The interaction of anti-inflammatory and anti-tumor components in the traditional Chinese medicine *Solanum lyratum* Thunb

Dian Zou[1,2,3#], Xiaohua Li[4#], Xinxin Zhou[1,2,3], Biaobiao Luo[1,2,3], Mohammad Omar Faruque[6], Sheng Hu[5], Jian Chen[5*], Xuebo Hu[1,2,3*]

[1]Intitute of Medicinal Plants, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China;

[2]National & Local Joint Engineering Research Center for Medicinal Plant Breeding and Cultivation, Wuhan, 430070, China;

[3]Hubei Provincial Engineering Research Center for Medicinal Plants, Wuhan, 430070, China;

[4]College of Life Science and technology, Wuhan Polytechnic University, Wuhan, 430023, China;

[5]Department of Head and Neck Surgery, Hubei Cancer Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430079, China;

[6]Ethnobotany and Pharmacognosy Lab, Department of Botany, University of Chittagong, Chattogram, 4331, Bangladesh.

[#]These two contributed equally to the work.

*__Correspondence__ J.C. (chenjian2003@aliyun.com) and X.H. (xuebohu@mail.hzau.edu.cn).

Abstract

Solanum lyratum Thunb is a traditional Chinese medicinal with a significant clinical outcome for tumor treatment; however, chemicals or fractions separated from the herb did not exhibit strong and comparable efficacy. To investigate the potential synergy or antagonism among chemicals in the extract, we obtained the compounds solavetivone (SO), tigogenin (TI), and friedelin (FR) from the herb. The anti-tumor effects of these three monomer compounds alone or in combination with the anti-inflammatory compound DRG were also tested in this study. SO, FR, and TI used alone did not inhibit the proliferation of A549 and HepG2 cells, but the combination of the three achieved 40% inhibition. *In vitro* anti-inflammatory analysis showed that DRG had a stronger anti-inflammatory effect than TS at the same concentration, and the combination of DRG with SO, FR, or TI inhibited the anti-tumor effect of DRG. This is the first study that documented the synergistic and antagonistic interactions between different compounds in a single herb.

Key words: *Solanum lyratum* Thunb; anti-tumor; anti-inflammation; synergism; antagonism.

Experimental

Plant materials

The herb of *Solanum lyratum* Thunb was used in this study as herbal material. The traditional Chinese medicine *Solanum lyratum* Thunb was purchased from Hubei Tianji traditional Chinese medicine decoction Co., Ltd. and identified as *Solanum lyratum* Thunb of Solanaceae by Professor Xuebo Hu of the Institute of medicinal plants of Huazhong Agricultural University, Wuhan China. The sample was stored in the laboratory of natural medicine and molecular engineering of Huazhong University. The voucher specimen number was 20170202001. The reference specimen is WUK 0198806 from plant plus of China.

Extraction and isolation

The air-dried whole plant of *S. lyratum* (10.0 kg) was cut and extracted three times (1 h each time) with refluxing EtOH (80 L each time). Evaporation of the solvent under reduced pressure provided the ethanolic extract (1.88 kg), which was dissolved and suspended in H_2O (5 L) and then partitioned with CH_2Cl_2 (5×5 L) and EtOAc (5×5 L). The CH_2Cl_2 fraction (250.0 g) was subjected initially to silica gel column (120×15 cm) chromatography (100-200 mesh, 2.5 kg) and eluted with petroleum ether-ethyl acetate at 100:0, 95:5, 90:10, 85:15, 82:20, 75:25, 60:40, 50:50, 46:60, 30:70, 20:80, and 0:100 to produce 38 fractions, which were numbered DA.1-38. Fraction DA.9 (5 g) was separated over silica gel (200-300 mesh) and eluted by petroleum ether-ethyl acetate (20:1, 15:1, 10:1, and 0:1) to obtain DA.9 (1-5). Fraction DA.9-5 (1.3 g) was separated by CC over silica gel (200-300 mesh) and eluted by petroleum ether–ethyl acetate (30:1, 20:1, 10:1, and 0:1) to obtain compound **2** (20 mg). Fraction DA.12 (3.4 g) was separated by CC over silica gel (200-300 mesh) and eluted by petroleum ether-ethyl acetate (6:1, 4:1, 2:1, and 0:1) to yield DA.12 (1-3). Fractions DA.12-2 and 12-3 was separated by Sephadex LH-20 (40 g, eluted with DCM-EtOH, 50:50, v/v) to produce compound **3** (30 mg). We mixed fraction DA.5-6 (9.2 g) and eluted by petroleum ether–ethyl acetate (100:0, 80:1, 50:1, 20:1, 10:1, 5:1, and 0:100) to obtain compound **14** (15 mg).

Extract total saponin and alkaloids

The air-dried whole plant of *S. lyratum* (500 g) was powdered and soaked in 95% ethanol overnight and extracted three times with refluxing EtOH at 60 °C. The solvent was filtered and ethanol was removed by a rotary evaporator to give the crude ethanol extract. was We dissolved and suspended the extract in purified water, added HCl (5%) until the pH was 3, stirred well, and let stand overnight to precipitate insoluble acid components. After filtration and removal of insoluble matter, petroleum ether was added three times to remove fat-soluble impurities. We modulated the acid solution was with ammonium hydroxide until the pH was 10, extracted with dichloromethane 5-10 times until the organic phase had no obvious color, and removed dichloromethane at 45℃ to obtain fat-soluble alkaloids. The alkaline aqueous solution was extracted with n-butanol and n-butanol was recovered under decompression to obtain water-soluble alkaloids.

For total saponin extraction, the air-dried whole plant of *S. lyratum* (100 g) was soaked in 95% ethanol overnight and extracted three times (30 min each time) using an ultrasonic machine (60 ℃, 90 W). Evaporation of the solvent under reduced pressure provided the ethanolic extract. The extract was dissolved and suspended in $H_2O$and partitioned with petroleum ether first and then with n-butanol. Finally, total saponin was obtained after evaporating n-butanol under reduced pressure.

HPLC analysis of DRG content in total saponin

The content of diosgenin3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosiduronic acid (DRG) in the total saponins, which is a steroidal saponin that was extracted in our previous research (to be published), was determined by high performance liquid chromatography (HPLC). DRG was dissolved and diluted into a series of solutions that covered the concentrations from 25 ppm to 100 ppm. Total saponins powder (100 mg) was dissolved in 1 ml methanol and configured into a 100 mg/ml sample solution. The separation was done on a ZORBAX SB-C18 column (4.6 mm×250 mm, 5 micron) with a C_{18} guard column. The column temperature was set at 40 °C with a detection wavelength of 210 nm and an injection volume of 10 μL. Two eluents were used for the mobile phase. Eluent A was methanol and eluent B was water. The program of

eluent A was 10% (0 min) → 30% (10 min) → 40% (25 min) → 60% (35 min) → 80% (40 min) → 100% (65 min). The flow rate was 1.0 mL/min.

Cell culture

HepG2 and A549 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured separately in 25 cm^2 culture flasks in DMEM medium (GibcoTM, Invitrogen Corp. USA) that were supplemented with 5% FBS, 100 U/ml penicillin, and 100 μg/mL streptomycin at 37 ℃ in an atmosphere of 95% humidity and 5% CO_2. HMEC-1 cells were cultured in MCDB131 medium that included 10% FBS, 1 μg/ml hydrocortisone, 10 ng/ml recombinant human epidermal growth factor, 100 U/ml penicillin, and 1 μg/mL streptomycin at 37˚C in 5% CO_2.

Determination of cell viability

The viability of cells after treatment with various chemicals was evaluated using MTT assay. Cells were plated at a density of 2×10^4/well into 96-well plates with DMEM medium. Subsequent to 24 h incubation, the cells were treated with different concentrations of compounds alone or in combination. Twenty-four hours after treatment, the drug-containing medium was removed and replaced by MTT solution, and the cells were cultured at 37˚C in 5% CO_2 for 4 h. MTT solution was then removed and 150 μl DMSO was added to each well. The optical density was then determined at 570 nm and 630 nm with a microplate reader.

Determination of the anti-inflammatory activity of DRG and total saponins

A validation model based on the ICAM-1 signaling pathway has been established in our laboratory (Zhang, et al., 2018). With the same DRG concentration, we used this model to determine the anti-inflammatory effects of total saponins and DRG.

Statistical analysis

All data were presented as the mean ± standard deviation. A one-way analysis of variance using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) determined whether the results showed statistical significance between groups. The significance level was expressed as * if P was <0.05, expressed as ** if P was 0.01-0.001, and expressed as *** if P was <0.001.

Compound 3: Tigogenin, white crystals, $C_{27}H_{44}O_3$, ^{1}H-NMR (400MHz, $CDCl_3$): δ_H 0.75 (3H, s, 18-CH_3), 0.77 (3H, d, J=6.3Hz, 27-CH_3), 0.81 (3H, s, 19-CH_3), 0.95 (3H, d, J=6.0Hz, 21-CH_3), 3.38-3.40 (1H, m, H-26α), 3.56-3.58 (1H, m, H-26β), 4.41 (1H, dd, J=7.2, 7.8Hz, H-16). ^{13}C-NMR (150MHz, $CDCl_3$): 12.37 (C-19), 14.51 (C-21), 16.51 (C-18), 17.15 (C-27), 21.08 (C-11), 28.62 (C-6), 28.81 (C-24), 30.31 (C-25), 31.38 (C-2), 31.52 (C-23), 31.78 (C-15), 32.26 (C-7), 35.13 (C-8), 35.59 (C-10), 36.97 (C-1), 38.21 (C-4), 40.08 (C-12), 40.58 (C-13), 41.62 (C-20), 44.84 (C-5), 54.36 (C-9), 56.32 (C-14), 62.19 (C-17), 66.85 (C-26), 71.31 (C-3), 80.86 (C-16), 109.26 (C-22).

Compound 2: Solavetivone, $C_{15}H_{22}O$, yellow oily liquid, EI-MS: m/z 219 $[M+H]^+$. ^{1}H-NMR (400MHz, $CDCl_3$): δ_H 5.69 (1H, brs, H-1), 2.15 (1H, dd, J =16.7, 4.4Hz, H-3α), 2.59 (1H, dd, J=16.7, 4.4Hz, H-3β), δ 4.69 (1H, s, H-12α), 4.67(1H, s, H-12β), 1.70 (3H, brs, H-13), 1.88 (3H, brs, H-14), 0.93 (3H, d, J=6.9Hz, H-15). ^{13}C-NMR (150MHz, $CDCl_3$): 15.9 (C-15), 20.92 (C-13), 21.29 (C-14), 32.77 (C-8), 34.4 (C-9), 39.27 (C-4), 40.83 (C-6), 42.99 (C-3), 46.57 (C-7), 50.12 (C-5), 109.4 (C-12), 125.52 (C-1), 147.16 (C-11), 166.64 (C-10), 199.12 (C-2).

Compound 1: Friedelin, $C_{30}H_{50}O$, white crystals; EI-MS: m/z 427 $[M+H]^+$. ^{1}H-NMR (400MHz, $CDCl_3$): δ_H 0.73 (3H, s), 0.87 (3H , s), 0.88 (3H, d), 0.95 (3H, s), 1.00 (3H , s), 1.01 (3H, s), 1.05 (3H, s), 1.18 (3H, s), 2.39 (1H, dd, J =13.4, 3.7 Hz), 2.30 (1H, dd, J =13.4, 5.9Hz), 2.25 (1H, q). ^{13}C-NMR (150MHz, $CDCl_3$): 6.84 (C-23), 14.67 (C-24), 17.96 (C-25), 18.25 (C-7), 18.68 (C-27), 20.27 (C-26), 22.29 (C-1), 28.18 (C-20), 30 (C-17), 30.51 (C-12), 31.79 (C-30), 32.1 (C-28), 32.43 (C-15), 32.77 (C-21), 35.04 (C-29), 35.35 (C-19), 35.63 (C-11), 36.01 (C-16), 37.45 (C-9), 38.3 (C-14), 39.26 (C-22), 39.71 (C-13), 41.29 (C-6), 41.54 (C-2), 42.16 (C-5), 42.79 (C-18), 53.11 (C-8), 58.23 (C-4), 59.48 (C-10), 213.27 (C-3).

Figures

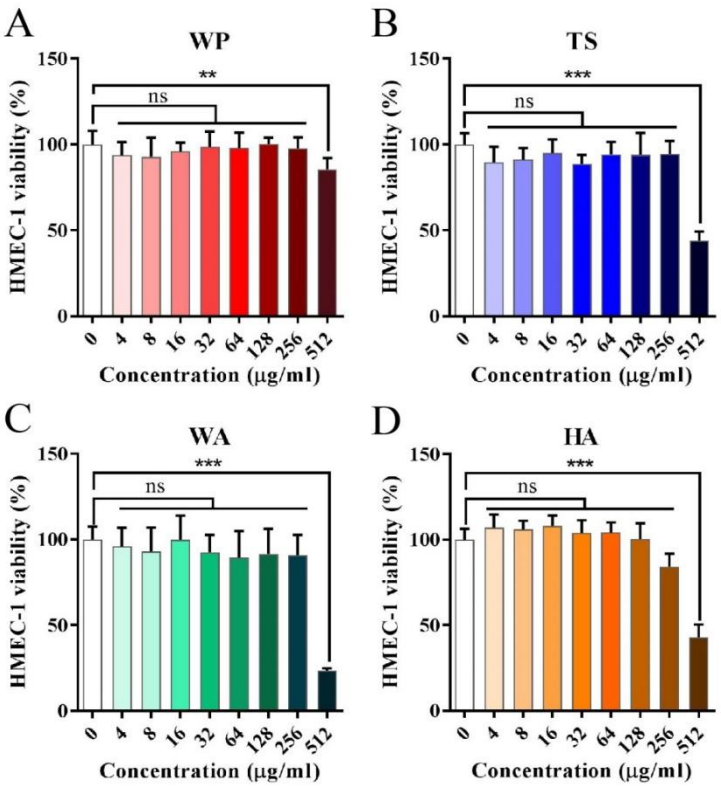
Fig S1. Effect of whole plant extract (WP), total saponin (TS), water-soluble alkaloid (WA), and hydrophobic alkaloid (HA) from *Solanum lyratum* Thunb. on the viability of HMEC-1 cells. Asterisks denote (** $p < 0.01$ and *** $p < 0.001$, n=6) statistically

significant differences between the treatment groups and the blank control; ns indicates no significant difference.

Fig S2. Effect of the monomeric compounds separated from *Solanum lyratum* Thunb. on the viability of HepG2 and A549 cells. Asterisks denote (* $p < 0.05$ and *** $p < 0.001$, n=6) statistically significant differences between the treatment groups and the blank control; ns indicates no significant difference.

Fig S3. Combinatorial action of solavetivone, tigogenin, and friedelin on the viability of HepG2 and A549 cells. Asterisks denote (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, n=6) statistically significant differences between the treatment groups and the blank control; ns indicates no significant difference.

Fig S4. A) Inflammation index of DRG and TS. B) Anti-tumor activity analysis of DRG on the proliferation of HepG2 cells after drug exposure for 24h. C) Anti-tumor activity analysis of DRG alone or combined with SO, TI, or FR on HepG2 cells. Asterisks denote (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, n=6) statistically significant differences between the treatment groups and the blank control; ns indicates no significant difference.



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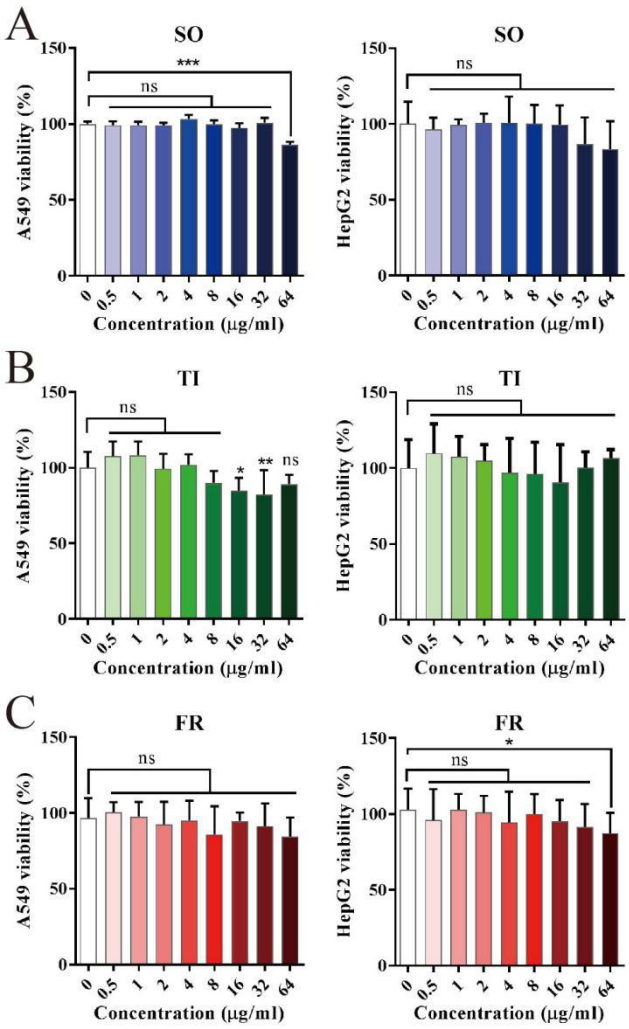

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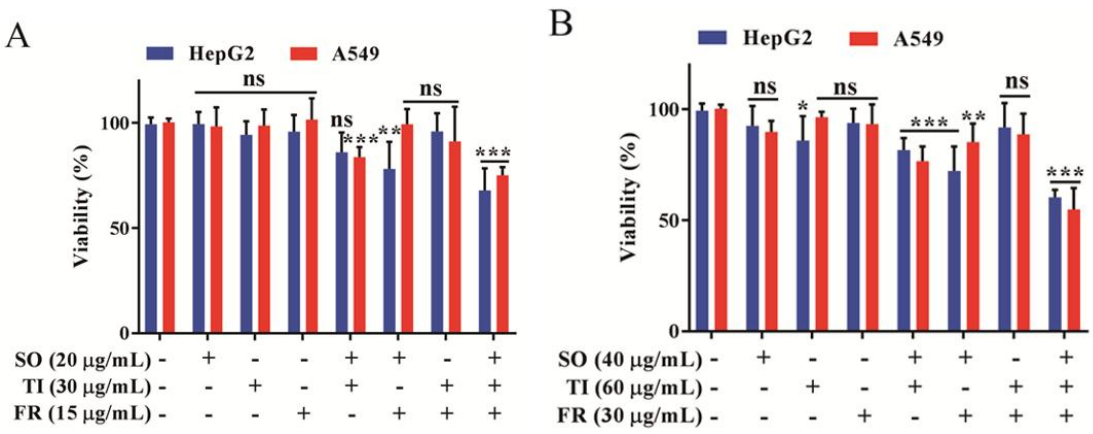

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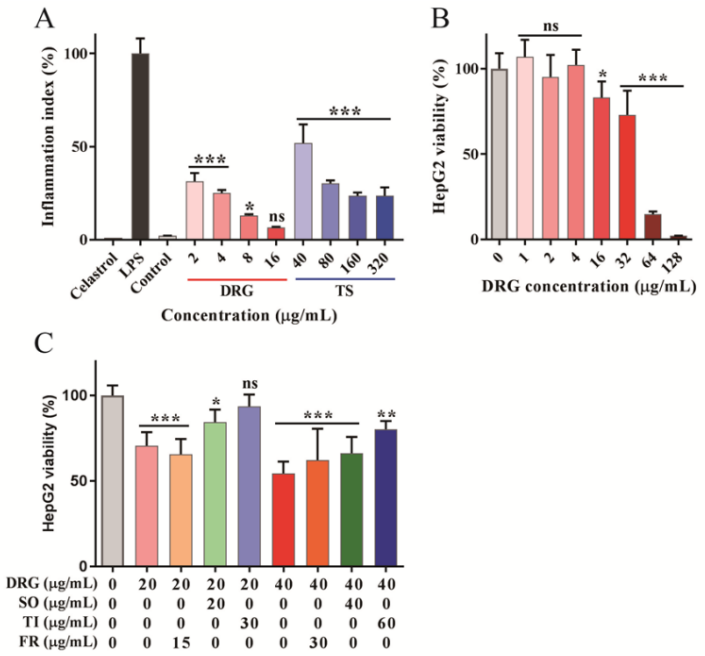

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