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Full Length Research Paper

Anti-tumor activity of triterpenoid-rich extract from bamboo shavings (*Caulis bamfusae* in Taeniam)

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Bamboo shavings are a kind of Chinese traditional medicine, which have been certificated as a material of functional food by the Ministry of Health in China. The anti-tumor activities of a triterpenoid-rich extract of bamboo shavings (EBS) and its main component, friedelin were evaluated in the present study. It was proved that EBS could inhibit the growth of P388 and A549 cancer cell lines effectively by SRB and MTT assay. Meanwhile, EBS had notable inhibitory effect on the sarcoma-loaded mice S180 model, which led to a depressed trend of tumor weight, compared to cyclophosphamide. Furthermore, the anti-tumor activity of friedelin monomer, a main triterpenoid separated from EBS, was tested by MTT assay and results showed that friedelin displayed rather strong anti-tumor activities on the proliferation of four cancer lines, A375, L929, Hela and THP-1, with a time-dose relationship compared to de-methyl-cantharidin, respectively. Therefore, it is suggested that EBS has a great potential to be applied in functional food for its anti-tumor activity, in which friedelin was one of the most important active factors.

Key words: Extract of bamboo shavings, anti-tumor, friedelin, triterpenoid (*Caulis bamfusae* in Taeniam).

INTRODUCTION

Bamboo shavings (*Caulis Bamfusae* in Taeniam), which are the intermediate layer of the stems of *Bambusa tuldoides* Munro, *Sinocalamus beecheyanus* var. *pubescens* P. F. Li or *Phyllostachys nigra* (Lodd.) Munro var. *henonis* (Mitf.) Stapf ex Rendle, are a perennial plant of *Gramineae* family in plant kingdom. Bamboo shavings can be obtained by scraping off the coat from bamboo stems, cutting the stems into slices and binding them together by drying in shadowy places. They have been used as a clinical Chinese traditional medicine mainly to lessen or cure stomach ache, diarrhea and vomiting, chest diaphragm inflammation, restlessness and excessive thirst and its efficacy had been recorded in the material media of past dynasties in Chinese history.

It is well known that there are abundant biological active compounds in bamboo shavings such as triterpenoids, saponins and sterols. Triterpenoids and their saponins, which are important biological active components in natural kingdom, can be divided into triterpenoid saponins and steroid saponins according to their different chemical structures (Yao, 2001). The triterpenoid-rich extract from Chinese bamboo shavings (EBS), which mainly contains a group of pentacyclic triterpenoids such as friedelin, friedelinol, lupenone and lupenol, etc (Figure 1), is a low polar component extracted from bamboo shavings using the CO_2 supercritical fluid extraction (SFE) technique.

Many researchers have reported that triterpenoids have many physiological functions such as anti-sepsis, anti-virus, anti-tumor, anti-fatigue, anti- hyperlipidemia, anti-hypertension, anti- hyperglycemia, etc (Yao, 2001). Research data about triterpenoids published in many countries refer to their prominent physiological functions especially on anti-tumor.

The reports on the biological activities of triterpenoids

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Abbreviations: EBS, Extract of bamboo shavings; **TBP,** triterpenes of betula platyphylla; **SRB,** Sulforhodamine B; **MTT,** microculture tetrazolium; **SFE,** supercritical fluid extraction; **IC_{50},** half-maximal inhibition; **FCS,** fetal calf serum; **HCPT,** hydroxycamptothecine; **DMSO,** dimethyl sulfoxide; **GC-MS,** gas chromatography-mass spectrometry; **EBV-EA,** Epstein-Barr virus early antigen; **TBP,** triterpenes of betula platyphylla.

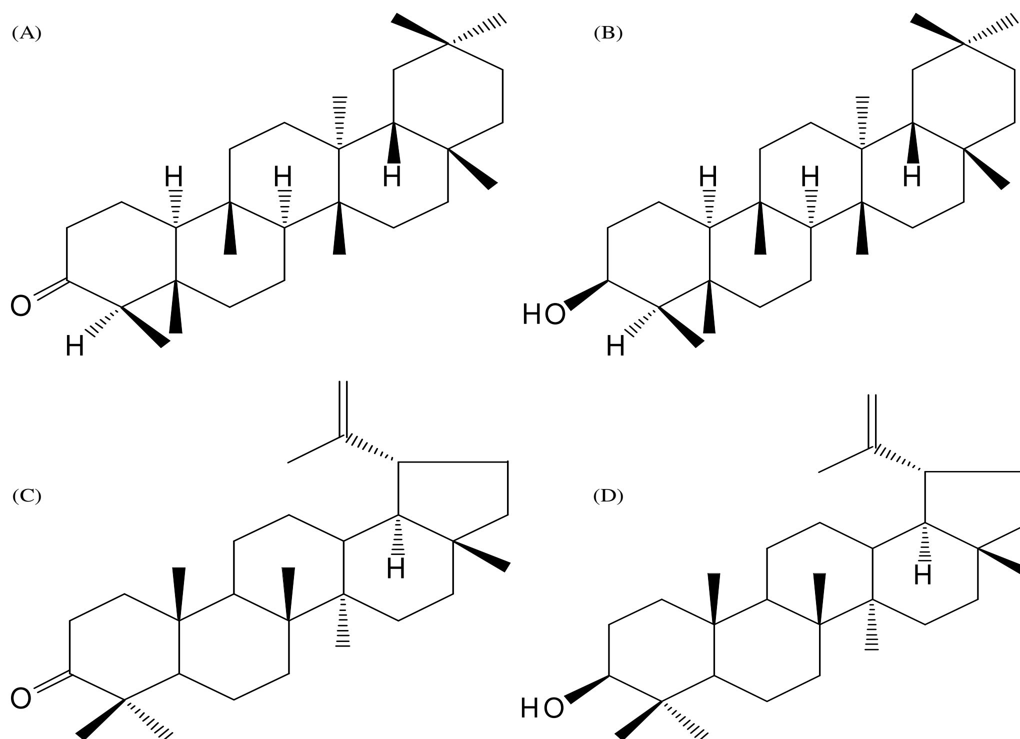

Figure 1. Chemical structure of mainly triterpenoids in EBS. (A) Friedelin, (B) Friedelinol, (C) Lupenone AND (D) Lupenol.

in bamboo shavings and physical data on the chemical composition are limited. In the preliminary tests, it was shown that EBS and friedelin have great potential for its anti-fatigue activity (Zhang et al., 2006), anti-hyperlipidemic activity and anti-hypertensive activity (Jiao et al., 2007). On the other hand, many researchers have reported the anti-tumor activity of various triterpenoids and their derivatives (Fukuda et al., 2005, 2006). However, there was no systematic report to investigate anti-tumor studies on the triterpenoid-rich extract from Chinese bamboo shavings, especially its main component, friedelin.

In the present study, the filtration tests of EBS and its mainly active monomer, friedelin, *in vitro* with the research objects of mouse leukemia (P388) cells, human lung adenocarcinoma (A549) cells, human malanoma (A375) cells, mouse lung epithelial tumor (L929) cells, human cervical tumor (Hela) cells, human macrophage tumor (THP-1) cells and sarcoma-loaded mice S180 model were systematically discussed so that the anti-tumor activities of EBS and friedelin were primarily evaluated.

MATERIALS AND METHODS

Plant material

Bamboo shavings were collected from the intermediate layer of the stems of *Phyllostachys nigra* var. *henonis* in Anji District (Huzhou, Zhejiang, China). This sample was authenticated by the Research Institute of the Subtropical Forestry of Chinese Academy of Forestry (Hangzhou, China) (Zhang et al., 2004). A voucher specimen EBS was deposited at the Herbarium of the Laboratory of Research and Development of Natural Products in Department of Food Science and Nutrition (Zhejiang University, Hangzhou, Zhejiang, China).

Preparation of EBS and identification of triterpenoids

Triterpenoid-rich extract was prepared from bamboo shavings (*P. nigra* var. *henonis*) identified by Research Institute of Subtropical Forestry of the Chinese Academy of Forestry (Hangzhou, China). Briefly, fresh bamboo shavings were adequately washed with water and dried in the air. The coarse powder of bamboo shavings was obtained after comminution and filtration (10 ~ 20 mesh). 5.5 kg powder was circularly extracted using the carbon dioxide SFE technique. Finally, the extract of bamboo shavings (EBS) was collected. Results showed that EBS was a kind of yellow or yellowish green powder with the melting point range from 74 to 79℃ and its carbon dioxide SFE yield was about 2%. The main triterpenoids in EBS were identified by GC-MS (Hewlett- Packard 6890 GC and MS Engine 5973 mass spectrometer, Agilent Inc., USA) and analyzed by GC (Agilent 6890N and FID detector, Agilent Inc., USA) (Yao et al., 2004). The chromatographic separation was performed with a HP5-MS column (30 m × 0.25 mm i.d.) coated with a phenyl-methyl polysiloxane stationary phase (film thickness: 0.2 µm). Helium was used as a carrier gas at a flow rate of 1 mL/min. 1 µL EBS sample dissolved in dichloromethane was injected using a splitless injection mode with the injection port temperature at 280℃. The column temperature was held at 100℃ for 2 min and then programmed from 100 to 270℃ at a rate of 20℃ /min, holding for 20 min. The mass selective detector was operated in the electron impact ionization mode with ionization energy of 70

eV. The ion source temperature was 230℃. Full scan mode was used with the MS scan range from 18 to 500 m/z. Data were analysed by Database NIST 98.

Anti-tumor active filtration of EBS *in vitro*

Filtration methods

Sulforhodamine B (SRB) protein staining method were used to evaluated the growth inhibitory effect of the adherent tumor cells (P388 mouse leukemia cells) by EBS and microculture tetrozolium (MTT) reducing method were used to evaluated the growth inhibitory effect of the suspended tumor cells (A549 human lung adenocarcinoma cells) by EBS, respectively. The effect time was 48 and 72 h compared to the control, Vincristin, respectively.

SRB assay

SRB assay of inhibitory effect by EBS was carried out according to the programs by Xiao et al. (2001). Briefly, adherent cells in 0.1 mL media were plated in each well of 96-well plates and allowed to attach for 24 h. EBS was added to the wells to produce the desired final concentrations and the plates were cultured at 37°C for 72 h. Cells were then fixed by gentle addition of 100 µL of cold (4°C) 10% trichloroacetic acid to each well. Plates were washed with deionised water five times and allowed to air dry. Cells were then stained by addition of 100 µL SRB solution [0.4% SRB (w/v) in 1% acetic acid (v/v)] to wells for 15 min. Following staining, plates were quickly washed five times with 1% acetic acid to remove any unbound dye and allowed to air dry. Bound dye was solubilized with 10 mmol/L Tris base (pH 10.5) prior to reading the plates. The optical density (OD) was read on a plate reader at a wavelength of 515 nm with Molecular Devices (model # VERSAmax). The inhibitory effect of HCPT on cultured cells was expressed as IC_{50}. Finally, the mean IC_{50} was calculated according to the data from three replicate tests.

MTT assay

Stock solutions of complexes were freshly prepared in 10% DMSO and diluted to the required concentration with culture when used. Tumor cells were grown in RPMI 1640 medium supplemented with 10% freshly inactivated fetal calf serum (FCS) and antibiotics. The cells harvested from exponential phase (2×10^5 per mL) were seeded equivalently into a 96-well plate. Then the compounds studied were added in a concentration gradient and the final concentrations were maintained at 1000, 100, 10, 1 and 0.1 µM, respectively. The plates were kept at 37°C in a humidified atmosphere of 5% CO_2 and cultured for 48 h; then MTT solution of an appropriate concentration (1 mg/mL) was added to each well and the plates were cultured at 37°C for 4 h. The measurements of absorbance of the solutions related to the number of live cells were performed on an ELISA spectrophotometer at 570 nm (Zhou et al., 2001).

Effect on tumor- bearing mice S180 model by EBS

S180 sarcoma-loaded mice were killed by cervical dislocation before the experiment and were saturated 8 - 10 min in the benzalkonium bromide solution. Then, several tumor blocks were isolated in asepsis operational condition and were made to be a homogenate mixing with the injection physiological saline according to the proportion of 1: 3 (3 mL physiological brine for each 1 g tumor block). Each experimental mouse was injected with 0.2 mL homogenate solution in axilla endermic tissue after partially disinfecting by ethanol iodine. They were divided into several groups at random 24 h later and were accepted oral administration continuously 10 days (once for each day) as follows: (i) control, water supply, 20 mL/kg (0.4 mL/20g), (ii) positive control, cyclophosphamide supply, 30 mg/kg, ip, CTX, (iii) EBS high dose, 1.6 g/kg, (iv) EBS intermediate dose, 0.8 g/kg and (v) EBS low dose, 0.4 g/kg. Mice were killed next day when stopping oral administration. The body was weighed and the corresponding tumor block was isolated and weighed for each mouse. Finally, the tumor inhibition rate was calculated.

Anti-tumor test of friedelin extracted from EBS

The Silica Gel Column Chromatography and Countercurrent Chromatography preparation techniques were used to obtain friedelin monomer isolated from EBS for the sake of evaluating its anti-tumor activity further. The purity of this monomer determined by GC analysis was 90.5%. MTT method was used to demonstrate the anti-tumor activity of friedelin monomer *in vitro*.

A375, L929, Hela and THP-1 were used for active filtration test in this experiment. Among these cell lines, A375, L929 and Hela were adherent cells while THP-1 was a kind of suspended cell. All cells were maintained in the RPMI-1640 culture medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin and 0.2% $NaHCO_3$. These supplements were dissolved in deionized water and 0.2 µm filtration membrane was used for filter sterilization. The fetal calf serum was inactivated 30 min at 56°C prior to an experiment. Cells were cultured in a humidified incubator of 5% CO_2 at 37°C.

A375, L929 and Hela cells in logarithmic growth phases were seeded at a cell density of approximately 5×10^4 cells/mL in 96-well plates (100 µL/well). In addition, THP-1 cell was seeded at a cell density of approximately 1×10^5 per mL because it was smaller than others. Samples were added in suspended cells after culturing 4 h and in adherent cells after culturing 12 h. Friedelin was dissolved in DMSO in advance and then in the culture medium with the ultrasonic treatment, where the final concentration of the solvent in the culture medium was < 0.1% (v/v). Sample was added for 7.5, 15, 30, 60, 120, 240, 480 and 960 µmol/L. Each level had four parallel pores and a negative control. The cells were cultured for 24 and 48 h and then 15 µL/well MTT solvent (5 mg/mL) was added.

After culturing for 48 h, the cells were centrifugated at 1500 rpm for 5 min. The supernatant was removed and 150 µL/well DMSO was added, following by micro-vibrator shocking for 10 min and crystal dissolving completely. The absorbance (OD value) was measured by ELISA spectrophotometer at 492 nm. Consequently, the inhibition rate of cell proliferation and the IC_{50} value of friedelin were counted by Bliss method. The de-methyl-cantharidin was took as a positive control and the inhibition rate of A375, L929 and Hela cells were measured by adding de-methyl-cantharidin (120 µmol/L) for 24 and 48 h. Finally, the corresponding data of friedelin were counted.

RESULTS

Identification of triterpenoids in EBS

Some pentacyclic triterpenoids, such as friedelin, friedelinol, lupenone and lupenol, were identified by GC-MS and the chemical structures of these triterpenoids are shown in Figure 1. Among these compounds, friedelin could be regarded as a representative triterpenoid in EBS because friedelin was determined as a dominant compound in the active fraction. Figure 2 shows the GC-

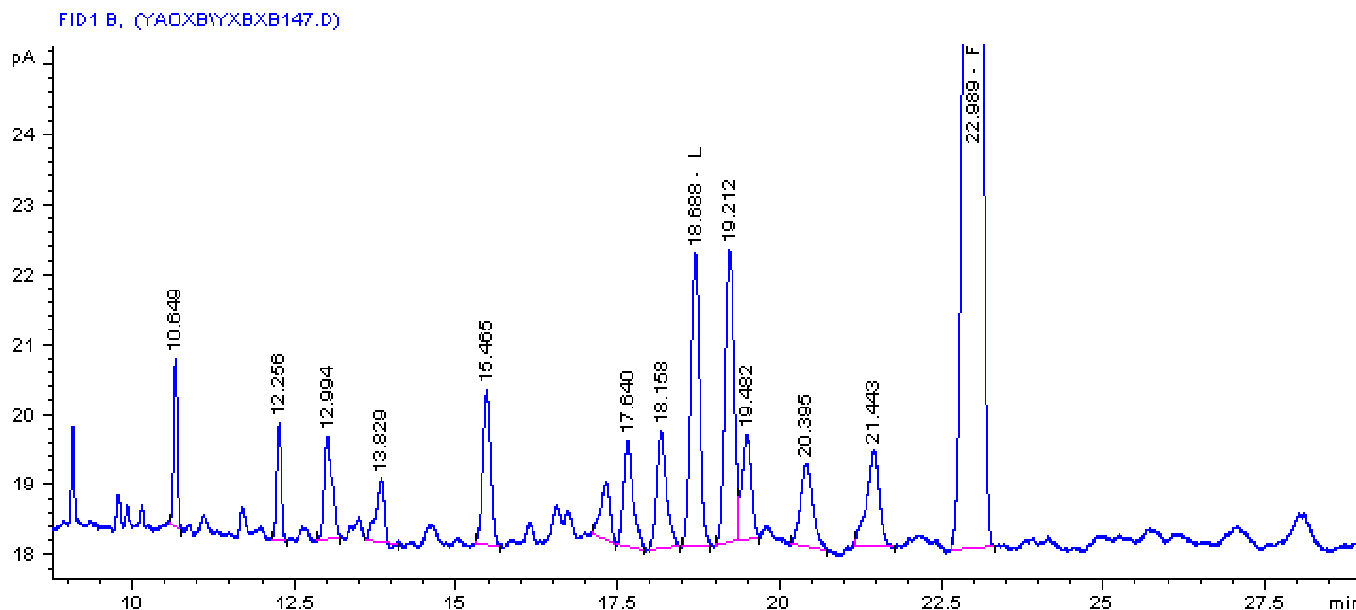

Figure 2. GC-MS chromatogram of EBS. The chromatographic conditions are given in Section 'Preparation of EBS and identification of triterpenoids'.

Table 1. Inhibitory effects on the growth of P388 mouse leukemia cells by EBS*.

Sample level	Inhibition rate (%)					Effects
Vincristin (mol/L)	10^{-4} 93.1	10^{-5} 94.3	10^{-6} 94.8	10^{-7} 94.7	10^{-8} 93.2	Strong effects
EBS (mg/mL)	1 90.7	0.25 98.2	0.063 90.4	0.016 57.7	0.004 0	A little effects

MTT reducing method. Effect time: 48 h.

Table 2. Inhibitory effects on the growth of A549 human lung adenocarcinoma cells by EBS*.

Sample	Inhibition rate (%)					Effects
Vincristin (mol/L)	10^{-4} 71.3	10^{-5} 71.6	10^{-6} 66.5	10^{-7} 44.3	10^{-8} 9.6	Weak effects
EBS (mg/mL)	1 70.2	0.25 90.6	0.063 24.8	0.016 0	0.004 0	A little effects

SRB protein staining method. Effect time: 72 h.

MS chromatogram describing the chemical profile of EBS.

Anti-tumor active filtration of EBS *in vitro*

The SRB protein staining and MTT reducing method were used to measure the growth inhibitory effects of P388 and A549 tumor cells, respectively. Results were shown in Tables 1 and 2. Vincristin had notable inhibitory effect on P388 cells while a little weaker effect on A549 cells. The inhibition rate of P388 was 57.7% with an EBS level of 0.016 mg/mL while the inhibition rate of A549 was 24.8% with an EBS level of 0.063 mg/mL. Further evidence suggested that EBS had shown inhibitory effect on P388 and A549 cell lines and had anti-tumor activity.

Inhibitory effects on the tumor-bearing mice S180 model by EBS

Results showed that both cyclophosphamide and EBS

Table 3. Effects on the sarcoma-loaded mice S180 model by EBS.

Group	Dosage (mg/kg)	Animal number	Tumor weight (g)	Tumor inhibition rate (%)
Control	-	10	1.81±0.60	-
CPA*	30	10	0.06±0.02**	96.68
EBS	1600	10	1.13±0.44*	37.57
EBS	800	10	1.14±0.41*	37.02
EBS	400	10	1.50±0.69	17.13

* Compared to control group, **P<0.01, *P<0.05; CPA, cyclophosphamide, single ip administration.

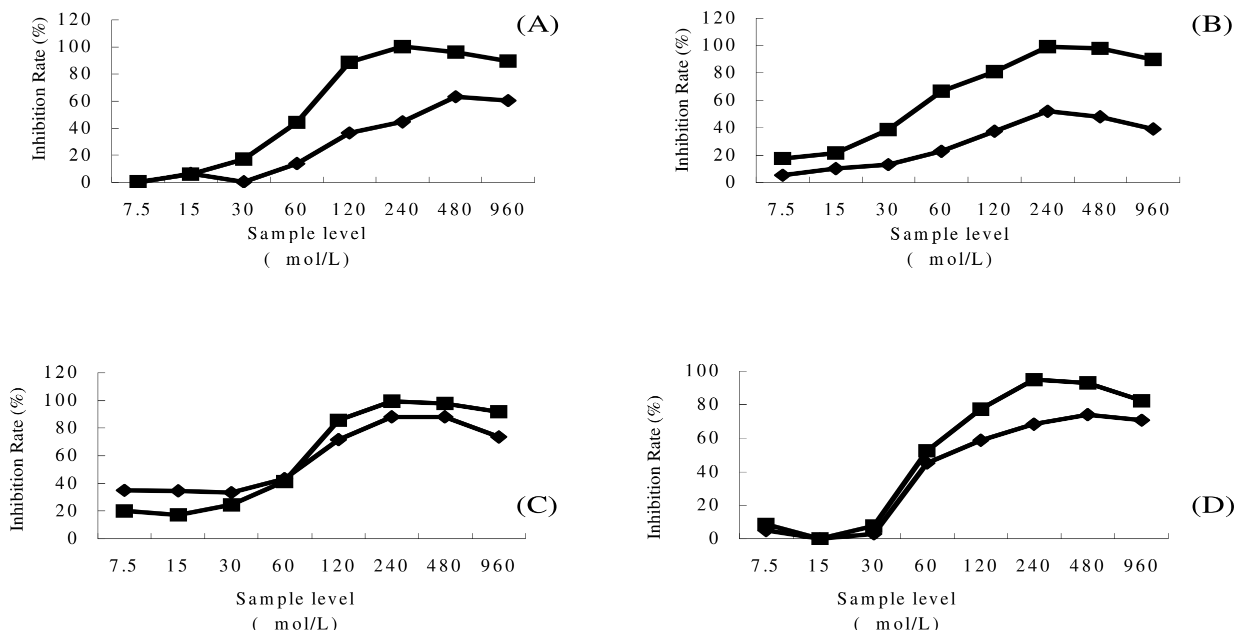

Figure 3. The inhibition rate and IC_{50} values of four tumor cells by Friedelin isolated from EBS. (A) A375 cell lines, (∅) Effect time: 24 h, (IC_{50} value: 356.54 μmol/L), (■) Effect time: 48 h, (IC_{50} value: 61.52 μmol/L); (B) L929 cell lines, (∅) Effect time: 24 h, (IC_{50} value: 665.42 μmol/L), (■) Effect time: 48 h, (IC_{50} value: 36.94 μmol/L); (C) Hela cell lines, (∅) Effect time: 24 h, (IC_{50} value: 61.25 μmol/L), (■) Effect time: 48 h, (IC_{50} value: 64.69 μmol/L); (D) THP-1 cell lines, (∅) Effect time: 24 h, (IC_{50} value: 85.10 μmol/L), (■) Effect time: 48 h, (IC_{50} value: 58.04 μmol/L).

had notable inhibitory effects on the sarcoma-loaded mice S180 model, which led to a depressed trend of tumor weights. The tumor inhibition rate of cyclophosphamide, EBS high dose, intermediate dose and low dose groups was 96.68, 37.57, 37.02 and 17.13%, respectively. In addition, it was notable for 30 mg/kg dose of cyclophosphamide and 1.6 g/kg dose, 0.8 g/kg dose of EBS to have statistical significance on inhibitory effects of tumor weights with Student's *t* test method compared to the control group ($P < 0.05$ or $P < 0.01$, Table 3).

Anti-tumor test of friedelin isolated from EBS *in vitro*

Figure 3 showed the inhibition rate of four tumor cells and the IC_{50} value by friedelin sample. From these data, it was proved that friedelin had notable inhibitory effect on Hela and THP-1 cells at 24 h. The inhibition rate of two cells had risen to 50% when the sample level was 60 μmol/L, which suggested that friedelin had higher sensitivity on these cells. On the other hand, friedelin had a little weaker inhibitory effect on A_{375} and L_{929} cells. It did not have notable effect until the effect time was up to 48 h. The inhibition rate of two cells had risen to 50% when the sample level was added to 240 μmol/L and the effect time was 24 h, which suggested that friedelin had weaker sensitivity on these two cells.

Figure 4 showed the inhibition rate of three adherent tumor cells compared to positive control, de-methyl-cantharidin. It could be demonstrated that friedelin and its positive control had similar inhibitory ability of A375, L929 and Hela adherent cells when the effect time was 24 h, while friedelin had stronger inhibitory effect on these three cells than de-methyl-cantharidin when the effect

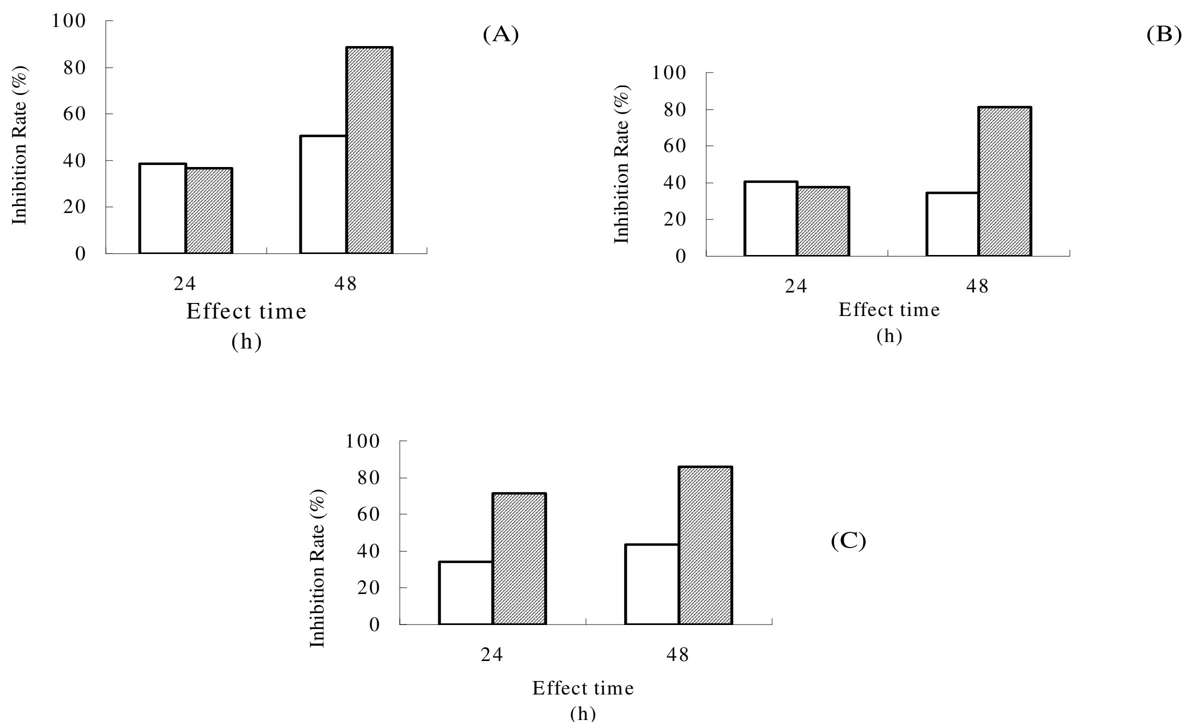

Figure 4. The inhibition rate of three adherent tumor cells by friedelin compared to de-methyl- cantharidin. (A) A375 cell, (B) L929 cell and (C) Hela cell. The concentrations of both samples were 120 μmol/L.

time was 48 h, which suggested that friedelin displayed a rather strong anti-tumor activity.

DISCUSSION

Bamboo shavings, which can be used as traditional Chinese medicinal materials, has mainly been applied for the field of Chinese medicine so far while it has not good application in other countries. However, the development of its extraction process and biological functions has not been focused on yet.

Some previous studies demonstrated the primary anti-tumor activities of bamboo leaf (Seki et al., 2008; Kim et al., 2007) or bamboo grass extracts (Tsunoda et al., 1998; Nagasawa et al., 2000), which have a bit important significance for our study. For instance, the extract of bamboo leaves could significantly inhibit tumor growth in S-180 and C38 tumor models and significantly prolonged overall survival of rats (Seki et al., 2008). The acetone fraction of bamboo leaf could enhanced leukemia cell differentiation (Kim et al., 2007).The extract of bamboo grass leaves could be a promising agent for the protection and therapy of breast and other types of tumors (Tsunoda et al., 1998). Research data in the present study primarily showed the anti-tumor activity of EBS. Therefore, it can be accepted that there may have some anti-tumor components which are widely distributed in different parts of bamboos. However, it is still uncertain whether the active compounds exerting the anti-tumor activity are similar with each other in bamboo leaves, bamboo grass or bamboo shavings.

Study on the anti-tumor effect of triterpenoids, which has become mainly biological active components in bamboo shavings, is widely concerned. Researches reported by Akinisa et al. (2001) suggested that 38 of multiflorane-type triterpenoid derivatives had been evaluated for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-O-tetradecanoylphorbol-13- acetate in Raji cells as a primary screening test for anti-tumor promoters. On the other hand, our study about anti-tumor activity of EBS and its monomer showed that friedelin monomer isolated from EBS had inhibitory effects on the proliferation of four tumor cells, A375, L929, Hela and THP-1 to a different extent, with a time-dose resistant relationship. Furthermore, friedelin had notable inhibitory effects on Hela and THP-1 cells at 24 h, which suggested that friedelin had higher sensitivity on these cells, but it had weaker sensitivity on A375 and L929 cells. Therefore, EBS had notable inhibitory effects on the sarcoma-loaded mice S180 model, which led to a depressed trend of tumor weights. It was notable for 1.6 and 0.8 g/kg dose of EBS to have statistical significance on inhibitory effects of tumor weights with Student's *t*-test method compared to the control group.

Recently, many triterpenoids isolated from natural plants have anti-tumor effects. Li et al. (2000) studied triterpenes

of betula platyphylla (TBP) which showed potent anti-tumor effects on melanoma B_{16} and sarcoma S_{180} and demonstrated that inducing apoptosis and arresting tumor cell in G_0/G_1 phase was one of anti-tumor mechanism of TBP. The triterpenoid fraction from the rhizomes of *Astilbe chinensis* could not only significantly inhibit the growth of mice transplantable tumor, but also remarkably increase splenocytes proliferation, NK cells activity and the level of IL-2 secreted by splenocytes in tumor-bearing mice, promote the DTH reaction and enhance anti-SRBC antibody level in naive mice (Tu et al., 2008). Furthermore, triterpenoids isolated from natural plants could decrease tumor cell proliferation and induce apoptosis (Mujoo et al., 2001; Ikeda et al., 2003) and the similar effect of some novel triterpenoids had been reported (Suh et al., 1999; Lapillonne et al., 2003) just as triterpenoids isolated from bamboo shavings in the present study.

Pentacyclic triterpenes from *Chrysobalanceae* species had notable inhibitory effects on P388 leukemia cell lines (Fernandes et al., 2003). Our results in this study suggested that EBS extracted from bamboo had good anti-tumor effects. On one hand, the inhibition rate of P388 was 57.7% with an EBS level of 0.016 mg/mL. Therefore, it had notable inhibitory effects. On the other hand, friedelin displayed a rather strong comparing with its positive control, de-methyl-cantharidin. Compared with other chemical medicines, the EBS and friedelin have lower anti-tumor activity, but the safety of EBS had been evaluated, it was confirmed that EBS is safety and has no side-effect (Zhang et al., 2004). Therefore, this extract of bamboo shavings had a great potential to be developed as anti-tumor health foods and natural medicine. Therefore, it can be concluded that friedelin can be regarded as one of the most important active factors in EBS though it is infrequent to report the physiological function of friedelin. But it is still unknown whether other terpenoids in EBS have anti-tumor effects and their corresponding mechanism. Overall, it can be predicted that EBS has a great potential to be applied in corresponding fields for its anti-tumor activity.

ACKNOWLEDGEMENT

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