Anti-proliferative activity of *Bupleurum scorzonerifolium* in A549 human lung cancer cells in vitro and in vivo

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Abstract

Nan-Chai-Hu, the root of *Bupleurum scorzonerifolium*, is a traditional Chinese herb used in treatment of liver diseases such as hepatitis and cirrhosis. We recently reported that the acetone extract of *B. scorzonerifolium* (BS-AE) could inhibit proliferation and induce apoptosis in A549 human lung cancer cells. We further examined its anti-proliferative mechanisms and in vivo anticancer effect. Our results showed that BS-AE had the ability to cause cell cycle arrest in G2/M phase, inducing tubulin polymerization, and activating caspase-3 and -9 in A549 cells. BS-AE-induced apoptosis could be blocked by the broad caspase inhibitor z-VAD-fmk in majority. The result of in vivo study showed that BS-AE could suppress growth in A549 subcutaneous xenograft tumors. These results indicate that BS-AE exerts antiproliferative effects on A549 cells in vitro and in vivo, and prompted us to further evaluate and elucidate the chemical composition profile of BS-AE.

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1. Introduction

Cancer is still a serious clinical problem and has a significant social and economic impact on the human health care system. Despite modern advancements in diagnosis, prevention and therapy, the disease still
affects millions of patients worldwide, reduces their quality of life and one of the leading causes of death in the world.

Natural products including plants, microorganisms and marines provide rich resources for anticancer drug discovery [1]. Based on ancient and modern Chinese herbal medicine books such as Shen Nung Pen Tsao Ching (200 AD) and Pharmacopoeia of China, there are many other anticancer plants or herbal formulations which should provide a guide, along with clinical evidence, for the identification of new anti-cancer compounds or a source of alternative cancer therapy, and receive increasing scientific attention recently [2–6].

As the different components in a herb may have synergistic activities or buffering toxic effects, mixtures or extracts of herbs might have more therapeutic or preventive activity than alone [5,7]. Several studies have demonstrated that extracts from several herbal medicines or mixtures had an anticancer potential in vitro or in vivo. Hu et al. [3] demonstrated that an alcohol extract of *Ganoderma lucidum* could induce apoptosis in MCF-7 human breast cancer cells, and Lee et al. [8] demonstrated that a water extract of *Paeoniae lactiflora* could induce apoptosis in HepG2 and Hep3B hepatoma cells. Kao et al. [4] reported that a water extract of Bu-Zhong-Yi-Qi-Tang (mixture of 10 herbs) could induce apoptosis in hepatoma cells. Yano et al. [6] demonstrated that the water-soluble ingredients of Sho-Saiko-To (mixture of seven herbs) inhibited the proliferation of KIM-1 human hepatoma cells and KMC-1 cholangiocarcinoma cells. PE-SPES (mixture of eight herbs) had been developed for clinical treatment of prostate cancer [7].

Nan-Chai-Hu, the root of *Bupleurum scorzonerifolium* Willd. (Umbelliferae), is a common and important Chinese herb and frequently prescribed in combination with other herbs to treat common cold, febrile disorders, malaria, and chronic liver disorders in China, Japan, and many other parts of Asia [9]. It is also a major ingredient of several Chinese herbal prescriptions such as Sho-Saiko-To (TJ9 in Japan) and Bu-Zhong-Yi-Qi-Tang that were reported to have anticancer activities [4,6]. Investigation of the possible anticancer activities of *B. scorzonerifolium* is one of the major research programs in our laboratories. Recently, we have reported that the acetone extract of *B. scorzonerifolium* (BS-AE) can inhibit telomerase activity and induce apoptosis in A549 human lung cancer cells [10]. These results prompted us to further evaluate the anticancer activity in vivo and clarify the mechanism of BS-AE.

### 2. Material and methods

#### 2.1. Chemicals and reagents

RPMI medium 1640, Eagle’s minimum essential medium, FBS, penicillin/streptomycin, trypsin/EDTA, and the NuPAGE Bis–Tris Electrophoresis System (pre-cast polyacrylamide mini-gel) were purchased from Life Technologies, Inc. (Grand Island, NY, USA). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromid (MTT), propidium iodine (PI), RNase A, paclitaxel, vinblastine and anti-β-tubulin antibody were purchased from Sigma Chemical Co. (St Louis, MO, USA). Mycoplasma Removal Reagent was from Dainippon Pharmaceutical Co. (Osaka, Japan). Annexin V-FLOUS Staining Kit was from Roche Molecular Biochemicals (Mannheim, Germany). A goat antimouse IgG-FITC antibody was purchased from Santa Cruz biotechnologies (Santa Cruz, CA, USA). Western Lightning Chemiluminescence Reagent Plus was from Perkin–Elmer Life Science (Boston, MA, USA). BSA Protein Assay Kit was from Pierce (IL, USA). Caspase activity assay kits, including the substrates of caspase-1 (YVAD-AFC), caspase-3 (DEVD-AFC), caspase-8 (IETD-AFC), caspase-9 (LEHD-AFC) and the broad-spectrum caspase inhibitor, z-VAD-fmk, were purchased from R&D Systems, Inc. (Minneapolis, MN, USA).

#### 2.2. Cell lines and culture

Five common human solid tumor cell lines, A549 (lung adenocarcinoma), MCF-7 (breast cancer), HT-29 (colon cancer), HepG2 (hepatocellular carcinoma) and OVCAR-3 (ovarian cancer) were obtained from American Type Culture Collection (Rockville, MD, USA). A549, HT-29 and OVCAR-3 cancer cells were maintained in RPMI medium supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin and 100 μg/ml streptomycin. MCF-7 and HepG2 cancer
cells were maintained in Eagle’s minimum essential medium with 10% heat-inactivated FBS, 100 units/ml penicillin and 100 μg/ml streptomycin. All cultured cells were incubated at 37 °C in a humidified atmosphere containing 5% carbon dioxide. Cells were fed with fresh cultured medium every 2–3 times per week and subcultured when 80% confluent. All cultures were free of mycoplasma.

2.3. Preparation of BS-AE

The roots of *B. scorzonerifolium* were supplied from Chung-Yuan Co., Taipei, and were identified by Prof. Lin. A voucher herbarium specimen was deposited at the School of Pharmacy, National Defense Medical Center (NDMC No. 900801). The crude extract BS-AE was prepared as described previously [10]. Briefly, the dried and powdered roots of *B. scorzonerifolium* (2.0 kg) were extracted with acetone (5 l, 3 times) at room temperature for 4 h. The extracts were concentrated and dried under vacuum to yield an acetone extract (BS-AE). The extract was stored at 4 °C before each experiment.

2.4. Cytotoxicity and cell viability analysis

Viability of control and treated cells were evaluated using the MTT assay in triplicate [10]. Briefly, cancer cells (5 × 10^3) were incubated in 96-well plates containing 200 μl of the growth medium. Cells were permitted to adhere for 16–18 h, washed with PBS, and then treated with agents dissolved in medium. After treatment, the drug-containing medium was replaced by fresh medium. Then cells in each well were incubated at 37 °C in 50 μl of MTT (5 mg/ml) for 4 h. After the medium and MTT were removed, 200 μl of DMSO and 25 μl of glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) were added to each well. Absorbance at 570 nm of the mixture was detected using a microplate ELISA reader (MRX II, DYNEX Technologies, Chantilly, VA, USA). The absorbance of untreated cells was considered as 100%. The results were determined by three independent experiments.

2.5. Cell cycle analysis

Analysis of the cell cycle of control and treated cancer cells was determined. Using standard methods [11], the DNA of cells were stained with PI, and the proportion of non-apoptotic cells in different phases of the cell cycle was recorded. Briefly, 5 × 10^5 cancer cells were treated with BS-AE, and washed twice with PBS. The cells were fixed overnight with cold 70% ethanol, and then stained with PI solution consisting of 45 μg/ml PI, 10 μg/ml RNase A, and 0.1% Triton X-100. After a 1-h incubation at room temperature in the dark, fluorescence-activated cells were sorted in a FACScan flow cytometer (equipped with a 488-nm argon laser) using CellQuest 3.0.1 software (Becton Dickinson, Franklin Lakes, NJ), and the data were analyzed using ModFitLT V2.0 software. In the non-apoptotic population (as 100% after excluded sub-G1 population), the percentage of cells in each phase of the cell cycle was determined as least triplicate and expressed as mean ± SD.

2.6. Detection of apoptosis

A modified method used for detection of PS (phosphatidylserine) in adherent cell lines was described by van Engeland et al. [12]. Externalization of PS and membrane integrity was quantified using an Annexin V-FLOUS Staining Kit. In brief, 10^6 cells were grown in 35 mm diameter plates before treatment. Treated and control cancer cells were labeled with Annexin V-FLOUS (10 μg/ml) and PI (20 μg/ml) prior to harvesting. After labeling, all plates were washed with binding buffer and harvested by scraping. Cells were resuspended in binding buffer at a concentration of 2 × 10^5 cells/ml before analysis by flow cytometry (FACScan). The data were analyzed using WinMDI V2.8 software. The percentage of cells undergoing apoptosis was determined by three independent experiments.

2.7. Caspase activity assay

Activity of caspase-1, -3, -8 or -9 was detected by using a fluorometric assay kits according to the manufacturer’s protocol. In brief, 2 × 10^6 control or treated cells were lysed in 50 μl of cold lysis buffer and incubated in ice for 10 min. Fifty microliters cell lysates added to 50 μl of reaction buffer and 5 μl of fluorescent report substrates specific for caspase-1, -3, -8, or -9 in a 96-well microplate. After incubation at 37 °C for 1 h, the fluorescence from the cleaved
AFC side chain was detected by a fluorescence microplate reader (Fluoroskan Ascent; Labsystems) with excitation at 400 nm and emission at 505 nm.

2.8. Immunofluorescence and confocal microscopy

The immunostaining method was described by Yoon et al. [13]. Briefly, cells were cultured on glass slides in six-well plates. Control and treated cells growing on the slides were fixed with cold 3.7% formaldehyde in PBS for 30 min. The fixed cells were washed twice in PBS, and then incubated in cold permeabilization solution (0.1% Triton X-100 + 0.1% sodium citrate) for 10 min. The cells were washed with PBS three times and incubated with anti-β-tubulin antibody diluted with PBS (1:200) at 37 °C for 1 h. After washing with PBS, the cells were blocked with 5% serum. The cells were washed with PBS twice and then treated with antimouse IgG linked to FITC for 30 min at 37 °C. After then, the cells were washed with PBS three times, and the images of tubulin conjugated with FITC staining were taken with an LSM-510 confocal laser scanning microscope system (Carl Zeiss, Oberkochen, Germany).

2.9. Tubulin polymerization by immunoblot assay

A simple assay for quantitation of tubulin polymerization as described by Giannakakou et al. was used [14]. Briefly, 2 × 10⁶ cells were cultured in six-well plates and treated in the following day. After treatment, cells were washed twice with PBS and lysed with 100 µl of hypotonic buffer (1 mM MgCl₂, 2 mM EGTA, 1% NP40, 2 mM phenylmethysulfonyl fluoride, 1 µg/ml aprotinin, 2 µg/ml pepstatin, and 20 mM Tris–HCl, pH 6.8) at 37 °C for 5 min. The cytosolic and cytoskeletal fractions were separated by high-speed centrifugation for 10 min. The supernatant contained the soluble cytosolic form and was collected. The pellet contained polymerized form and was dissolved in 100 µl of hypotonic buffer. After addition of 20 µl sample buffer (45% glycerol, 20% β-mercaptoethanol, 9.2% SDS, 0.04% bromphenol blue, and 0.3 M Tris–HCL, pH 6.8) to each 100 µl sample, the samples were vortexed and boiled for 5 min. Electrophoresis was performed using a NuPAGE Bis–Tris Electrophoresis System (4–12% NuPAGE Bis–Tris gels) using 20 µl of each sample. Immunoblotting was performed using monoclonal anti-β-tubulin antibody conjugated to antimouse IgG-HRP antibody, and detected by the Western Lightning™ Chemiluminescence Reagent Plus. The expressions of β-tubulin were quantified using a densitometer. The experiment was repeated three times with similar results.

2.10. Antitumor activity in vivo

Female congenital athymic BALB/c nude (nu/nu) mice were purchased from Charles River Laboratories (Boston, MA, USA). They were maintained under specific pathogen-free conditions and provided with sterile food and water. The Committee on Animal Research approved all procedures. All experiments were carried out using 6–8-week-old mice weighting 18–22 g. Murine tumor models were used as previously described [15,16]. In vitro cultured A549 cancer cells (1 × 10⁷) were injected s.c. into the back of mice. When the tumor reached 80–120 mm³ in volume, animals were divided randomly into test groups consisting of 6–8 mice per group (day 0). Daily i.p. administration of BS-AE, dissolved by 10% Tween 80 in normal saline (v/v), from day 0 to day 4 was performed. The mice were weighed three times a week up to days 21–28 to monitor toxic effects. Tumor response was determined by measurement of the length (L) and width (W) of the tumor mass three times a week up to days 21–28. The tumor volume at day n (TVₙ) was calculated as TV (mm³) = (L × W²)/2. The relative tumor volume at day n (RTVₙ) was expressed according to the following formula: RTVₙ = TVₙ/TV₀. Tumor regression (T/C%) in treated versus control mice was expressed as follows: T/C% = (mean RTV of treated group)/(mean RTV of control group) × 100. Xenograft tumors of treated and control mice were harvested and fixed in 4% formalin, embedded in paraffin, and cut in 4-µm sections for histologic study.

2.11. Statistical analysis

The data are shown as mean ± SD. Statistical significance of differences were analyzed using the Student’s t-test for normally distributed values and by the non-parametric Mann–Whitney U-test for
valuables that were not normally distributed. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Antiproliferative effect of BS-AE on human solid tumor cells

The antiproliferative effect of BS-AE was on various human cancer cell lines was determined. Cells were treated at different concentrations of BS-AE (0–200 µg/ml) for 24 h. Cells incubated with 0.2% DMSO was used as a control. The $IC_{50}$ values of BS-AE tested in these cancer cells were ranged from 50 to 80 µg/ml after 24-h treatment (Table 1). HepG2 cells showed the highest susceptibility ($IC_{50} = 46–58$ µg/ml), and MCF-7 cells displayed the lowest susceptibility to BS-AE ($IC_{50} = 79–82$ µg/ml). After exposure of BS-AE (60 µg/ml) on these cancer cell lines for 12, 24 or 48 h, the cell viability was significantly suppressed after 24-h treatment (Fig. 1). The results indicated that BS-AE-induced growth inhibition presented with a time-dependent manner on these cancer cells.

3.2. Cell cycle analysis

The cell cycle were analyzed in A549 cells treated with BS-AE (60 µg/ml) for 24 or 48 h. The percentage of cells in each phase of cell cycle in control (exposed to 0.2% DMSO) and treated cells were determined by flow cytometry and the results were showed in Fig. 2. The sub-G1 population indicated apoptotic-associated chromatin degradation [17]. As compared to

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Origin</th>
<th>$IC_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>Lung adenocarcinima</td>
<td>60 ± 5</td>
</tr>
<tr>
<td>MCF7</td>
<td>Breast carcinoma</td>
<td>74 ± 6</td>
</tr>
<tr>
<td>HT-29</td>
<td>Colon carcinoma</td>
<td>55 ± 6</td>
</tr>
<tr>
<td>HepG2</td>
<td>Hepatocellular carcinoma</td>
<td>52 ± 5</td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>Ovarian carcinoma</td>
<td>58 ± 5</td>
</tr>
</tbody>
</table>

The values of $IC_{50}$ are result after 24-h treatment (mean ± SD from three independent experiments).

Fig. 1. Anti-proliferative activity of BS-AE on A549, MCF-7, HT-29, HepG2 or OVCAR-3 cells detected by MTT assay after 12, 24 or 48 h treatment. The control is cells treated with solvent (0.2% DMSO). Values are Mean ± SD (means of three experiments). BS-AE shows a time-dependent antiproliferative activity in these cancer cells. *$P < 0.05$ versus the control.
control, the sub-G1 group significantly increased after cells cultured with BS-AE (24 h: 12.6 ± 3.2%; 48 h: 21.8 ± 4%). In non-apoptotic population, we observed that A549 cells accumulated in G2/M phase after administration of BS-AE for 24 and 48 h (42.9 ± 3 and 75.2 ± 4%, respectively). On the other hand, BS-AE caused S phase attenuation after 48 h treatment (control: 31.3 ± 3%; 24 h: 24.5 ± 3%; 48 h: 11.5 ± 2%). These results suggested that BS-AE can induce cell cycle arrest in G2/M phase and apoptosis in A549 cells with a time-dependent manner.

3.3. Apoptosis assay

In our previous study, BS-AE induced chromatin change in A549 cells [10]. Shrinkage of cells and disorganization of chromatin might be related to apoptosis [18]. Simultaneous staining with Annexin V-FLOUS and PI distinguished between intact cells, early apoptosis, late apoptosis or cell death [19]. In A549 control culture (exposed to 0.2% DMSO for 48 h), 92 ± 3% of the cells were viable, 5 ± 2% were in early apoptosis and 3 ± 1% were in late apoptosis or cell death (Fig. 3A). In A549 cells treated with BS-AE (60 μg/ml) for 48 h, 29 ± 3% were in early apoptosis, and 5 ± 2% were in late apoptosis or cell death, respectively (Fig. 3B). Moreover, after A549 cells exposed to different dosages of BS-AE (10, 50, 100 or 200 μg/ml) for 48 h, the apoptotic cells were significantly increased with a dose-dependent (data not shown). These results suggested that BS-AE can induce apoptosis in A549 cells.

3.4. Caspase activity assay

For evaluation the molecular effector pathway of apoptosis induced by BS-AE, activity of caspase-1, -3, -8, and -9 were measured using specific fluorogenic peptide substrates after addition of 60 μg/ml BS-AE to A549 cells for 6, 12, 24 or 48 h. As shown in Fig. 4A, activation of caspase-3 and caspase-9 were significant in BS-AE-treated cells since 12 h of treatment and continued to rise until 48 h. But BS-AE did not activate caspase-1 or -8. For further definition, the role of caspases in BS-AE-induced apoptosis, A549 cells were pretreated with the broad-spectrum caspase inhibitor, z-VAD-fmk. A549 cells were treated with 100 μM of z-VAD-fmk 2 h prior BS-AE treatment. The apoptosis was detected 24 or 48 h after treatment. As shown in Fig. 4B, z-VAD-fmk attenuated BS-AE-induced apoptosis at both 24 and 48 h. But z-VAD-fmk did not inhibit BS-AE-induced apoptosis completely. Otherwise, these results indicated that activation of caspases involved BS-AE-mediated apoptosis.
3.5. In situ fluorescent staining of β-tubulin

For β-tubulin immunofluorescent staining, A549 cells were treated with BS-AE (60 μg/ml) for 24 h. Using confocal microscopy, compared to control cells (Fig. 5A), we found that BS-AE can influence microtubule dynamic stability by increasing tubulin polymers in A549 cells (Fig. 5B).

3.6. Tubulin polymerization

The distribution of tubulin in soluble or polymerized forms was analyzed by immunoblotting. After separation, the soluble tubulin (S) was contained in the cytosolic fraction, and the polymerized tubulin (P) was in the cytoskeletal fraction.

Tubulin in the cytosolic and cytoskeletal fractions from A549 cells exposed to BS-AE (60 μg/ml), paclitaxel (100 nM) or vinblastine (50 nM) were isolated and analyzed by immunoblotting for β-tubulin (Fig. 5C). These results showed that BS-AE caused significant shifts of soluble tubulin to the polymerized form, thus stabilizing microtubules in A549 cells. This shift contributes to G2/M arrest caused by BS-AE.

3.7. Anticancer activity in vivo

To evaluate the antitumor activity of BS-AE in vivo, we created human lung cancer xenografts by s.c.
injection of A549 cells into nude mice. After daily i.p.
administration of 100, 300 or 500 mg/kg of BS-AE on
day 0 to day 4, a significant and dose-dependent
suppression of growth was found on BS-AE-treated
A549 xenografts ($P < 0.001$), but not in that of control
group (administration of 100 μl 10% Tween 80)
(Fig. 6A). Furthermore, BS-AE could also induce
regression of A549 tumors at dosages 300 and
500 mg/kg. Regarding to general toxicity, body
weight of the tumor-bearing mice had significantly
decreased at dosage of BS-AE 500 mg/kg before day
6, and recovered progressively after then. Otherwise,
there was not significant change in body weight at
dosages less than 300 mg/kg (Fig. 6B). Compared to
the control tumor-bearing mice, the histology showed
that a massive necrosis and infiltration of inflamma-
tory cells in the BS-AE-treated A549 xenograft tumor
was found (300 mg/kg, D9; Fig. 6C and D). These
results demonstrated that BS-AE had antitumor
activity in nude mice bearing A549 lung cancer
xenografts.

4. Discussion

In the present study, we found that BS-AE had
antiproliferative effect to human cancer cells. BS-AE
can cause cell cycle arrest in G2/M phase, induce
apoptosis, shift tubulin to a polymerized form and
activate caspases in A549 cells. Furthermore, BS-AE
can inhibit tumor growth and induce tumor regression
in an A549 xenograft model, and a dose-dependent
manner was also found. In vivo toxicity was also
roughly examined by body weight change and
histologic study of vital organs (data not shown),
and there were no significant toxic effects below a
dose of 500 mg/kg of BS-AE.

Apoptosis is an important homeostatic mechanism
that balances cell division and cell death and
maintaining the appropriate cell number in the body.
Disturbances of apoptosis in cancer cells have been
studied in detail, and induction of apoptosis in cancer
cells was one of the strategies for anticancer drug
development [20,21]. There are several crucial
cellular and molecular biological features involving apoptosis, including cell shrinkage, disorganization of chromatin, externalization of PS, and activation of caspase [22,24]. Activation of the family of caspases was known as a crucial mechanism for induction of death signals to apoptosis. Caspases are a family of cysteine proteases in mammalian cells that cleave following an aspartic acid residue [23]. Caspases can be divided into initiator and effector caspases. Initiator caspases (e.g. caspase-1, -2, -8, -9 and -10) containing a long prodomain involve in early stages of the proteolytic cascade, whatever effector caspases (e.g. caspase-3, -6, and -7) containing a short prodomain act downstream in cleavage of specific intracellular substrates (e.g. poly-ADP-ribose polymerase, focal adhesion kinase) resulting programmed cell death [23–25]. PS externalization is an early feature if apoptosis and can be detected by the binding of annexin V to PS on the cell surface [12]. In our results, BS-AE can cause externalization of PS and activate caspase-3 and -9. Elevation of caspase-3 and -9 by BS-AE was initiated since 6 h of BS-AE exposure and maintained to 48 h. Otherwise, our results also showed that BS-AE-induced apoptosis on A549 cells could be inhibited after broad caspase inhibitor z-VAD-fmk pretreated. It indicated that BS-AE-induced apoptosis is mediated through both caspase-dependent pathways.

Disturbance of the cancer cell cycle is one of therapeutic targets for development of new anticancer drugs [26]. The result of cell cycle analysis evaluated by flow cytometry analysis showed that BS-AE can induce G2/M arrest on A549 cells. Targeting regulatory molecules involving G2/M transition (e.g. cdc2, cyclin A or B), or interfering with cytoskeletal function could be contributed this effect [26,27]. Our results also demonstrated that BS-AE can cause polymerization of tubulin and, thus, interferes with mitotic spindle formation. This plays a major role in the molecular basis of BS-AE-induced cell cycle arrest in G2/M. These effects resemble those of the microtubule-stabilizing cancer chemotherapy agents, such as Taxans and epothilone B, but the detailed molecular mechanisms need for further evaluated.
Using interference with tubulin’s biological functions causing mitotic arrest and apoptosis in cancer cells was one of the strategies for anticancer drug discovery [27]. Our results showed that BS-AE can cause tubulin polymerization which interrupts cell mitosis and commits the cell to apoptosis, and might play a critical role in BS-AE-induced cell cycle arrest as well as apoptosis.

Saikosaponins were reported as the major components of *B. scorzonerifolium* [28]. Most saikosaponins isolated from Bupleuri Radix have been reported to have pharmacological activities involving anti-inflammatory, antiviral or antihepatotoxic activity [29,30]. The antiproliferative effect in cancer cells of saikosaponins was not valid. The components in BS-AE belonged to non-polar characteristics, but saikosaponins were more polar compounds [31]. The molecular mechanisms responsible for the anticancer effects of BS-AE may encourage us for further evaluation and application of BS-AE and elucidate its chemical composition profile to identify its active anticancer components.

In summary, our study showed that BS-AE could proliferation of A549 cells via causing cell cycle arrest in G2/M phase, inducing apoptosis, and increasing microtubule stabilization. BS-AE also exhibited antitumor activity in vivo. *B. scorzonerifolium* was widely used clinically in other diseases rather than antitumor application. The in vitro and in vivo antitumor effects were reported here, and it is valuable for further investigation including elucidation of active components.

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