http://dx.doi.org/10.4314/ajtcam.v10i5.6

A STUDY ON ANTICANCER ACTIVITY OF *CAULIS SPATHOLOBI* EXTRACT ON HUMAN OSTEOSARCOMA SAOS-2 CELLS

Bo Liu [1], Jinlan Liu[2¥], Jing Chen [3], Dongmei Zhu [1], Huajiang Zhou [1], Ximing Wang [1]*

3rd Orthopaedics Department, The Fourth Hospital of Jinan,Jinan,250031,China ;[2], Department of Joint Surgery, Affiliated Hospital of Weifang Medical University, Weifang, 261031, China;[3] Department of ICU, The Fourth Hospital of Jinan,Jinan,250031,China
***E-mail:** wangximing1312@163.com

Abstract

The objective of the present study was to investigate the anticancer activity of Chinese medicine *Caulis Spatholobi* extract on multicentric osteosarcoma cells. Ultraviolet spectrophotometry was used to determine the total flavonoid content in each sample; vanillin sulphuric acid assay was used to determine the condensed tannin content in each sample; and the varying degrees of inhibitory activities of ethanol, ethyl acetate and n-butanol extracts of *Caulis Spatholobi* on human osteosarcoma Saos-2 cells were studied. The results showed that the inhibitory activity of ethyl acetate extract was the highest among the four extracts. The condensed tannin contents of 1.2 mg/mL *Caulis Spatholobi* water extract, ethanol extract, ethyl acetate extract and petroleum ether extract were 26.23%, 48.36%, 70.18% and 40.51% respectively; and condensed tannin content of 1.5 mg/mL *Caulis Spatholobi* water extract, ethanol extract, ethyl acetate extract and petroleum ether extract were 4.15%, 5.81%, 8.76% and 7.30% respectively.

Keywords: *Caulis Spatholobi* , Inhibitory activities, Osteosarcoma cells

Introduction

Caulis Spatholobi is the dried vine stem of *Spatholobus suberectus Dunn.* (Leguminosae). Clinically, it is mainly know to have the anti-tumour, anti-viral, immunomodulatory, anti-inflammatory, antioxidant, sedative and hypnotic efficacies (Fu., 2003; Gou et al., 2010; Zhang and Wang, 2011; Zeng et al., 2011). Compounds that have already been found from *Caulis Spatholobi:* include flavonoids, terpenes, sterols, anthraquinones, lactones, volatile oils and other types of compounds, of which the flavonoid constituent has been relatively widely studied. The flavonoid constituent has a direct anti-tumour effect, and cell cycle arrest is one of its pharmacodynamic mechanisms of action (Tang et al., 2007). However, the constituent yet has no bone marrow suppressive effect, and has some promoting effect on erythropoiesis. At present, studies on the effect of *Caulis Spatholobi* extract on human osteosarcoma cells are scarce, so this paper studies the inhibitory activities of different solvent extracts of *Caulis Spatholobi* on human osteosarcoma Saos-2 cells.

Materials and Methods

Materials

Human osteosarcoma Saos-2 cell lines were purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS. MTT, DMSO and 0.25% trypsin were purchased from Sigma. PI, was purchased from Invitrogen, USA, and prepared to a concentration of 50 μg/ml. AnnexinV/FITC apoptosis kit was purchased from Invitrogen, USA. CO_2 incubator and -80 ℃ cryogenic refrigerator, SANYO, Japan, clean bench: Sartorius, Germany, electronic balance, Milli-Q ultrapure water system: Millipore, and inverted microscope: Olympus constitute other materials used in the experiment.

http://dx.doi.org/10.4314/ajtcam.v10i5.6

Extraction of active constituents

15g of *Caulis Spatholobi* powder (sifted through a 20 mesh sieve) was weighed in quadruplicate, added with water, 75% ethanol, petroleum ether and ethyl acetate as extraction solvents at a 1:10 (W:V) ratio respectively, and extracted by heat reflux for 45 min. Each item of this experiment was extracted twice repeatedly. After filtration of extracts, the filtrates were combined and *Caulis Spatholobi* extracts were successively concentrated to dryness using rotary evaporator and vacuum oven, and then weighed. The extract solutions were prepared at a concentration of 3.5 mg/mL, and DMSO was used to assist in dissolving.

Cell cultivation (Wu et al., 2010; Wang and Wei., 2011**)**

The human osteosarcoma Saos-2 cell lines were maintained in DMEM medium supplemented with 10% FBS, penicillin (100 IU/ml) and streptomycin (10 mg/L), and cultured in a CO_2 thermostat incubator. The medium was replaced once every two days. After 6 days from cultivation, the cells reached the logarithmic growth phase, with a density of about $4*10^5$ cells/ml. After trypsin digestion, cells were collected, counted, and centrifuged at 1000 r/min for 5 min to prepare a $5*10^6$~$5*10^7$ cell suspension and cryopreserved for later use. After Saos-2 cells were grown to logarithmic phase, they were digested with trypsin to prepare cell suspensions. During dosing, the logarithmic growth phase cells were taken and seeded in 96-well culture plates at a concentration of $4*10^3$ per well. The volume of each well was 200 µl, and the aqueous solutions of ethanol extract, petroleum ether extract and ethyl acetate extract were added respectively; their concentrations were all 3.5 mg/ml. Each sample was prepared at three different concentrations, which were 3.5, 1.75 and 0.875 mg/ml respectively.

Determination of condensed tannin content in the extracts (Cheng et al., 2011**)**

53.12 mg of catechin reference substance was accurately weighed, placed in a 50 mL volumetric flask, dissolved in methanol and diluted to the mark, shaken well to serve as a 1.0624 mg/ml reference solution. About 2.5 mg of four extracts were weighed separately, placed in 5 ml volumetric flasks, added with methanol, ultrasonically dissolved and diluted to the mark, then shaken well to serve as the test solutions, respectively. During measurement, 0.5 ml of test solutions were taken and added to the dark test tubes, 2 ml of 3% vanillin-methanol solution and 2.5 mL of 30% concentrated sulphuric acid-methanol solution were added separately, and shaken well. After colour development in dark condition for 25 min, A_{500nm} were measured.

Determination of total flavonoid content in the extracts (Huang., 2011; Wang et al., 2011**)**

Formononetin is mainly used as the quantitative reference substance of total flavonoids in the extract. 4.8 mg of formononetin reference substance was accurately weighed, dissolved in a small amount of 95% ethanol, and then placed in a 20 ml volumetric flask. It was diluted to the mark and shaken well to serve as the reference solution. Its concentration was 0.240 mg/ml and total flavonoid content was determined by measuring the absorbance at 250 nm. During measurement, approximately 1.5 mg of ethanol extract was accurately weighed, placed in a 15 ml volumetric flask, added with 1 ml of 95% ethanol, ultrasonically dissolved and diluted to the mark, and then shaken well to obtain the test solution. A_{250nm} were measured, and their total flavonoid contents were calculated according to the standard curves.

Results

Anticancer activity of the extracts

As can be seen from Figure 1 and Table 1, the inhibitory activity of ethyl acetate extract is the highest among the four

http://dx.doi.org/10.4314/ajtcam.v10i5.6

extracts, and this result may be directly related to the higher total flavonoid content within it, as can be found in Table 3. Inhibitory activities of ethanol extract and petroleum ether extract are slightly lower, and the aqueous extract has the lowest activity.

Table 1: Inhibitory activity of the four extracts

Name	Inhibition rate (%)		
	Concentration (mg/ml)		
	0.875	1.75	3.5
Aqueous extract	8.5	10.1	30.8
Ethanol extract	14.7	35.2	48.1
Ethyl acetate extract	21.9	41.2	65.4
Petroleum ether extract	12.8	26.9	52.3

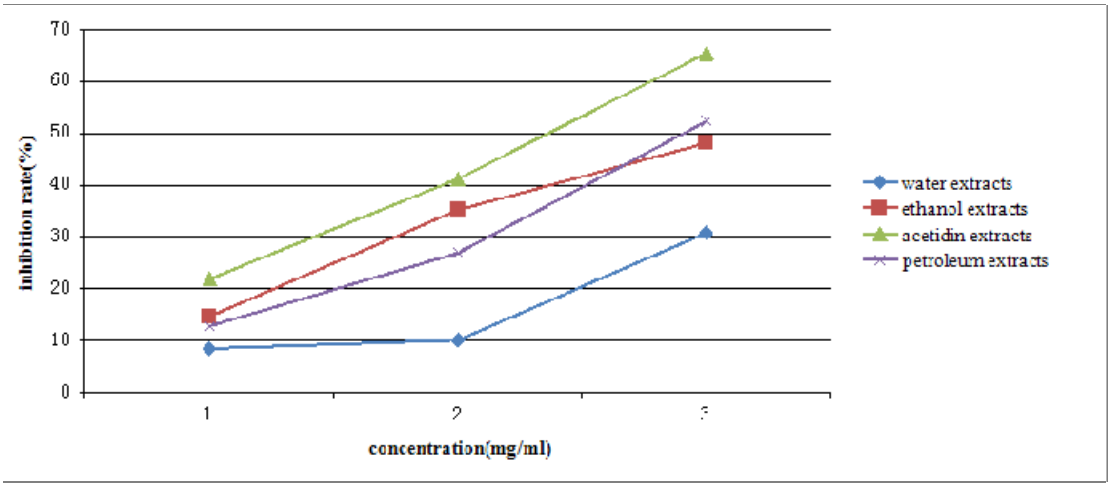

Figure 1: Inhibitory activity of the four extracts

Results of the condensed tannin content in the extracts

Different absorbance values were obtained according to the different concentrations of reference solutions in order to get the standard curve: Y=2.1564X-0.2314, R^2=0.9997. Linearity range was 0.1062~0.5312 mg/ml. *Caulis Spatholobi* aqueous extract, ethanol extract, ethyl acetate extract and petroleum ether extract were weighed respectively, and their tannin contents were determined. The results showed that the condensed tannin contents of the 1.2 mg/mL *Caulis Spatholobi* aqueous extract, ethanol extract, ethyl acetate extract and petroleum ether extract were 26.23%, 48.36%, 70.18% and 40.51% respectively (Table 2).

Table 2: Results of the condensed tannin content in the extracts

Extract (1.2 mg/mL)	Condensed tannin content (%)	Standard curve
Aqueous extract	26. 23	Y=2.1564X-0.2314, R^2= 0.9997, linearity range of 0.1062~0.5312 mg/ml
Ethanol extract	48. 36	
Ethyl acetate extract	70. 18	
Petroleum ether extract	40. 51	

http://dx.doi.org/10.4314/ajtcam.v10i5.6

Total flavonoid content

The results showed that the linear relationship was good within the concentration range of total flavonoids of 2.40~12 mg/ml. Standard curve was determined to be: Y=0.1208X-0.1724, R^2=0.9996. *Caulis Spatholobi* aqueous extract, ethanol extract, ethyl acetate extract and petroleum ether extract were weighed respectively, and their formononetin contents were determined. The results showed that the total flavonoid contents of the 1.5 mg/mL *Caulis Spatholobi* aqueous extract, ethanol extract, ethyl acetate extract and petroleum ether extract were 4.15%, 5.81%, 8.76% and 7.30% respectively(Table 3)

Table 3: Results of the total flavonoid content in the extracts

Extract (1.5 mg/mL)	Total flavonoids content	Standard curve
Aqueous extract	4. 15	
Ethanol extract	5. 81	Y = 0.1208X -0.1724, R^2=0.9996, R2= 0.9997,
Ethyl acetate extract	8. 76	linearity range of 2.40~12 mg/ml
Petroleum ether extract	7. 3	

Discussion

In this paper, different extracts are obtained with water and three other different solvents by heat reflux method, and the condensed tannin content as well as the total flavonoid content with formononetin as reference substance in each extract are determined using UV spectrophotometric method and by plotting standard curves. The inhibition rates of four different extracts against human osteosarcoma cells under three different concentrations are determined by MTT assay respectively, and the inhibition curves are plotted. The results show that the ethyl acetate extract has the highest total flavonoid contents, as well as the highest inhibition rate against human osteosarcoma Saos-2 cells. Its inhibitory activity is between 21.9%~65.4% at the concentration range of 0.875~3.5 mg/ml. The inhibition rate of petroleum ether extract is between 12.8%~52.3%, and ethanol extract between 14.7%~48.1%. The inhibition rate of aqueous extract is the lowest, which is between 8.5%~30.8%.

At present, Chinese medicines used in the study of human osteosarcoma Saos-2 cell suppressive activity include *Angelica decursiva*, *Nigella sativa*, etc., *Angelica decursiva* induces the suppression of cell growth and cell apoptosis in Saos2 human osteogenic sarcoma cells (Lee et al., 2009); thymoquinone extract from *Nigella sativa* significantly downregulates NF-κB DNA-binding activity, XIAP, survivin and VEGF in SaOS-2 cells (Lei et al., 2013).

Caulis Spatholobi has relatively good inhibitory activity against many cancer cells. For instance, the flavonoid component of *Caulis Spatholobi* has significant growth inhibitory effect on human lung cancer A549 and human colorectal cancer HT-29 cell lines, with IC50 of 70.17 ± 12.17 μg·mL^{-1} and 126.81 ± 43.00 μg·mL^{-1} respectively (Tang et al., 2007). A study used a mouse model of transplanted Lewis lung carcinoma to observe the in-vivo anti-tumour and anti-metastatic effects of certain *Caulis Spatholobi* extract in mice. It explored its anti-tumour mechanism of action by detecting the cell cycle and apoptosis status. The results showed that the inhibition rate against mouse Lewis lung cancer is the highest, which is 28.6%. It also had anti-metastatic effect, acting cell cycle was G1 phase, and the apoptosis rates were not significantly different among the groups (Fu et al., 2009). In addition to the anticancer activity, *Caulis Spatholobi* also has the antithrombotic efficacy, as thrombosis is the pathological basis of ischemic cardio-cerebrovascular diseases. Another study has also shown that *Caulis Spatholobi* has an obvious platelet aggregation inhibitory effect, and can significantly reduce the thrombus wet weight (Wang et al., 2005).

http://dx.doi.org/10.4314/ajtcam.v10i5.6

References

1. Cheng Yue, Chen Jia-sheng, Chen Jian-ping, Wang Dong-mei. (2011). Determination of different types of tannins in Radix Sanguisorbae extract. Chinese Traditional Patent Medicine, **05**: 852-857.
2. Fu Xiu-hua. (2003). Effects of Chinese Herbal Medicine on Immune Function in Mice. Nanjing Agricultural University.
3. Fu Qi, Tang Yong, Luo Xiao-qin, Yang Guo-wang, He Wei, Wang Xiao-min. (2009). Anti-tumor activity and mechanism with SSCE of Spatholobus suberctus. China Journal of Chinese Materia Medica, **12**: 1570-1573.
4. Gou Ming-yue, Liu Liang, Zhang Chun-zhi. (2010). Determination of Antioxidant Activity in 26 Plants by DPPH Method. Food and Fermentation Industries, **03**: 148-150.
5. Huang Xiao-dong. (2011). Total flavonoid contents, antibacterial activities and antioxidant activities of four extracts from Dimocarpus longan seeds. Food Science, **11**: 43-47.
6. Lei Peng, An Liu, Yue Shen, Hua-Zi Xu et al. (2013). Antitumor and anti-angiogenesis effects of thymoquinone on osteosarcoma through the NF-κB pathway. Oncology reports, **29(2):** 571-578.
7. Lee, Sook-Young, Chun Sung Kim, Seon-Ho Cho, et al. (2009). The effects of Angelica decursiva extract in the inhibition of cell proliferation and in the induction of apoptosis in osteogenic sarcoma cells; Journal of Medicinal Plants Research, **3(4)**: 241-245.
8. Tang Yong, He Wei, Wang Yu-zhi, Zhang Gan-lin, Wang Xiao-min. (2007). Studies on the Anti-tumor Activity of the Extract of Spatholobus suberctus Dunn. Chinese Journal of Experimental Traditional Medical Formulae, **(02)**: 51-54.
9. Wang Jun, Wei Yi-yong. (2011). The effects of Ligustrazine on proliferation and VEGF gene expression of osteosarcoma SaOS-2 cell lines. Acta Universitatis Traditionis Medicalis Sinensis Pharmacologiaeque Shanghai, **03**: 92-94.
10. Wang Yan, Xie Yuan-yuan, Sun Jia-hong, Wang Rui-jie, Yuan Dan. (2011). Quantitative determination of the total flavonoids and organic acids of Chrysanthemum morifolium extract. Journal of Shenyang Pharmaceutical University, **02:** 124-129.
11. Wang Xiu-hua, Liu Ai-dong, Xu Cai-yun. (2005). Study on antithrombotic effect of Caulis Spatholobi. Journal of Changchun University of Traditional Chinese Medicine, **04**: 41.
12. Wu Yan-feng, Liang Xin-jun, Guo Yu, Xia Kan, Xia Ren-yun, Chen Hui-yong.(2010). The effect of curcuma on proliferation and apoptosis of Saos-2. Chinese Pharmacological Bulletin, **09**: 1150-1153.
13. Zeng Fan-li, Cheng Yue, Chen Jian-ping, Fu Ying, Wang Qiao-li, Yang De-po, Chen Zhen-ping, Xiang Yang-fei, Wang Yi-fei, Wang Dong-mei.(2011). Study on in vitro Antiviral Activities of Different Extracts from Caulis Spatholobi. Traditional Chinese Drug Research and Clinical Pharmacology, **01**: 16-20.
14. Zhang Xiao-nan, Wang Jia. (2011). An overview of the pharmacological effects of Caulis Spatholobi. Heilongjiang Science and Technology Information, **(12)**: 28.