

Extraction And Characterization Of Cycloartenol Isolated From Stems And Leaves of *Coix Lacryma-Jobi* L. And Its Potential Cytotoxic Activity

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Research

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Abstract

[Background] *Coix lacryma-jobi* L. is a nourishing food and a traditional Chinese medicine and has been used for the treatment of neuralgia, inflammatory diseases, and rheumatism. Little is known about the anti-tumor of *Coix lacryma-jobi* L.. In this study, the cytotoxic effects of *Coix lacryma-jobi* L. on HeLa, HepG2, and SGC-7901 were evaluated.

[Methods] The cytotoxic active compounds were isolated and extraction from the stems and leaves of *Coix lacryma-jobi* L. The structural identification of the compound was determined using ultraviolet spectroscopy, Fourier transform infrared spectroscopy and nuclear magnetic resonance spectroscopy. The cytotoxic activity *in vitro* effect of the compound was determined using CCK-8, Flow cytometry, and DNA Topo I inhibition experiments.

[Results] A compound F2 was isolated and purified from the petroleum ether extract of *Coix lacryma-jobi* L. stems and leaves. It was identified as the cycloartenol. The minimum IC_{50} values of HeLa, HepG2 and SGC-7901 cells for this compound were 500, 537.7, and 336.8 $\mu\text{g}/\text{mL}$, respectively. The compound had pro-apoptotic effects on three types of tumor cells, and had a significant inhibitory effect on DNA topoisomerase I.

[Conclusion] This study demonstrates that cycloartenol has good cytotoxic activity *in vitro*, suggesting that cycloartenol could be a potential candidate as a natural antitumor drug.

Introduction

Coix lacryma-jobi L. is an annual or perennial herbaceous plant of Graminae family. It is a nourishing food and a traditional Chinese medicine and has been used for the treatment of neuralgia, inflammatory diseases, rheumatism, osteoporosis, and as a diuretic (Yang et al., 2013). The *Coix lacryma-jobi* L. plant has significant medicinal value in human (Xi et al., 2016). It is widely cultivated in China, Taiwan, Japan, Thailand and Korea. Coix seeds contain polysaccharides, flavonoids, phenols, proteins, fibers, vitamins and oils. Among them, the polysaccharides and polyphenols of *Coix lacryma-jobi* L. are considered to be the major active components and have immunomodulatory, anti-oxidant and anti-inflammatory effects (Li et al., 2019; Wang et al., 2012; Yao et al., 2015). Phenolic compounds have a variety of biological activities in antioxidant activity and xanthine oxidase inhibitor activity (Xu et al., 2017). Polysaccharides have immune potentiation effects on macrophages and should serve as a beneficial health food. Coixol shows an inhibitory action to cancer cells and a composure and demulcent action with the central nervous system, and has anti-inflammatory, health and beauty functions (Amen et al., 2017; Hu et al., 2020; Son et al., 2017).

In recent years, people are paying more attention to the pharmacological and health functions of coix seeds. For example, the coix seed constituents have strong anti-oxidant, anti-inflammatory, and anti-obesity activities, and can stimulate reproductive hormones, promote uterine contraction and modulate gut microbiota (Devaraj et al., 2020). The study on the anti-tumor effect of coix seeds is in-depth, but

there are few studies on the pharmacological effects of other parts of *Coix lachryma-jobi* L. Lee et al. (2008) showed that five active compounds that could inhibit tumor cells were isolated from *Coix lachryma-jobi* L. var. *ma-yuen* Stapf, and their structures and activities *in vitro* were characterized. All the compounds were tested for their anti-proliferative effect on A549, HT-29 and COLO205 cells. These compounds showed anti-tumor activities. Chang et al. (2018) suggested that the anti-tumor effects of the hexane fraction of the *Coix lachryma-jobi* L. var. *ma-yuen* Stapf testa ethanolic extracts on the human uterine sarcoma cancer cell line MES-SA. Manosroi et al. (2016) showed that the anti-cancer activities of *Coix lachryma-jobi* L. extracts on human colon adenocarcinoma *in vitro*. Overall, *Coix lachryma-jobi* L. has been used as an herbal medicine in China. Many studies of this plant have reported anti-tumor effects on human tumor cell lines.

In previous study, we observed that the aqueous and ethanol extracts of *Coix lachryma-jobi* L. stems and leaves could inhibit the growth of HeLa, HepG2 and SGC-7901 cell lines (Lin et al., 2018; Zhu et al., 2015). The extracts also could inhibit S180 sarcoma cells in mice, and protect the liver, spleen, thymus, and other immune organs of mice (Lin et al., 2015). Interestingly, the petroleum ether extracts of stems and leaves of *Coix lachryma-jobi* L. had the similar inhibitory effect on HeLa, HepG2 and SGC-7901 cell lines *in vitro*.

Recent studies have shown that *Coix lachryma-jobi* L. stems and leaves may inhibit tumor cells growth, but only a few anti-tumor specific compounds have been identified. In this study, the compound F2 from stems and leaves of *Coix lachryma-jobi* L. was isolated and purified. The structure of compound F2 was identified using ultraviolet (UV), Fourier transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy. Its cytotoxic activity was evaluated using CCK8, flow cytometry and DNA topoisomerase I (DNA Topo I) inhibition assay. In addition, the structure-activity relationship and action mechanism were explored, to provide a certain reference for the synthesis and development of anti-tumor drugs.

Material And Methods

2.1 Plant and cell

The stems and leaves of *Coix lacryma-jobi* L. were collected from Zubie Yao and Miao Ethnic Township in Xilin County of the Guangxi Zhuang Autonomous Region of China, in October 2017, and identified by professor Qin Daoguang from Teaching and Research Section for Ethnic Medicine of Youjiang Medical University for Nationalities as *Coix lacryma-jobi* L. HeLa, HepG2, and SGC-7901 cell lines were purchased from the Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China).

2.2 Plant material preparation and extraction

Plant material preparation and extraction process was shown in Fig. 1. The air-dried *Coix lacryma-jobi* L. stems and leaves (~ 50 kg) were crushed separately, and extracted three times with 200 L 95% ethanol (Chengdu Kelong Chemical Co., Ltd., Chengdu, Sichuan, China) and heated under reflux for 2 h (Chiang et al., 2020; Zhang et al., 2019) to yield ~ 1 kg of a crude extract, which was suspended in 4 L ultrapure water (Milli-Q A10, Merck Millipore, Billerica, MA, USA), extracted with petroleum ether (4 × 10 L) (Chengdu Kelong Chemical Co.), and the petroleum ether extract was combined and concentrated using a rotary

evaporator (BC-R501C, Bekai, Shanghai, China) at 40°C to obtain ~ 50 g of petroleum ether extract. Part of the extract was separated using silica gel column chromatography (200–300 mesh, Qingdao Wave Silica Gel Desiccant Co., Ltd., Qingdao, Shandong, China). Gradient elution (15:1→10:1→5:1) was carried out with petroleum ether-ethyl acetate as the mobile phase, and the eluent was examined using thin layer chromatography (TLC) Developing agent (petroleum ether:ethyl acetate = 10:1) (v/v). The TLC plate (GF₂₅₄ plate, Qingdao Wave Silica Gel Desiccant) was sprayed using 10% (v/v) sulfuric acid in ethanol, and then heated at 110°C for 5 min. According to the TLC analysis, 11 fractions were obtained (Fr. 1–1 to Fr. 1–11). Based on the previous experimental results (Lin et al., 2018), the Fr. 1–7 fraction (extracted from petroleum ether-ethyl acetate, 15:1) was further separated using silica gel column chromatography. The eluent was dichloromethane (CH₂Cl₂) (Chengdu Kelong Chemical) as the mobile phase, and the eluent was again examined using TLC. According to the TLC analysis, 6 fractions were obtained (Fr. 2–1 to Fr. 2–6). Fr. 2–2 was used for further study.

Fr. 2–2 was scanned from 190–600 nm (HPLC2695, Waters Co., Milford, MA, USA). The maximum absorptions were at 200 and 240 nm. Fr. 2–2 was separated and purified using preparative high performance liquid chromatography (NP7060C, Hanbon Science & Technology Co., Huaian, Jiangsu, China). The chromatographic conditions were as follows: C₁₈ column, diameter 8 cm, length 65 cm. Gradient elution was carried out with methanol as the mobile phase, flow rate of 140 mL/min, wavelength of 210 nm and evaporative light scattering detector (ELSD-2000, Alltech, Chicago, IL, USA) to obtain the fraction Fr. 3–2 (t_R = 34 min). Fr. 3–2 was concentrated at 50°C, and crystallized with methanol-water (100:1, v/v) to obtain the compound referred to as F2 (250 mg).

2.3 Structural identification of the compound F2

2.3.1 Ultraviolet (UV) analysis

F2 (5 mg) was dissolved in 5 mL deionized water (Sigma-Aldrich, St. Louis, MO, USA) for scanning between 100 and 500 nm using a UV spectrophotometer (1260, Agilent, Palo Alto, CA, USA).

2.3.2 Fourier-transform infrared (FT-IR) spectrum analysis

The FT-IR spectrum analysis of the compound F2 was done using a FT-IR spectrometer (Nicolet 6700, Thermo Scientific Co., Waltham, MA, USA) at room temperature. Sample pellets were obtained by mixing the lyophilized (Alpha 1–2 LD plus, Christ, Osterode, Germany) compound F2 (5 mg) powder with KBr (500 mg) (Sigma-Aldrich), and compressed into tablets prior for scanning in the frequency range of 400 to 4000 cm⁻¹ and at a resolution ratio of 1 cm⁻¹.

2.3.3 Nuclear magnetic resonance (NMR) spectroscopy analysis

F2 structure was measured using NMR analysis using a NMR spectrometer (Avance II-600, Bruker, Karlsruhe, Germany). F2 (25 g) was dissolved in 0.55 mL deuterium oxide (D₂O) (Sigma-Aldrich), lyophilized, and redissolved in D₂O for ¹H NMR (600 MHz) and ¹³C NMR (150 MHz) spectrometry. All data were processed and analyzed using MestReNova software, Version 11.0.4 (Mestrelab Research, Santiago de Compostela, Spain).

2.4 Cell viability

Cell viability was detected using a colorimetric Cell Counting Kit (CCK, CK04, Dojindo Laboratories, Kyushu, Japan) assay based on the manufacturer's instructions. Briefly, The tumor cells were seeded in a 96-well plate (1.0×10^4 cells/well). The cells was cultured overnight under different treatments, the medium was exchanged with 90 μ L of fresh medium supplemented with 10 μ L CCK8 and incubated for 3 h at 37°C. Subsequently, the absorbance was measured at 450 nm with a microplate reader (SpectraMax Plus 384, MD, USA). The data were obtained from 6 independent experiments.

2.5 Flow cytometry

Cells were digested with trypsin and then washed with phosphate-buffered saline (PBS) (Gibico). An Annexin V-FITC Apoptosis Detection Kit (AP101-100-kit, Hangzhou MultiSciences Biotech Co., Ltd., Zhejiang, China) was used to practice cell apoptosis in line with the manufacturer's instructions. The apoptotic cells were dual-stained with propidium iodide (PI) and Annexin V-FITC using an Annexin V-FITC Kit (Hangzhou MultiSciences Biotech). Analysis was carried out by Flow Cytometer (ACCURI C6, Becton, Dickinson, Franklin, NJ, USA).

2.6 Inhibitory effect of the compound F2 on DNA Topo I

Different concentrations of a positive control drug 10-HCPT (Selleck Chemicals, Houston, TX, USA) and different concentrations of the compound F2 were brought to 20 μ L of the ultrapure water system (Merck Millipore), reacted at 37°C for 30 min, then 2 μ L 10% SDS was added to stop the reaction. Proteinase K (1 μ L, RT403-01, Tiangen Biotech (Beijing) Co., Ltd., Beijing, China) and put in a water bath at 37°C for digestion for 15 min, extracted with phenol:chloroform:isoamyl alcohol (1:1:1, v/v/v) (Sangon Biotech). The aqueous phase (15 μ L) was added to 3 μ L of 6 \times loading buffer, Twelve μ L with the λ -Hind III digest as a marker and 1% agarose gel was used for electrophoresis (EPS-600, Tanon Science & Technology Co., Ltd., Shanghai, China) at 5 V/cm for 2 h. DNA was visualized with nucleic acid stain (10,000 \times) for 15 min, decolorized using ultrapure water for 15 min, and quantification was done by a gel imaging system (Gel doc XR+, Bio-Rad, Hercules, CA, USA).

2.7 Statistical analysis

Statistical analysis was done with one-way analysis of variance using the Statistical Package for the Social Sciences (SPSS) software, version. 22.0 (SPSS Inc., Chicago, IL, USA). Group means were compared, using the least significant differences and $p < 0.05$ or $p < 0.01$ were considered to be statistically significant.

Results

3.1 Structural analysis of the compound F2

F2 was obtained using the extraction and purification of the petroleum ether extract from *Coix lacryma-jobi* L. stems and leaves. F2 was a white powder. The UV absorption peak $\lambda_{\max} = 202$ nm (Fig. 2a) suggested that there is no color-enhancing group in the molecular structure, there may be isolated double

bonds. The FT-IR spectra (Fig. 2b) of the compound F2 shows the presence of characteristic signal peaks, such as hydroxyl groups (3412 cm^{-1}), methyl groups ($2959, 2927, 2867\text{ cm}^{-1}$) and double bond (1638 cm^{-1}).

According to signals $\delta\text{H} 1.60, \text{s}; 1.684, \text{s}; 0.887, \text{s}; 0.808, \text{s}; 0.959, \text{s}; 0.962, \text{s}$ in $^1\text{H-NMR}$ ($600\text{ MHz}, \text{CDCl}_3$) (Fig. 3a), there are 6 methyl signals, and $\delta\text{H} 5.10, \text{m}$ is a separate double bond hydrogen signal, and $\delta\text{H} 3.280, \text{m}$ is a carbon signal connected to oxygen atom.

On the basis of $^{13}\text{C-NMR}$ ($150\text{ MHz}, \text{CDCl}_3$) and distortionless enhancement by polarization transfer (DEPT) at 135° (Fig. 3b), it can be inferred that the compound has a total of 30 carbon atom signals, in which 7 are methyls (CH_3), 11 methylenes (CH_2), 6 methines (CH), and 6 quaternary carbons (C). These data inferred that there are 30 carbon atoms and 49 hydrogen atoms; and there is only one carbon signal connected to oxygen atom $\delta\text{C} 78.85$, indicating that the carbon is related to hydroxyl (OH). Therefore, it can be inferred that the molecular equation of this compound most likely was $\text{C}_{30}\text{H}_{50}\text{O}$, it may be a triterpenoid or steroidal compound. By calculation, the unsaturation is 6. After removing the unsaturation of a double bond, there are 5 unsaturations, so there may be 5 rings. $\delta\text{C} 125.26, 130.79$ are double bond signal.

Using the carbon-hydrogen signal one to one with HSQC 2D NMR spectra (Fig. 3c), and combining $\delta\text{H} 5.10, \text{m}$ and $\delta\text{H} 2.041, \text{ol}$, $\delta\text{H} 2.041, \text{ol}$ and $\delta\text{H} 1.437, \text{ol}$, $\delta\text{H} 1.437, \text{ol}$ and $\delta\text{H} 1.383, \text{ol}$, $\delta\text{H} 1.383, \text{ol}$ and $\delta\text{H} 0.879, \text{d}$, $J = 7.3\text{ Hz}$ with COSY 2D NMR spectra (Fig. 3d) and $\delta\text{C} 130.89$ and $\delta\text{H} 5.10, \text{m}; 1.60, \text{s}; 1.684, \text{s}$ with HMBC 2D NMR spectra (Fig. 3e), all are interrelated, so it can be inferred that there is a functional group of (1,5-dimethyl-4-ene)-hexyl, that is, 8 carbon atoms, and the other carbon atoms may be pentacyclic triterpenes or pentacyclic sterols composed of 22 carbons, including 4 methyls, and the remaining 18 carbons forming a five-ring structure. Using the similar structural data in literature (Radics et al., 1975; Teresa et al., 1987), the compound F2 was determined to be cycloartenol. The molecular weight (MW) of the compound F2 was $\sim 426\text{ kDa}$. The structure of compound F2 is shown in Fig. 4.

3.2 Cell viability assay

The cell viability of compound F2 with the HeLa, HepG2 and SGC-7901 cell lines is shown in Fig. 5 after 24, 48 and 72 h, respectively. These results showed that compound F2 inhibited the proliferation of the three cell lines at different time points. The cytotoxic active by compound F2 of the three cell lines was dose-dependent. The cell survival rate declined with the increase of drug concentration, and the cytotoxic active of the drugs was proportional to the drug concentration. At 72 h, the IC_{50} values of the compound F2 to the three tumor cells were 500, 537.7 and 336.8 $\mu\text{g/mL}$, respectively. It can be seen that the compound F2 was sensitive to SGC-7901, and cytotoxic active was much lower than the 10-HCPT.

3.3 Induction of apoptosis by compound F2

From Fig. 6, it can be suggested that the apoptotic rate of the three tumor cell lines increased significantly after treatment with compound F2 and 10-HCPT. The apoptotic rates of compound F2 on HeLa, HepG2 and SGC-7901 cells were $56.67 \pm 0.24\%$, $69.09 \pm 0.55\%$ and $67.94 \pm 1.84\%$, respectively. It can be

suggested that compound F2 had high pro-apoptotic effect on three tumor cell lines. Interestingly, the pro-apoptotic effect of compound F2 on HepG2 and SCG-7901 cells was higher than that of 10-HCPT at the same concentration. These results suggested that compound F2 could be a new promising agent for tumor treatment.

3.4 Inhibitory effect of compound F2 on DNA Topo I

Figure 7 shows that 10-HCPT and compound F2 of at different concentration of the positive control group had significant inhibition of the DNA Topo I, and showed a concentration-dependent effect. The inhibitory effect of compound F2 on DNA Topo I was lower than that of the positive control 10-HCPT. These results showed that they have similar effects.

Discussions

Taking the anti-tumor activity as the separation guide, a anti-tumor active compound was isolated from the stems and leaves of *Coix lacryma-jobi* L. Cycloartenol, a phytosterol compound, is one of the key precursor substances for biosynthesis of numerous sterol compounds. Cycloartenol has a variety of pharmacological activities such as anti-inflammatory, anti-tumor, antioxidant, antibiosis and anti-alzheimer's disease. Cycloartenol also plays an important role in the process of plant growth and development (Niu et al., 2018; Zhang et al., 2017). In this study, we demonstrated that cycloartenol exhibited anti-tumor activities via inhibiting suppression of cell proliferation, and induction of apoptosis *in vitro*.

According to the experimental results and the results of previous studies about similar chemical components or compounds, we discussed the structure-activity relationship and mechanism of anti-tumor effects of cycloartenol. It is inferred that the structure-activity relationship and mechanism of cycloartenol. Some studied found that extracts from stems and leaves of *Coix lacryma-jobi* L. have cytotoxic active. Zhu et al. (2014) used water and ethanol extracts of stems and leaves of *Coix lacryma-jobi* L. to act on HeLa, HepG2 and SGC-7901 cells. These suggested that the water and ethanol extracts of the stems and leaves of *Coix lacryma-jobi* L. can inhibit these cells. Lin et al. (2018) studied the *in vitro* anti-tumor activity of stems and leaves of *Coix lacryma-jobi* L. petroleum and suggested that this compound can inhibit the growth of HeLa, HepG2 and SGC-7901 cells. On the other hand, Son et al. (2017, 2019) observed that *Coix lacryma-jobi* L. sprout extract significantly inhibited the cell proliferation in human cervical cancer HeLa and colon cancer cells *in vitro*. These results confirmed the cytotoxic active of cycloartenol.

The cytotoxic active of cycloartenol may be related to the spatial structure of the mother nucleus of the cycloartane, C3-OH and double bonds (Al Muqarrabun et al., 2014; Reddy et al., 2017; Xia et al., 2018). Among the 12 cycloartane triterpenes and 4 chromones, it was suggested that two compounds showed dramatic inhibitory activities against human breast cancer cells (Li et al., 2018). In addition, Chumkaew et al. observed that a new cytotoxic sterol, stigmast-5-ene-3beta,17alpha-diol from the fruits of *Syzygium*

siamense. This compound has cytotoxic activity against human oral epidermoid carcinoma, human breast cancer and human small cell lung cancer cell lines. In summary, they have similar structures.

Cycloartenol can inhibit the proliferation and promote apoptosis of HeLa cells using blocking the cells at the G2/M phase (Dai et al., 2017; Niu et al., 2018). They may inhibit the degradation, invasion and metastasis of HeLa cell-associated matrix using specifically blocking the gelatinase in HeLa cells and inhibiting the matrix metalloproteinase 2 (MMP-2), and independently promote cell apoptosis. They can also induce apoptosis from exogenous pathways using induced prostate apoptosis response-4 (Par-4) activation of downstream caspase-3, activating Fas-mediated signaling pathways. Withaferin A (3-azido Withaferin A) can inhibit ERK and Akt phosphorylation in HeLa cells, accordingly inhibiting cell proliferation and promoting apoptosis of HeLa cells (Rah et al., 2012). On the other hand, cycloartenol can inhibit the proliferation of HepG2 and promote its apoptosis. It can block cells at the G2/M phase and induced apoptosis using endogenous mitochondrial pathway, then reduce lysosomal degradation activity, accordingly leading to impaired autophagic flux of HepG2 cells, or possibly connected with regulating the expression of p53, cdc2 and caspases family proteins in the cells (Sun et al., 2015). In addition, cycloartenol can inhibit the proliferation of SGC7901 cells using blocking SGC7901 cells at the G2/M phase. It also promoted the apoptosis of SGC7901 cells using up-regulating the pro-apoptotic protein Bax and down-regulating the expression of apoptotic protein Bcl-2, or possibly using regulating the expression of TIMP-1, TIMP-2, MMP-2, MMP-9, and CD147 to inhibit cell invasion (Niu et al., 2018). Using up-regulating ATG5-ATG12 conjugate protein, it can induced autophagy, and using inhibiting PI3K/Akt/mTOR/p70S6K1 signaling pathway, it can induced apoptosis and autophagy (Liu et al., 2011).

The 10-HCPT is a typical antineoplastic drug. As a Topo I inhibitor, it blockades the normal binding of Topo I to DNA and inhibits the rapid proliferation of tumor cells, thus exerting its antineoplastic activity (Hu et al., 2012). The inhibitory effect of compound F2 on DNA topo I activity may be related to its carbonyl, double bond, and hydroxyl groups (Huang & Liu, 2017).

Conclusions

In summary, the cycloartenol was isolated and purified from the petroleum ether extract of *Coix lacryma-jobi* L. stems and leaves. CCK8, flow cytometry and DNA Topo I inhibition experiments have confirmed that cycloartenol has good cytotoxic active *in vitro*. However, its anti-tumor molecular mechanism still needs further study.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Conceptualization, Qiaorong Yu and Suoyi Huang; Data curation, Qiaorong Yu and Tong Li; Formal analysis, Qiaorong Yu and Rong Li; Funding acquisition, Suoyi Huang; Investigation, Qiaorong Yu, Guangbin Ye, Rong Li and Tong Li; Methodology, Qiaorong Yu, Rong Li and Tong Li; Supervision, Suoyi Huang; Writing-original draft, Qiaorong Yu and Guangbin Ye; Writing-review & editing, Guangbin Ye and Suoyi Huang. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

List of abbreviations

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

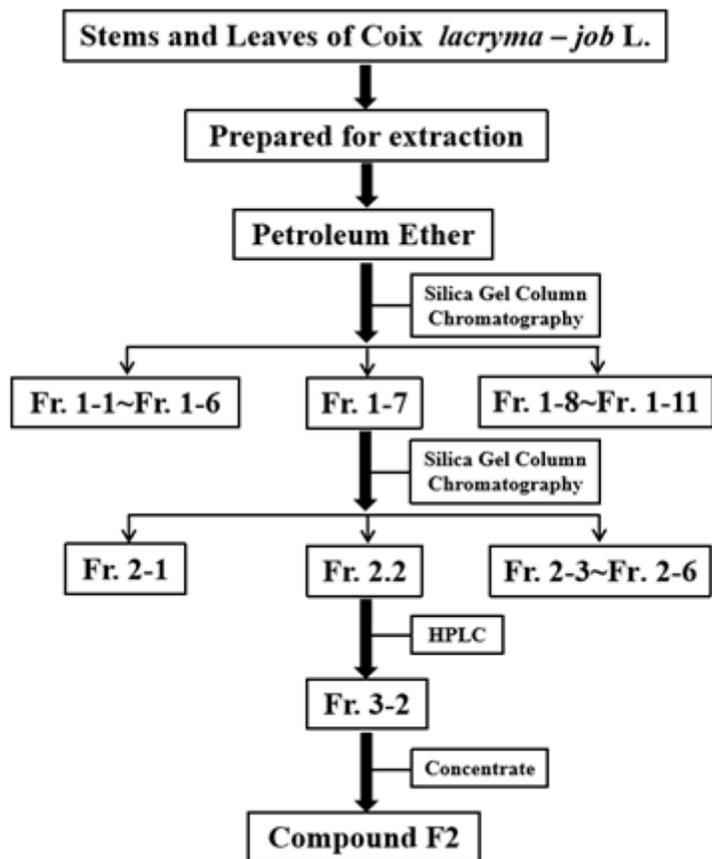


Figure 1

Flow chart showing plant material preparation and extraction process.

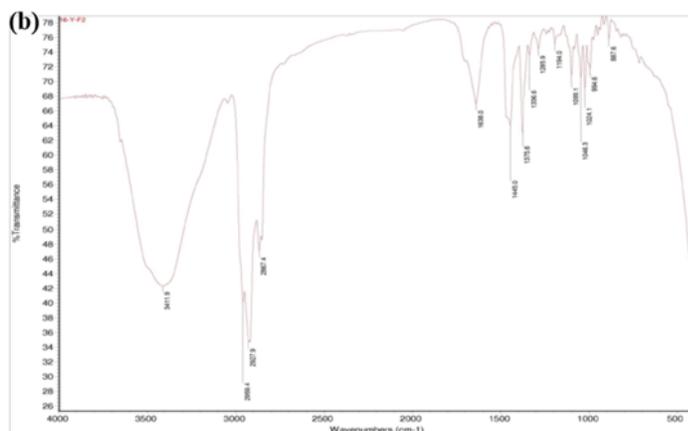
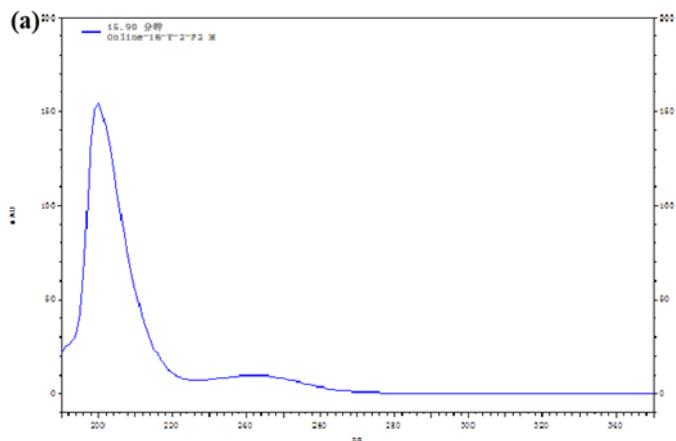


Figure 2

UV (a), and FT-IR (b) chromatogram of the compound F2 from *Coix lacryma-jobi* L. stems and leaves.

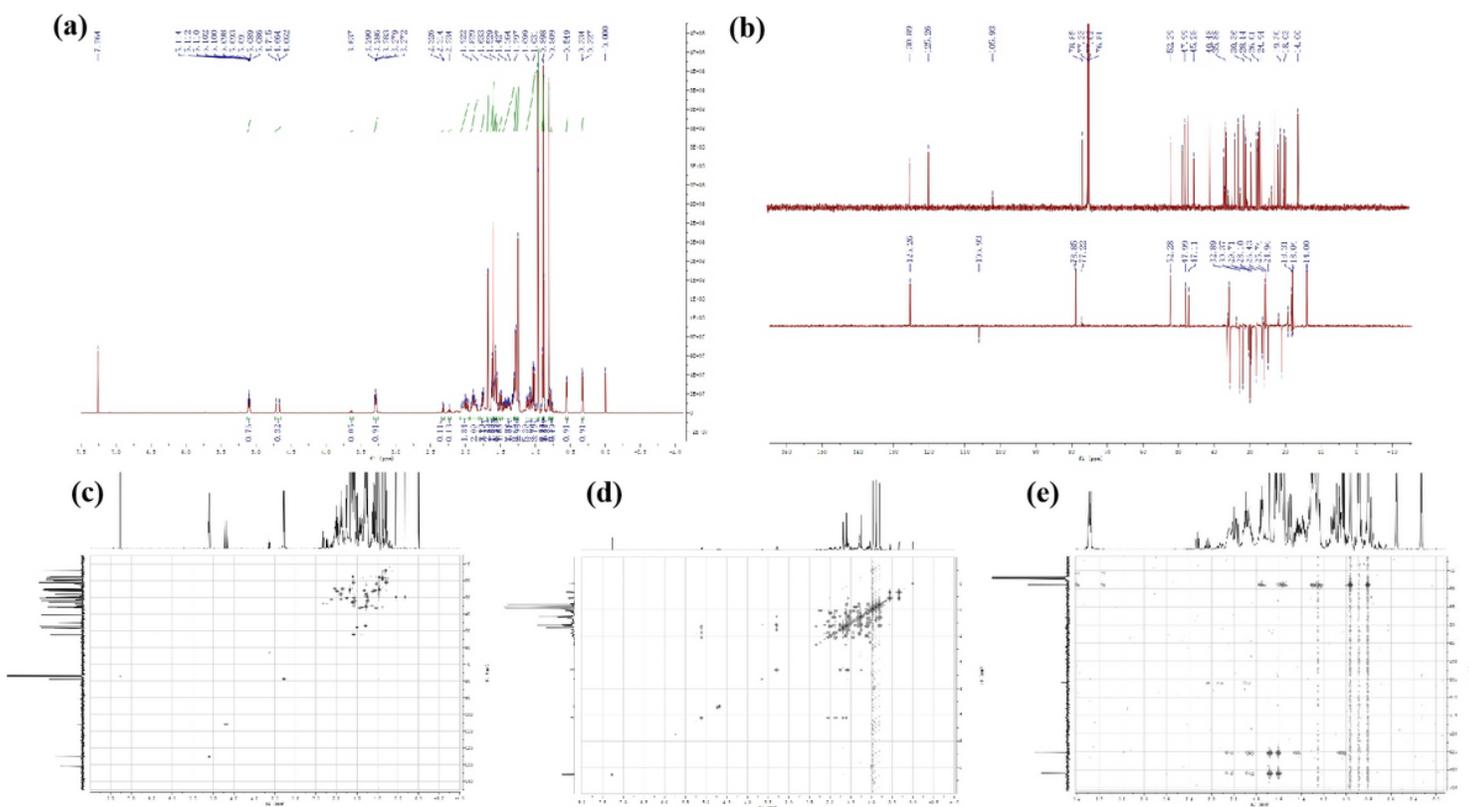


Figure 3

1D and 2D NMR spectra of compound F2 from *Coix lacryma-jobi* L. stems and leaves. (a) ¹H-NMR; (b) ¹³C-NMR; (c) HSQC 2D NMR; (d) COSY 2D NMR; (e) HMBC 2D NMR.

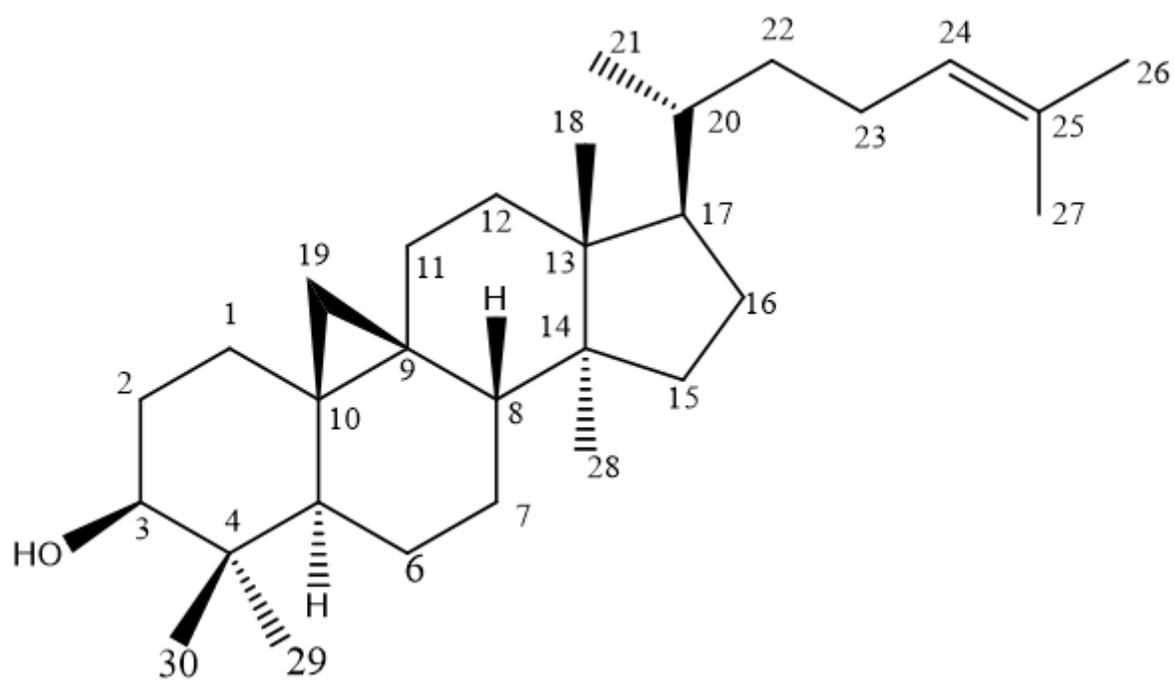


Figure 4

The structure of the compound F2.

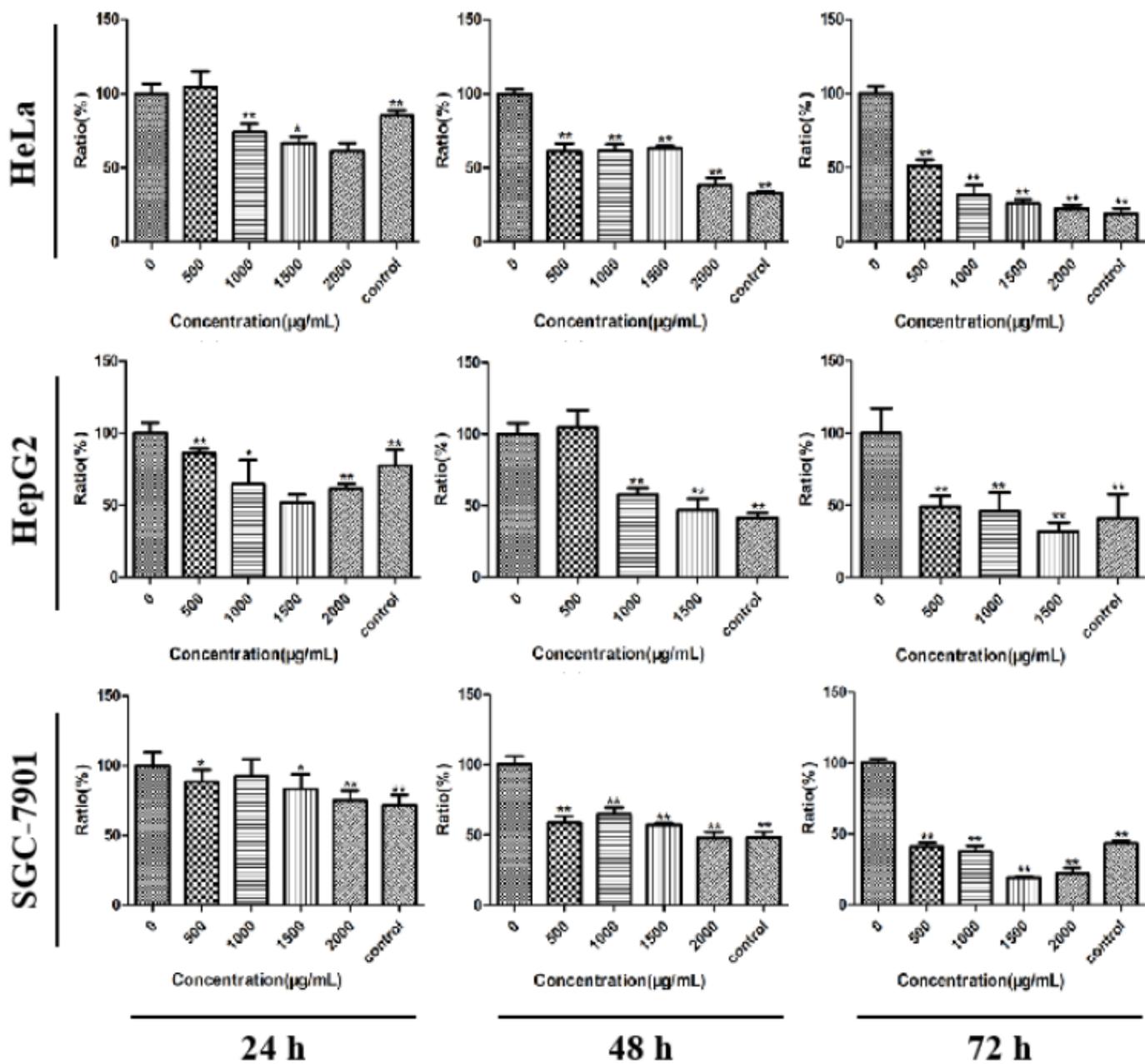


Figure 5

Effect of compound F2 on the viability of HeLa, HepG2 and SGC-7901 cell lines. The cell activity of compound F2 treatment for 24, 48 and 72 h, respectively. Compared with no treatment group, **: $p < 0.01$, *: $p < 0.05$, control; 10-10CP.

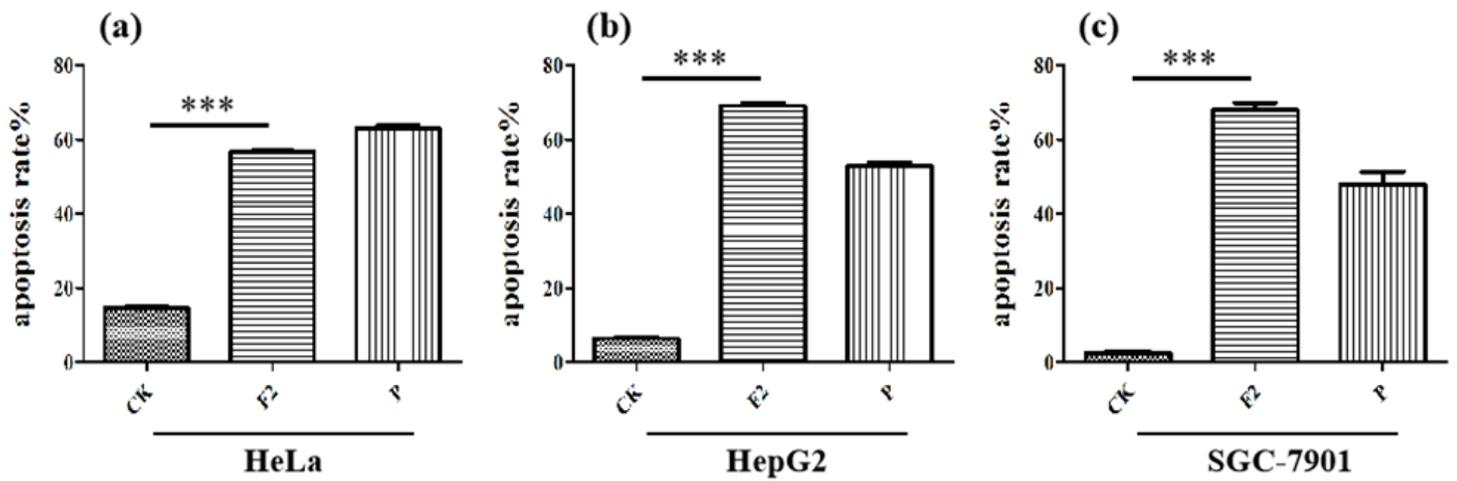


Figure 6

Apoptotic effect of compound F2 on HeLa (a), HepG2 (b) and SGC-7901 (c) cell lines. Bar charts depicting percentage of apoptotic rate for the treatments on the corresponding cell lines. Values are mean \pm SD (n = 3). CK, control; P, 10-HCPT; ***, p < 0.01.

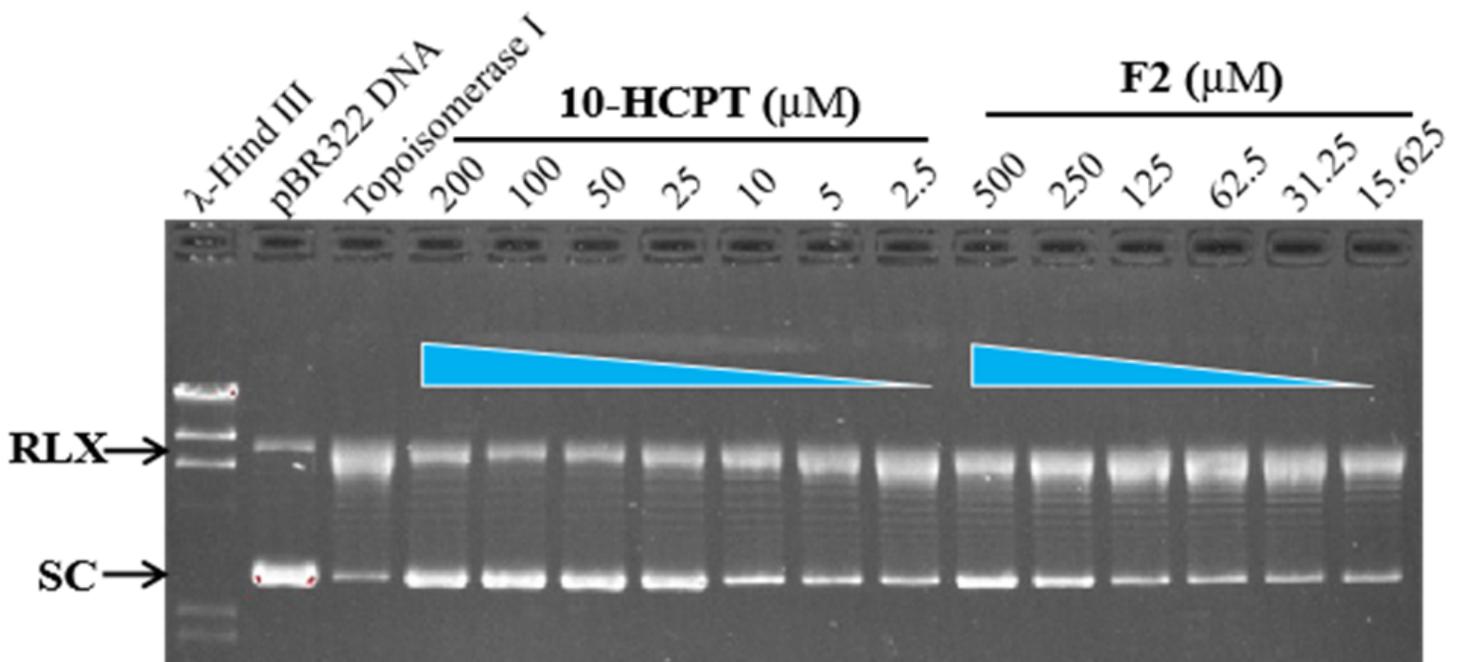


Figure 7

Effect of compound F2 on DNA Topo I. From left to right: the first lane is λ -Hind III digest as a marker; the second lane is pBR322 DNA alone; the third lane is pBR322 DNA and DNA Topo I, but no compound F2; the fourth to tenth lanes are 10-HCPT with final concentration of 200~2.5 μ M; the eleventh to sixteenth lanes are compound F2 with a final concentration of 500~15.63 μ M; SC is a supercoiled plasmid and RLX is relaxed DNA.