Anti-tumor activity of sulfated polysaccharides from Sargassum fusiforme

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ARTICLE INFO
Article history:
Available online 28 April 2017

Keywords:
Sargassum fusiforme polysaccharide
Sulfation
MTT method
Anti-tumor

ABSTRACT
In this study, our purpose is to discover the correlation between polysaccharides sulfated structure and anti-tumor activity. Sulfated polysaccharide from Sargassum fusiforme were synthesized with the chlorosulfonic acid pyridine method. The inhibitory effect of Sargassum fusiforme polysaccharides and the application of MTT assay before and after chemical modification on the proliferation of hepatocellular carcinoma HepG-2 cells in vitro were studied. Sulfated polysaccharide from sargassum fusiforme DS is 0.803. The modified polysaccharide has certain inhibitory effect on HepG-2 cells, and its inhibition on the cells growth has improved compared with the original SFPs. The sulfated polysaccharide from Sargassum fusiforme has the ability to enhance anti-tumor activities.

1. Introduction
Sargassum fusiforme is cold, bitter, with a soft solid loose knot and can be used in purging heat and water swelling, and reducing phlegm (Xie et al., 2014; Samad et al., 2017). Sargassum can regulate thyroid function, soothe blood coagulation, reduce blood pressure, reduce blood fat, lower blood sugar effect, enhance cellular antioxidant activity and enhance the body's immunity (Ma et al., 2004). Polysaccharide is not only a nutrient, but can also be used as a drug, due to its widely recognized safety and effectiveness. Polysaccharides have very obvious pharmacological effects in improving immunity and treating tumors with no side effects and is therefore one of the alternatives to treat some common diseases instead of alkaloid drugs (Nawaz et al., 2017; Han and Ji, 2014; Li et al., 2014). The sulfated polysaccharide is a semi-synthetic chemical compound and contains sulfate groups of natural polysaccharide derivatives. It is anti-virus, anti coagulation, anti-tumor, antioxidant, and may enhance the immune system and biological activities (Zaidi et al., 2017). After sulfated polysaccharides were found to have anti-tumor effects, it attracted a lot of attention from researchers. The research on polysaccharide has become a hot spot.

2. Material and methods
2.1. Instruments
Electric thermostatic water bath (Jiangsu Jintan Medical Instrument Co., Ltd), FTIR-8001 IR (Shimadzu), CO2-150 type CO2 incubator (America NBS company), Super clean bench (DL-CJ-1N medical) (Beijing East Kazakhstan Instrument Manufacturing Co., Ltd), 680 type enzyme standard instrument (U.S. Eliasa Bio-Rad Co., Ltd), pH Albert (Shanghai Instrument Co., Ltd).

2.2. Drugs
Dextran G-200 (Pharmacia), sargassum fusiforme polysaccharides (SFPs) (Life Science and Environmental Science Research Center of Harbin University of Commerce), sulfated polysaccharide from Sargassum fusiforme (Life Science and Environmental Science Research Center of Harbin University of Commerce), Adriamycin (Pfizer), Pancreatin and RPMI1640 medium (Gibco), Fetal bovine serum (Hangzhou Sijiqing Biological Engineering Co., Ltd), MTT (Sigma), Dimethyl Sulfoxide (Cangzhou Dongli Fine Chemical Co., Ltd).

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Peer review under responsibility of King Saud University.
2.3. Sulfation of polysaccharides from Sargassum fusiforme

Add 100 mg SFPs into 10 ml N,N-dimethylformamide (DMF), let the mixture go through magnetic stirring for 20 min. After it’s dissolved, transfer it into a configured esterification reagent bottle. After completion, transfer it into a water bath at a constant temperature of 85 °C for oscillation for 3 h at a constant. Cool it down to room temperature and then add 50 ml of iced water. Then add 2.5 mol/L NaOH to adjust the pH to the neutral level. Add three times volume of ethanol and keep it at 4 °C overnight. Then filter it for precipitation. Then dissolve the precipitation in distilled water for running water dialysis for 2 days, and for distilled water for 1 day. After post-dialysis contraction and dry freezing, the sulfated polysaccharide from Sargassum fusiforme is obtained.

2.4. Measuring of the content of sulfuric acid

2.4.1. Standard curve drawing

Extract 0.02, 0.06, 0.10, 0.14, 0.18, 0.20 ml of standard solution. Supplement those less than 0.2 ml by using 1 mol/L hydrochloric acid solution to 0.2 ml, and with 0.2 ml of hydrochloric acid solution as the blank reference. Add 3.8 ml of 3% trichloroacetic acid and 1.0 ml of barium chloride gelatin solution, into each tube, fully shake the tubes, then keep the tubes static at room temperature for 15 min. The absorbance measured at 360 nm wavelength is A1. Then use the 1 ml gelatin solution to replace barium chloride gelatin solution, and measure the absorbance as A2 according to the method above. Make three parallel samples at each point and obtain the mean value. In order to draw the standard curve of the vertical coordinate, make the quality of sulfuric acid (mg) as the horizontal coordinate, and the absorbance (A1−A2) as the vertical coordinate.

2.4.2. Measurement of the content of sulfuric acid

Respectively extract 0.2 ml of the sample solution, measure the (A1−A2) value according to the standard curve method, then calculate the sulfate content with the standard curve and the conversion factor. Parallel 3 times to calculate average value.

SO4\(^{2−}\) content (%) = (f × C × d/W) × 100%

Substitution degree formula: D.S. = (1.62 × S)/(S × 32 – 1.02)

In the formula, S is the content of sulfur in the sample, f is the conversion factor, C is the measured value of the sample (mg/ml), D is the dilution factor, and W is the mass of the sample (mg).

2.5. Infrared chromatographic analysis

Obtain 2 mg of polysaccharide sample, mix it with 400 mg of dry KBr. Grind the mixture in the agate mortar for 5–10 min. Tablet the mixture, and put the tablets in the infrared spectra to measure the infrared spectra of 4000–400 cm\(^{-1}\) in infrared spectrometer.

2.6. Thermal weight loss test

Respectively weigh 1.5 mg of SFPs and sulfated polysaccharide from Sargassum fusiforme, add Al\(_2\)O\(_3\) and put them into the cauldron. Increase the temperature from 30 °C to 400 °C at 10 K/min., and obtain the thermal weight loss spectra.

2.7. Measuring the survival rate of tumor cells using the MTT method

After culturing the cells for 72 h, extract the liquid from every hole. In a dark environment, Add 100 μL of 0.5 mg/ml MTT liquid into each hole, put the 96 hole plate into the incubator for another 4 h culture (Gohar et al., 2017). After 4 h, take out the 96 hole plate and add 150 μL of dimethyl sulfoxide to each hole in a dark environment and then use a micro oscillator to oscillate the solution even. Afterwards, measure the value of optical density with enzyme labeller. The reference wavelength is 490 nm, the detection wavelength is 570 nm. The following formula is used to calculate the inhibitory rate of the drug on tumor cells, and to calculate the IC\(_{50}\).

Inhibition rate = (OD\(_1\) − OD\(_2\))/OD\(_1\) × 100%

OD\(_1\): the average OD value of control group
OD\(_2\): the drug group OD.

3. Result analysis

3.1. Measuring results of polysaccharide sulfate modification

3.1.1. Sulfuric acid based standard curve drawing

As shown in Fig. 1, the measured sulfuric acid based standard curve Y = 0.8887X + 0.0042, \(R^2 = 0.9996\), the sulfate mass is within the range of 0–1.4 mg, and the polysaccharide sulfate and the absorption have a good linear correlation.

3.1.2. Measuring result of the content of sulfuric acid in the sample

See Table 1.

3.2. Measuring results by infrared spectroscopy

SFPs, sulfated polysaccharides were analyzed using IR in 4000–4000 cm\(^{-1}\), and the infrared spectra was obtained. As shown in Figs. 2 and 3, from the infrared spectra, it can be seen that the scanning result of sulfated polysaccharide indicates that the O−H stretching vibration of sulfated polysaccharides prepared at about 3400 cm\(^{-1}\) leans toward higher wave number, and that the S=O stretching vibration at about 1240 cm\(^{-1}\) OSO\(^3−\) absorption is added. These are the characteristics absorption peak of sulfuric acid ester, suggesting that the replacement of sulfuric acid ester is a success.

3.3. Heat loss test results

As shown in Figs. 4 and 5, at about 230 °C, the mass of sulfated polysaccharides decreases rapidly; at about 215 °C, the mass of SFPs decreases rapidly. This suggests that modified SFPs argalis has a more stable structure than the original fusiforme polysaccharide.

![Fig. 1. The standard curve of sulfate group.](image-url)
3.4. MTT assay of Sargassum fusiforme sulfated polysaccharide’s impact on the proliferation of tumor cells, inhibition of polysaccharide solution on gastric cancer HepG-2 cells rate curve

As shown in Fig. 6, the sulfated polysaccharide has certain inhibitory effect on HepG-2 cells, and the inhibition is significantly enhanced compared with that of SFPs. After the chemical modification of polysaccharides, the structure of Sargassum fusiforme polysaccharide has changed to some extent, enhancing the stability of the structure and having more impact on the anti-tumor effects of polysaccharide.

4. Discussion

From the infrared spectra, it can be seen that the scanning result of sulfated polysaccharide suggests that the O–H stretching vibration of sulfated polysaccharides prepared at about 3400 cm$^{-1}$ leans toward higher wave number, and that the S=O stretching vibration

| Measure times | 1   | 2   | 3   | 4   | 5   |
|---------------|-----|-----|-----|-----|-----|
| Absorbance ($A_1 - A_2$) | 0.079 | 0.084 | 0.083 | 0.079 | 0.083 |
| Average value (%) | 0.0816 |     |     |     |     |
| DS            | 0.803 |     |     |     |     |

Table 1

Contents of sulfate radical.
at about 1240 cm$^{-1}$ OSO$_3^-$ absorption is added (Zaheer et al., 2017). These are the characteristics absorption peak of sulfuric acid ester, suggesting that the replacement of sulfuric acid ester is a success.

TG test results showed that the mass of sulfated polysaccharide decreases rapidly at about 230°C while that of SFPs decreases rapidly at about 215°C, suggesting that the structure of modified polysaccharides is more stable than that of the original sulfated polysaccharide and the modification of polysaccharides can be done by using different substituents.

In this experiment, we use the MTT method to test of inhibition effects of sargassum fusiforme polysaccharide and sulfatied sargassum fusiforme polysaccharide on the proliferation of hepatoma HepG-2 cells (Muhammad et al., 2017). The experimental results show that SFPs has no obvious inhibitory effect on the proliferation of the HepG-2 cells, and so does the modified sargassum polysaccharide, but the effects of the modified polysaccharide has significantly improved than SFPs.
5. Conclusions

Polysaccharide is a kind of polymer material which is very common in our life. The cheap polysaccharide has many biological effects on human bodies. It is small and has many other advantages. It is widely used in various fields related with medicine (Kelly et al., 2014; Yang et al., 2014). The modification of the tumor cells showed effective inhibition and killing effects, and polysaccharide was regarded as the next generation of anti-tumor drugs (Yan et al., 2014). In this experiment, with MTT, we tested the effects of SFPs, sulfated polysaccharides in cultured hepatoma HepG-2 cell proliferation inhibition. The results showed that SFPs showed no obvious inhibitory effect on the growth and that the inhibitory effect of sulfated polysaccharides was not very evident, either but was better than that of SFPs. After being sulfated, polysaccharides has had some changes. The addition and variation of the substituents and the enhanced stability in the structure all improve the pharmacological effects of polysaccharides. However, through the experiment, the basic structure of high sulfated polysaccharide has hardly changed. This is the reason why the anti-tumor effect of modified polysaccharide has improved but not much. In the future, we will conduct further research on other chemical, physical and biological modification of SFPs.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (Grant No. 81274067), the Postdoctoral Foundation of China (Grant No. 2015MS81467), the Natural Science Foundation of Heilongjiang Province (Grant No. D201138), the Postdoctoral Foundation of Hei Long Jiang Province (Grant No. LBH-Z15107), the Science and Technology Innovation Team Program in Higher Education Institutions of Heilongjiang Province (2014TD009), the Harbin Special Foundation for Young Technological Innovative Talented Person (2013RFQXJ150) and the Training Program for Young Innovative Talented Person of Heilongjiang Province (UNPYSCT-2015071).

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