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In vitro polysaccharide extraction from *Cipangopaludina cathayensis* and its pharmacological potential

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Abstract

Cipangopaludina cathayensis (mudsnails) *is* a snail-like animal. This study intends to reveal pharmacological potential of polysaccharide extracted from mudsnails. Crude soluble polysaccharide of mudsnails was extracted using hot water extraction and ethanol precipitation method. The crude polysaccharide was purified successively using Sevag method. Applying Fenton reactions, it was found that antioxidant potential efficiency of polysaccharide extracted from mudsnail was dose-dependent and reached up to 44.29% at 4 mg ml^{-1} concentration. Most significantly, polysaccharide extracted from mudsnail could efficiently inhibit lung cancer cell line A-549 proliferation *in vitro* and over 50% cells were killed on applying 300 mg ml^{-1} of polysaccharide after 24 hrs of post treatment. It implies that mudsnail polysaccharide is a potential anti-tumor agent.

Key words

Antioxidant potential, Anti-tumor properties, *Cipangopaludina cathayensis*, Polysaccharide

Introduction

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units, bound together by glycosidic linkages and on hydrolysis give constituent monosaccharides or oligosaccharides. Recently, polysaccharides have been extensively studied, and it had been found that they play an important role in cell membrane function and immunity. Due to varied biological activities, polysaccharides are widely applied in the field of health care and pharmacy. *Cipangopaludina cathayensis* (mudsnails), commonly known as Spiral Lion belongs to family Cipangopahudina Hannibal and is an edible freshwater snail. The flesh of snail-like animals is widely employed in Chinese traditional medicine to improve urination or to cure jaundice, hemorrhoids and otitis.

Recent studies indicate that mollusks such as snail-like animals and shellfish are rich in polysaccharides. In the present study efficient antioxidant potential was found in the mudsnail polysaccharides and suggests that it play a promising role in pharmacology and provides an alternative choice to utilize and control this invasive alien species in South China.

Materials and Methods

Mudsnails were purchased from Nanchang which were grown and cultured in freshwater (pH=7) at 24°C. Mudsnails were kept in fresh water for 2-3 day and water was changed several times. Hulled snail meat was weighed (171 g), and then using the homogenizer stirred into homogenate and added methanol (5% of the volume ratio). The homogenate was defatted for 12 hrs. and then centrifuged at 4000 rpm for 15 mins. Distilled water was added to the residue extract for 6 hrs in the Thermostatic magnetic mixer at 60°C and centrifuged at 4000 rpm for 40 mins. The supernatant was collected and dissolved in anhydrous ethanol (the final ethanol concentration is of 75%). The resulting solution was kept in refrigerator at 4°C for overnight. On the second day, the

solution was centrifuged and the precipitate was collected and washed with anhydrous ethanol, ether and acetone and degreased two times, for 20 mins, followed by centrifugation at 4000 rpm for 40 mins. Finally, the crude polysaccharides of mudsnails was obtained.

Identification of polysaccharide: In the present study, Molish analysis was employed to identify the polysaccharide. The mudsnail polysaccharide was white, tasteless crystal soluble in water but insoluble ethanol.

Quantification of total polysaccharide : Using anthrone-sulfuric acid method, total polysaccharide was quantified. First, a glucose standard curve was prepared. Glucose (0.25 g) was dissolved in pure water and diluted to a concentration of 1 mg ml^{-1}. A 2, 4, 6, 8 and 10 ml of glucose solution was diluted by distilled water to a final volume of 100 ml for preparing a series of concentration gradients standard glucose solution. Anthrone-sulfuric acid mixture was added to the standard glucose solution. The mixtures were first incubated in ice water and then boiled for 7 mins. After boiling, the mixtures were cooled down to room temperature for 10 mins and absorbance of the specimens was measured at 580 nm. According to the absorbance and related concentrations of the standard glucose solutions regression analysis was performed. The glucose standard curve is shown in Fig. 1. The regression equation is represented as Y = 0.1352X - 0.0054 (Y and X stand for the glucose concentration and absorbance, repectively), and R^2=0.999.

Mudsnail polysaccharides (0.006 g) was dissolved in pure water to a final volume of 60 ml. The absorbance of the solution was determined and concentration was calculated by the formula given below:

$$\text{Polysaccharide quantification (\%)} = (C \times D)/W \times 100$$

where, W and D represent the quality of polysaccharide and dilution coefficient, respectively.

Antioxidant potential analysis : Ferrous ammonium sulfate hexahydrate (166.81 mg) was dissolved in pure water to make a final volume of 100 ml. Salicylic acid (82.87 mg) and 30% hydrogen peroxide (68 µl) was added in pure water to make a final volume of 100 ml. A series of standard Vitamin C solution 0.1, 0.2, 0.3, 0.4 and 0.5 mg ml^{-1} were first diluted in distilled water to make a final volume of 3.2 ml. After dilution, Fenton reaction was employed to prepare an antioxidant potential standard curve. For antioxidant potential of mudsnail polysaccharide, the polysaccharide (0.5 g) was dissolved in water to a final volume of 50 ml. The polysaccharide solution was diluted by water to a final concentration of 0.5, 1, 2, 3, 4 mg ml^{-1}, respectively. After dilution, Fenton reactions were performed and antioxidant potential for hydroxyl radicals of mudsnail polysaccharide were then determined. The absorbance values were determined at 510 nm.

Anti-tumor properties analysis : A-549 cells were first digested by trypsin and then collected. Cell activation was determined by MTT cell proliferation assay. Anti-tumor index was calculated by the formula given below:

Antitumor index (%) = [(absorbance of the control – absorbance of the undermined specimen /absorbance of the control)] × 100. The absorbance values were determined at 510 nm.

Results and Discussion

Mudsnails are rich in polysaccharides: 1 ml of 10% aqueous solution of mudsnail polysaccharide were taken into a small tube, added a few drops of α - naphthol. After shaking, dropped 1 ml of the concentrated sulphuric acid slowly along the wall pipe, red purple ring appeared at the junction of two liquids. It indicated that the polysaccharide is present (Fig. 2).

Using anthrone-sulfuric acid method, total polysaccharide was quantified. It was found that the total polysaccharide of mudsnail was 66.4%, which revealed that mudsnails are rich in polysaccharides.

Through UV detection, a series of absorbance values were obtained. A generated data was applied to the

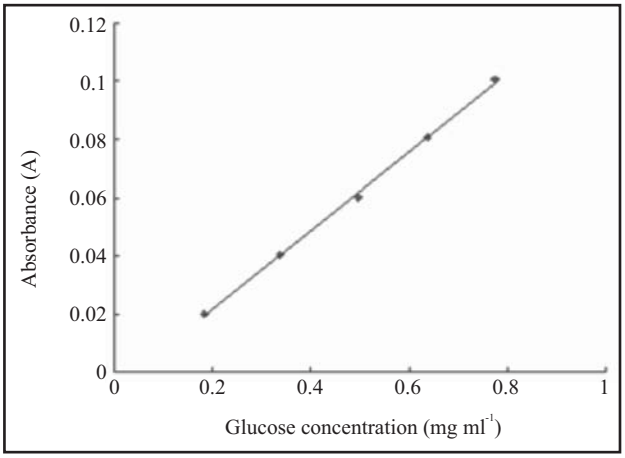

Fig. 1 : Glucose standard curve

formula for obtaining mudsnail polysaccharide antioxidant (Fig. 3).

As evident from Fig. 3, the mudsnail polysaccharide showed good antioxidant activity, when the concentration reached 4 mg ml^{-1}, the inhibition rate of hydroxyl free radical reached 44.29%. The antioxidant activity increased with increased in the concentration of polysaccharides. As observed from the curves, a good test result was noted. The antioxidant efficiency showed a linear increase on increasing the concentration of polysaccharide from 0 to 4mg ml^{-1}. Study on the inhibition rate on free radical of polysaccharide in higher concentration is still in progress. The theory will reach a suppression of good rate and then tends to be gentle. In general, meeting the requirements of the experiment, it is indicated that the mudsnail polysaccharide has good antioxidation ability.

Numerous natural polysaccharides isolated from mushroom have been used as a source of therapeutic agents to cure various diseases including cancer . The result of the present study indicate that mudsnails are rich in polysaccharides. Therefore, it is interesting to find out whether the mudsnail polysaccharide can inhibit carcinoma cell proliferation. Lung carcinoma cell line A-549 was treated with mudsnails polysaccharide at the indicated concentrations. As shown in Table 1, the antitumor effect was time and dose-dependent, and over half of the cancer cells were killed at 24 hrs at 300 μg ml^{-1}. Therefore, the results suggested that mudsnail polysaccharide is a promising of anti-tumor agent.

Mushroom polysaccharides have been shown to play multiple important role in human health. However, the biological function of animal-derived polysaccharide is still unknown. Recent studies have indicated that snail-like animals are rich in polysaccharide. Important pharmacological properties such as antivirus and antitumor are found in these polysaccharides. They are also widely used in pharmacy in combination with some drug to from various

Table 1 : Mudsnail polysaccharide can efficiently inhibit cancer cell proliferation

Concentration (mg ml^{-1})	4 hr	8 hr	24 hr
20	11.00%	13.40%	26.98%
40	16.27%	25.00%	32.93%
160	27.11%	33.99%	40.10%
300	41.27%	43.97%	51.32%

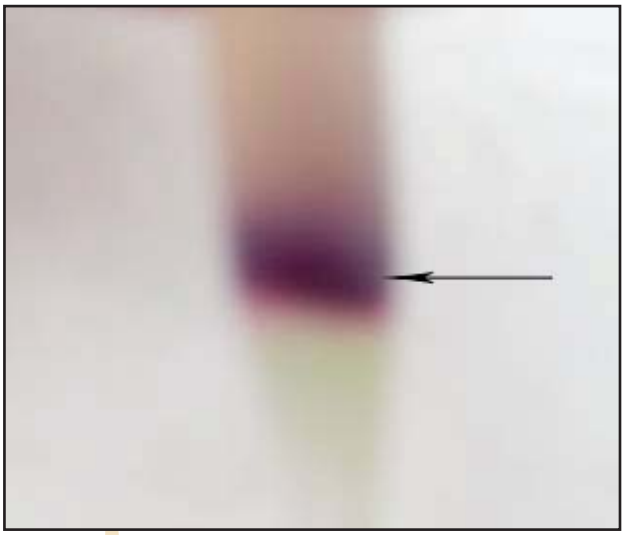

Fig. 2 : Identification of polysaccharides

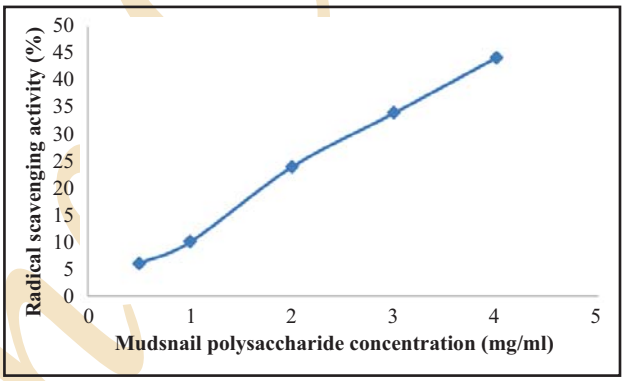

Fig. 3 : The inhibition rate of mudsnails polysaccharide and hydroxyl radical

formulations, such as tablets and films. The total polysaccharide component of mudsnail flesh powder is up to 66.4%. Mudsnails are extremely polyphagous, fast growing and multiply quickly, therefore, it can be considered as an important source of polysaccharide. The biological function of mudsnail polysaccharides is still uncharacterized. Using Fenton reactions, efficient antioxidant potential was found in mudsnail polysaccharide. However, further study is needed to identify which polysaccharides are responsible for antitumor activity.

Acknowledgments

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