

Full Length Research Paper

Antioxidant and cytotoxic activity of the acetone extracts of root of *Euphorbia hylonoma* and its ellagic acid derivatives

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The acetone extracts and fractions prepared from the roots of *Euphorbia hylonoma* were investigated for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, superoxide anion and hydroxyl radical scavenging activity and cytotoxic activity against the human hepatoma cell line SMMC-7721, human cervix epitheloid carcinoma cell line HeLa and the human gastric cancer cell line SGC-7901. The acetone extract and fraction of EtOAc showed high antioxidant activities. The acetone extract and fraction of water showed a stronger cytotoxic effect on all tested cells, with IC₅₀ values being less than 20 µg/mL. The acetone extract and fraction of water induced cell cycle arrest in G1 phase in SMMC-7721 cells. The major compounds isolated from the acetone extract were ellagic acid derivatives and showed weak cytotoxic activities on the tested cell lines, and these compound were 3,3',4-tri-O-methylellagic acid (1), 3,3'-di-O-methylellagic acid (2), 3-O-methylellagic acid (3) and 3,3'-di-O-methylellagic acid-4'-O-β-dxylopyranosid (4). Compound 3 showed better obvious radical scavenging activity than other tested compounds.

Key words: *Euphorbia hylonorma* Hand-Mazz, antioxidant activity, cytotoxic activity, chemical constituents.

INTRODUCTION

Euphorbia hylonoma Hand-Mazz (Euphorbiaceae), commonly known as Jiu Niuzao, has long been used in folk medicine in China. It grows wildly in Northwestern China and is used as a folk medicine for antineoplastic intervention, and in the treatment of hepatocirrhosis, oedema, and retention of urine (Jiangsu, 1977). Recent studies have indicated that several *Euphorbia* species have also been employed in the world as traditional medicines for the treatment of numerous diseases including skin diseases, gonorrhoea, migraine (Chaabi et al., 2007) and cancerous conditions (Claudia et al., 2004; Zhang et al., 1998; Yasukawa et al., 2000). There have been many reports on the bioactive and chemical constituents from the members of the *Euphorbia* genus

(WHeLan et al., 2003; Bruni et al., 2004; Hore et al., 2006; Shi et al., 2005; Ruan et al., 2006; Cao et al., 1996). Earlier investigators have reported the isolation of the chemical constituents from this plant, these constituents being tannins, ferulic acid esters, and sesquiterpenoids (Guo et al., 1995; Ruan et al., 2007).

There have also been reports on the pharmacognosy of this plant (Guo et al., 1996; Guo et al., 1997). However, the reports on anticancer effects of this plant are scarce. Therefore, in the present study, the antioxidant and the cytotoxic activities of different polarity extractions were tested to get the most active extraction and then this extraction was isolated, and we also tested the antioxidant and cytotoxic activities of all isolated compounds to determine the anticancer agents. The antioxidant effect was measured by DPPH free radical, superoxide anion and hydroxyl radical scavenging assay, and the cytotoxic activity was determined by using thiazolyl tetrazolium (MTT) (3-(3, 4-dimethyl thiazol-yl)-2, 5-diphenyltetrazolium bromide) assays. The human hepatoma cell line SMMC-7721 cells, human cervix epitheloid carcinoma cell line HeLa and the human gastric cancer cell line SGC-7901 were adopted in

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Abbreviations: DPPH, 1, 1-Diphenyl-2-picrylhydrazyl; MTT, thiazolyl tetrazolium; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; IC₅₀, inhibition concentration; FBS, fetal bovine serum; PBS, phosphate buffer saline.

cytotoxic activity determination.

MATERIALS AND METHODS

Plant material

The roots of *E. hylonoma* were collected from the Taibai Mountains, Shaanxi Province, China, in September 2005, and were identified by Professor Juxian Lu, the Faculty of Pharmacy, Medical School of Xi'an Jiaotong University. The voucher specimen was retained at the Faculty of Pharmacy, Medical School of Xi'an Jiaotong University for future reference.

Extraction and isolation procedures

The acetone extracts and their fractions from the roots of *E. hylonoma* were prepared as described below. The dried and powdered roots (1 kg) of plant were extracted with acetone three times for 24 h at room temperature resulting in 212 g of acetone extracts. Part of acetone extracts (100 g) was taken to get suspended in water, and then petroleum ether, chloroform and EtOAc were added and partitioned and concentrated using rotary evaporator. Therefore, we obtained the fraction of water (32 g), fraction of petroleum ether (7.5 g), fraction of chloroform (8.6 g), and the fraction of EtOAc (12.5 g), respectively. Then these extracts and fractions were vacuum-dried and stored in a refrigerator until further tests. Part of acetone extracts (20 g) was chromatographed on silica gel (500 g) column. The column was eluted by stepwise gradient elution with petroleum ether-ethyl acetate (100:1 to 1:100) and methanol. Each eluting was 250 mL and underwent TLC inspection. The fractions with the same TLC spectrum behavior were combined. There were seven fractions A-G. Then, fraction D (4.3 g) was further isolated on silica gel column eluted with petroleum-ether acetate (7:3), and compound 1 (0.8 g) and 2 (0.6 g) were obtained. Further column chromatography of sub-fraction b (1.2 g) from fraction D on Sephadex-LH₂₀ eluted with methanol resulted in compounds 3 (0.4 g) and 4 (0.6 g). Identification of these compounds was performed through analysis of the spectroscopic features.

DPPH radical scavenging activity

In order to measure antioxidant activity, the DPPH radical scavenging assays were carried out according to references (Wang et al., 1998; Yen et al., 1994). IC₅₀ values were calculated from a regression curve, which denotes the concentration of sample required to scavenge 50% of DPPH radicals. The samples of the extracts of *E. hylonoma* for tests were dissolved in dimethyl sulfoxide (DMSO) by sonication. EtOH solution of DPPH (1 × 10⁻⁴ mM) was added, and the reaction mixture was shaken vigorously. The absorbance of samples was measured at the spectrophotometer (Hitachi U-3310 Spectrophotometer, Japan) at 517 nm against a blank of EtOH. The DPPH scavenging activity was calculated as follows: scavenging activity (%) = (1 - absorbance of sample/absorbance of control) × 100. Calculate 50% inhibition concentration (IC₅₀). Ascorbic acid was adopted as positive control.

Superoxide anion scavenging assay

The superoxide anion scavenging activity was measured by the method of Robak and Gryglewski (1988) with a minor modification. The reaction mixture contained 1.5 mL of 0.1 M Tris-HCl buffer (pH = 8.2, 2 mM ethylenediaminetetraacetic acid) (EDTA), sample

solution a mL (a = 0.00 (control), 0.005, 0.01, 0.02, 0.04, 0.07, 0.1), 0.3 mL of 10 mM HCl (zero modulation group) or 0.3 mL of 6 mM pyrogallol hydrochloric acid solution (observation group). After 5 min of incubation at 25°C the absorbance was measured at 420 nm. The superoxide anion scavenging activity was calculated as follows: scavenging activity (%) = (1 - absorbance of sample/absorbance of control) × 100. Calculate 50% (IC₅₀). The positive control was also ascorbic acid.

Hydroxyl radical scavenging assay

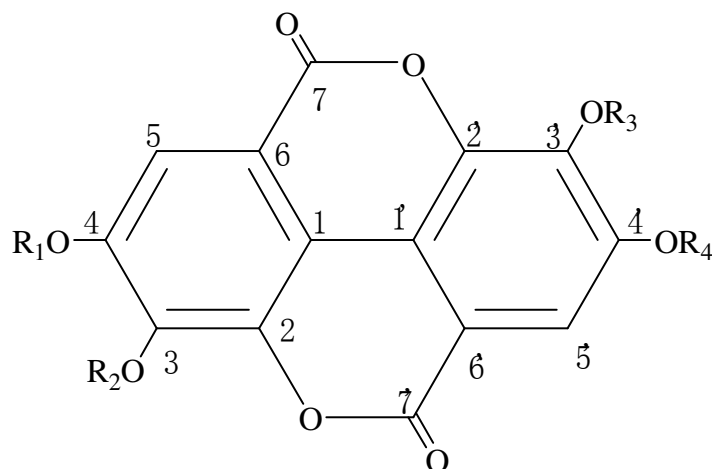
The ability of different *E. hylonoma* fractions and its Ellagic acid derivatives constituents to scavenge the hydroxyl radical generated by Fenton reaction was measured according to the modified method of (Chung et al., 1997). The Fenton reaction mixture contained 1.5 mL of 0.3 M PBS (pH = 7.4), 0.1 mL of 7.5 mM FeSO₄, 0.1 mL of 7.5 mM orthophenanthroline solution, sample solution a mL (a = 0.00 (control), 0.005, 0.01, 0.02, 0.04, 0.07 and 0.1), 0.3 mL of 0.1% H₂O₂ only in observation group and water to a total volume of 4 mL. After 1h of incubation at 37°C the absorbance was measured at 536 nm. The hydroxyl radical scavenging activity was calculated as follows: scavenging activity (%) = (1 - absorbance of sample/absorbance of control) × 100. Calculate 50% (IC₅₀). The positive control was also ascorbic acid.

MTT assay of cell viability and cytotoxic activity

Cell viability was estimated by the MTT assay, which is based on the cleavage of a tetrazolium salt by mitochondrial dehydrogenases in viable cells (Rajesh et al., 2002; Lee et al., 2005). Cytotoxicity was measured by the improved MTT method for the human hepatoma cell line SMMC-7721 cells, human cervix epitheloid carcinoma cell line HeLa cells and the human gastric cancer cell line SGC-7901 cells (These tumor cell lines were obtained from the Center of Medicinal Biology of Xi'an Jiaotong University). Cells were harvested by centrifugation at 250 × g for 5 min and resuspended in RPMI-1640 + 10% fetal bovine serum (FBS) to make a stock cell suspension containing 1 × 10⁵ cells/mL. 100 μL of this stock cell suspension was then added to the wells of a 96-well plate. Various concentrations of the test samples were added to the wells, respectively. Controls were performed in which only culture media was added into wells containing cells. Media was then added to bring the total volume of each well to 200 μL. The cells were then incubated at 37°C in a 5% CO₂, 95% air atmosphere. After 24, 48, and 72 h of incubation, the culture medium was removed and the cells washed twice with phosphate buffer saline (PBS). Then 20 μL of 5 mg/mL MTT was added to each well. The cells were further incubated at 37°C for 4 h. The supernatant was discarded and 150 μL of DMSO was added to each well. The mixture was shaken on a micro-vibrator for 5 min and the absorbance was measured at 490 nm that serves as a measure of cell viability (Polastar Optima, BMG Germany). The IC₅₀ values were obtained by the concentration-response curves of the percentage viable cell versus extract concentrations using Bliss program (Zhu et al., 2005).

Flow cytometry assay

SMMC-7721 cells were harvested by centrifugation at 250 × g for 5 min and resuspended in RPMI-1640 + 10% FBS to make a stock cell suspension containing 2 × 10⁵ cells/mL. 1 mL of this stock cell suspension was then added to the wells of a 6-well plate. SMMC-7721 cells were treated with various concentrations of acetone extracts and fraction of water for 48 h, respectively. Cells were then washed with PBS buffer three times. The cells were stored in prechilled 70% alcohol at 4°C for 12 h. Then alcohol was removed



| | R ₁ | R ₂ | R ₃ | R ₄ |
|------------|----------------|----------------|----------------|----------------|
| Compound 1 | Me | Me | H | Me |
| Compound 2 | Me | H | H | Me |
| Compound 3 | Me | H | H | H |
| Compound 4 | Me | Xyl | H | Me |

Figure 1. The structure of compound 1 to 4.

and the cells washed twice with PBS. PI and RNase solution were added. Then, this solution was put in a darkroom for 30 min. Flow cytometry (Facsccalibur, BD USA) was performed to detect the expression of cell cycle (Frey et al., 2001).

Data handling

All the data were expressed as means \pm standard deviations of five determinations.

RESULTS AND DISCUSSION

Identification of isolated compounds

Compounds 1 to 4 were obtained by combining silica gel and Sephadex LH-20 separation. These compounds were identified by its spectroscopic features and compared with those reported in the literature; the structures are shown in Figure 1. Compound 3 was first obtained from this plant. Compound 1 is amber powder, m.p. 286~288°C. ¹H-NMR (500 MHz, DMSO-*d*₆) δ ppm: δ 10.79(1H, s, OH-4), δ 7.49(1H, s, H-5), δ 7.57(1H, s, H-5'), δ 4.03 (3H, s, Me-3), δ 4.04(3H, s, Me-3'), δ 3.98(3H, s, H-4'); ¹³C-NMR (125MHz, DMSO-*d*₆) δ ppm: 111.11(C-1), 141.40(C-2), 140.74(C-3), 152.58(C-4), 111.63(C-5), 111.83(C-6), 158.23(C-7), 112.42(C-1'), 141.40(C-2'), 140.15(C-3'), 153.74(C-4'), 107.40(C-5'), 113.27(C-6'),

158.41(C-7'), 60.68(OCH₃-3), 61.26(OCH₃-3') and 56.68(OCH₃-4'). By comparing these spectral data with those reported in the literature (Liu et al., 2002; Duc et al., 1990), compound 1 was identified as 3, 3', 4-tri-*O*-methylellagic acid. Compound 2 is amber powder, m.p. > 300°C. ¹H-NMR (500 MHz, DMSO-*d*₆) δ ppm: 4.05 (6 H, s, 2xOCH₃), 7.54(2 H, s, H-5, H-5'), 10.79(2 H, s, OH-4, OH-4'); ¹³C-NMR (125 MHz, DMSO-*d*₆) δ ppm: 111.46(C-1, C-1'), 141.25(C-2, C-2'), 140.23(C-3, C-3'), 152.23(C-4, C-4'), 111.69(C-5, C-5'), 112.18(C-6, C-6'), 158.52(C-7, C-7') and 61.00 (2xOCH₃). After these spectral data were compared with those reported in the literature (Liu et al., 2002; Grzegorz et al., 1996), Compound 2 was identified as 3, 3'-di-*O*-methylellagic acid.

Compound 3 is amber powder, m.p. > 300°C. ¹H-NMR (500 MHz, DMSO-*d*₆) δ ppm: 7.45(1H, s, H-5), 7.50(1H, s, H-5'), 4.03(3 H, s, 3-OCH₃), 10.78(1H, s, 4-OH), 10.73(1H, s, 4'-OH), 10.56(1H, s, 3'-OH); ¹³C-NMR (125 MHz, DMSO-*d*₆) δ ppm: 112.29 (C-1), 140.23(C-2), 139.52(C-3), 152.20(C-4), 110.24(C-5), 111.67(C-6), 159.10(C-7), 112.14(C-1'), 140.22(C-2'), 136.37(C-3'), 148.09 (C-4'), 111.45 (C-5'), 107.66 (C-6'), 158.47 (C-7') and 60.97 (3-OCH₃). After the comparison of these spectral data with those reported in the literature (Liu et al., 2002), Compound 3 was identified as 3-*O*-methylellagic acid. Compound 4 is white powder, m.p.

Table 1. Antioxidant activity of different *E. hylonoma* fractions and compounds 1 to 4.

| Samples | IC ₅₀ (mean±SD, n = 5) | | |
|-----------------------------|-----------------------------------|--------------------------------------|---------------|
| | DPPH· (µg/mL) | ·O ₂ ⁻ (mg/mL) | ·OH (mg/mL) |
| Acetone extracts | 6.80±0.65 | 0.180±0.013 | 0.0507±0.0043 |
| Fraction of Water | 17.76±0.94 | 0.504±0.042 | 0.2172±0.0234 |
| Fraction of petroleum ether | 48.21±3.76 | 0.649±0.078 | 0.1293±0.0152 |
| Fraction of Chloroform | 25.19±1.65 | 0.975±0.082 | 0.4064±0.0373 |
| Fraction of EtOAc | 6.62±0.29 | 0.560±0.054 | 0.1322±0.0147 |
| Compound 1 | 142.48±6.39 | 0.250±0.023 | 0.0322±0.0045 |
| Compound 2 | 233.22±11.46 | 0.457±0.034 | 0.0745±0.0080 |
| Compound 3 | 97.48±5.27 | 0.152±0.027 | 0.0167±0.0023 |
| Compound 4 | -- | -- | -- |
| Ascorbic acid (control) | 3.55±0.27 | 0.035±0.012 | 0.2682±0.0132 |

--: no significant free radical scavenging activity.

241-243°C. ¹H NMR (500 MHz, DMSO-*d*₆) δppm: δ7.48 (1H, d, *J*=5.3, H-5), δ7.73 (1H, d, *J*=3.2, H-5), δ4.05(1H, s, 3-OCH₃), 4.08(1H, s, 3'-OCH₃), δ10. 8(1H, s, 4-OH); ¹³C-NMR(125 MHz, DMSO-*d*₆) δppm: 111.07(C-1), 140.94(C-2), 140.19(C-3), 151.26(C-4), 111.65(C-5), 111.82(C-6), 158.37(C-7), 114.19(C-1'), 141.58(C-2'), 141.98(C-3'), 152.84(C-4'), 112.02(C-5'), 112.72(C-6'), 152.40(C-7'), 101.94(C-1''), 73.09(C-2''), 76.17(C-3''), 69.31(C-4''), 65.84(C-5''), 61.48(3-OCH₃) and 61.02(3'-OCH₃). After the comparison of these spectral data with those reported in the literature (Liu et al., 2002; Yan et al., 2004), Compound 4 was identified as 3, 3'-di-*O*-methyl ellagic acid-4-*O*-β-dxylopyranosid.

Antioxidant activity

The role of free radical reactions in biology has become an area of intense interest. It is generally accepted that free radicals play an important role in the development of tissue damage and pathological events in living organisms (Velazquez et al., 2003). The results of antioxidant activity of different *E. hylonoma* fractions and compounds 1 to 4 are shown in Table 1. The acetone extracts and fraction of EtOAc showed a relatively high DPPH radical, superoxide anion and hydroxyl radical scavenging activity. Compound 3 showed a more obvious radical scavenging activity than other tested compounds. Concerning the radical scavenging activity of acetone extracts and fraction of EtOAc results indicated that extracts and fractions of different polarities exhibit different bioactivities, and the phenolic compounds of the plant constitute one of the major groups of compounds acting as primary antioxidants and free radical terminators by donating hydrogen from the phenolic hydroxyl groups (Maher et al., 2006). It suggests that the antioxidant activity in the extracts suppresses the hydrogen atom abstraction from the tannins and other

compounds. The compounds ellagic acid derivatives showed that more phenol hydroxyl exhibits greater radical scavenging activity. Compound 3 has three active hydrogens, so it showed stronger antioxidant activity. This result is consistent with the previous findings in the researches on other different herbal extracts (Yildirm et al., 2003).

Cytotoxicity activity assay

The extract of *E. hylonoma* stimulated cellular proliferation and decreased cellular viability as determined by the MTT assay. The results of the cytotoxication tests are displayed in Table 2. It presents half of IC₅₀ (µg/mL) of acetone extracts and other fractions and four compounds of *E. hylonoma* on SMMC-7721 cells, HeLa cells, SGC-7901 cells for 24, 48, 72 h treatment. In the US NCI plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity if the IC₅₀ value (concentration that causes a 50% cell kill) in KB carcinoma cells, following incubation between 48 and 72 h, is less than 20 µg/mL, while it is less than 4 µg/mL for pure compounds (Lee et al., 2005). As shown in Table 2, acetone extracts and fraction of water showed a significant cytotoxic activity against all tested cells, fraction of EtOAc displayed a significant cytotoxic activity in HeLa cells with the IC₅₀ value less than 20 µg/mL on 48 h. Fraction of petroleum ether showed a modest cytotoxic activity against SGC-7901 cells. Fraction of chloroform displayed a modest cytotoxic activity in all cells. The table clearly indicates the antitumor potency of acetone extracts and other fractions on all tested cells as follows:

Water> Acetone> Ethyl-acetate >Chloroform> Petroleum ether.

These results suggest that the cytotoxic activity of the

Table 2. The cytotoxic activity (IC₅₀, µg/mL) of acetone extracts and four fractions and compounds 1 to 4 from *E.hylonorma* Hand-Mazz on different cells.

| Samples | SMMC-7721 | | | HeLa | | | | SGC-7901 | |
|-----------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------|
| | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 |
| Acetone extracts | 30.7±0.4 | 15.1±0.1 | 10.5±0.8 | 42.5±0.5 | 22.2±0.5 | 13.0±0.2 | 28.8±0.2 | 18.3±0.6 | 13.2±0.3 |
| Fraction of water | 11.5±0.9 | 8.2±0.8 | 6.4±0.2 | 84.7±1.3 | 18.7±0.9 | 10.9±0.2 | 47.7±0.5 | 5.0±0.5 | 0.26±0.2 |
| Fraction of petroleum ether | 371.6±1.3 | 240.8±1.5 | 184.7±0.3 | 762.2±2.1 | 356.7±1.1 | 194.2±0.5 | 165.2±2.1 | 65.0±0.8 | 28.3±0.5 |
| Fraction of chloroform | 228.4±2.2 | 84.7±0.8 | 32.0±0.2 | 54.7±1.5 | 32.3±0.9 | 13.7±1.3 | 64.6±0.3 | 51.0±0.2 | 37.2±0.1 |
| Fraction of EtOAc | 80.0±0.8 | 39.4±0.6 | 23.2±0.9 | 52.7±1.0 | 11.9±1.3 | 7.6±0.6 | 101.0±1.0 | 43.8±0.7 | 20.3±0.3 |
| Compound 1 | 205.7±0.9 | 172.4±0.3 | 91.5±0.2 | 175.2±0.4 | 129.6±0.1 | 69.2±0.3 | 190.2±0.6 | 124.3±0.2 | 56.2±0.5 |
| Compound 2 | 186.9±0.8 | 135.2±0.5 | 57.6±0.3 | 171.6±0.2 | 127.8±0.3 | 66.8±0.4 | 185.3±1.2 | 132.1±1.1 | 49.2±0.6 |
| Compound 3 | 182.4±1.7 | 132.5±0.2 | 59.6±0.4 | 154.3±0.3 | 146.2±0.5 | 81.2±0.3 | 131.9±0.6 | 88.6±0.3 | 52.1±0.4 |
| Compound 4 | 188.6±1.4 | 136.3±0.6 | 60.1±0.2 | 164.4±0.4 | 143.2±0.2 | 79.1±0.4 | 156.2±1.3 | 79.5±0.4 | 35.6±0.2 |

IC₅₀ values are the mean±standard deviation from five independent experiments.

compounds from *E. hylonoma* is mainly present in acetone extracts and fraction of water. In this case the acetone extracts were further isolated and obtained four ellagic acid derivative compounds 1 to 4. In preclinical studies, ellagic acid was reported to have a wide range of chemopreventive activities, inhibiting chemically induced carcinogenesis in the esophagus, lungs, tongue, colon and skin. The relatively low toxicity of ellagic acid ellagitannins throughout the plant has led to many investigations related to cancer chemoprevention (Khallouki et al., 2007). In this investigation, the free ellagic acid was not detected in the extracts, but rather its various O-methyl derivatives compounds 1 to 4 were obtained. The MTT was used to assay cytotoxic activity of these compounds. Compounds 1 to 4 showed a weak activity against all tested cells, the IC₅₀ value was higher than 50 µg/mL on 48 h. It is clear that there should be another compound or compounds in the extracts that displays a good cytotoxic activity to inhibit these tumor cells. The water fraction exhibits the highest cytotoxic activity in all the tested cells which indicates that the active cytotoxic compounds maybe the polarity compounds. It also showed why the plant is taken as antineoplastic agent in traditional use in China.

Expression of cell cycle by flow cytometry

In order to study the mechanism of the folk medicine in antineoplastic treatment, the cell cycle of SMMC-7721 cells was analyzed using flow cytometry after exposure to 50, 80, 100 µg/mL of acetone extracts and fraction of water for 48 h, respectively. As shown in Figure 2, it indicated that the SMMC-7721 cells growth was arrested at G₀/G₁ phase. Cells were treated with 50 and 100 µg/mL of fraction extracts. Then, the percentage of the G₁ phase cells increased (control: 64.45 ± 5.23%, 50 µg/mL: 70.7 ± 6.47%, 80 µg/mL: 76.05 ± 8.32%, 100 µg/mL: 76.85 ± 7.29%), and for acetone extracts, the percentage of G₁ phase cells also increased (control:

64.45 ± 5.23%, 50 µg/mL: 73.58 ± 6.92%, 80 µg/mL: 80.15 ± 7.12%, 100 µg/mL: 79.30 ± 6.49%). In non-apoptotic population, the portion of cells in G₁ phase increased with a decrease of cells in S phase (acetone extract: control: 34.11 ± 2.73%, 50 µg/mL: 26.16 ± 2.06%, 80 µg/mL: 23.87 ± 3.01%, 100 µg/mL: 15.89 ± 0.98%; fraction of water: control: 34.11 ± 2.73%, 50 µg/mL: 24.82 ± 2.23%, 80 µg/mL: 18.58 ± 1.76%, 100 µg/mL: 19.49 ± 2.09%). G₂/M phase cells percentage did not have significant changes. These results suggest that acetone extracts and fraction of water can induce cell cycle arrest in G₁ phase in SMMC-7721 cells.

Conclusion

This study has indicated that extracts and fractions from *E. hylonoma* of different polarities exhibit different bioactivities. Our research found that the acetone extracts and fraction of EtOAc showed a relatively high antioxidant activity. The acetone extracts and fraction of water showed a stronger cytotoxic effect on SMMC-7721 cells, HeLa cells, and SGC-7901 cells, with IC₅₀ values being less than 20 µg/mL on 48 h. Therefore, we chose acetone fraction as the subject for further research. Acetone fraction as isolated and four ellagic acid derivatives were obtained, 3,3',4-tri-O-methylellagic acid (1), 3,3'-di-O-methylellagic acid (2), 3-O-methylellagic acid (3) and 3,3'-di-O-methylellagic acid-4'-O-β-dxylopyranosid (4). Compound 3 showed a more obvious radical scavenging activity than other tests compounds. All of these compounds showed weak cytotoxic activity on the tested cells. Flow cytometry assay showed that acetone extracts and fraction of water can induce cell cycle arrest in G₁ phase in SMMC-7721 cells.

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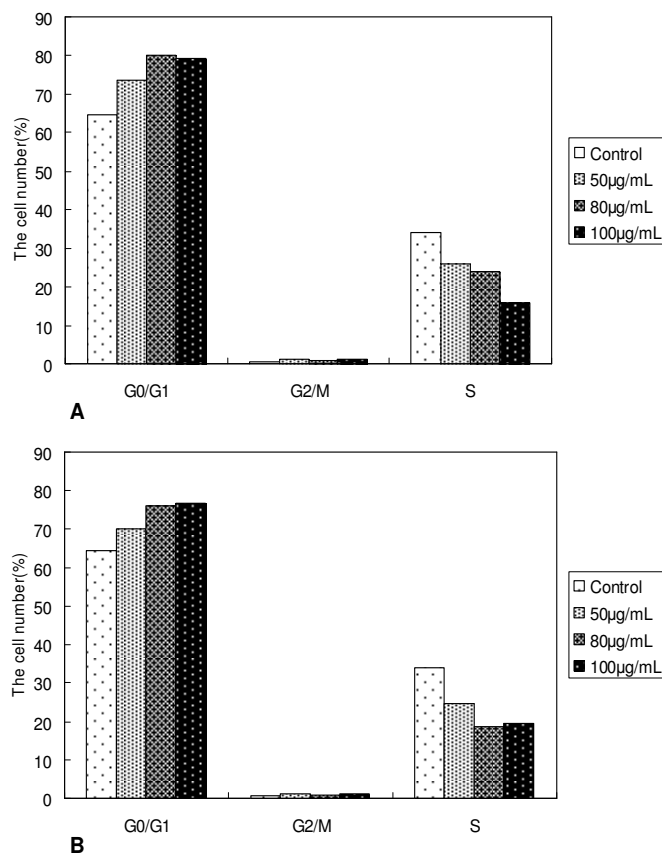


Figure 2. The effect of acetone extracts (A), fraction of water (B) on cell cycle distribution in SMMC-7721 cells.

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