Salvia bowleyana Dunn root is a novel source of salvianolic acid B and displays antitumor effects against gastric cancer cells

BINGHUA CHEN[1*], CHAOQUN HUANG[1,2*], YUANYAN ZHANG[1], XIAOQIONG TANG[1], SUHUAN LI[1], QINGSHUI WANG[1] and YAO LIN[1]

[1]College of Life Sciences, Fujian Normal University, Fuzhou, Fujian 350117; [2]Central Laboratory, The Fifth Hospital of Xiamen, Xiamen, Fujian 361101, P.R. China

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Abstract. Salvianolic acid B (Sal-B) is widely used in China for the treatment of numerous diseases. Currently, *Salvia miltiorrhiza* Bunge is the main source of this compound, but *Salvia bowleyana* Dunn, a surrogate of *S. miltiorrhiza* Bge, may provide a novel source for obtaining more Sal-B. In the present study, a simple method for separation and purification of phenolic compounds from *S. bowleyana* Dunn roots was employed. Sal-B was subsequently purified and its inhibitory effect on the gastric cancer HGC-27 and AGS cell lines was investigated. Sal-B extracted from *S. bowleyana* Dunn displayed significant antitumor activity in proliferation and apoptosis assays. Overall, it was found that *S. bowleyana* Dunn has a higher Sal-B content than *S. miltiorrhiza* Bge and may be used as a novel source of this potential anti-gastric cancer compound.

Introduction

Salvianolic acid B (Sal-B) is a water-soluble component of *Salvia miltiorrhiza* Bunge with a wide spectrum of effects, including anti-inflammatory effects, inhibition of new vessel formation and atherogenesis, and relief of chronic hepatitis and liver fibrosis, as well as antioxidant and tumor-modulating effects (1-4). A previous study demonstrated that this compound inhibits cell proliferation in head and neck squamous cell carcinoma (5). In addition, it has been reported to decrease viability of U87 cells in a dose- and time-dependent manner (6).

S. miltiorrhiza Bge is the main source of Sal-B (7-10) and it also possesses another active ingredient, tanshinone, that is widely used clinically. Tanshinone has been reported to inhibit oxidation of low-density lipoproteins, improve lipid metabolism, protect endothelial cells and prevent myocardial ischemia (11-13). In addition, this compound displays preventive effects on cardiovascular diseases, such as atherosclerosis, and reduces the area of myocardial infarction and the oxygen consumption of the myocardium (14-17). Unfortunately, the current extraction methods of Sal-B result in the loss of fat-soluble tanshinone. Following tanshinone extraction, the residue can be used for Sal-B extraction by process modification; however, the yield is low and the process is time-consuming and laborious (18). Therefore, *S. miltiorrhiza* Bge is mainly used in the extraction of tanshinone rather than Sal-B. However, *S. miltiorrhiza* Bge is very expensive, and its accessibility is limited due to its regional distribution. It is therefore imperative to look for alternative sources of Sal-B to replace *S. miltiorrhiza* Bge. In this regard, *Salvia bowleyana* Dunn has been suggested as an alternative source since it is often used as a surrogate of *S. miltiorrhiza* Bge. This species is abundant in areas that exhibit high incidences of gastric cancer in China, such as Fujian (19).

In the present study, the water-soluble components of *S. miltiorrhiza* Bge and *S. bowleyana* Dunn were extracted and assayed for antitumor effects on gastric cancer cell lines. Since there have only been a few studies on Sal-B for the prevention and treatment of gastric cancer (2,20-23), the present study aimed to explore the potential of these species in treating gastric cancer.

Materials and methods

Determination of Sal-B in S. bowleyana Dunn roots. S. bowleyana Dunn plants were collected from Lianjiang (Fuzhou, China) in July 2014 (E, 119˚20'; N, 20˚11'; Alt, 57 m), while *S. miltiorrhiza* Bge (produced in Anhui, China, in 2015) was purchased from Hui Chun Pharmacy (Fuzhou, China). The roots of *S. bowleyana* Dunn and *S. miltiorrhiza* Bge were washed, dried, ground to a fine powder and passed through a 425-μm sieve respectively. Sal-B in the roots of the two plants was purified by the following experimental steps. A total of 1 g of the resulting powder was placed in an Erlenmeyer flask with 20 ml 60% ethanol and left at room temperature. The rest of the powder was stored at ≤-20˚C for subsequent use.

Correspondence to: Professor Yao Lin, College of Life Sciences, Fujian Normal University, 1 Technology Road, Fuzhou, Fujian 350117, P.R. China
E-mail: yaolin@fjnu.edu.cn

*Contributed equally

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After 6-8 h, the solution was exposed to ultrasound at 40 kHz for ~35 min and centrifuged for 15 min at 8,000 x g, and the supernatant was collected at room temperature. Subsequently, 20 ml 60% ethanol was added to the Erlenmeyer flask, and the solution was exposed to ultrasound at 40 kHz for 35 min and centrifuged at 8,000 x g for 15 min at room temperature. The resultant supernatant was collected and mixed with the supernatant collected in the first phase. Finally, the supernatant was topped up to 1,000 ml using ultra-pure water and stored at 4˚C for use in subsequent experiments.

The extract was analyzed using Waters 2695 Alliance HPLC high-performance liquid chromatography (Waters Corporation). A Sal-B standard sample (≥98.3%) was purchased from Nanjing Chunqiu Biological Engineering Co., Ltd., and was used for analysis on ZORBAX Eclipse XDB-C18 (4.6x250 mm, 5 μm) chromatography columns (Agilent Technologies, Inc.). The (4.6x250 mm) are the diameter and length of the column, respectively. The particle size of the particles is 5 μm in the column, which is the composition of the solid phase. Results were detected using the Ultrospec™ 2100 pro UV-Vis variable wavelength detector (Amersham; Cytiva) at a wavelength of 286 nm. The analysis involved a mobile phase with 0.5% carboxylic acid, 99.9% acetonitrile and 99.9% methanol in the ratio 48:7:45 (V:V:V). Other conditions included a 0.8-ml/min flow rate and a column temperature of 28˚C for a sample volume of 10 μl. The analyzed Sal-B contents were imaged and quantified using Empower System Suitability (24-26). Sal-B in *S. miltiorrhiza* Bge root was not used in the remaining experiments

Purification and verification of Sal-B. A 20-g sample of powder from *S. bowleyana* Dunn roots was used in the extraction of plant components as aforementioned. The obtained supernatant was concentrated under reduced pressure at 45˚C for ethanol removal, and the solution was subsequently diluted to 500 ml with ultra-pure water and divided into 10 bottles (500 ml/bottle). A total of 4 g X-5 resin (Nankai University Chemical Plant) was added into each bottle, followed by 12 h of shaking at 110 r min^{-1} in a rotary incubator at room temperature to allow full absorption of the compound by the resin. Subsequently, any resin impurities on the surface were washed off by running water, and the rest of the solution in the bottle was discarded. The mixture absorbed by the resin was eluted using 60% ethanol and shaken for 1-2 h at 110 r min^{-1} in a rotary incubator at room temperature until the resin became colorless. The ethanol solution was concentrated under reduced pressure and freeze-dried to obtain a powder. The powder was dissolved in 10% ethanol solution and filtered using a 0.45 μm microporous membrane. Subsequently, the filtered solution was purified via column chromatography. Sephadex LH-20 (Amersham; Cytiva) was used for chromatographic media in the columns (Φ1.6x70 cm) and ethanol solutions of different volume percentages (10, 30 and 50%) were used for eluting the columns in sequence. Finally, the solution was concentrated to dryness in a rotary evaporator under reduced pressure conditions, and the yield was calculated. The purified compound powder was stored at -20˚C before HPLC and nuclear magnetic resonance (NMR) analyses.

A total of 2 mg of the powder was dissolved in 2 ml methanol, and the filtered sterile solution was placed into a sample bottle. Components of this purified powder were identified by liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry ($LC\text{-}MS^2$) using the aforementioned HPLC chromatographic conditions. Parameters for mass spectrometry (G6520B; Agilent Technologies, Inc.) were as follows: A negative ionization mode, fragmentor set at 100 V, mass spectra scanning range of 200-1,200 m/z, a two-stage mass spectrometry scanning range of 50-800 m/z, nebulizer pressure 40 psi (10 l/min), nitrogen gas temperature of 350˚C and the energy of collision chamber of 10, 20 and 40 eV (27,28). A total of 8 mg powder was obtained and dissolved in 0.7 ml D_2O by ultrasound in a 1-ml clean centrifugal tube for ^1H NMR test using the UNITY-400 NMR spectrometer (Varian).

Establishment of cell culture. The human gastric cancer HGC-27 and AGS cell lines were obtained from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences for use in the present study. All cells were tested by short tandem repeat genotyping and confirmed to be mycoplasma negative. HGC-27 cells were grown in RPMI-1640 medium, whereas AGS cells were maintained in DMEM F12 (both Biological Industries). The cultures were supplemented with 10% FBS (Biological Industries), 100 U/ml penicillin G and 100 μg/ml streptomycin (BBI Life Sciences Corporation). All cells were grown at 37˚C in a humidified incubator containing 5% CO_2.

Sulforhodamine B (SRB) assay. Cells (100 μl) were seeded into 96-well plates at concentrations of 10,000 and 8,000 cells/well for AGS and HGC-27, respectively. After 24 h of incubation, the gastric cancer cells were treated with different concentrations of Sal-B (100, 200, 400, 600 and 800 μM) for 48 h at 37˚C. The culture media was removed, cells were fixed with 3% trichloroacetic acid and stained with 0.057% SRB (both Sigma-Aldrich; Merck KGaA) (29). SRB was solubilized in 10 mM Tris base solution, and its fluorescence was quantified using the Synergy HT Multi-Mode Microplate Reader (Agilent Technologies, Inc.) at a wavelength of 510 nm. The wells treated with Sal-B were compared with control wells (30,31). At least three biological replicates were performed for each assay.

Cell Counting Kit-8 (CCK-8) assay. Cells (100 μl) were seeded into 96-well plates at concentrations of 10,000 and 8,000 cells/well for AGS and HGC-27, respectively. After 24 h, the gastric cancer cells were treated with different concentrations of Sal-B as described for the SRB assay for 48 h at 37˚C. After treatment, 10 μl CCK-8 solution (TransGen Biotech Co., Ltd.) was added to each well, according to the manufacturer's protocol, and the cells were incubated at 37˚C for 2 h. Subsequently, optical density values were measured at 450 nm using the Synergy HT Multi-Mode Microplate Reader. Drug-treated wells were compared with solvent-controlled wells using results obtained from three independent experiments.

Colony formation assay. The ability of cells to form colonies was analyzed for HGC-27 and AGS cells in the exponential phase. Each cell line was divided into 3 groups with 3 replicate

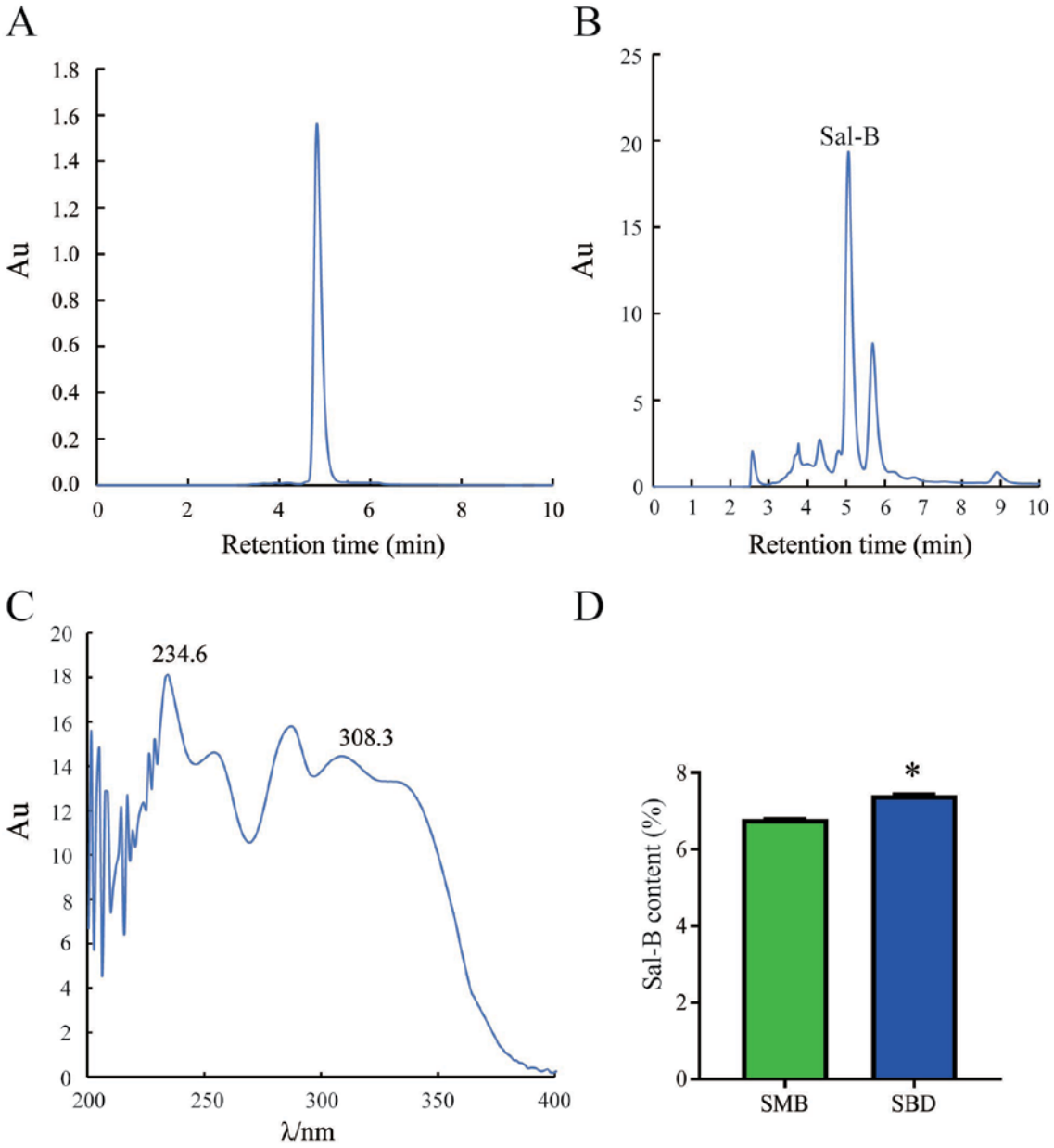

Figure 1. Sal-B content in SBD and SMB roots. (A) Standard sample of Sal-B analyzed via HPLC. (B) Chromatogram and (C) UV spectrum of HPLC registered with a diode array detector at 286 nm for SBD root. (D) Quantification of Sal-B content. Data are shown as the mean ± SEM (n=3). *P<0.05 vs. SMB. Sal-B, salvianolic acid B; HPLC, high-performance liquid chromatography; SMB, *Salvia miltiorrhiza* Bunge; SBD, *Salvia bowleyana* Dunn.

wells in each group and seeded into 6-well plates ($1x10^7$/well), and were subsequently treated with 0, 400 or 800 μM Sal-B when the monolayer cell density reached 70% confluency. After 48 h, adhered cells were disassociated with trypsin and cells from each well were seeded into 6-well plates. Additionally, 500 cells/well of AGS and HGC-27 were seeded to continue culture in complete medium without Sal-B treatment. The cells were grown for 7 to 14 days to allow colony formation from viable clonogenic cells. After culture, the medium was removed, and 70% methanol was added to fix the cells for 15 min, then methanol was discarded and stained with 5% Giemsa (Sigma-Aldrich; Merck KGaA) for 10 min after air drying at room temperature. Subsequently, the plates were washed with PBS and captured to calculate colony numbers, using a Canon EOS 70D (https://www.canon.com.cn/product/70d). Statistical results were obtained from three independent experiments.

Protection from oxidative DNA damage induced by 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH). AAPH (Sigma-Aldrich; Merck KGaA) is an oxidizing agent, and its formation of cationic free radicals can occur at the first level. The ability of the extracted Sal-B to protect the supercoiled pUC18 plasmid from AAPH was measured according to the method previously described by Zhang and Omaye (32), with some modifications. Briefly, 2 μl of intact pUC18 plasmid (0.1 μg/μl) was mixed with various concentrations (0.017 mmol/l, 0.03, 0.06 and 0.13 mmol/l) of Sal-B samples (7 μl) and 12.5 mM AAPH (6 μl) in PBS (pH 7.4), and then the mixture was incubated at 37˚C. After 1 h of incubation, the samples were electrophoresed on a 0.8% agarose gel for 30 min and subsequently stained for 3-5 min with 0.5 μg/ml ethidium bromide. Analysis of DNA damage was performed on images taken using a GelDoc EZ gel image analysis system (Bio-Rad Laboratories, Inc.) (33).

Western blot analysis. Proteins were extracted from cells using RIPA buffer composed of 50 mM Tris HCl (pH 8.0), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS. Protein concentration was measured using the Pierce BCA Protein assay kit (Thermo Fisher Scientific, Inc.), 30 μg protein/lane separated via SDS-PAGE on a 10% gel and transferred to a nitrocellulose membrane (GE Healthcare Life Sciences). After blocking with 5% skimmed milk powder for 1 h at room temperature, the nitrocellulose membrane was incubated with Caspase3 mouse mAb (1:1,000; cat. no. 9668) and GAPDH rabbit mAb (1: 1,000; cat. no. 2118; both from Cell Signaling Technology, Inc.) overnight at 4˚C. Subsequently, the blots were stained with the IRDye® 680RD goat-anti-rabbit IgG (cat. no. 926-68071) and IRDye® 800CW goat anti-mouse IgG (cat. no. 926-32210; both 1:5,000 and from LI-COR Biosciences) secondary antibodies labeled with fluorescence in a cassette at room temperature for 1 h. Subsequently, the blots were directly imaged and semi-quantified using the Odyssey Infrared Imaging system (model no. 9140; LI-COR Biosciences) (34).

Flow cytometry. Cells were seeded in 6-well plates at a density of $2x10^5$ cells/well. After 24 h, the cells were treated with Sal-B (0, 400 and 800 μM) at 37˚C for 48 h and assessed for cellular apoptosis using the AnnexinV-FITC/PI Apoptosis Detection kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. Apoptotic cells were analyzed using a flow cytometer and FlowJo software (v10; FlowJo LLC).

Statistical analysis. Data were analyzed using one-way ANOVA with a Dunnett's post hoc test. Analyses were performed in GraphPad Prism v7 (GraphPad Software, Inc.). Data are presented as the mean ± SEM. $P<0.05$ was considered to indicate a statistically significant difference. The IC_{50} value was calculated by Dr Fit 1.042 (35).

Results

S. bowleyana Dunn roots contain high Sal-B content. Analysis of the peak area of the standard sample resulting from HPLC as well as linear regression [y (peak area)=9480.2x (mg/ml)-181.39 (R^2=0.9998)] allowed detection of Sal-B in the extract (Fig. 1A). The content of Sal-B was imaged and quantified using Empower System Suitability (Fig. 1B and C). Quantification revealed a significantly higher Sal-B content in *S. bowleyana* Dunn roots (7.42%) than in *S. miltiorrhiza* Bge root (6.79%; $P<0.05$; Fig. 1D). After purification, the yield of Sal-B was 4.26% and the purity of Sal-B was 93.26% by HPLC analysis.

Sal-B in S. bowleyana Dunn roots is verified by LC-MS and LC-MS². The purified component extracted from *S. bowleyana* Dunn roots was verified by LC-MS and LC-MS^2. Separation and purification procedures allowed the identification of a substance with molecular formula $C_{36}H_{30}O_{16}$ and molecular mass of 718. The compound can lose one proton to be negatively charged under the condition of negative ion full-wave scanning mass spectrometry. Thus, its base peak is the molecular ion peak and the m/z value was 717.14 (Fig. 2A). Ion fragments of the compound from different collision energies were collected and detected, including those at m/z 519, 339, 321 and 295 (Fig. 2B-D). These fragments were consistent with the standard sample of Sal-B and with a previous report (36). The current results indicated that Sal-B was formed by condensation of lithospermic acid and danshensu, and that its two ester bonds were the most prone to breaking. Therefore, Sal-B is liable to lose two danshensu units and form two different m/z 519 substances (paths a and b; Fig. 2E). Subsequently, two substances, m/z 339 (paths a_1 and b_2) and m/z 321 (paths a_2 and b_1), are formed, with m/z 339 having two possible structures (Fig. 2E). The ion fragments of m/z 321 are formed by removing monomolecular danshensu. The ion fragments of the two m/z 339 structures can additionally form two different m/z 321 structures (paths a_3 and b_3; Fig. 2E). The chemical structure of Sal-B was identified according to its ^{1}H NMR data. Compared with the data in the literature (9), the data of the compound was the following: ^{1}H NMR (400 MHz, D_2O), δ/ppm: 6.78 (m, 7 H; H 1), 6.63 (m, 2 H; H 2), 6.27 (d, J=8 Hz, 1 H; H3), 6.08 (d, J=2 Hz, 1 H; H 4), 5.92 (dd, 1 H; H 5), 5.80 (d, J=6 Hz, 1 H; H 6), 5.68 (d, J=16 Hz, 1 H; H 7), 4.85 (dd, 2 H; H 8), 4.75 (dd, 9 H; H 9), 4.09 (d, J=6 Hz, 1H; H10), 2.97 (dd, 1 H; H 11), 2.80 (m, 2 H; H 12) and 2.41 (dd, 1 H; H 13) (the H number corresponds to the structure shown in Fig. 2F). The current results indicated that the purified product corresponded to Sal-B.

Sal-B effectively inhibits proliferation of gastric cancer cells. A number of biological experiments were conducted to examine the anti-gastric cancer effect of purified Sal-B extracted from *S. bowleyana* Dunn. Treating AGS and HGC-27 cells with different concentrations of purified Sal-B inhibited cell proliferation. This is based on the different IC_{50} values obtained from the SRB (AGS, ~824 μM; HGC, ~576 μM; Fig. 3A) and CCK-8 assays (AGS, ~615 μM; HGC, ~511 μM; Fig. 3B).

A colony formation assay comparing Sal-B-treated HGC-27 cells with control cells did not reveal significant differences (Fig. 4A and B). However, colony formation in AGS cells was strongly suppressed at 400 and 800 μM (Fig. 4C and D). Western blot analysis of AGS and HGC-27 cells treated with varying concentrations of Sal-B revealed that the compound had a significant effect on the expression levels of cleaved caspase-3 only when treating HGC-27 cells with 800 μM Sal-B (Fig. 5A-D). Alternative assessment of apoptosis using flow cytometry revealed similar results in AGS and HGC-27 cells (Fig. 5E-H).

Finally, the ability of Sal-B from *S. bowleyana* Dunn to prevent oxidative DNA damage was examined (Fig. 6). It was observed that oxidative DNA damage was gradually diminished with increasing Sal-B concentration. Oxidative DNA damage was rarely observed at 0.06 mmol/l Sal-B; this concentration may effectively prevent DNA from being sheared. To some extent, the low level of apoptosis may be associated with the protective effect of Sal-B on DNA.

Discussion

The molecular weight of purified monomeric compound extracted from *S. bowleyana* Dunn was successfully detected using HPLC-MS, while ion fragments were analyzed via HPLC-MS^2. Results from ion fragments were consistent with the decomposition product of Sal-B, and Sal-B content from

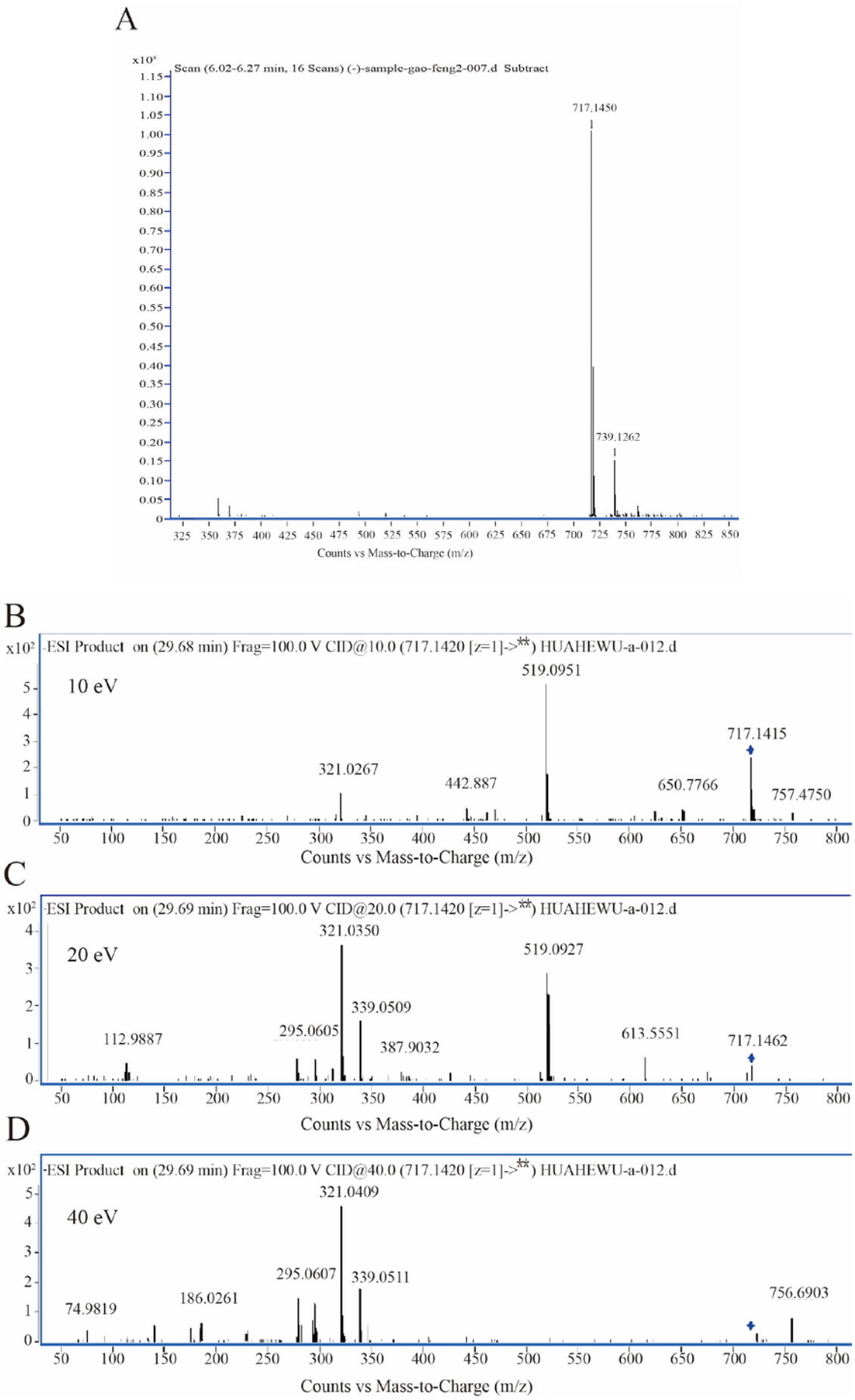

Figure 2. Sal-B identification via liquid chromatography-mass spectrometry, LC-MS^2 and ^{1}H NMR spectrum. (A-D) Mass spectrum via negative ESI for Sal-B. LC-MS^2, liquid chromatography-tandem mass spectrometry; NMR, nuclear magnetic resonance; ESI, electrospray ionization; Sal-B, salvianolic acid B.

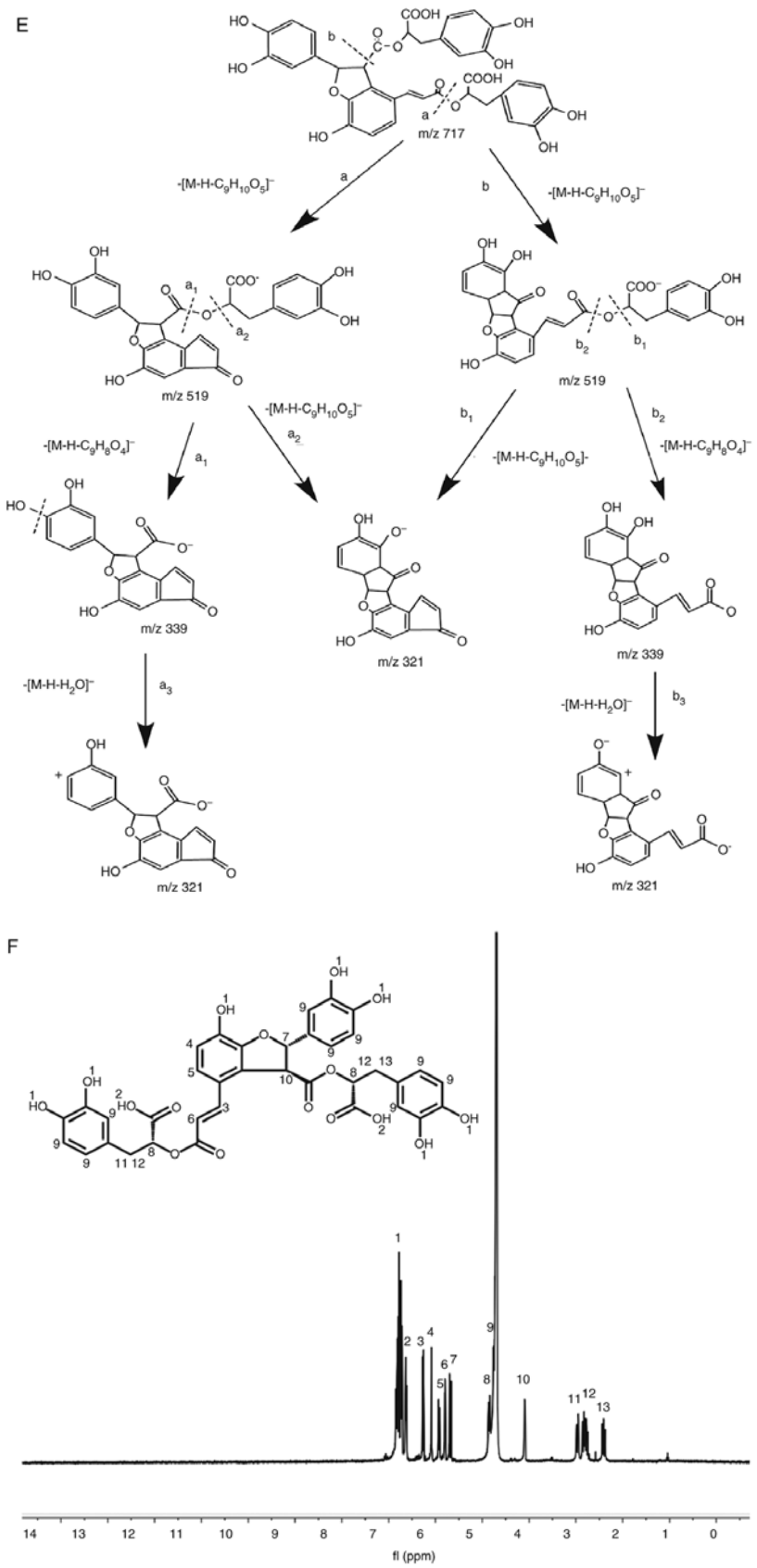

Figure 2. Continued. Sal-B identification via liquid chromatography-mass spectrometry, LC-MS^2 and ^{1}H NMR spectrum. (E) Proposed ESI-MS^2 fragmentation pathway of Sal-B. (F) ^{1}H NMR spectrum of Sal-B (D_2O). LC-MS^2, liquid chromatography-tandem mass spectrometry; NMR, nuclear magnetic resonance; ESI, electrospray ionization; Sal-B, salvianolic acid B.

S. bowleyana Dunn roots was significantly higher than that from *S. miltiorrhiza* Bge roots. The current extraction method was therefore improved and relatively simplified compared with previous methods (37,38).

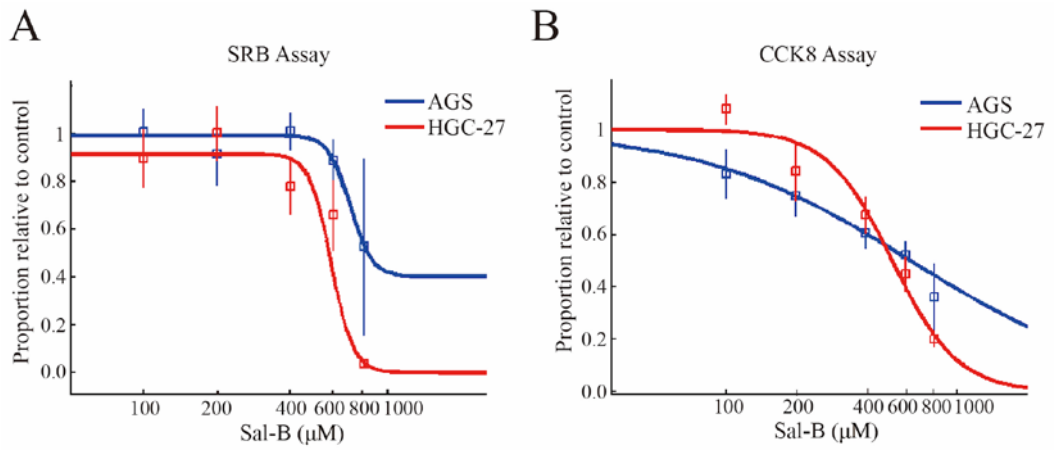

Figure 3. Sal-B suppresses gastric cancer cell growth *in vitro*. Cell proliferation was assessed by (A) SRB and (B) CCK-8 assays. Percentage cell viability was compared with parallel untreated cells. Data are shown as the mean ± SEM (n=3). Sal-B, salvianolic acid B; SRB, sulforhodamine B.

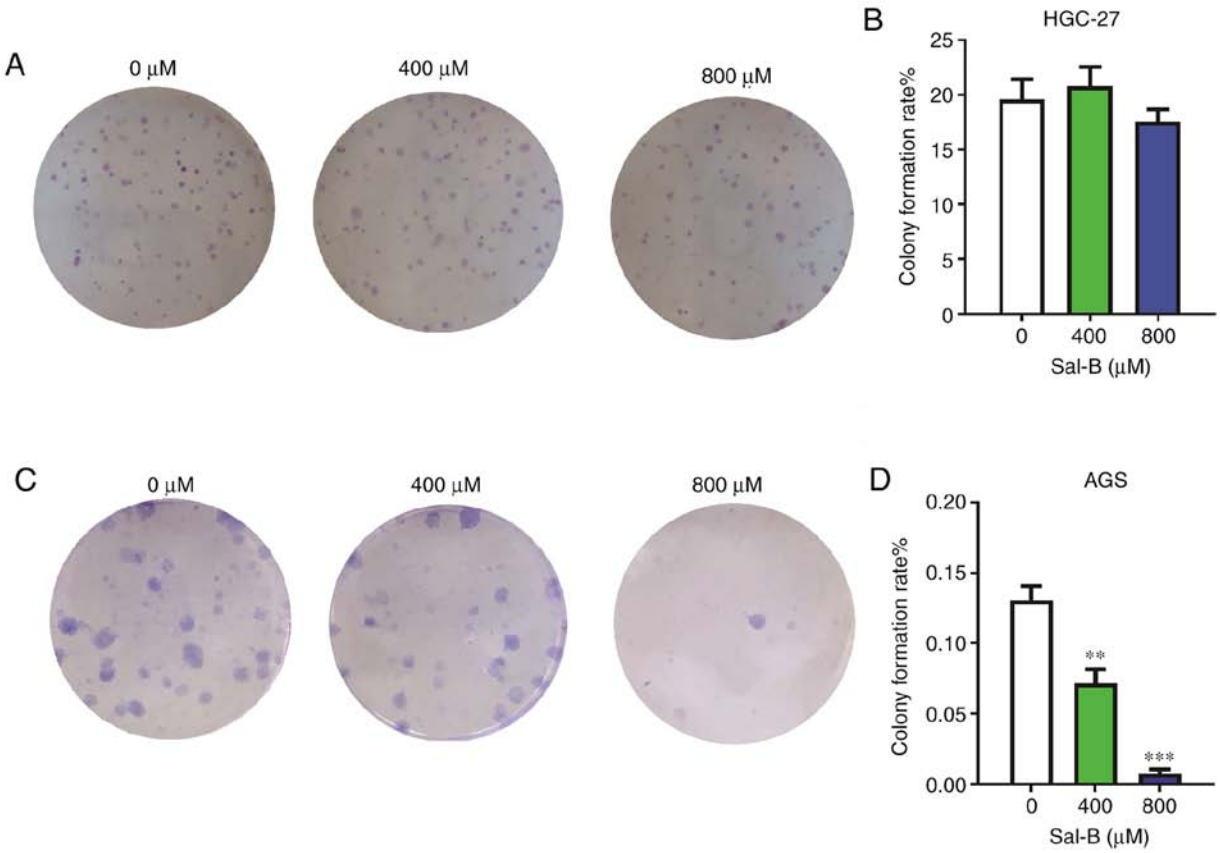

Figure 4. Effect of Sal-B on colony formation in gastric cancer cells. (A) Representative images and (B) quantification of colony forming assays in HGC-27 cells with or without Sal-B treatment. (C) Representative images and (D) quantification of colony forming assays in AGS cells with or without Sal-B treatment. Data are shown as the mean ± SEM (n=3). **P<0.01; ***P<0.001 vs. 0 μM Sal-B. Sal-B, salvianolic acid B. The images were captured using a Canon camera (EOS 70D).

S. bowleyana Dunn is widely distributed in the Fujian province, where there is a high incidence of gastric cancer (39). The extraction of compounds from *S. bowleyana* Dunn and the potential identification of anti-gastric cancer activities may result in a beneficial use of the plant in this region. The findings of the present study add substantial knowledge to the few reports regarding the anti-gastric cancer effects of Sal-B (40-43). In the current study, Sal-B extracted from *S. bowleyana* Dunn had an inhibitory effect on the proliferation of AGS and HGC cells. The ability of AGS cells to form colonies was strongly inhibited when cells were treated with high concentrations of Sal-B, while no significant inhibition was observed in HGC-27 cells. In addition, no significant differences were observed in the apoptotic marker cleaved caspase-3 when AGS and HGC-27 cells were treated with different concentrations of Sal-B. Although there was a significant difference in apoptosis between the control group and HGC-27 cells treated with

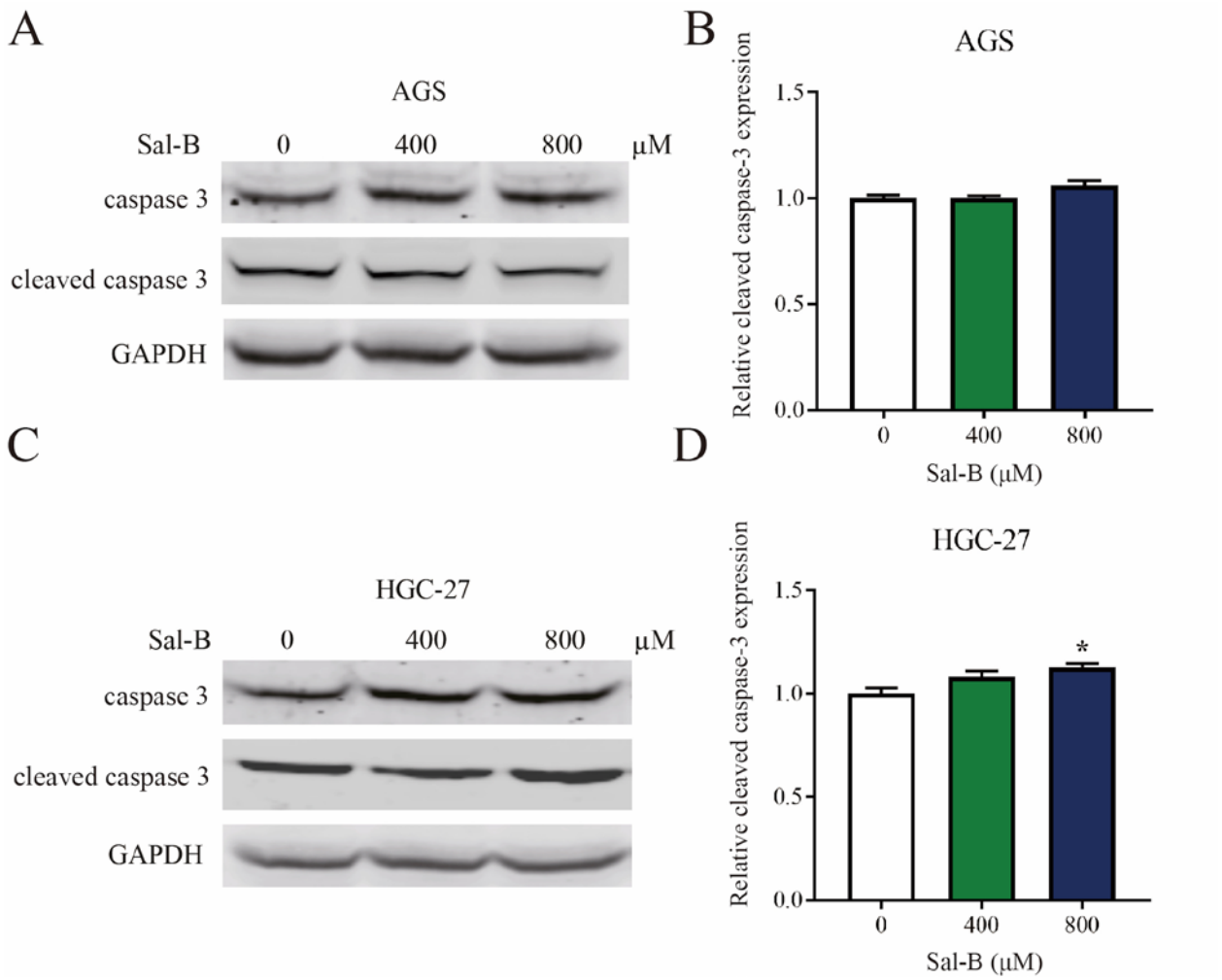

Figure 5. Effect of Sal-B on the apoptosis of gastric cancer cells. AGS and HGC-27 cells were exposed to Sal-B at concentrations of 0, 400 and 800 μM for 48 h. Expression levels of cleaved caspase-3 were evaluated by western blotting. (A) Representative western blot images and (B) semi-quantification of cleaved caspase-3 in AGS cells. (C) Representative western blot images and (D) semi-quantification of cleaved caspase-3 in HGC-27 cells. Additionally, the rate of apoptosis in AGS and HGC-27 cells was assessed by flow cytometry. Data are shown as the mean ± SEM (n=3). *P<0.05 vs. 0 μM Sal-B. Sal-B, salvianolic acid B; PI, propidium iodide.

800 μM Sal-B, the number of apoptotic cells was low. Sal-B may inhibit apoptosis by decreasing oxidative damage to mitochondrial DNA and protecting mitochondrial function. Additionally, Sal-B has been demonstrated to decrease the release of cytochrome *c* from mitochondrial cells into the cytosol, thus inhibiting activated caspase-3 (44). However, in the present study Sal-B inhibited the proliferation of AGS and HGC-27 cells, suggesting that they may be directly killed. Sal-B is easily degraded within a few hours of exposure to an alkaline solution, which may be one of the causes of this phenomenon (45-47). Furthermore, different tumor cells have different levels of resistance to the same reagent; therefore, the development and assessment of stable Sal-B (alone or in combination with other drugs) may open more frontiers on its application. Further analyses to unravel the underlying mechanism of action for the inhibition of proliferation should be performed in future research.

Overall, the findings of the present study revealed that *S. bowleyana* Dunn contains a higher Sal-B content than *S. miltiorrhiza* Bge, and that it may be a novel source of this potentially anti-gastric cancer compound.

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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 upon reasonable request.

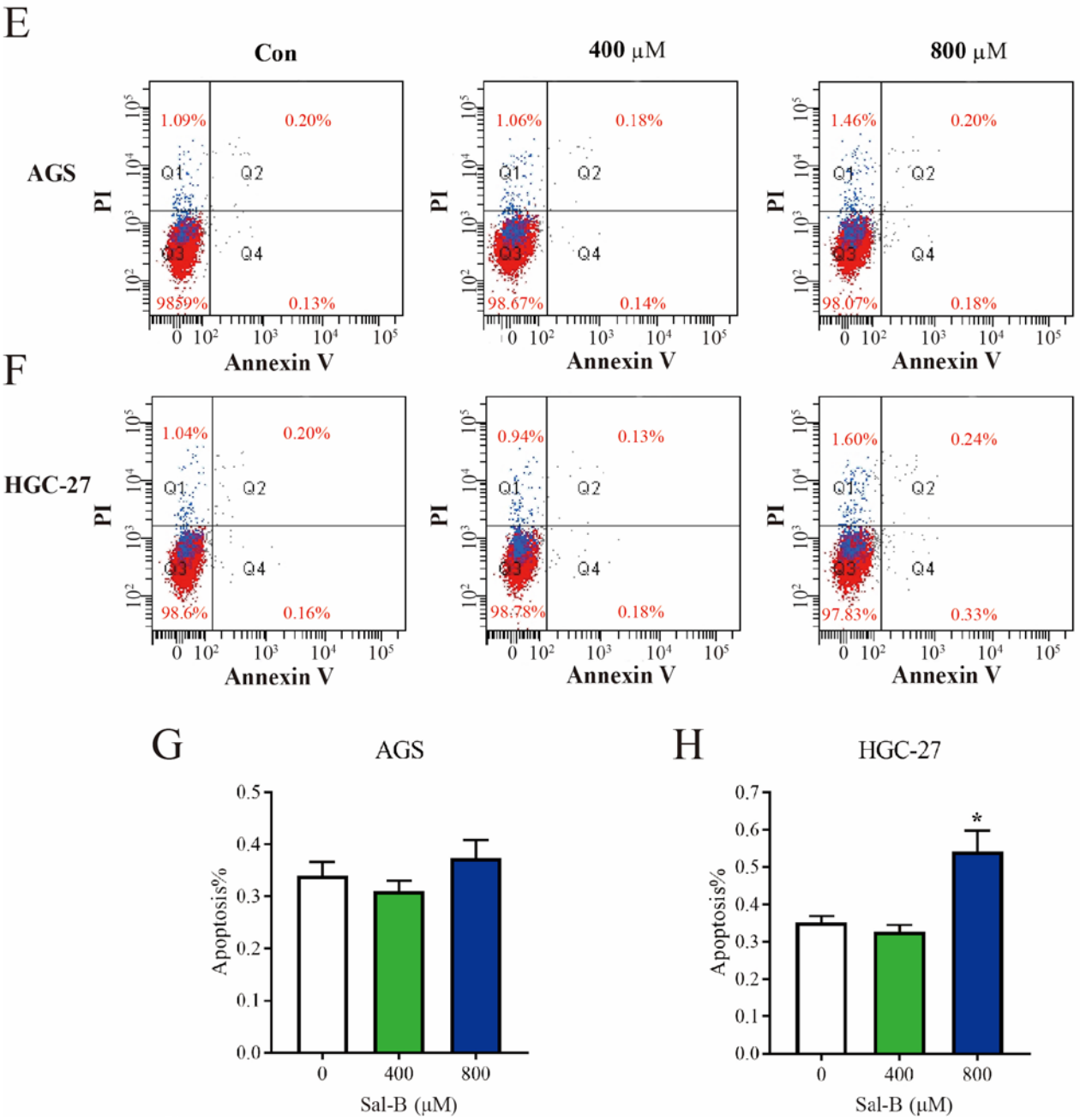

Figure 5. Continued. Effect of Sal-B on the apoptosis of gastric cancer cells. AGS and HGC-27 cells were exposed to Sal-B at concentrations of 0, 400 and 800 μM for 48 h. Expression levels of cleaved caspase-3 were evaluated by western blotting. Representative scatterplots are provided for (E) AGS and (F) HGC-27 cells, and quantification of apoptosis in (G) AGS and (H) HGC-27 cells. Data are shown as the mean ± SEM (n=3). *P<0.05 vs. 0 μM Sal-B. Sal-B, salvianolic acid B; PI, propidium iodide.

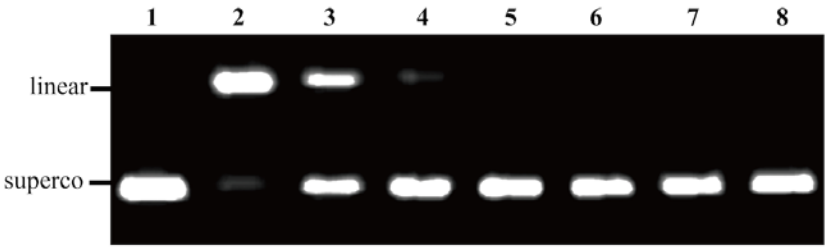

Figure 6. Protective effects of Sal-B from *Salvia bowleyana* Dunn on oxidative DNA damage induced by AAPH. Lane 1, native DNA; lane 2, DNA treated with AAPH and solvent; lane 3, DNA treated with AAPH and 0.004 mmol/l Sal-B; lane 4-7, DNA treated with AAPH and 0.017, 0.03, 0.06 and 0.13 mmol/l Sal-B, respectively; lane 8, DNA treated with AAPH and 0.08 mmol/l rutin. A total of 100 ng pUC18 DNA and 12.5 mmol/l AAPH were used in every appropriate lane. Sal-B, salvianolic acid B; AAPH, 2,2'-azobis (2-methylpropionamidine) dihydrochloride; superco, supercoiled.

Authors' contributions

BC designed and performed the experiments, and prepared the figures. CH designed and performed the experiments, drafted the initial manuscript, and prepared the figures. YZ extracted Sal-B and performed partial verification experiments. XT, SL and QW performed the experiments and analyzed the data. YL conceived and designed the experiments and drafted the initial manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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