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Synthesis and Characterization of Seleno-*Lilium davidii var. Unicolor Salisb* Polysaccharides

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Lilium davidii var. unicolor Salisb polysaccharides (LP) were extracted from *Lilium davidii var. unicolor Salisb* by distilled water, precipitated with ethanol. Seleno-LP were prepared by employing sodium selenite to modify the *Lilium davidii var. unicolor Salisb* polysaccharides under the catalysis of nitric acid-barium chloride. The favor reaction conditions were at 80 ºC for 14 h in the 0.8 % (v/v) nitric acid. The selenic content of seleno-polysaccharide determined by ICP- AES was 3.561 mg/g. The structural and the binding energy of oxygen differences between polysaccharide and seleno-polysaccharide were examined by FT-IR and XPS, respectively. The title products were characterized by differential thermal analysis (DTA) and thermogravimetric analysis (TG).

Key Words: *Lilium davidii var. unicolor Salisb*, Polysaccharide, Synthesis, Characterization.

INTRODUCTION

Selenium is well known for its photoelectrical and semiconductor properties and has been successfully used in solar cells, rectifiers, photographic exposure meters and xerography[1]. Selenium is also one of the essential elements for humans. It has been confirmed that selenium can improve the activity of the seleno-enzyme, glutathione peroxidase and prevent free radicals from damaging cells and tissues *in vivo*[2,3]. Selenium could delay the process of decrepitude, prevent cancer, cardiovascular disease, Keshan disease prevention, Kashin-Beck disease, hemolytic anemia and liver necrosis, improve the vision etc.[3]. Thus, selenium has been used to nourishment and medicine. Recently, selenium is attracting more and more attention due to their excellent photoelectric performance and high biological activity[4,5] and much effort has been devoted to the fabrication of selenium polysaccharides. The chemical methods based on solution-phase procedures seem to provide an excellent route to fabricate selenium polysaccharides, although a number of routine techniques are

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capable of synthesizing selenium polysaccharides. The multifold chemical methods for the synthesis of selenium polysaccharides have been developed by Gates *et al*[4,6,7], Gao *et al.*[5], Nandhakumar *et al.*[8], Abdelouas *et al*.[9] and Mees *et al*.[10]. Smith and Cheatham[11], Zhang *et al.*[12] and Zhao *et al*.[13] have shown that *Lilium davidii var. unicolor Salisb* is growing for its edible bulbs, which is exceptionally esculent and sweet compared with the other three prominent lilies in China (Yixing in Jiangsu, Luoyang in Henan, Longya in Hunan, Lanzhou in Gansu)[14]. Traditionally, *Lilium davidii* is considered as a vegetable with high nutritious value. Scientific researches have been focused on the area of breed appraisal, planting practice, evaluation of chemical components, cytology, molecular biology, health care exploitation, cross-fertilization and tissue culture[15-18]. Recently, there are observations that some botanic polysaccharides from lily bulbs have antioxidation properties, antitumor activities, immunomodulatory effect and anti-HIV function[19]. Because the bulbs of *Lilium davidii* contained high content of polysaccharides of which have been considered as having some medicinal value.

Selenium is a trace element essential to human body. However, *Lilium davidii var. unicolor Salisb* polysaccharides (LP) has the remarkable biological activity. So we expect that selenium combine with LP will have the double functions which will produce the special functions in medicine. Therefore, the combining of seleno-polysaccharides would have important theoretical and realistic meaning.

EXPERIMENTAL

The materials includes sodium selenite, ascorbic acid, sodium carbonate, sodium sulfate, nitric acid and barium chloride, were purchased from Chemical Reagent (Tianjin, China) and were used as received without further purification. The aqueous solutions of the materials were obtained as routine method. Instruments included a UV1100 Ultraviolet spectrophotometer (Beijing, China), ICP-AES DV4300 (PE Company, USA) spectrometer, a Nicolet AVATAR 360 FT-IR Spectrometer (Thermo Electron Inc., USA), XPS (PH15702, USA) spectrometer, a DE-6300 DAT/TG (Thermo Electron Co, USA).

Selenylation: Seleno-LP was performed as follows: the LP powder (1.00 g) was dissolved in 0.8 % (v/v) nitric acid 100 mL then heated by oil-bath. The sodium selenite (0.8 g) and barium chloride (1.44 g) were added when the temperature got to 80 ºC. At the end of this period, the mixture was allowed to cool to room temperature and then removed the unreacted by air pump filtration. The filtrate was adjusted to pH 5-6 by adding sodium carbonate, and sodium sulfate was added to removal of the barium ion. The obtained supernatant by centrifugation dialyzed for 12 h with tap water in dialysis tubing then with distilled water. It is not stopping dialyzing until the solution is colorless when ascorbic acid was added. The dialysate was concentrated under vacuum distillation at 60 ºC, then, precipitated with anhydrous alcohol. The precipitate was collected by centrifugation after freeze-drying in vacuum.

The UV-vis absorption spectra were obtained with dilute aqueous solution on a UV-vis spectrophotometer. The FT-IR spectra of the samples were recorded with the KBr discs in the range of 4000-400 cm^{-1} [20-21]. The ICP-AES were obtained with LP and seleno-LP dissolving in mixing acid solution perchloric acid, hydrochloride and nitric acid. Operating conditions for the instrument are presented in Table-1.

TABLE-1
OPERATION CONDITIONS FOR ICP-AES

Item	Parameter
Pump rate	20 rpm min^{-1}
R. F. power	1200 w
Nebulizer	Type of V, attached peristalic pump
Integration time	5 s
Cooling gas flux	12.0 L min^{-1}
Auxiliary gas flow rate	0.75 L min^{-1}
Solution uptake rate	1.0 mL min^{-1}
Observation height	5 mm
Observation width	0.06 mm

The surface state of the LP and seleno-LP were examined by XPS on a model of PH15702 spectrometer using Al, Ka radiation. The C 1s (E_b = 285.0 eV) peak was chosen as a reference line for the calibration of the energy scale. The magnetic measurements were performed at room temperature using a Quantum Design MPMS SQUID magnetometer. Error in weighing the samples for magnetic measurements did not exceed 0.5 %.

The thermal decomposition of LP and seleno-LP were measured on a DTA/TG device by using thermogravimetry (TG) and differential thermogravimetry (DTG). The samples were purged with nitrogen. The heat flow rate was recorded from 20 to 750 ºC at a heating rate of 10 ºC/min. Alumina was used as the standard reference material to calibrate the temperature and energy scales of the DSC instrument.

RESULTS AND DISCUSSION

Reaction principle: In the synthetic process, nitric acid had catalytic action, which is protonic acid. Therefore it could provide H^+ for reaction solution, which made the hydroxy bind with free carboxyl group more easily, while barium chloride may be selected as chelating agent.

Ultraviolet spectrum qualitative analysis of the seleno-LP [22,23]: LP (0.02 g) and seleno-LP(0.02 g) samples were added at the 50 mL-conical flask, respectively, then perchloric acid, sulfate and nitric acid (v:v = 1:1:4) 2 mL were added, The solution was cooled and transferred to the 10 mL of volumetric flask and fixed volume until it is clarification. After treated with the method of Qingtang Liu[23], the UV-vis absorption spectra of the product was obtained by spectrum scan in range of wavelength 290 to 650 nm. The result was shown in Figs. 1 and 2. In Fig. 2, there is a peak in λ = 334 nm compared with Fig. 1, which was a characteristics peak of selenium reported in the literature. The result indicated the synthetic product might contain selenium.

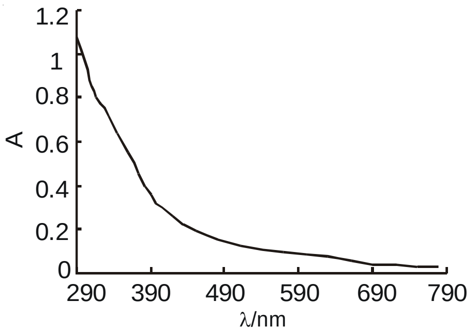

Fig.1. Ultraviolet spectrum of LP

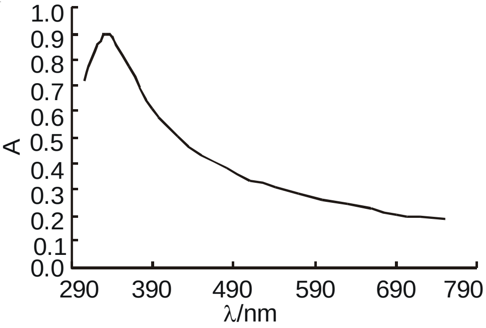

Fig. 2. Ultraviolet spectrum of seleno-LP

Determination the selenic content by ICP-AES: LP(0.02 g) and seleno-LP (0.02 g) samples were placed at the 50 mL-conical flask, respectively, then perchloric acid, hydrochloric and nitric acid(v:v = 1:1:4) 2 mL were added. The solution was cooled and transferred to the 10 mL volumetric flask and fixed volume until it is clarification. The selenic content determined by ICP-AES of LP and seleno-LP was 0 mg/g and 3.561 mg/g, which basically tallied with the UV analyses.

As shown in Fig. 3, the FTIR spectra of LP and its derivatives were found to be similar. The band between 3600 and 3200 cm^{-1} (LP: 3378 cm^{-1}; seleno-LP: 3390 cm^{-1}) represented the stretching vibration of hydroxyl. The small absorption band at around 2927 cm^{-1} was associated with stretching vibration of C–H in the sugar ring. All the absorptions were characteristic peaks of polysaccharides. The absorption

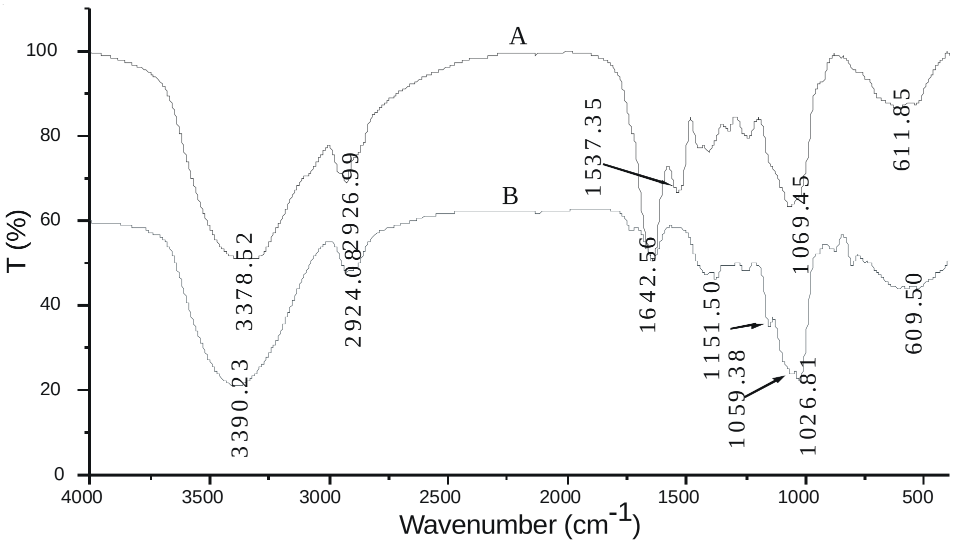

Fig. 3. FTIR spectra of LP(A) and seleno-LP(B)

at around 1643 cm^{-1} represented the amide linkage. However, there are some differences between LP and seleno-LP. The band at 1643 cm^{-1} weakened obviously after selenylation, which showed that protein decreased after selenylation. While Se only substituted S in mercapto of Met and Cys when it reacted with amino acid[24]. But formed disulfide bonds between mercaptos in Cys. So it is very difficult to form secysteine and semethionine in reaction. But it needs to be further explained through experiment.

Seleno-LP had weak peaks at 1150 cm^{-1}, but in LP this band is absent. According to previous report[25], the band between 1100 and 1130 cm^{-1} represented the stretching vibration of Si–O–Si in the organics. According to similarities between silicon and selenium, it is presumed that the peak of 1150 cm^{-1} is the stretching vibration of Se–O–Se. Fig. 3A showed the prominent band 1070 cm^{-1} was representative the vibration of hydroxyl. But in Fig. 3B, the concentration of the hydroxy group decreased because of the hydroxy group combining with carboxyl group.

Figs. 4 and 5 show the full XPS spectra of LP and seleno-LP, respectively. In XPS spectra, the order of the peaks were electrons of carbon kll, oxygen 1*s* and carbon 1*s* by which excitated the peaks, but the selenium 3*d* XPS spectra was not observed. The detection limit of XPS is 0.001 mol/g and which detects 2-8 nm in surface of substance only. But the binding energy of oxygen 1s is change greatly after the reaction. Figs. 6 and 7 showed the oxygen 1s core-level spectra for LP and seleno-LP. It was shown that the oxygen 1s for seleno-LP was higher than that of LP, but sulfur 2p XPS spectra was not observed. So we could not analyze the binding energy of sulfur 2p before and after reaction, nor determined the selenium substituted sulfur.

LP is a hydroxy aldehyde. The oxygen of hydroxyl is binding with hydrogen (–OH) before reaction, but binding with selenium (O–Se–O) after reaction. The sharing of electrons biased toward oxygen, deviated from hydrogen when oxygen

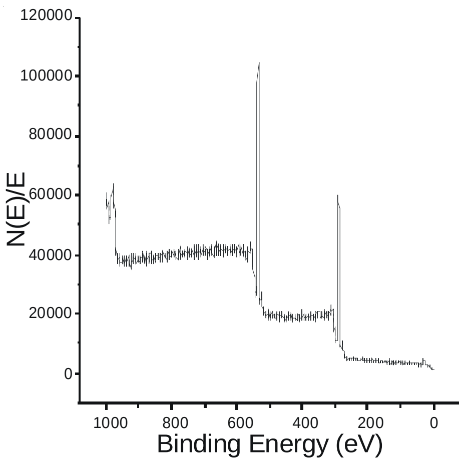

Fig. 4. XPS pattern of seleno-LP

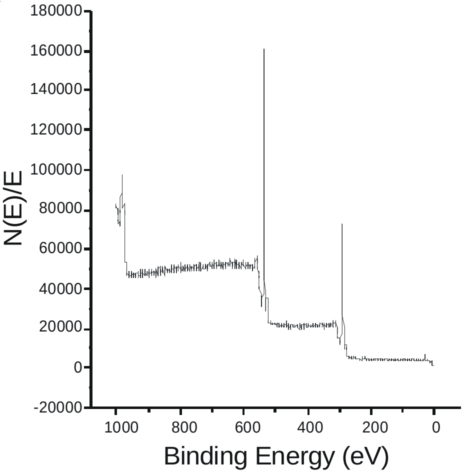

Fig. 5. XPS pattern of LP

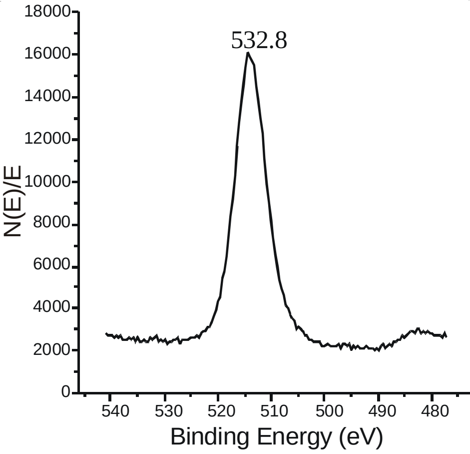

Fig. 6. Oxygen 1s core level spectra of selone-LP

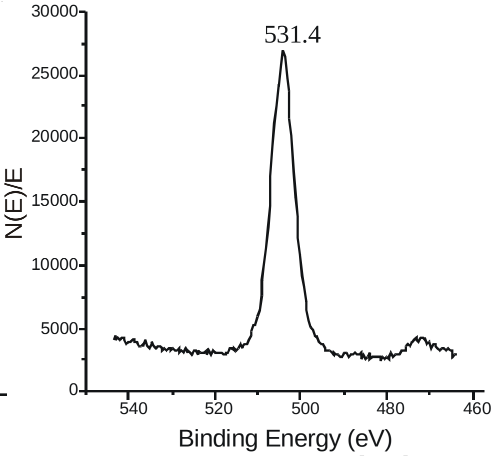

Fig. 7. Oxygen 1s core level spectra of LP

binding with hydrogen. After reaction, the oxygen binding with selenium, the binding electrons ability of selenium was stronger than hydrogen. The extent of sharing of electrons biased toward oxygen decreased, so electron density decreased around the oxygen. Therefore the binding energy of oxygen 1s increased, suggesting that the esterification is occurred between sodium selenite and LP, which was accordance with the result of UV, IR-FT and ICP-AES.

Thermal analysis: The mechanism of the thermal decomposition in nitrogen atmosphere of the dried LP and seleno-LP gels was studied by TG/DTA measurements. TG curves (Fig. 8) showed that the weight loss of LP and seleno-LP before 100 ºC were due to adsorbed water and crystal water. Difference was not obvious between LP and seleno-LP. When the temperature increased from 100 to 147 ºC, the seleno-LP began to decompose, seleno-LP decomposed completely in a moment, for synthetic ester content was very litter. Both of LP and seleno-LP began to decompose when the temperature reaches to 210 ºC. The main reason was that the long-chain polysaccharide fractured and protein and residual starch decomposed.

The initial decomposition temperature of LP and seleno-LP were 147 and 211 ºC, respectively, which were given in Table-2. From the range of the testing temperature, it is concluded that the seleno-LP stability is less than LP, due to ester formation.

TABLE-2
INITIAL DECOMPOSITION TEMPERATURE AND WEIGHT LOSS OF LP AND SELENO-LP

Sample	Initial decomposition temperature (ºC)	Weight loss rate at 320 ºC	Weight loss rate at 480 ºC	Weight loss rate at 700 ºC
LP	211	70.2	86.9	97.0
Seleno-LP	147	71.1	91.3	99.0

The DTA of LP and seleno-LP were showed in Fig. 9. The curve ordinate is temperature difference (ΔT) between sample and reference material, the curve shifted upward expressed endothermic reaction, downward expressed exothermic reaction. The two samples were similar and both of them showed three exothermic peaks and three endothermic peaks. But they are dissimilar. The difference was the first peak. The seleno-LP temperature was obvious lower than that of LP, which was an important relationship in forming esters. The three summit temperatures of endothermic peaks of seleno-LP were lower than those of LP, which was accordance with the stability between them. The DTA data of the two samples were showed in Table-3.

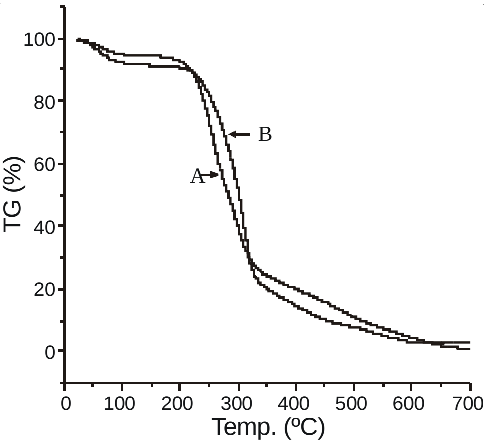

Fig. 8. TG curves recorded for the LP(A) and seleno-LP(B) gel

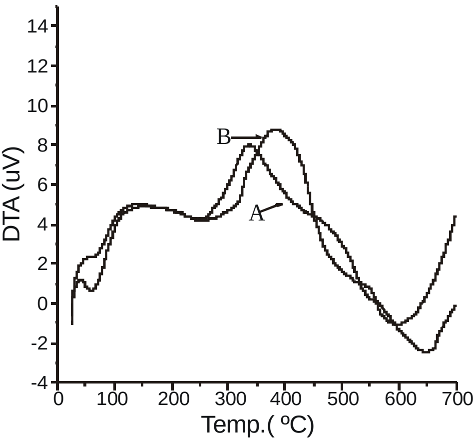

Fig. 9. DTA curves recorded for the LP(A) and seleno-LP(B) gel

TABLE-3
DIFFERENTIAL THERMAL DATE OF LP AND SELENO-LP

Sample	Temperature of exothermic peaks (ºC)	Temperature of endothermic peaks (ºC)
LP	40,158,382	61,257,597
Seleno-LP	24,142,337	57,251,647

Conclusion

For the first time, seleno-LP was synthesed by the method of nitric acid-barium chloride. The obtained seleno-LP samples were characterized by UV-Vis, ICP-AES, FT-IR, thermal analysis and XPS. It was demonstrated that there was selenium in LP by UV-Vis and ICP-AES. FT-IR and XPS showed that selenium exist in LP in form of ester, which was confirmed by thermal analysis. But failed to judge if the selenium substituted sulfur of Cys and Met and it would be carried out by our laboratory. From the results obtained, it could be concluded that seleno-LP was synthesized with the conditions: 80 ºC, 14 h, 0.8 % nitric acid (v/v) and the catalysis of nitric acid-barium chloride.

The selenic content of seleno-LP determined by ICP-AES was 3.561 mg/g, however, selenium is a trace element essential to human body and the daily intake of selenium was 50-250 μg[26]. Consequently, the seleno-LP could be potential value as drug or health products in medicine.

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