



ORIGINAL ARTICLE

Evaluation on bioactivities of triterpenes from *Bergenia emeiensis*



Siyuan Luo, Jiajia Li, Yiling Zhou, Li Liu, Shiling Feng, Tao Chen, Lijun Zhou, Chunbang Ding*

College of Life Science, Sichuan Agricultural University, Ya'an 625014, China

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Abstract *Bergenia emeiensis* is a traditional folk medicine in China. Most studies are focusing on the bioactivity of bergenin, neglecting other compounds. Therefore, in this present work, the antioxidant, antibacterial and anticancer abilities of triterpenes from *B. emeiensis* rhizomes (TBE) were comprehensively evaluated. The results showed that TBE could well scavenge DPPH with a lower EC₅₀ of 0.29 mg/mL and could improve the cell viability of CHO cells against H₂O₂ induced cell death. Additionally, TBE also enhanced the resistance of *C. elegans* under thermal stress, exhibiting a strong antioxidant potential *in vivo*. Moreover, TBE showed a certain antibacterial ability on five kind strains such as *Pseudomonas aeruginosa* while TBE could not significantly inhibit the growth of plant pathogenic fungi. Furthermore, TBE possessed an excellent suppression ability on the proliferation of cancer cells especially Hela and Hep G2 cells with an IC₅₀ of 41.79 µg/mL and 48.62 µg/mL at 48 h, respectively. After fractionally extracted with petroleum ether, ethyl acetate and ethanol, ethanol phase (EP) contained the most triterpenes, followed by ethyl acetate phase (EAP) and petroleum ether phase (PEP). Besides, EP and EAP exhibited a strong antioxidant capacity and also inhibited the proliferation of Hela and Hep G2 cells with the effect of pro-oxidation by inducing a high level of ROS and decreasing mitochondrial membrane potential, thus causing oxidative stress then inducing cell apoptosis. Therefore, these outcomes indicated the triterpenes from *B. emeiensis* possessed a strong antioxidant ability and an anticancer effect, thus making *B. emeiensis* become a promising potential alternative in pharmacological field.

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1. Introduction

Bergenia (family, Saxifragaceae) is commonly used as a folk medicine in Asia especially in India and the Himalayan region (Shikov et al., 2012). Many reports appeared for treating diabetes, fever, pulmonary and kidney stones (Uniyal et al., 2006; Ballabh et al., 2008). And it is well known as Pashanabheba used in indigenous system of medicine (Gurav and Gurav

* Corresponding author.

E-mail address: dcb@scau.edu.cn (C. Ding).

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2014). There are 10 kinds in *Bergenia*, and *B. ciliata*, *B. ligulate*, *B. crassifolia* and *B. ligulata* are the most frequently applied in treatment (Chernetsova et al., 2012). Among these, *B. emeiensis* is a special species in China. In the area of Mountain Emei, the rhizomes of *B. emeiensis* are a kind of specific drug on curing respiratory diseases. But the literature on the pharmacological activity of *B. emeiensis* is rarely documented.

The methanolic and aqueous extracts of *B. ciliata* rhizome exhibited a strong antioxidant ability on scavenging DPPH and hydroxyl radicals and also prevented lipid peroxidation (Maregesi et al., 2009). The crude aqueous-methanolic extract of *B. ligulata* rhizome could inhibit calcium oxalate crystal aggregation as well as crystal formation in the metastable solutions showing a strong antiurolithic effect (Bashir and Gilani, 2009). *B. ciliata* as a Nepalese medicinal herb exhibited an inhibition on α -glucosidase and α -amylase making it as a potential drug to treat diabetes (Bhandari et al., 2008). In our previous work, the triterpenes extract from the leaves of *B. emeiensis* could well suppress the proliferation of Hela cells via cell apoptosis (Luo et al., 2020b). And the triterpenes from the rhizomes of *B. emeiensis* showed a strong antioxidant ability on protecting CHO cells against H_2O_2 damage (Luo et al., 2020a). But whether the triterpenes from *B. emeiensis* show other bioactivities is still unknown.

B. emeiensis as an important medicinal plant in the region of southwest of China has not been reported on its utilization. Comprehensive acquaintance of the bioactivities of *B. emeiensis* is still unknown. There were many substances in the rhizomes of *B. emeiensis*, and a comprehensive component pretest of *B. emeiensis* could help to get a better understanding on the individual components of *B. emeiensis* so as to better separate those compounds. Therefore, in this present work, the antioxidant, antibacterial and anticancer abilities of the total triterpenes *B. emeiensis* were assessed. Then, different polar solvents used to fractionally extract total triterpenes from *B. emeiensis*, and subsequently three phases were obtained to assess the antioxidant and anti-cancer effect so as to get a better understanding on the triterpenes compounds from *B. emeiensis* for further pharmacological activities analysis and other industrial applications.

2. Materials and methods

2.1. Materials and chemicals

B. emeiensis was picked up on the Mount Emei, Sichuan, China at the altitude of 1300–1500 m. The rhizomes were washed with distilled water and then naturally air-dried. After grounded into fine powder, the rhizome powder was kept at $-20\text{ }^\circ\text{C}$ for following experiments.

2,2-Diphenyl-1-picryl-hydrazyl (DPPH), nitro blue tetrazolium (NBT), phenazine methosulfate (PMS) and nicotinamide adenine dinucleotide (NADH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol, perchloric acid, acetic acid, ferric chloride, and vanillin were purchased from the Chengdu Kelong Chemical Factory (Chengdu, China). Reactive Oxygen Species Assay Kit (ROS) kit was purchased from Beyotime Biotechnology (Shanghai, China). The total protein assay kit (with standard: BCA method) was bought from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Fetal bovine serum (FBS)

was obtained from Gibco. Dulbecco's modified eagle medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, PBS, digestive enzyme and Penicillin-Streptomycin solution (10X) were purchased from Hyclone (USA). Agar powder, phosphate buffer solution (PBS) (0.2 M), trypsin, 4',6-diamidino-2-phenylindole (DAPI), Rhodamine 123 and Hoechst 33,258 were obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Yeast extract and tryptone were purchased from English OXOID Co., Ltd. (UK). All chemicals were analytical grade.

2.2. Extraction of Triterpenes from *Bergenia emeiensis* (TBE)

0.5 kg rhizome powder was resolved in 3 L 85% ethanol with the assistance of ultrasound for 40 min at $45\text{ }^\circ\text{C}$. The extract solution was filtered twice by a buchner funnel then the filtrate was dried in a vacuum freeze drying machine for 4 days. Then the dried-objection was resolved in ethanol to remove impurities including those that did not dissolve to ethanol. After filtered again, the filtrate was dried to obtain triterpenes from *Bergenia emeiensis* (TBE). The content of triterpenes in TBE was higher than 75%.

2.3. Fractionation extraction of total triterpenes from *B. emeiensis*

TBE was packed in a filter paper. Then petroleum ether, ethyl acetate and ethanol were used to fractionally extract the total triterpenes from sample, respectively. Each extracted solution was concentrated and lyophilized to obtained petroleum ether phase (PEP), ethyl acetate phase (EAP) and ethanol phase (EP).

2.4. Determination of total triterpene content

The total triterpenes content was determined by vanillin-perchloric acid method (Zhou et al., 2020). Briefly, 50 μL sample solution was mixed with 100 μL 5% vanillin-acetic acid (w/v) and 200 μL perchloric acid, then the mixture was placed at $60\text{ }^\circ\text{C}$ for 15 min before adding 650 μL acetic acid. After standing in dark for 5 min, the absorbance of reaction solution was measured at 550 nm. The content of total triterpenes was calculated according to standard curve made with ursolic acid as reference. The content of total triterpenes was expressed as milligram ursolic acid equivalent per gram (mg GAE/g) of dry sample.

2.5. Spectroscopical analysis

Ethyl acetate phase (EAP) and ethanol phase (EP) were resolved in ethanol and added into quartz colorimetric utensils to measure the ultraviolet spectra from 200 to 400 nm taking ethanol as reference (UV-1800 ultraviolet spectrophotometer, Shimadzu Company, Japan). The fourier transform infrared (FT-IR) spectroscopy was determined with KBr pellet. 5 mg dry-samples were mixed with 500 mg KBr then grinded into fine powder and pressed to a slice. The range of infrared scanning and signal acquisition is $400\text{--}4000\text{ cm}^{-1}$ (Nicolet-380 fourier transform infrared spectroscopy, Thermo Fisher Company, USA).

2.6. The Ultra Performance Liquid Chromatography analysis (UPLC)

EP was resolved in ethanol and the chemical compositions were identified with UPLC (Waters ACQUITY UPLC + Waters XEVO G2-S QTOF). The chromatographic condition was shown in Table 1. And the mass spectrometer (Xevo G2-S QTOF MS) conditions were as collection quality range: 50–1500 Da; scanning time: 0.1 s; collection mode: ESI resolution mode; MS^E Lock mass: leucine enkephalin (LE) 1 ppm (0.3 s scan, interval: 15 s); capillary voltage: 2.5 KV (ESI-); taper voltage: 40 V; collision energy (eV): low CE: 6/High CE: 20–40; ionization source temperature: 120 °C; desolvation temperature: 500 °C; air flow rate of cone hole: 30 L/h; desolvated gas flow rate: 1000 L/h; data acquisition time: 20 min.

2.7. Antioxidant assays in vitro

2.7.1. Determination of scavenging ability on DPPH radicals

The clearance ability on DPPH free radicals was conducted according to previous method with some modifications (Luo et al., 2019). TBE was resolved in ethanol to different concentrations. 30 µL sample solution was mixed with 170 µL DPPH ethanol solution (0.8 mM) at dark for 15 min. Then the absorbance of the reaction solution was read at 517 nm. Ascorbic acid was used as the positive control. The clearance rate was calculated according to the following formula:

$$\text{Clearance rate (\%)} = 1 - A1/A0$$

where the A1 was the absorbance of the group with TBE and A0 was the absorbance of the group with ethanol instead of sample.

2.7.2. Determination of scavenging ability on superoxide anion radicals

The scavenging ability on superoxide anion free radicals was measured by the previous method with some modifications (Zhishen et al., 1999). Briefly, 0.2 mL sample solution was added into 0.2 mL NBT (156 µmol/L), 0.2 mL PMS (60 µmol/L) and 0.4 mL NADH (470 µmol/L). The reaction solution was placed at 37 °C for 20 min. Then absorbance of the solution was read at 540 nm. Ascorbic acid was used as the positive control. The clearance rate was calculated according to the following formula:

$$\text{Clearance rate (\%)} = 1 - A1/A0$$

where the A1 was the absorbance of the group with TBE and A0 was the absorbance of the group with ethanol instead of sample.

2.7.3. Determination of total reducing power

The reducing power was associated with the antioxidant ability. Therefore, the total reducing power of TBE was determined by the previous method with some modifications (Zeng et al., 2020). 0.2 mL TBE sample solution was mixed with 0.5 mL K₃[Fe(CN)₆] (1% w/v) and 0.5 mL phosphate buffer (0.2 M). The mixture was cooled at 0 °C after placed at 50 °C for 20 min. Subsequently 0.5 mL TCA (10% w/v) was added into for 5 min. Then, 0.1 mL aliquot of supernatant fluid was mixed with 0.5 mL distilled water and 0.1 mL FeCl₃ (0.1% w/v). The absorbance of the reaction solution was read at 700 nm. An ascending absorbance meant an increasing total reducing power.

2.7.4. The cell culture

Chinese hamster ovary cells (CHO cells) were kindly obtained from the Biotechnology Center of Sichuan University (Sichuan, China). CHO cells were cultured in DMEM containing 10% FBS at 37 °C in a 5% CO₂ atmosphere. When the density of CHO was about 80–90%, cells were digested from the flask and washed by PBS for 2 times. Then the cell suspension was transferred into microplates for following experiments.

2.7.5. The protection of TEB on CHO cells

TBE resolved in DMSO and the finally concentration of DMSO was lower than 0.5%. CHO cells suspension at the concentration of 10⁵ cells/mL was transferred into microplates for 12 h. Then TBE sample was added into each well for another 48 h at 37 °C. Subsequently, H₂O₂ was put into each well after removing the old medium for 5 h. Then new medium containing 10% CCK-8 kit replaced the solution and the plates was placed at 37 °C for 30 min. The cell viability was calculated according to the following formula:

$$\text{Cell viability (\%)} = A1/A0$$

where the A1 was the absorbance of the group with TBE and A0 was the absorbance of the group with PBS instead of sample.

2.7.6. Determination of ROS

Cells were adjusted to the concentration of 10⁵ cells/mL with DMEM medium. 1.8 mL cell suspension was added into 6-wells microplate for 24 h. Then TBE sample was transferred into each well. After 48 h incubation, all solution was removed and H₂O₂ was added into the plate for 5 h. Subsequently, PBS was used to wash the cell for twice before ROS assay kit was added into the plate for 20 min. Then the dying solution was removed and the cells were washed by PBS for twice. The fluorescence of each well was read by a microplate reader.

Table 1 The chromatographic condition for UPLC.

Chromatographic column: Waters BEH C18 (2.1 * 100 mm, 1.5 µm)				
Time (min)	Flow (mL/min)	A (0.1% HCOOH)	B (Methanol)	Curve
Initial	0.2	90.0	10.0	Initial
0.5	0.2	90.0	10.0	6
15.0	0.2	0.0	100.0	6
19.5	0.2	0.0	100.0	1
20.0	0.0	90.0	10.0	1

2.8. Antioxidant assays *in vivo*

2.8.1. The strains and the culture

Wild-type N2 *Caenorhabditis elegans* (*C. elegans*) and *Escherichia coli* OP50 strain were kindly provided from the *Caenorhabditis* Genetics Center (CGC). *C. elegans* was cultured on nematode growth medium (NGM) agar plates, with a layer of *E. coli* OP50 as food sources and maintained at 20 °C. The synchronized worms were obtained by the sodium hypochlorite method (Porta-de-la-Riva et al., 2012).

2.8.2. Food clearance assay

Synchronized worms were placed on NGM plate for 48 h then transferred into a 96-well plate (20–30 worms/well) containing different concentrations of TBE (DMSO < 0.5%), OP50 (dead) and 5-fluorouracil (25 µM). The plate was kept at 20 °C and the absorbance was read at 600 nm every day.

2.8.3. Thermal stress assay

The synchronized eggs were placed on the NGM plate with the presence and absence of TBE for 48 h. Then the worms were transferred to a new NGM plate keeping at 35 °C for 5 h and subsequently the survival was counted as the dead worm was identified with no response to gentle touch of platinum.

2.9. Anti-cancer assays

2.9.1. The cell culture conditions

Hela, A549 and Hep G2 cancer cells were kindly provided by the Biotechnology Center of Sichuan University (Sichuan, China). S180 cells were bought from Stem Cell Bank, Chinese Academy of Sciences. Hela and Hep G2 cells were maintained in DMEM medium. S180 and A549 cells were cultured in RPMI 1640 medium.

2.9.2. Determination of inhibition effect

When the cell density was about 70–80%, the cells were digested from the flask and washed with PBS for twice. Then the cell was suspended with fresh medium to the concentration of 10⁵ cells/mL. 90 µL cell solution was added in a 96-well microplate with edge filled by 100 µL PBS. After 12 h incubation, 10 µL sample solution was transferred into each well and 25 µg/mL of 5-fluorouracil (5-Fu) was used as the control group. Then the solution was removed and new medium containing 10% CCK-8 kit was added. The absorbance of each well was read at 450 nm after 30 min. The inhibition rate was calculated according to the following formula:

$$\text{Inhibition rate (\%)} = 1 - A1/A0$$

where the A1 was the absorbance of trial group with sample treatment and A0 was the absorbance of blank group.

2.9.3. DAPI and Hoechst 33258 dyeing methods to observe apoptosis

The cell apoptosis was observed with DAPI and Hoechst 33,258 staining methods. Briefly, cancer cells (10⁶ cells/mL) were seeded in 6-well plates for 12 h at 37 °C. Then sample solution was transferred into the plate for 48 h, and paraformaldehyde (4%) was used to fixed the cells for 30 min at 4 °C. Then, the fixation fluid was removed and cells

were washed twice with PBS. DAPI and Hoechst 33,258 working solution were added into to cover the cells for 10 min. Subsequently, the dyeing solution was removed and PBS adopted to wash cells again for twice before the plate was observed with a fluorescence microscope (Olympas-BX51TRF, Japan)

2.9.4. Determination of ROS level and MMP

Cell suspension was adjusted to the density of 10⁶ cells/mL then transferred into 6-well plate for 12 h. After incubation, sample solution was added into each well for another 24 h. Subsequently, the solution was removed and paraformaldehyde (4%) was used to fixed the cells for 30 min at 4 °C. Then the cells were washed with PBS for 3 times to remove paraformaldehyde. Then ROS kits and Rhodamine 123 (10 µM) were used to stain cell for 30 min, respectively. Finally, the dyeing liquor was removed and PBS was used to wash 2 times before the cells were observed with a fluorescence microscope (Olympas-BX51TRF, Japan).

2.10. Anti-microbial assays

2.10.1. The test-strains

*Salmonella typhimurium*I, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterococcus*, *Pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* were obtained from College of Life Sciences, Sichuan Agricultural University. All bacteria strains were cultured in LB medium. *Colletotrichum gloeosporioides*, *Fusarium graminearum* (Schw.) Petch, *Fusarium oxysporum* f. sp. *niveu* and *Colletotrichum acutatum* were kindly given by College of Agronomy, Sichuan Agricultural University. All fungal was cultivated in potato medium (PDA).

2.10.2. Determination of inhibition zones

The antibacterial ability was determined by the oxford cup method (Diao et al., 2014). Briefly, TBE was resolved in DMSO and the concentration of DMSO was lower than 0.5% finally. The strains were adjusted to the concentration of 10⁵ CFU. 1 mL suspension was spread on the LB plate until the solution was dried in the super-clean worktable. Then an oxford cup was placed on the plate with 200 µL sample solution in the hole. The plate was transferred to an incubator at 37 °C for 24 h. The inhibition zones were determined by crossing method.

2.10.3. Anti-fungal assay

The antifungal ability of TBE was determined by using plate growth rate method (Zhou et al., 2016). The strains were cultivated on the PDA medium at 25 °C. When the mycelium reached on wall of the plate, a block of strain was transferred on a new plate which was spread with a layer of sample solution. Then the plate was placed at the incubator. The diameter of mycelium was determined daily by crossing method.

2.11. Statistical analyses

The data obtained in this study were analyzed statistically by ANOVA (GraphPad Prism 8.0) (GraphPad Software, Inc., La Jolla, CA, USA). All experimental results were expressed as mean ± standard deviation (SD). P < 0.05 was considered to be significantly different.

3. Results

3.1. Antioxidant ability *in vitro*

Extracts from *Bergenia* had been pronounced to show an excellent antioxidant ability (Zafar et al., 2019; Kraujalienė et al., 2016). But relative researches about *B. emeiensis* were

rarely reported. This work could help to get a better understanding on the antioxidant ability of *B. emeiensis*. As shown in Fig. 1A, TBE could well clear DPPH free radicals within the concentrations of 0.2–1.0 mg/mL. And the clearance rate was equivalent with Vc when the concentration was higher than 0.8 mg/mL with an EC₅₀ of 0.29 mg/mL. A lower EC₅₀ meant a strong antioxidant property (Arya et al., 2012; Andriani et al., 2015). Meanwhile, at the lowest concen-

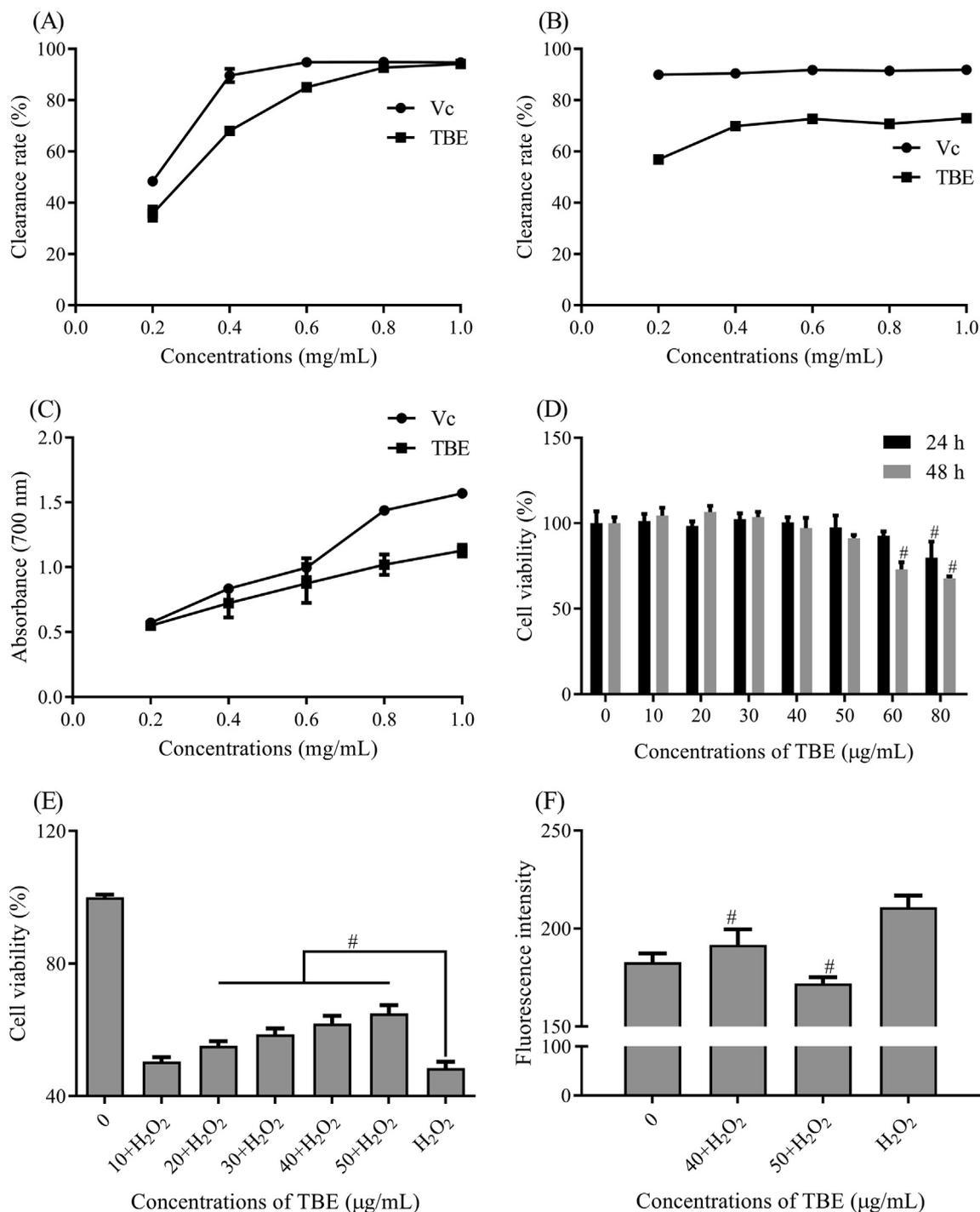


Fig. 1 The antioxidant ability of TBE *in vitro*. (A) the clearance ability on DPPH radicals (B) the scavenging ability on superoxide anion radicals and (C) the total reducing ability. After treated with TBE, (D) the cell viability of CHO cells. Under the oxidative stress, (E) the protective effect of TBE on CHO cells and (F) the ROS level in CHO cells. Notes: # means $p < 0.001$.

tration of 0.2 mg/mL, the clearance rate on superoxide anion was over 50% and the rate tended to be stable when the concentration was higher than 0.4 mg/mL (Fig. 1B). An ascending absorbance meant an increasing total reducing power which was associated with the antioxidant capacity to some degree. As shown in Fig. 1C, the absorbance of TBE at 700 nm was rising with the ascending concentration. Hence TBE possessed a strong total reducing power as well as a great antioxidant ability.

3.2. Protection on CHO cells against oxidative stress

3.2.1. The toxicity of TBE on CHO cells

When the concentration of drugs was high, the cell viability would be affected by osmotic pressure of cell or other negative factors. Hence a non-toxic concentration of TBE on CHO cells should be found. As shown in Fig. 1D, after treated with TBE for 24 h, the cell viability was significantly reduced when the concentration was over 80 $\mu\text{g/mL}$. And after 48 h treatment, the growth of CHO cells was obviously inhibited when the concentration of TBE was higher than 60 $\mu\text{g/mL}$. Therefore, for following experiments, the concentration of TBE was con-

trol under 50 $\mu\text{g/mL}$ so as to avoid the negative effect of TBE on CHO cells.

3.2.2. The protective effect of TBE on CHO cells

When the cells were under the condition of H_2O_2 , the cell viability was reduced (Fig. 1E). After treated with 0–50 $\mu\text{g/mL}$ of TBE for 48 h, the cell viability was promoted as the increasing concentration of TBE compared with the injury group. Among them, 50 $\mu\text{g/mL}$ of TBE could enhance the cell viability to the highest showing the strongest protection (Fig. 1E). Therefore, 40 and 50 $\mu\text{g/mL}$ of TBE were selected for the following experiment.

3.2.3. The ROS level under oxidative stress

H_2O_2 is a trigger for ROS. CHO cells were in the environment of H_2O_2 , the inner level of ROS was increased (Fig. 1F). A high level of ROS lead to the cell death. Hence the injury group survived less (Fig. 1E). To evaluate the effect of TBE on the ROS level, CHO cells were treated with different concentration of TBE for 48 h and then determined the content of ROS. The results showed that the ROS level in trial group was significantly lower than that in injury group. And 50 $\mu\text{g/}$

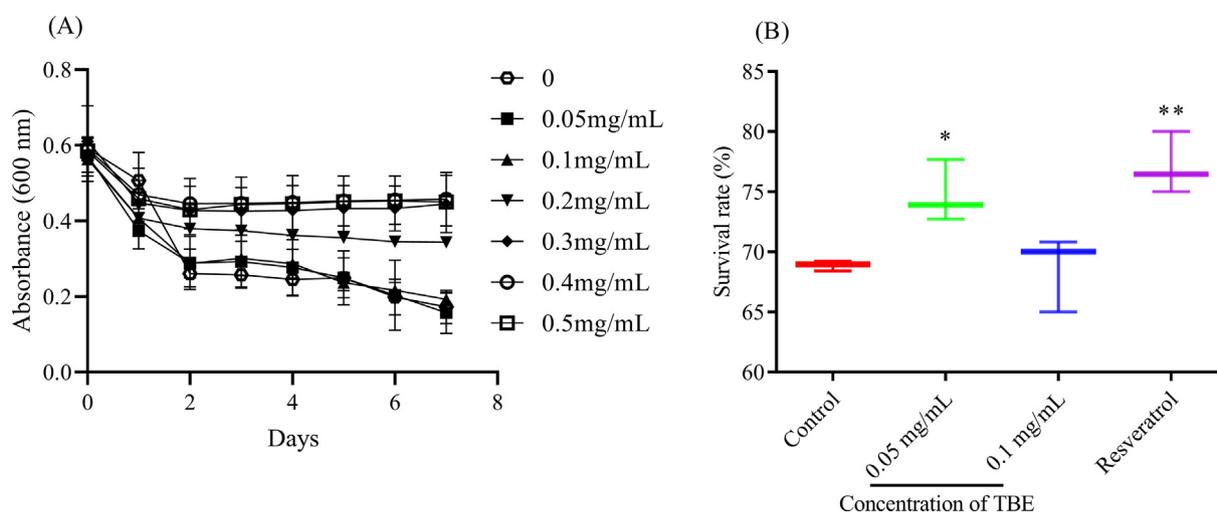


Fig. 2 The antioxidant potential of TBE *in vivo*. (A) the effect of TBE on the intake ability of *C. elegans* and (B) the survival of *C. elegans* under thermal condition. Note: * means $p < 0.05$ and ** means $p < 0.01$.

Table 2 The inhibition zones of eight tested strains.

Strains	Inhibition zones (mm)				
	100 $\mu\text{g/mL}$ Ampicillin Sodium	2.5 mg/mL TBE	5 mg/mL TBE	10 mg/mL TBE	Blank Control
<i>Salmonella typhimurium</i>	14.75 \pm 0.43	8.00 \pm 0.00	8.00 \pm 0.00	8.00 \pm 0.00	8.00 \pm 0.00
<i>Bacillus subtilis</i>	16.25 \pm 1.09	10.75 \pm 0.43	12.25 \pm 0.43	14.00 \pm 0.71	
<i>Pseudomonas aeruginosa</i>	16.75 \pm 2.95	11.88 \pm 1.34	13.75 \pm 1.30	16.00 \pm 1.22	
<i>Bacillus cereus</i>	24.25 \pm 0.83	8.00 \pm 0.00	8.00 \pm 0.00	8.00 \pm 0.00	
<i>Enterococcus</i>	16.50 \pm 0.50	11.25 \pm 1.30	12.75 \pm 0.83	14.25 \pm 1.30	
<i>Pneumoniae</i>	15.75 \pm 0.43	10.38 \pm 1.56	12.75 \pm 1.92	14.00 \pm 1.73	
<i>Staphylococcus aureus</i>	17.50 \pm 1.12	11.25 \pm 0.83	12.63 \pm 0.82	14.50 \pm 0.87	
<i>Escherichia coli</i>	14.63 \pm 1.98	8.00 \pm 0.00	8.00 \pm 0.00	8.00 \pm 0.00	

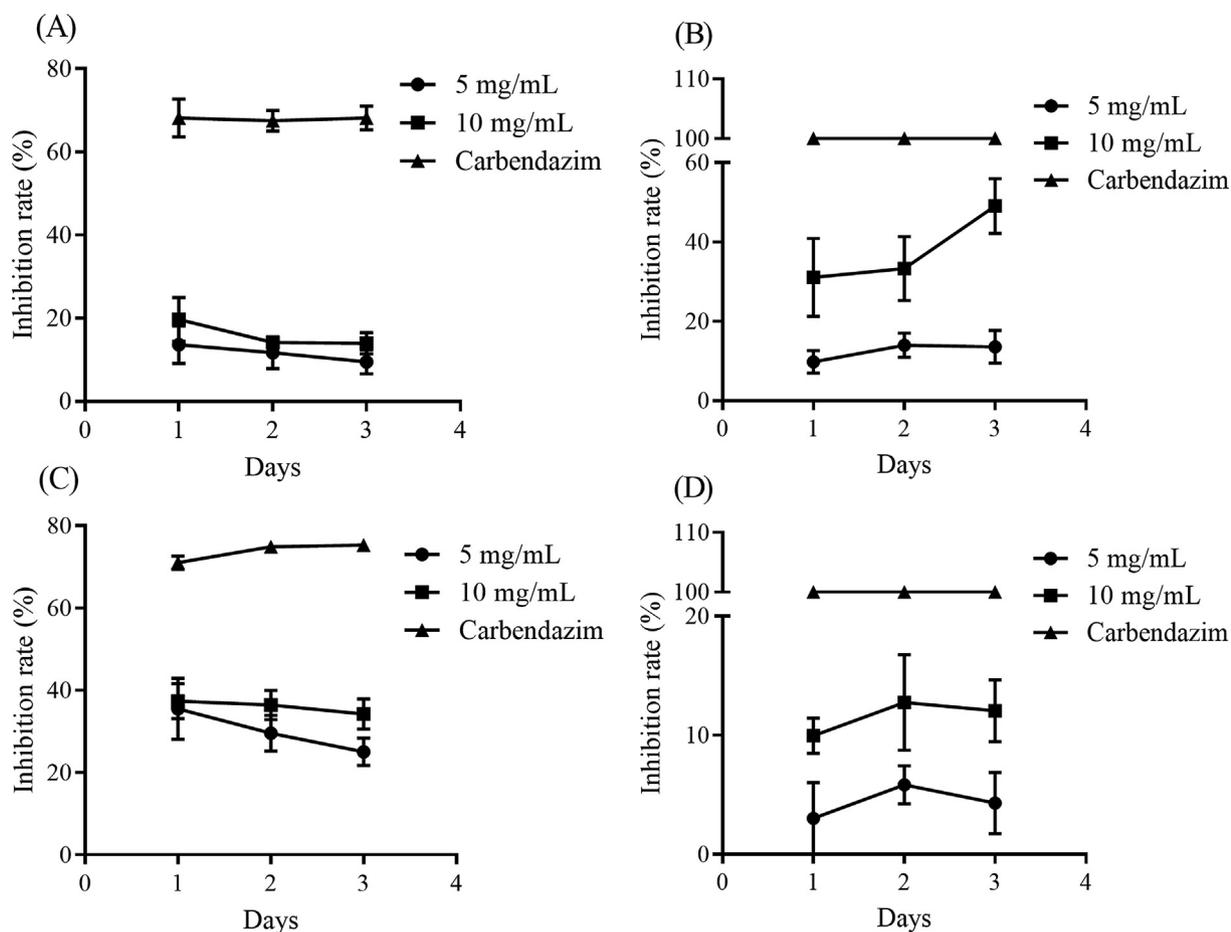


Fig. 3 The effect of different concentrations of TBE on the growth of (A) *Colletotrichum gloeosporioides*, (B) *Botrytis cinerea*, (C) *Fusarium oxysporum* and (D) *Colletotrichum acutatum*.

mL of TBE could even make the ROS content lower than blank control (Fig. 1F). Thereby, TBE could reduce the increasing ROS level in CHO cells under oxidative stress.

3.3. Antioxidant ability *in vivo*

But the inner environment is complex. Whether TBE still exhibited a strong antioxidant ability *in vivo*? The model organism *C. elegans* was applied to evaluate the antioxidant capacity of TBE *in vivo*. First, food clearance assay was performed to select a suitable concentration of TBE on *C. elegans*. In a limited environment, the worms could consume the food OP50 quickly, hence as shown in Fig. 2A, 0.2 and 0.4 mg/mL TBE had a negative effect on the intake of *C. elegans* on OP50, while 0–0.1 mg/mL of TBE showed no significant effect on the intake. Hence, 0–0.1 mg/mL of TBE was used as the no-toxic concentration for following assays. When the worms were shifted to a higher temperature, *C. elegans* would undergo a stress and only the part of the worms could survive. As shown in Fig. 2B, after pretreated with TBE for 48 h, 0.05 mg/mL of TBE obviously promoted the survival of *C. elegans* to 5.91% while 0.1 mg/mL TBE exhibited no positive effect on increasing the resistance of *C. elegans* ($p > 0.05$). Therefore, TBE also exhibited a strong antioxidant capacity *in vivo*.

3.4. Antibacterial ability

Bergenia had been pronounced to possess a certain bacteriostatic ability (Singh et al., 2017; Żbikowska et al., 2017). *Salmonella typhimurium* and *Escherichia coli* were gram-negative bacteria and *Bacillus cereus* was gram-positive bacteria. As shown in Table 2, the inhibition zones of *Salmonella typhimurium*, *Bacillus cereus* and *Escherichia coli* were 8.00 ± 0.00 mm indicating TBE exhibited no negative effect on the above three strains. With the increasing concentration of TBE, the diameter of zones was largening for the other five strains. Among them, the inhibition zones of *Pseudomonas Aeruginosa* were the largest 16.00 ± 1.22 mm at 10 mg/mL of TBE. TBE could well inhibit the growth of both gram-negative and gram-positive bacteria, and vice versa. Therefore, the antibacterial motive of TBE was not against a class of bacteria.

3.5. Antifungal ability

Although TBE could well inhibit the growth of some bacteria, the effect on pathogenic fungi was quite different (Fig. 3). The inhibition rates on *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* were lower than 20% showing a weak suppression ability (Fig. 3A and D). On the first day, TBE could restrain the growth of *Fusarium oxysporum* to about

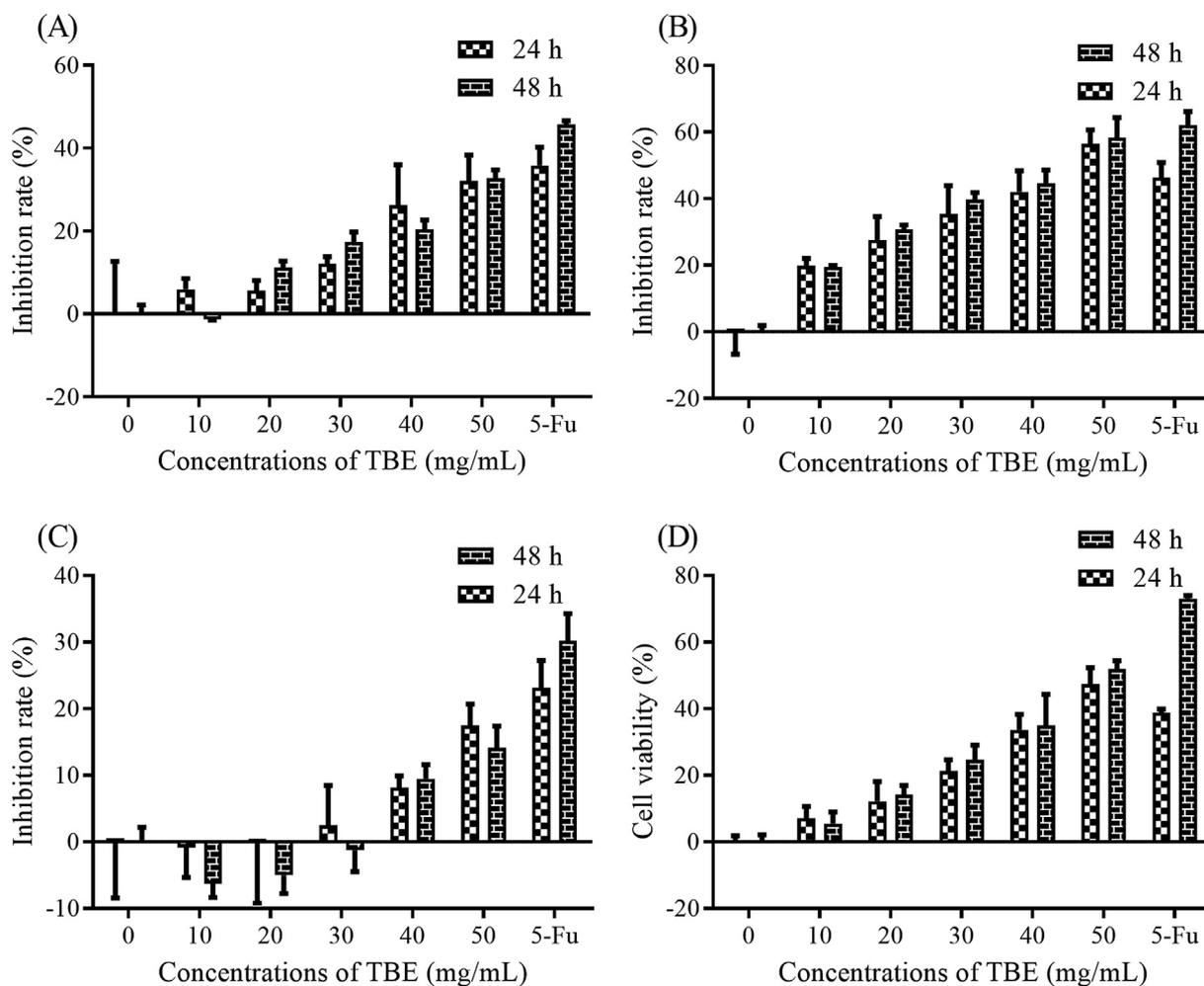


Fig. 4 The anticancer abilities of TBE on (A) A549 cells (B) Hela cells (C) S180 cells and (D) Hep G2 cells.

Table 3 The contents of total triterpenes in three phases.

Components	Contents (mg/g)
Petroleum ether phase	22.57 ± 9.48
Ethyl acetate phase	391.38 ± 12.36
Ethanol phase	743.02 ± 23.48

40%, but on the next and third day, the inhibition rate was reduced continuously (Fig. 3C). 5 mg/mL of TBE showed no obvious inhibition on *Botrytis cinerea* (Fig. 3B). Nevertheless, 10 mg/mL of TBE could significantly suppressed *Botrytis cinerea* to about 50% on the third day (Fig. 3B). Therefore, TBE exhibited an efficient inhibitory on *Botrytis cinerea* but except other three strains.

3.6. Anticancer ability

Based on the outcomes of the toxicity on CHO cells, the appropriate concentration of TBE used on cells was lower than 50 µg/mL. So different concentrations of TBE were added to treat four cancer cells. As shown in Fig. 4A, the proliferation

of A549 cells was declined with the increasing concentration of TBE. And the maximum inhibition rate was 32.7% at 50 µg/mL of TBE for 48 h. When TBE was higher than 10 µg/mL, the growth of Hela cells was obviously restrained. And the inhibition ability of 50 µg/mL was close to the positive group (Fig. 4B). The IC₅₀ was 44.19 µg/mL and 41.79 µg/mL for 24 and 48 h, respectively. Although with the increasing concentration of TBE, the inhibition rate on S180 was also ascending, the maximum inhibition rate was pretty low 17.50% (Fig. 4C). So, TBE might have no significantly negative effect on the growth of S180. As shown in Fig. 4D, the proliferative capacity of Hep G2 cells was suppressing as the concentration of TBE was increasing. The maximum inhibition rate was 51.87 µg/mL with the IC₅₀ of 51.09 µg/mL and 48.62 µg/mL for 24 and 48 h, respectively. Therefore, TBE exhibited a strong inhibition ability on selected cancer cells likewise Hela and Hep G2 cells, which was in accordance with our previous report (Luo et al., 2020b)

3.7. The content and characteristic of total triterpenes content in different extraction phases

Since TBE showed a strong antioxidant ability *in vitro*, thereby, fractionally extraction was applied to further explore

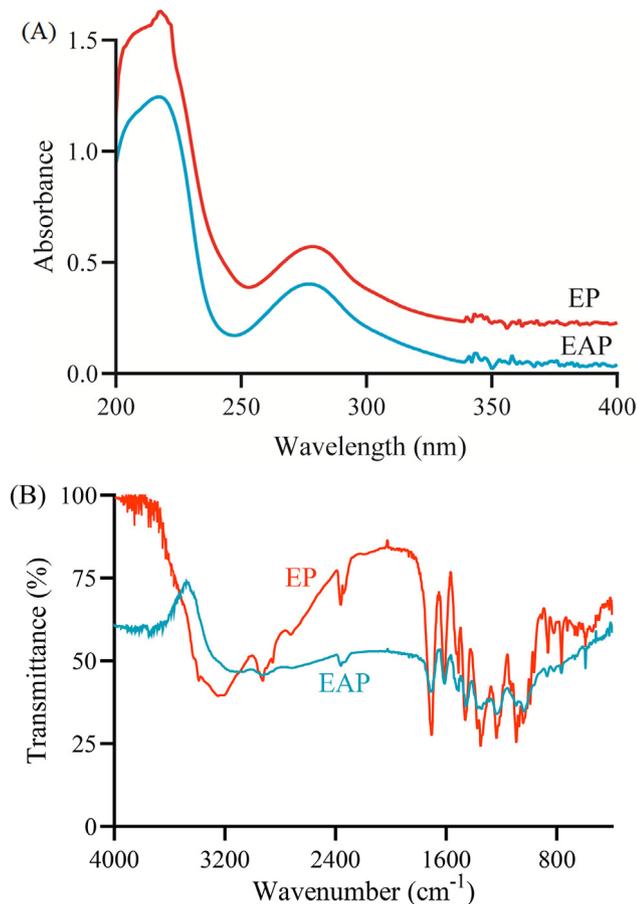


Fig. 5 The spectroscopic analysis of ethyl acetate phase and ethanol phase (A) the ultraviolet absorption spectrum (B) infrared absorption spectrum.

the substance with antioxidant capacity. As shown in Table 3, the ethanol extraction phase (EP) contained the highest level of total triterpenes (743.02 ± 23.48 mg/g) while petroleum ether phase (PEP) had the lowest (22.57 ± 9.48 mg/g). Therefore,

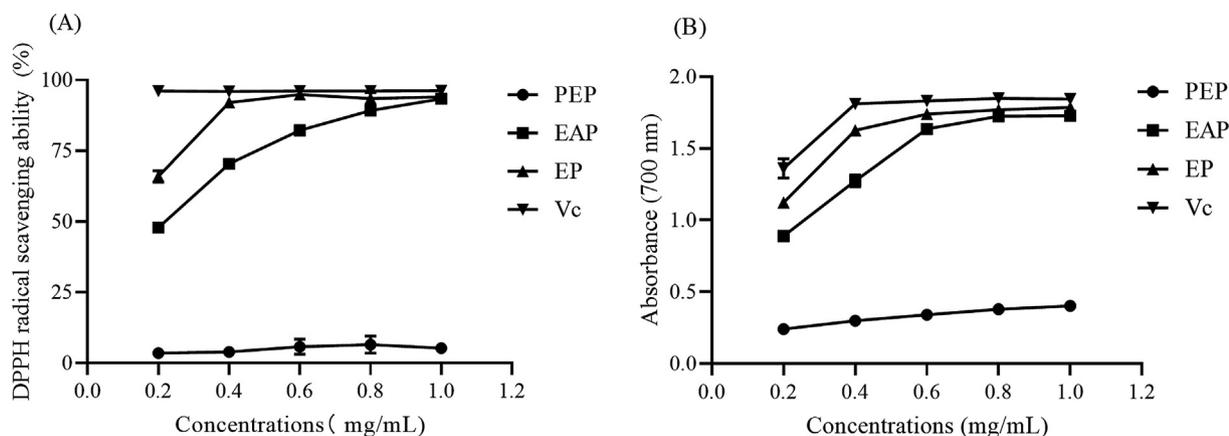


Fig. 6 The antioxidant ability of three phases (A) DPPH free radicals scavenging ability and (B) the total reducing power.

total triterpenes were highly existed in *Bergenia emeiensis* and were tending to a large polarity. Bergenin and other phenolic compounds were found in *Bergenia* plants (Singh et al., 2017). Nevertheless, this was the first time to report the presence of total triterpenes in *Bergenia*. The Fei et al. also used ethanol, petroleum and ethyl acetate to extract total triterpenes from *Pyrus ussuriensis*, respectively. And the content ranged from 66.01 to 256.33 mg/g, which was much lower than that in this work (Peng et al., 2020). The optimal yield of total triterpenoids from Jujube (*Ziziphus jujuba*) was 19.21 mg/g (Song et al., 2020). Chen et al. used five extraction methods to extract total triterpenes from *Ganoderma atrum* and the yields ranged from 1.51 to 5.11% (Chen et al., 2007). But those results were quite lower than that in the rhizomes of *B. emeiensis*. Hence, *B. emeiensis* contained a high level of triterpenes than other natural plants, and could be an abundant resource for triterpenes.

To further confirm the existence of total triterpenes in ethyl acetate (EAP) and ethanol phases, the UV and FT-IR scanning spectrum were conducted. Petroleum ether phase was viscous materials and contained the least triterpenes. Hence, the UV and FT-IR scanning spectrums were only applied to investigate the functional groups in ethyl acetate and ethanol phases. As shown in Fig. 5A, both phases had a strong absorbance at 215–220 nm, indicating there was a strong presence of conjugated dienes. And a faint peak at 270–280 meant the existence of ketones in EAP and EP. As shown in Fig. 5B, there were some O-H stretching absorbance at $3700\text{--}3500\text{ cm}^{-1}$, meaning there existed more hydrogen bonded hydroxy groups in EAP and EP. And the peaks from $3100\text{ to }2800\text{ cm}^{-1}$ was ascribed the stretching vibration of C-H. EP had stronger and more absorbance peaks at $1850\text{--}1660\text{ cm}^{-1}$ than EAP, indicating more carbonyl groups in EP. Therefore, the substances in EP and EAP were quite different, meaning EA and EAP might exhibit a different bioactivities.

3.8. The antioxidant ability of different extraction phases

Since the content of total triterpenes differently existed in three phases, the bioactivity could be different. As shown in Fig. 6A, PEP could hardly clear DPPH radicals, while EAP and EP could well scavenge DPPH. The clearacne rate of EP was

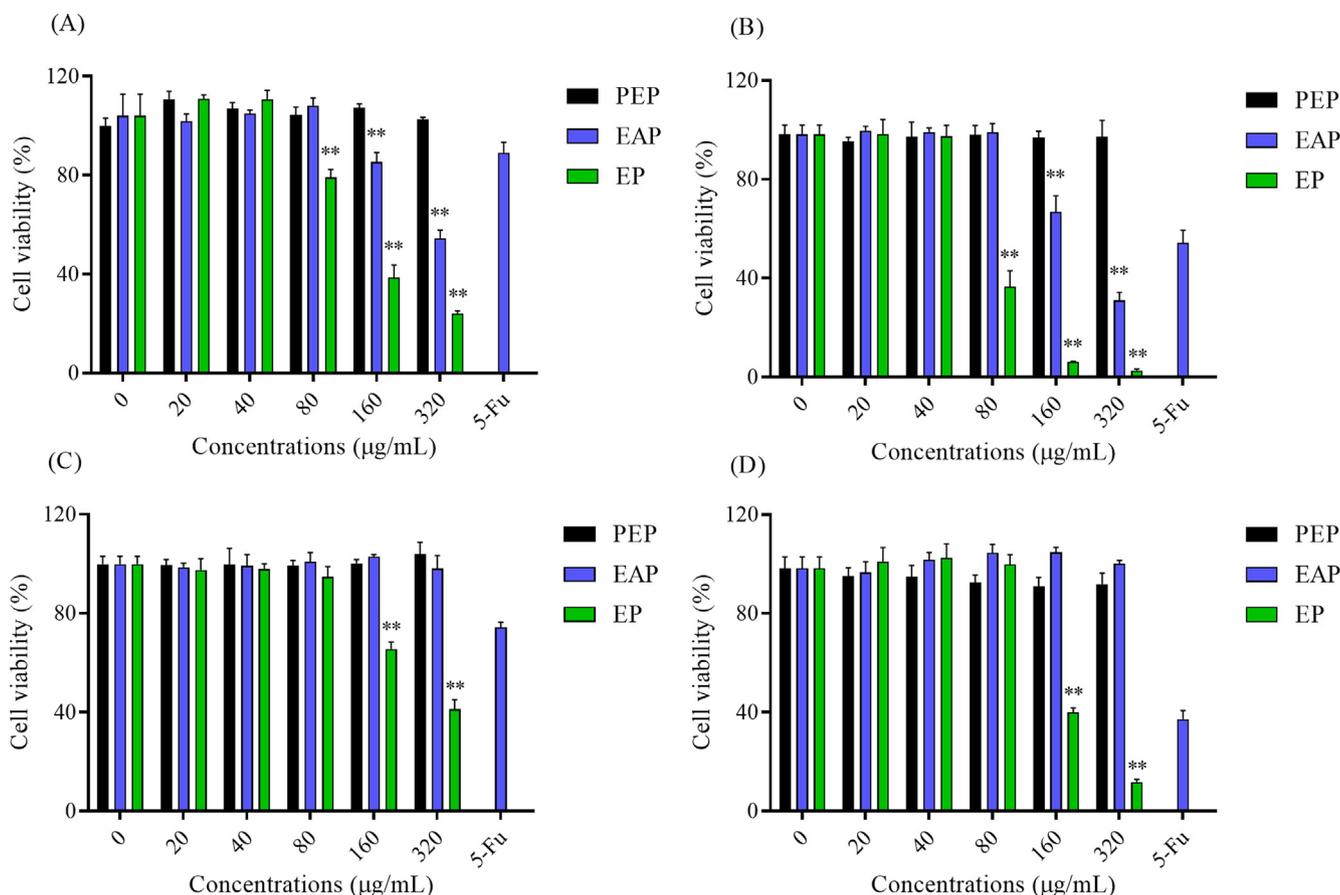


Fig. 7 The anticancer effect of two phases on HeLa and Hep G2 cells. The cell viability of HeLa cells treated with samples for (A) 24 h and (B) 48 h. The cell viability of Hep G2 treated with samples for (C) 24 h and (D) 48 h. The apoptotic bodies were stained by DAPI and Hoechst 33258 in (E) HeLa cells and (F) Hep G2 cells. The apoptosis cells were dyed in bright blue. Red bar meant 50 µm. Note: ** meant $p < 0.01$.

tended to be flat and close to Vc when the concentration was higher than 0.4 mg/mL. And the concentration of EAP was over 0.8 mg/mL, the scavenging capacity was close to Vc. The EC50 of EAP and EP was 0.31 mg/mL and 0.37 mg/mL, respectively. Hence, EP exhibited a greater DPPH radical scavenging ability, followed by EAP and PEP. As the increasing concentration, the total reducing power of three phases was also promoting, of which EP and EAP were close to Vc (Fig. 6B). Total triterpenoids from *Jatropha curcas* leaves could scavenge 79.51% DPPH free radicals at 3.2 mg/mL and for ABTS radical, the percentage inhibition was 65.25% with the concentration of 1.6 mg/mL (Wei et al., 2015). The EC50 of total triterpenoids from *Poria cocos* on DPPH radical was 0.79 mg/mL (Wang et al., 2017). The clearance was much lower than EAP and EP. Mariagrazia et al. found the crude extract from *Bergenia cordifolia* also showed a strong antioxidant ability while the specifically compounds were still unknown (Roselli et al., 2011). In this present work, the total triterpenes in EP and EAP were in a high level, maybe the total triterpenes was a main bioactivity constituents in *B. emeiensis*. Since EP contained more carbonyl than EAP (Fig. 6 B), maybe this is the reason for EP showed a stronger antioxidant ability (Liang et al., 2008). Hence, total triterpenes from *B. emeiensis* exhibited an excellent antioxidant capacity.

3.9. The anticancer effect of different extraction phases on HeLa and Hep G2 cells

Triterpenes has been proved to have a selective toxic effect on cancer cells showing a strong anti-tumor effect (Lei et al., 2014). Based on the results of anticancer assays (Fig. 4), in this work, TBE showed a strong inhibition effect on HeLa and Hep G2 cells. Therefore, the toxic effect of three phases from *B. emeiensis* was evaluated by determined the cell viability and cell apoptosis of HeLa and Hep G2 cells. As shown in Fig. 7A and B, PEP had no positive or negative effect on HeLa and Hep G2 cells. While as prolonging the time of treatment, EP and EAP greatly inhibited the growth of HeLa cells, and the effect of EP was better than EAP and the positive control. Nevertheless, EAP could not suppressed the proliferation of Hep G2 cells. Yet, EP still exhibited a significant inhibitory ability (Fig. 7C and D). To further explore the reason for low cell viability, two dyeing methods were applied to observe cell apoptosis. As shown in Fig. 7E, HeLa cells pretreated with EAP and EP were less than control group and the bright blue was more in cells, meaning EAP and EP induced the cells apoptosis in HeLa cells. Since EAP showed a weak inhibition on Hep G2 cells, the apoptosis cells were less than the group treated with EP (Fig. 7F). And the number of cells had

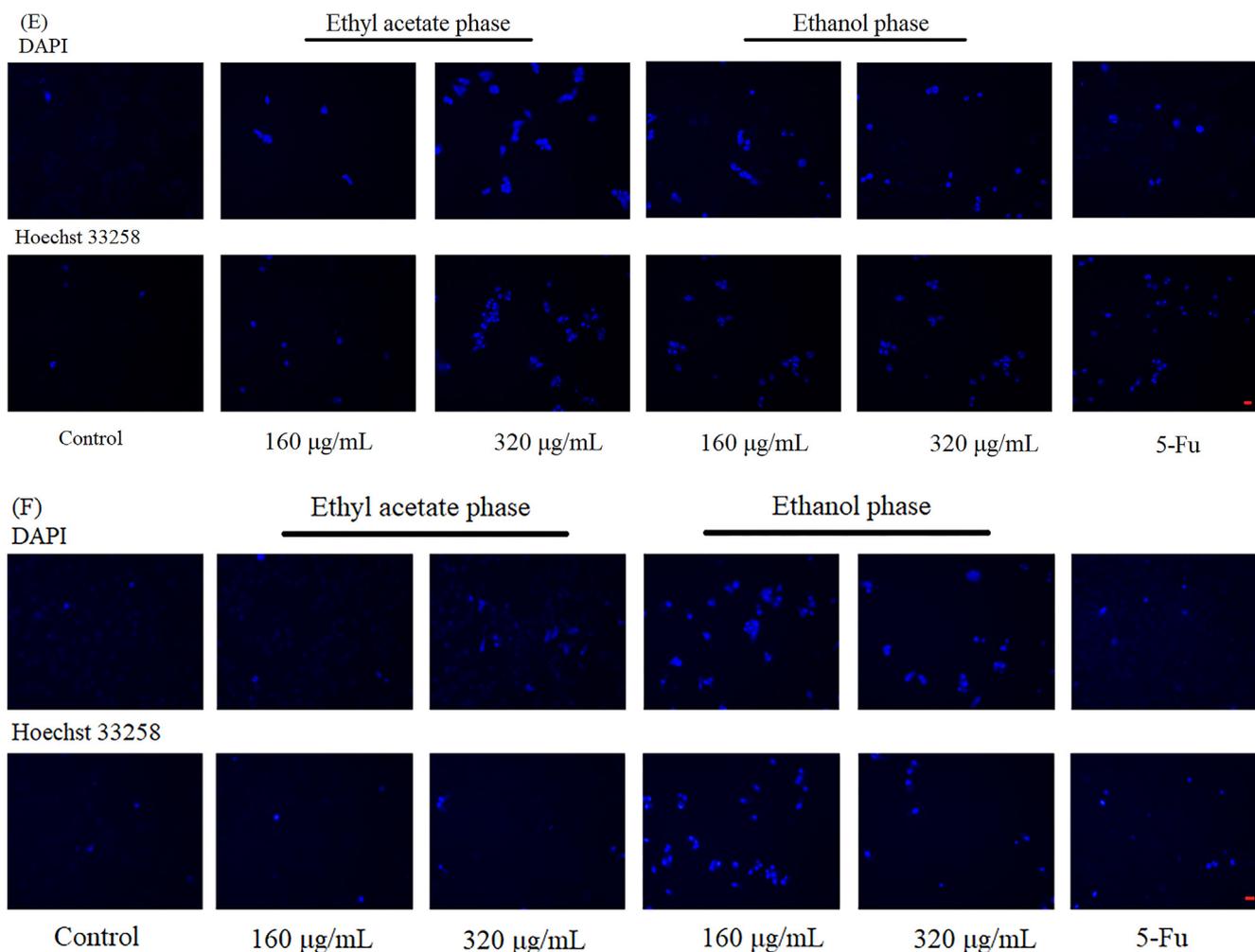


Fig. 7 (continued)

Table 4 The chemical components in EP identified with UPLC.

Chemical compositions	Chemical formula
(-)-Catechin gallate	$C_{22}H_{18}O_{10}$
Bengenin	$C_{14}H_{16}O_9$
Ganoderic acid H	$C_{31}H_{42}O_9$
Oleanic acid	$C_{30}H_{48}O_3$
Quercetagenin-7-O-glucoside	$C_{21}H_{20}O_{13}$
Rutin	$C_{27}H_{30}O_{16}$

Notes: the total ion chromatogram image and MS for each chemical composition was provided as [Supplemental files 1–7](#).

decreased after treated with EP. Other total triterpenes could also restrain the proliferation of cancer cells. Total triterpenes from *Ganoderma lucidum* could induce cells apoptosis in MCF-7 by down-regulating Bcl-2, Bcl-xL and up-regulating the levels of Bax and caspase-9 (Smina et al., 2017). Triterpenoid saponins from the *Anemone flaccida* also induced apoptosis via COX-2/PGE2 pathway on HeLa and Hep G2 cells (Han et al., 2013). Hence, total triterpenes from *B. emeiensis* (EAP

and EP) could significantly depress the growth of HeLa and Hep G2 cells by inducing cell apoptosis.

EP showed a strong anticancer ability. Therefore, UPLC was used to analyze the chemical components in EP. As shown in Table 4, six compositions were identified, among those, (-)-Catechin gallate, Ganoderic acid H and Oleanic acid were identified. (-)-Catechin gallate belonged to the bioactive polyphenols in green tea, and those polyphenols had exhibited an excellent inhibition effect on hepatic epithelial cells (Uesato et al., 2001; Lu and Hwang, 2008). Ganoderic acid was proved to be a promising anticancer agent (Upadhyay et al., 2014). Hence, we could assume that the suppression on the proliferation of cancer cells of EP might be results of the synergetic effect among those notably components. But, more further investigations should be carried out to elucidate the tumor suppression mechanism.

3.10. The ROS level and the mitochondrial membrane potential of HeLa and Hep G2 cells

On the cancer therapy, anticancer agents will induce a high level of ROS in cells then cause cell apoptosis owing to the pro-oxidation effect of anticancer agents, such as camp-

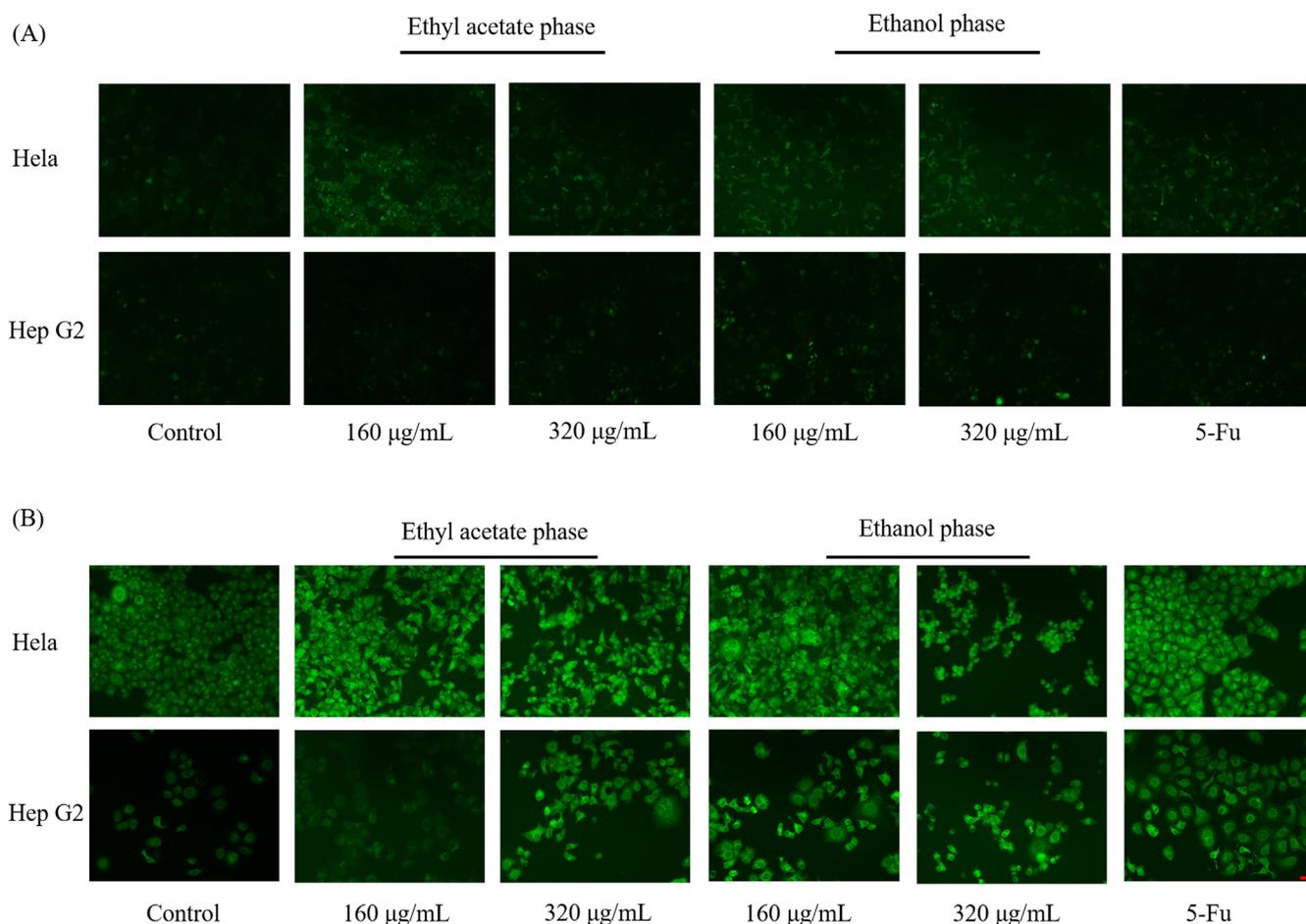


Fig. 8 The effect of two phases on (A and C) the ROS level and (B and D) the mitochondrial membrane potential. The relative fluorescence density was calculated based on the density of blank control group. Notes: * meant $p < 0.05$. ** meant $p < 0.01$. Red bar meant 50 μm .

tothecins and alkylating agents (Ozben, 2007; Conklin, 2004). Extra ROS in cells is proved to bring permanent damage to mitochondrial membrane then induce cell apoptosis (Kroemer et al., 2007). Therefore, to further explore the inhibition effect of total triterpenes from *B. emeiensis*, the ROS level and mitochondrial membrane potential were determined. As shown in Fig. 8A, the ROS level in cancer cells treated with triterpenes from *B. emeiensis* increased compared with the control group. And an increasing concentration of agents induced more ROS in cells (Fig. 8C).

When the cells undergo apoptosis, the integrity of the mitochondrial membrane is destroyed, and the mitochondrial membrane permeability transfer hole opens, causing the breakdown of the mitochondrial transmembrane potential, and Rh123 re-releases from mitochondria, thus emitting strong yellow-green fluorescence. A high fluorescence density hence means a decreasing mitochondrial membrane potential (MMP). The fluorescence images demonstrated the MMP in HeLa and Hep G2 cells treated with triterpenes was changed (Fig. 8B). Since EAP could not suppress the proliferation of Hep G2 cells, the ROS and MMP were not significantly changed (Fig. 8D) while 320 $\mu\text{g/mL}$ of EAP could significantly change the MMP of Hep G2 cells. *Antrodia salmonea*, a traditional Chinese medicine was also proved to increase the ROS

level in human promyelocytic leukemia cells and then induce cell apoptosis (Hseu et al., 2014). Therefore, it could be presumed that the anticancer effect of total triterpenes from *B. emeiensis* was mediated via increasing ROS level and decreasing MMP.

4. Discussions

Extracts from *Bergenia* had been proved to possess an antioxidant ability. 70% Ethanol extracts from *Bergenia crassifolia* was a typical acceptor of peroxy radicals (Shilova et al., 2006). But those researches were rarely reported and only focused on the antioxidant ability on free radicals *in vitro*. More importantly, the components responsible for the antioxidant capacity were not still identified yet. In this work, the antioxidant of total triterpenes from *B. emeiensis* was evaluated *in vitro* and *in vivo*. The high clearance rates on DPPH and superoxide anion radicals showed that TBE could well scavenge free radicals. And in cellular level, TBE reduced the ROS level in CHO cells under oxidative stress. Hence the survival of treated cells was enhanced. Both *in vitro* assays demonstrated TBE could clear free radical and alleviate the oxidative damage, thus exhibiting a strong antioxidant ability *in vitro* in accordance with the previous report (Pozharitskaya et al.,

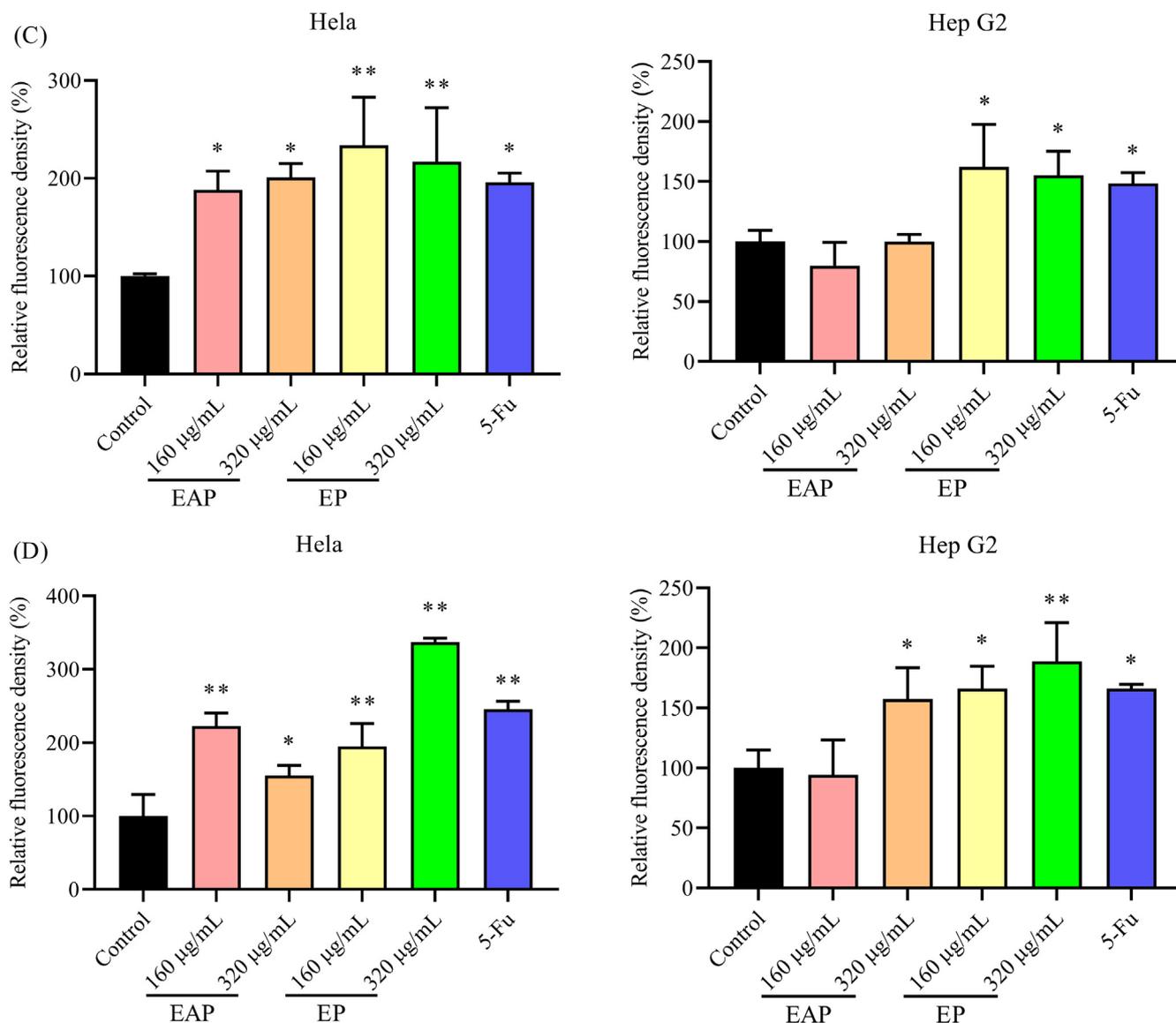


Fig. 8 (continued)

2007). But the environment *in vivo* was complex, hence, *C. elegans* was applied to further investigate the effect of TBE in a whole organism. As shown in Fig. 2, TBE could enhance the resistance of *C. elegans* under thermal stress, reflecting a great antioxidant *in vivo*. In our previous work, the triterpenes extract from *B. emeiensis* also showed a strong antioxidant ability that the suitable concentration of extract on *C. elegans* was 0.1 mg/mL (Luo et al., 2020a). While in this work, 0.05 mg/mL could extend the survival of *C. elegans*. In the previous work, the content of triterpenes was about 40–45%, but the content of triterpenes in TBE was 75–80%. Hence, it was easily concluded that a high content of triterpenes might cause a certain toxicity on a whole organism, therefore, the suitable concentration decreased. As the content of triterpenes increased in this work, the antioxidant ability was stronger than our previous work, indicating the triterpenes in TBE might contribute to the excellent antioxidant capacity.

The antimicrobial ability of *Bergenia* was also reported in previous researches. The ethanol extract from *Bergenia crassifolia* showed a bacteriostatic ability on five bacteria with the

IC₅₀ ranging from 15.63 to 62.50 mg/mL and the rhizome extract exhibited a better capacity than that of Aerial part (Kokoska et al., 2002). Extract from *Bergenia ligulata* also exhibited antimicrobial activity against six bacteria with the zone of inhibition ranging from 2.17 to 4.78 mm (Agnihotri et al., 2014). And in this study, we found TBE from *B. emeiensis* also showed a certain antimicrobial capacity against five bacteria except for *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium*. However, *Bergenia* was found to inhibit *Bacillus cereus* and *Escherichia coli* at the minimum inhibitory concentration of 62.50 mg/mL while in this work the concentration of TBE was lower than 10.00 mg/mL (Rossing et al., 2003). Hence, maybe the concentration of drug was not enough to suppress the two strains. Moreover, *B. ligulata* leaf extracts showed some certain inhibition effect on fungal such as *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium citrinum*, *Trichoderma viride* and *Trametes hirsute* with weak inhibition zones (Agnihotri et al., 2014). In this present work, TBE only could suppress the growth of *Botrytis cinerea* but exhibited no obviously positive or negative effect on *Colletotrichum*

gloeosporioides, *Fusarium oxysporum* and *Colletotrichum acutatum*, showing a weak anti-fungal capacity. This result was in accordance with the previous report (Agnihotri et al., 2014). In this work, the concentration of triterpenes was higher than 75%, hence we used a low dose to conduct the antibacterial and antifungal experiments. But the results showed the antibacterial and antifungal capacity of TBE were weak. Therefore, we assumed that the antibacterial and antifungal effects of plants from *Bergenia* was connected with a higher concentration and other compounds expect triterpenes.

Although *Bergenia* was pronounced to possess the property of antiviral (Dulta et al., 2020), the anticancer capacity was rare reported. *Bergenia* ciliate rhizome extract showed a remarkable selective cytotoxicity against the Human Cervical cancer (HeLa) and Human colon cancer (HT-29) cell lines (Molnar et al., 2006). TBE could well inhibit the growth of HeLa and Hep G2 cancer cells and also showed a certain negative effect on the proliferation of S180 and A549 cells *in vitro*. Natural triterpenes from plants had been proved to exhibit a strong inhibition on cancer cells (Tundis et al., 2014; Fang et al., 2010). Cycloartane triterpenoids deriving from *Cimicifuga yunnanensis* induced the apoptosis of MCF7 cancer cells via p53-dependent mitochondrial pathway (Peng et al., 2020). Other triterpenoids also could inhibit cancer cells such as Prostate ADPC, PC3 and so on by inducing apoptosis (Fang et al., 2010). In our previous work, the triterpenes from the leaves of *B. emeiensis* could inhibit the growth of HeLa cells via mitochondrial signaling pathway then induce cell apoptosis (Luo et al., 2020b). In this work, after fractionally extraction, EP showed a strong suppression on the growth of Hep G2 and HeLa cells via causing pro-oxidation and activating cell apoptosis. Hence, the triterpense from *B. emeiensis* rhizomes might become a promising anticancer agent.

Some antioxidants lead to cell apoptosis during cancer therapy (Tomris, 2007). In this work, TBE showed a strong antioxidant ability both *in vitro* and *in vivo*. additionally, TBE exhibited a strong inhibition effect on the growth of HeLa and Hep G2 cells via causing a high content of ROS. In previous work, antioxidant may enhance the effects of chemotherapy, while preventing free-radical-induced side effects (Lamson and Brignall, 1999). And considerable existing data show increased effectiveness of many chemotherapeutic agents, as well as a decrease in toxic adverse effects, when given concurrently with antioxidants (Drisko et al., 2003; Lamson and Brignall, 2000). As TBE showed a certain antibacterial ability, which might be the reason why the local people in rural area of Asia mashed the fresh of *Bergenia* to prevent wound infection. Therefore, TBE is a promising agent in treating cancer cells for a low toxicity and a low ability of infection.

5. Conclusion

B. emeiensis as a common medicine in the area of southwest of China, was rarely assessed its bioactivity. Hence, in this work, the antioxidant, antibacterial and anticancer abilities of triterpenes from *B. emeiensis* were thoroughly evaluated. The results demonstrated TBE could well scavenge free radicals and showed a strong protection on CHO cells against H₂O₂ damage. In addition, TBE also enhanced the resistance of *C. elegans* under thermal stress. More evidence suggested TBE

possessed a certain antibacterial capacity against selective strains such as *Pseudomonas Aeruginosa*. While TBE could not inhibit the growth of plant pathogenic fungi. Furthermore, TBE showed a strong anticancer effect on HeLa, Hep G2 cells. After fractionally extraction, the triterpenes were highly existed in *B. emeiensis*, and the ethanol phase (EP) contained the most, followed by ethyl acetate phase (EAP) and petroleum ether phase (PEP). EP could well inhibit the growth of HeLa and Hep G2 cells while EAP only suppressed the cell viability of HeLa cells and PEP showed no anticancer effect. In addition, EP and EAP could induce cell apoptosis to restrain the proliferation of HeLa and Hep G2 cells via increasing ROS level and decreasing MMP. Therefore, the total triterpenes from *B. emeiensis* exhibited an excellent antioxidant capacity and anticancer effect, indicating *B. emeiensis* could become a potential candidate for an alternatively natural antioxidant and anticarcinogen. And it was promising to further isolate triterpenes from *B. emeiensis* for a potential anticancer agent.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.arabjc.2021.103225>.

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