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A STUDY ON THE EXTRACTION PROCESS OF ACTIVE INGREDIENTS FROM *AKEBIA STEM* AND AN ANALYSIS OF THEIR ANTI-GASTRIC CANCER ACTIVITY

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Abstract

The study investigated the extraction process of active ingredients from *akebia stem* and an analysis of their anti-gastric cancer activity. Three different extraction methods were used to obtain extracts, namely the decoction method (group A), reflux extraction method (group B), and maceration method (group C), of which reflux extraction method and maceration method used ethanol as the extraction solvent, while decoction method used distilled water for extraction. The differences in anti-gastric cancer activity of the three extracts were compared. MTT assay was used to test and compare the inhibitory effects of extracts obtained in A, B, and C groups on gastric cancer cells. The results showed that the dry extract obtained by heat reflux extraction with "water-ethanol" ratio of 1:2, extractant volume of 70 ml, with ethanol as extraction solvent presented the best inhibitory activity on gastric cancer SGC-7901 cells in this study. Its inhibitory effect did not change over time, and was directly proportional to the concentration.

Keywords: SGC-7901 cell, *Akebia trifoliata (Thunb.)*, extraction process

Introduction

Gastric cancer is a common malignant disease of the human digestive system with high mortality rate. Therefore, the development of one or a group of new drugs that will be effective on the human body with very little side effects in the treatment of gastric cancer is needed. A variety of Western medicines has a lot of side effects in the treatment of cancers, one of the traditional Chinese medicine — Akebia Stem is the woody stem vine of *Akebia trifoliata* (Thunb.) Koidz., a plant in the Lardizabalaceae family. It is grown in hillsides, shrubs or ditches, and distributed in places such as Hebei, Shanxi, Shandong, and Anhui. Its stem vines are harvested in summer and autumn, dried in the sun to get the Akebia Stem crude drugs. It has a wide range of pharmacological activities (Xu, 2001).

Materials and Methods

Materials

Gastric cancer SGC-7901 cells were purchased from the Shanghai Institute of Biochemistry and Cell Biology, CAS. SGC-7901 cells were seeded in RPMI-1640 medium containing 10% foetal bovine serum, 100 U·ml-1 penicillin and 100 U·ml-1 streptomycin, and cultured in an incubator set at 37 ℃, 5% CO_2, with saturated humidity. Culture medium was replaced every 2~3 days. After a monolayer of cells was formed, cells were serially subcultured by conventional trypsin digestion method. Other materials used for the experiment include the following: Chinese medicine Akebia Stem decoction pieces, purchased from Henan Guoyao Medicine Co., Ltd.; foetal bovine serum, Hangzhou Sijiqing Bioengineering Co., Ltd.;

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foetal calf serum (GIBCO, USA), double antibiotics (penicillin, streptomycin, purchased from Gibco, USA); trypsin (purchased from Amresco, USA); MTT, DMSO, Sino-American Biotechnology Co., Ltd.; RPMI 1640 medium, GIBCO. Microplate reader, BIO-RAD; Thermo incubator, Thermo, USA; KQ50DA ultrasonic cleaner, Zibo Saike Experimental Instrument Co., Ltd.; NIKON-Ti-U inverted fluorescence microscope, NIKON, Japan.

Preliminary sample treatment

200 g of herbal material was weighed, crushed, and passed over a 20-mesh sieve. Then 40 g of powders was weighed in triplicate respectively for later use.

Decoction method

40 g of group A sample was placed in a 200 ml beaker, added with 100 ml of distilled water, and boiled for 1 h, while stirring constantly with a glass rod. 1 h later, 50 ml of distilled water was added, and the boiling was continued for another 0.5 h. After that, 25 ml of distilled water was added, and the boiling was again continued for another 0.5 h. The sample obtained was suction filtered, and the resulting filtrate was placed in a 500 ml beaker. After the evaporation of large amounts of water by electric furnace heating, water bath was used instead. The extract solution obtained was placed in several evaporating dishes, and further evaporated by water-bath heating. The final resulting extract is just the product obtained by the decoction method.

Reflux extraction method (Wang, 2011)

40 g of Akebia Stem powder was weighed, and extracted by reflux extraction at 100 ℃ for l h with different ratios (1:1, 1:2, 2:1, v/v) of water and anhydrous ethanol as the extractants, where the volumes of extractants were all 70 ml. The extracts were suction filtered to remove the ethanol. Excess water was evaporated by water-bath heating to obtain dry extracts, and the amounts of dry extracts obtained by different extractants were compared.

Maceration method

40 g of Akebia Stem powder was weighed, placed in a 200 ml beaker, added with 70 ml of ethanol, and soaked for 1 h, while stirring with a stirrer so as to obtain more effective extracts.

Cell growth curve (Chen et al, 2011)

Gastric cancer cells in logarithmic growth phase were collected, digested into a single cell suspension with trypsin, and seeded in 96-well plates with an inoculum concentration of 1×10^5 cells/ml. Cells of control group were cultured in RPMI-1640 medium containing 10% foetal bovine serum. Culture medium was replaced once every 2~3 days, and cell growth status was observed under an inverted microscope. 3 flasks of cells were collected from each group every day. After digestion, the cells were stained with trypan blue, counted using cell counting chamber, and averaged to plot growth curves. This procedure lasted 9 days.

Activity screening (Liu et al, 2011)

Cells on the 5th day from thawing were selected and used in the activity screening experiment. Under the premise that cells were in a good state when observed under an inverted microscope, the cells in the culture flask were digested with

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trypsin, diluted to a concentration of 1×10^4 using 10% FBS-containing RPMI 1640 medium, seeded in 96-well plates, and cultured for 24 h. The cells were then added with the sample. The dry extracts obtained in 3 groups were formulated into 2.5 g/ml aqueous solution respectively. 5 µl of drug solution and 95 µl of cell medium (serum-free) were added to each well. After that, test was performed in accordance with the MTT assay procedure.

Results

Decoction method

The product obtained by this method was evaporated by high-temperature heating, and then by water-bath low-temperature (relative to the high temperature) heating. The weight of resulting dry extract was 23.5 g; dry extract yield was 58.7%.

Reflux extraction method

As can be seen from Figure 1, dry extract obtained with water as extractant had the lowest yield, which is possibly due to the too high polarity of water. When the water-ethanol ratio was 1:2, the yield of dry extract was the highest; therefore, it is speculated that the active ingredients it contains should be the highest. So, we selected the dry extract under this ratio as the sample for activity experiment.

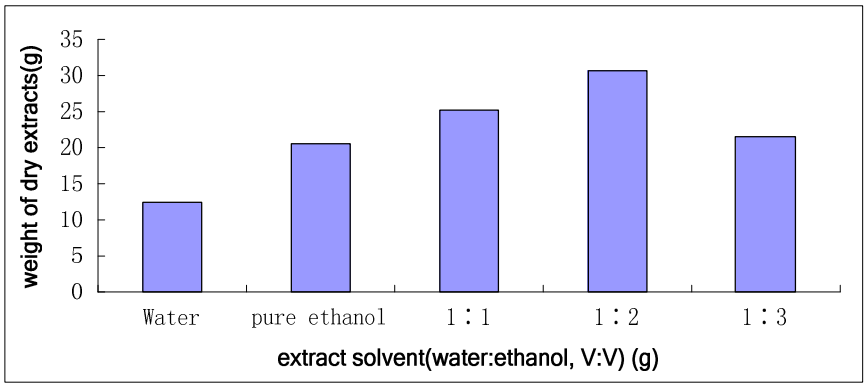

Figure 1: Weight of dry extracts obtained by ethanol reflux extraction under different solvents

Cell growth curve

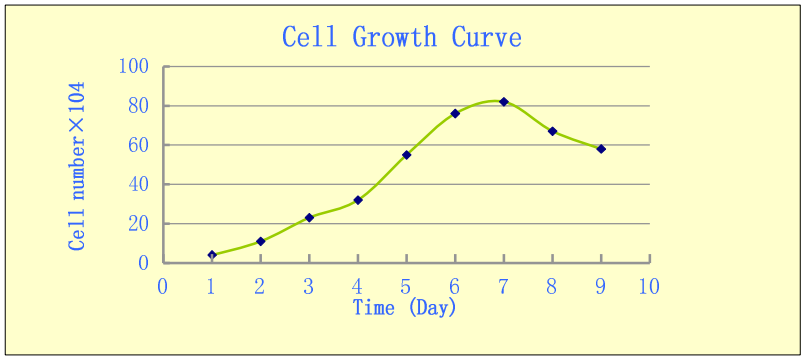

Figure 2: SGC-7901 cell growth curve

As can be seen from the results, cells had very fast proliferation rates on the 4th-6th day. So, we selected the cells which had the fastest proliferation rate on 4th day, that is, cells on 6th day from culturing after thawing, cells in this phase have strong viability, so are chosen as the cells used in the anticancer activity screening experiment.

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Activity screening (Ji et al, 2012)

Tables 1, 2 and 3 are the results for the inhibitory effects of three dry extracts obtained by decoction method, ethanol reflux extraction method and ethanol maceration method on SGC-7901 cells by MTT assay. As can be seen from Table 1, inhibition rates by decoction method were moderate, but the inhibition rates decreased over time. They were greatly influenced by the concentration and were directly proportional to the concentration. The inhibition rates were low compared with the 2.5 g/ml concentration group in Table 2. The dry extract obtained by maceration method had the lowest inhibition rates, which was about 30% at maximum. Therefore, we can conclude that in this experimental study, the dry extract obtained by ethanol heat reflux extraction is the product having the most obvious inhibitory effect on gastric cancer cell proliferation.

Table 1: Inhibitory effect of group A on SGC-7901 cells by MTT assay

		Inhibition rate %		
Group	Dose g/ml	24h	48h	72h
Control	—	0.1±0.0	0.2±0.1	0.3±0.1
	2.5	36.5±5.3	39.3±6.1	42.7±6.9
Group A	1.25	26.1±4.3	27.2±5.2	28.9±5.5
	0.63	16.5±2.5	23.8±4.8	36.4±6.2

Table 2: Inhibitory effect of group B on SGC-7901 cells by MTT assay

Group	Dose g/ml	24h	48h	72h
Control	—	0.1±0.1	0.1±0.2	0.2±0.0
	2.5	54.1±4.1	56.9±5.3	60.2±5.8
Group B	1.25	38.1±3.7	39.2±3.1	41.5±3.2
	0.63	11.4±1.2	15.8±1.3	14.8±1.4

Table 3: Inhibitory effect of group C on SGC-7901 cells by MTT assay

Group	Dose g/ml	24h	48h	72h
Control	—	0.2±0.1	0.3±0.1	0.1±0.1
	2.5	26.1±2.3	27.5±3.7	30.1±4.1
Group C	1.25	15.8±2.2	27.2±2.4	28.9±3.1
	0.63	16.5±1.8	23.8±2.5	36.4±3.7

Discussion

This study is mainly divided into two major parts: Akebia Stem extraction process and anti-gastric cancer activity. In the extraction process, decoction method, ethanol reflux extraction method and ethanol maceration method were used, thus obtaining three different products, and comparing anticancer activities of these different products. The results show that the extract obtained by reflux extraction has the most obvious inhibitory effect, and the effect is more pronounced with increasing concentration.

Akebia Stem extract contains a variety of saccharides (He et al, 2004). It has certain inhibitory effects on mouse sarcoma 180 and 37, human cervical cancer JTC26 cells, and Hela cells (Gu & Gu, 2009), indicating that Akebia Stem extract must be rich in a certain kind of active compound or a group of mixtures that exerts anticancer activity. So far, researches on Akebia Stem are not much, most of which mainly focused on the isolation of monomer compounds. The activity assay of Akebia Stem, especially its anti-cancer activity assay, has rarely been conducted. Therefore, the design of

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this study has some novelty.

Monomer compounds isolated from Akebia Stem include palmitic acid, sitosterol, daucosterol, hederagenin-glucopyranoside, etc. Doctors of traditional Chinese medicine have proposed the "Spleen-nourishing and Qi-regulating" principle for the treatment of liver cancer, and many of them formulated the spleen-nourishing and qi-regulating prescription with Radix Codonopsis, Rhizoma Atractylodis Macrocephalae, Poria cocos, Akebia Stem, etc. (Cai & Li, 2005). A large number of clinical cases have shown that the prescription has a certain efficacy on the treatment of liver cancer. Therefore, some studies have demonstrated that one of the reasons for its efficacy is due to up-regulated bax gene protein expression, as we know that bax is closely related to apoptosis of tumour cells. While the inhibitory effect of trichostatin A on gastric cancer cells is conducted through caspase-independent pathway, this process involves mitochondrial release of AIF and EndoG, and transfer to the nucleus (Wu, 2007). Chinese patent medicine, Weikang Granule, which is formulated with *Radix Codonopsis, Rhizoma Atractylodis, Radix Curcumae, Actinidia sinensis* and *Rhodiola rosea*, also has a good inhibitory effect on gastric cancer cells, where the growth of gastric cancer cells is inhibited mainly through the induction of autophagy, intracellular vacuolisation, etc. Therefore, further research on Akebia Stem extract as well as its anti-gastric cancer mechanism is needed in order to accumulate valuable and sufficient materials for future studies (Jiege et al, 2012; Zhang et al, 2011).

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