ 0)}$$

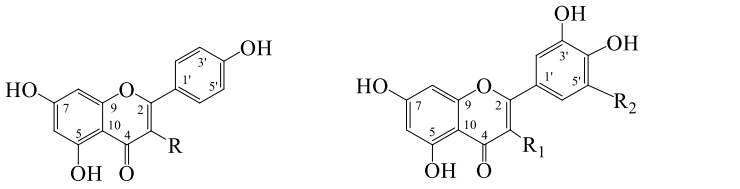
The tests were repeated 3 times to ensure accuracy. Ellipticine (Sigma-Aldrich, USA) at concentrations of 10 μg/mL; 2 μg/mL; 0.4 μg/mL; 0.08 μg/mL was always used as a positive control. DMSO 10% was always used as a negative control. The IC_{50} value (concentration that inhibits 50% of growth) was determined using TableCurve 2Dv4 computer software (System Software Inc., San Jose, California, USA).

3. Results and Discussions

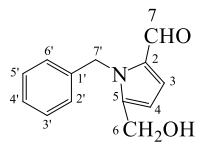
3.1 Chemical structure of compounds

The chemical structure of six flavonoid glycosides (**1**)-(**6**) and two alkaloids (**7**)-(**8**) (Figure 1) was determined by NMR, MS spectra and compared with the reported data. Spectroscopic data of compounds (**1**)-(**6**) were reported in the previous literature [7].

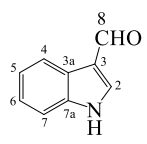
Spectroscopic data of compound (**7**), and (**8**):



(**1**) R = *O*-*a*-*L*-rhamnopyranoside
(**2**) R = *O*-*b*-*D*-glucopyranoside
(**3**) R = *O*-*a*-*L*-arabinopyranoside
(**4**) R_1 = *O*-*a*-*L*-rhamnopyranoside, R_2 = H
(**5**) R_1 = *O*-*b*-*D*-galactopyranoside, R_2 = H
(**6**) R_1 = *O*-*a*-*L*-rhamnopyranoside, R_2 = OH



(**7**)



(**8**)

*Figure 1: Chemical structure of compounds **(1)-(8)***

1-Benzyl-5-(hydroxymethyl)-1*H*-pyrrole-2-carbaldehyde (7): **Colorless oil.** HR-ESI-MS: *m/z* 238.0842 $[M+Na]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 6.96 (1H, d, *J* = 4.0 Hz, H-3), 6.31 (1H, d, *J* = 4.0 Hz, H-4), 4.57 (2H, s, H-6); 9,57 (1H, s, H-7); 7,00 (2H, d, *J* = 7,0 Hz, H-1′, H-6′), 7.28 (2H, t, *J* = 7.0 Hz, H-3′, H-5′), 7.24 (1H, t, *J* = 7.0 Hz, H-4′); 5.76 (2H, s, H-7′). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 132.9 (C-2), 124.2 (C-3), 110.8 (C-4), 141.9 (C-5), 56.7 (C-6), 179.8 (C-7), 137.8 (C-1′), 126.1 (C-2′), 128.8 (C-3′), 127.4 (C-4′), 128.8 (C-5′), 126.1 (C-6′), 48.6 (C-7′) [13].

Indole-3-aldehyde (8): Yellow needle-shaped crystals. HR-ESI-MS: *m/z* 146.1657 $[M]^+$ (calcd for C_9H_7NO, 146.1659). ^{1}H-NMR ($CDCl_3$), δ (ppm): 8.11 (1H, s, H-2), 8.18 (1H, d, *J* = 7.5 Hz, H-4), 7.25 (1H, dd, *J* = 7.5, 8.0 Hz, H-5), 7.30 (1H, dd, *J* = 7.5, 8.0 Hz, H-6); 7.50 (1H, d, *J* = 8.0 Hz, H-7), 9.91 (1H, s, H-8). ^{13}C-NMR ($CDCl_3$), δ (ppm): 139.7 (C-2), 120.2 (C-3); 125.7 (C-3a), 122.4 (C-4), 123.6 (C-5), 125.0 (C-6), 113.1 (C-7), 139.0 (C-7a), 187.4 (C-8) [14].

3.2. Evaluation of the cytotoxic activity of compounds

The results of evaluating the cytotoxic activity of six flavonoid glycosides (**1**)-(**6**) and two alkaloids (**7**)-(**8**) from the male *Carica papaya* flowers on tested three cell lines: A549 (lung cancer), MCF-7 (breast cancer), and Hep3B (liver cancer) are presented in Table 1. Flavonoid glycosides (**1**), (**3**), (**6**), an alkaloid (**7**) exhibited medium cytotoxic activity on these cancer cells with IC_{50} values from 26.72±0.76 to 64.37±3.42 µg/mL. Flavonoid glycosides (**2**), (**4**), and (**5**) showed weak cytotoxic activity on these cancer cells with IC_{50} values from 71.52±3.27 to 91.37±3.40 µg/mL. Alkaloid (**8**) did not display cytotoxic activity on these cancer cells. Ellipticine was a positive control.

Table 1: The cytotoxic activity of compounds (**1**)-(**8**)

Compound	IC_{50} (µg/mL)		
	A549	MCF-7	Hep3B
Flavonoid glycoside			
(**1**)	61.72±3.00	47.79±2.12	64.37±3.42
(**2**)	71.52±3.27	81.84±4.72	80.13±2.76
(**3**)	34.67±2.21	26.72±0.76	44.13±3.61
(**4**)	91.37±3.40	75.27±4.95	90.50±2.74
(**5**)	71.70±3.87	85.61±4.43	74.90±3.13
(**6**)	36.02±1.69	35.92±1.13	45.49±4.29
Alkaloid			
(**7**)	44.58±4.04	55.91±3.08	49.87±3.80
(**8**)	>100	>100	>100
Ellipticine	0.43±0.04	0.37±0.03	0.50±0.04

Ellipticine: The positive control, which acts stably in the experiment.

Flavonoid compounds in papaya have been reported to have antioxidant, anti-inflammatory, anti-tumor, and cytotoxic activity [15], [16]. At the same time, it has been reported that phenolic and flavonoid compounds in papaya leaves have cytotoxic activity with different mechanisms [17]. Six flavonoid glycosides (**1**)-(**6**) all had inhibitory effects on all three cancer cell lines A549, MCF-7, Hep3B with IC_{50} values in the range of 26.72±0.76 to 91.37±3.40 µg/mL.

Alkaloids are compounds with biological activity, especially cytotoxic activity in human cancer [18-20]. Some alkaloids have been used to treat cancer [21], [22]. Compound 1-benzyl-5-(hydroxymethyl)-1H-pyrrole-2-carbaldehyde (**7**) is a pyrrole alkaloid first reported to be isolated from natural sources. It is the first time this alkaloid can inhibit inhibiting three cancer cell lines A549, MCF-7, and Hep3B. This result is consistent with the study of Bhardwaj, V. et al. [23], which reported the cytotoxic activity of pyrrole-nucleated alkaloids. Research by Staub, R. E. et al. [24] showed that the alkaloid indole-3-carbinol isolated from the genus *Brassica* could inhibit MCF-7 cancer cells, indole alkaloids isolated from the *Muntafara sesilifolia* plant also has extreme MRC-5 lung cancer cytotoxic activity with IC_{50} from 0.47 to 1.89 µM [18]. Meanwhile, indole-3-aldehyde (**8**), an indole alkaloid

isolated from the male *Carica papaya* flowers, did not show cytotoxicity on all three tested cell lines, proving structural differences lead to differences in activity. In addition, the study of Ashour, M. A. et al. [14] also showed that the compound indole-3-aldehyde has an inhibitory action on the L5178Y cell line (T-cell lymphoma) from DBA female mice.

4. Conclusion

In summary, most flavonoid glycosides and alkaloids isolated from the male *Carica papaya* flowers exhibited cytotoxic activity against tested cancer cell lines (A549, MCF-7, Hep3B) under *in vitro* conditions. The flavonoid glycosides (**1**), (**3**), (**6**), an alkaloid (**7**) exhibited medium cytotoxic activity on these cancer cells with IC_{50} values from 26.72±0.76 to 64.37±3.42 µg/mL. Flavonoid glycosides (**2**), (**4**), and (**5**) showed weak cytotoxic activity on these cancer cells with IC_{50} values from 71.52±3.27 to 91.37±3.40 µg/mL. Alkaloid (**8**) did not display cytotoxic activity on these cancer cells with ellipticine was a positive control.

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