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Isolation of Polysaccharide from Rootstalks of *Polygonatum odoratum* and Its Characteristics

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Abstract After rootstalks of *Polygonatum odoratum* was washed with water three times, with distillated water three times, and then extracted with distillated water at the temperature of 100℃ for 30 minutes three times, the optimal conditions for the isolation of insoluble high molecular polysaccharide from the sediments was established. First of all, the influences of the temperature, volume of solvent, period that affect extraction of high molecular polysaccharide were clarified by considering the yield of polysaccharide and the effect on the number of acidocyte and blood sugar. When the sample was extracted with water (the ratio between solid and liquid =1∶8) at the temperature of 80℃ for 30 minutes, the yield of polysaccharide was the most and the number of acidocyte decreased significantly and blood sugar increased. The isolated polysaccharide formed white precipitate when alcohol was added and it became rosy yellow by reaction with phenol-sulphuric acid. When confirmed the constituent sugars of hydrolysate of the polysaccharide by PC, one spot appeared including arabinose, mannose, glucose and galactose. The constituent sugars and their composition ratio (arabinose, mannose, glucose and galactose are (0.92±0.01), (64.0±1.01), (7.3±0.2) and (0.82±0.03)%, respectively) are analyzed by infrared spectrum and GS-MS and the molecular weight was confirmed to be 100 000～150 000 by gelchromatography.

Key words *Polygonatum odoratum*, polysaccharide, acidocyte, blood sugar

Introduction

The great leader Comrade **Kim Jong Il** said as follows.

"We must not hesitate to introduce in curative and preventive work new medicines and modern medical appliances which are based on the latest achievements of medical science and technology, and raise our methods of diagnosis and treatment to the world standard as soon as possible."("ON THE FURTHER IMPROVEMENT OF THE HEALTH SERVICE" P. 13)

Solomon's seal is rootstalks of *Polygonatum odoratum* which belongs to Liliaceae.

It includes steroidsaponin, flavonoid, phlegmatic temperament, mucopolysaccharide, neutralpolysaccharide, glucose, arabinose, mannitol and etc [5－7].

So far it has been known that there is odorantan, polysaccharide which is soluble in water in *Polygonatum odoratum*. Its molecular weight is about 5 000 and its constituent sugars are fructose, mannose, glucose, galactronic acid and etc [6].

Polygonatum odoratum is used in the treatment of diabetes, dipsia, myocardial insufficiency, hyperglycemia, etc. not only in our country but also in other countries[4, 7, 8].

According to the recent research high molecular polysaccharide in *Polygonatum odoratum* is

effective in dermatopathy such psoriasis, eczema and etc [10].

While we were studying the pharmacological action of polysaccharide, we have discovered that the high molecular insoluble polysaccharide isolated from sediment of which odoratan is extracted decreases the number of acidocyte and increases blood sugar significantly and it is effective in dermatopathy such as psoriasis, eczema. Therefore we have studied on the isolation of polysaccharide from *Polygonatum odoratum* and clarification of its characteristics [1－3, 9, 10].

1. Materials and Methods

1.1. Materials

The rootstalk (loss on drying, 5%) of *Polygonatum odoratum*, glucose (A.P), arabinose (A.P), galactose (A.P) and mannose (A.P) were used in experiments.

1.2. Methods

1.2.1. The determination number of acidocyte and blood sugar

0.5mg/150g of polysaccharide isolated from *Polygonatum odoratum* was injected into tail vein of a mouse, 3 hours later, the number of acidocytes(dyeing with 1% water soluble eosin) and blood sugar (O-toluidine method) were determined and compared with the value before injection.

1.2.2. The determination of optimum extraction of high molecular polysaccharide from *Polygonatum odoratum*

The dried rootstalks severed into 1～2cm were washed with water three times and with distilled water three times.

After that they are extracted with water at 100℃, three times (each time for 30 minutes) and the sediments are crushed. The temperature and time of extraction, amounts of solvent (the ratio between solid and liquid) which affect extraction were examined by determining yield of polysaccharide and effects on the number of acidocyte and blood sugar.

1.2.3. The characteristics of high molecular polysaccharide isolated from *Polygonatum odoratum*

The isolated polysaccharide is identified by using the reaction with alcohol and phenol-sulphuric acid and by PC. Polysaccharide is assayed by phenol-sulphuric reaction its structure is determined by infrared spectrum, constituent sugars and their ratio is determined by GC-MS. Its molecular weight is determined by gel chromatography.

2. Results and Discussion

2.1. The determination of optimum extraction of high molecular polysaccharide from *Polygonatum odoratum*

2.1.1. Selection of the extraction temperature

When 10.0g of specimen are weighed accurately and extracted with 8 folds of water 3 times (30min each), the yield of polysaccharide, effect on the number of acidocyte and blood sugar according to the temperature of extraction are as follows.

Table 1. The yield of polysaccharide and number of acidocyte and blood sugar according to the temperature of extraction(n=5)

Extraction temperature /℃	Specimen /g	Yield /g	Number of acidocyte/(unit·μL^{-1})		Blood sugar/(mg·dL^{-1})	
			Before injection	After injection	Before injection	After injection
70	10.0	0.498	187.2±15.4	29.5**±6.1	77.6±3.1	118.4**±7.9
80	10.0	0.512	183.6±21.6	30.5**±5.6	78.4±4.3	119.6**±10.2
90	10.0	0.515	185.4±18.7	35.4**±7.3	79.5±5.6	117.5**±9.5
100	10.0	0.517	184.7±20.1	37.8**±8.2	80.4±3.8	113.5**±8.7

Table 1 clearly showed that at 80℃ the numbers of acidocyte decreases down to 29.5±6.1, blood sugar increases up to 118.4±7.9. On this temperature the yield of polysaccharide increased the most.

2.1.2. Selection of amounts of extraction solvent (ratio between solid and liquid)

After we weighed accurately 10.0g of specimen, we determined the yield of polysaccharide, effect on the number of acidocyte and blood sugar according to ratio between solid and liquid under the above condition (Table 2).

Table 2. The yield of polysaccharide, effect on the number of acidocyte and blood sugar according to amounts of extraction solvent (n=5)

Ratio of solid and liquid	Specimen /g	Yield /g	Number of acidocyte/(unit·μL^{-1})		Blood sugar/(mg·dL^{-1})	
			Before injection	After injection	Before injection	After injection
1:7	10.0	0.491	185.9±15.4	29.8**±3.5	80.1±5.9	122.7**±8.6
1:8	10.0	0.513	183.6±21.6	30.6**±4.9	78.4±9.1	120.1**±7.5
1:9	10.0	0.516	187.5±18.5	35.7**±5.7	80.2±8.6	117.4**±7.6
1:10	10.0	0.519	185.2±19.4	39.6**±6.3	81.3±8.7	114.5**±3.9

As seen in table 2, at the ratio of 1∶8 between the solid and liquid, the yield of polysaccharide was the highest, and the effect on the number of acidocyte and blood sugar was the best.

2.1.3. Selection of extraction time

After we accurately weighed 10.0g of specimen and extracted under the above condition, we investigated the yield of polysaccharide and effects on the number of acidocyte and blood sugar according to the time of extraction. The result is like the table 3.

Table 3. The yield of polysaccharide, effect on the number of acidocyte and blood sugar according to the time of extraction (n=5)

Period /min	Specimen /g	Yield /g	Number of acidocyte/(unit·μL^{-1})		Blood sugar/(mg·dL^{-1})	
			Before injection	After injection	Before injection	After injection
20	10.0	0.485	185.6±14.6	30.5**±7.2	78.2±7.9	120.2**±8.7
30	10.0	0.509	183.6±21.6	31.6**±7.2	79.4±9.1	118.6**±10.2
40	10.0	0.513	185.8±18.5	34.8**±5.3	81.2±8.6	115.3**±9.5
50	10.0	0.518	186.7±15.4	37.5**±4.7	82.3±8.7	111.6**±8.7

Table 3 showed that the numbers of acidocyte decreases down to 30.5±7.2 while blood sugar increases up to 120.2±8.7 significantly at the 30 minutes and yield of polysaccharide.

2.1.4. Isolation of insoluble high molecular polysaccharide from *Polygonatum odoratum*

First, 1kg of dry rootstalks are severed into 1～2cm of piece, washed with water 3 times and then distilled water 3 times. And then after it was extracted with distilled water at the temperature of 100℃ for 30 minutes, 3 times, the sediment was extracted with distilled water at the temperature of 80℃, the ratio between solid and liquid of 1:8, for 30 minutes and centrifuged with the speed of 2 000～3 000r/min, then filtered, finally if alcohol is added, polysaccharide is isolated.

2.2. The characteristics of insoluble high molecular polysaccharide isolated from *Polygonatum odoratum*

2.2.1. The identity of polysaccharide

The identity by chemical reaction After 0.15g of polysaccharide were weighed exactly and dissolved in distilled water, following reactions were carried out (Table 4).

Table 4. The identity of polysaccharide isolated from Polygonatum odoratum n=3

Reaction	Result
Reaction with alcohol	White precipitate
Reaction with phenol-sulphuric acid	Pale yellow

As seem table 4 the polysaccharide isolated from *Polygonatum odoratum* shows typical identity of polysaccharide.

The identity of constituent sugars by PC After polysaccharide is hydrolyzed with sulphuric acid, the hydrolyzate is identified by PC (Fig. 1).

As seen in Fig. 1, there were one spot besides glucose, arabinose, mannose and galactose.

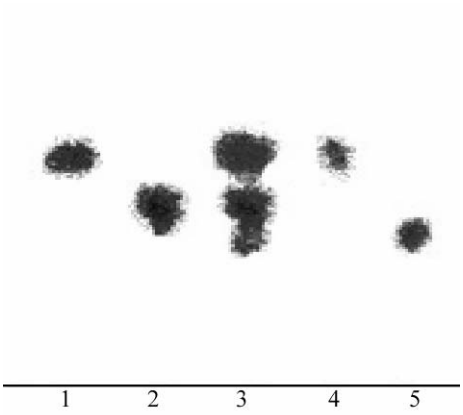

Fig. 1. PC of hydrolyzate
1－arabinose, 2－glucose, 3－spaceman, 4－mannose, 5－galactose, Elution; solvent-*n*-BuOH：pyridine：water= 6：4：3, developer－anilinphthalate

2.2.2. Assay of polysaccharide

The results of assay of polysaccharide by phenol-sulphuric acid are as follows (Table 5).

Table 5. The results of assay of polysaccharide by phenol-sulphuric acid(n=5)

Frequency	1	2	3	4	5	Average
Content/%	97.9	98.3	98.7	98.0	98.5	98.28

The result of assay showed that average deviation was 0.264, standard deviation was 0.335 and standard deviation of average value was 0.150.

2.2.3. Infrared spectrum of polysaccharide

When polysaccharide was analyzed by $\nu_{max}^{KBr}(cm^{-1})$ with infrared spectrum, it was α－glycoside with 3 470(ν_{OH}), 2 900(ν_{CH}), 1 730(ν_{CO}), 1 260, 1 055(ν_{CO}), 793.

2.2.4. Constituent sugars and their content

The constituent sugars and their content were determined by GC-MS.

Table 6 and Fig. 2 showed that constituent

Table 6. Constituent sugars and their contents

Kind	Concentration/(mg·mL^{-1})	Content/%
Arabinose	0.253±0.002	0.92±0.01
Mannose	17.6±0.3	64.0±1.01
Glucose	2.01±0.05	7.3±0.2
Galactose	0.225±0.009	0.82±0.03

sugars of polysaccharide isolated from *Polygonatum odoratum* are arabinose, mannose, glucose, galactose and their ratio is (0.92±0.01), (64.0±1.01), (7.3±0.2), (0.82±0.03)% respectively.

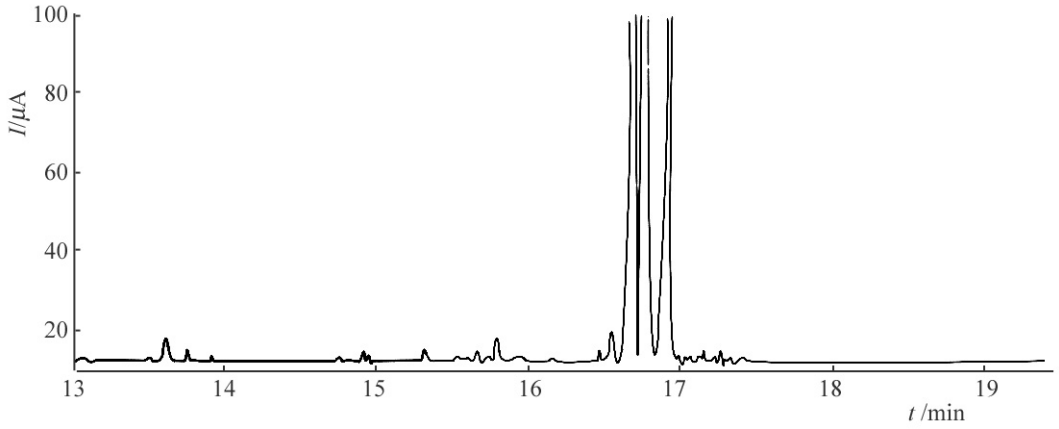

Fig. 2. GC-MS of polysaccharide

2.2.5. The determination of molecular weight of polysaccharide isolated from *Polygonatum odoratum*

When we draw elution volume according to molecular weight using different standard of dextran, T-10, T-20, T-40, equation of lg$M$=－1.014 5V + 6.153 4 is obtained (correlation coefficient, r=0.996 6).

The absorbencies according to the elution volume of the different specimen solution are determined.

As seen in Fig. 3, elution volume of the maximum absorbance of specimen solution was 20, 60mL. Therefore, the molecular weight of polysaccharide is 100 000～150 000.

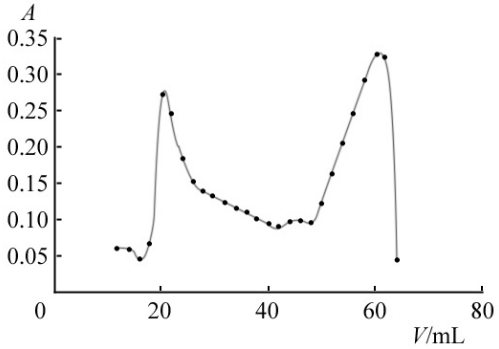

Fig. 3. Absorbencies according to the elution volume of the different specimen solution

Conclusion

First, we isolated polysaccharide form rootstalk of *Polygonatum odoratum*.

Optimum extraction conditions are as follows: temperature is 80℃, the ratio between solid and liquid－1∶8, extraction time－30min.

Second, the characteristic of polysaccharide isolated form rootstalk of *Polygonatum odoratum* are clarified.

Constituent sugars of polysaccharide are arabinose, mannose, glucose, galactose and their respective content is (0.92±0.01), (64.0±1.01), (7.3±0.2), (0.82 ±0.03)%.

Its molecular weight is 100 000～150 000.

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