Comparison of the Polysaccharide Content of Cultivated *Dioscorea nipponica* Makino and Research on Pharmacologic Activity of Polysaccharide

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Abstract: *Dioscorea nipponica Makino, a perennial herb growing in mountainous areas of the Chinese Changbai mountains and Korean peninsula, has long been used as a folk medicine for bronchitis, asthma, and rheumatoid arthritis. Due to these therapeutic properties, wild Dioscorea nipponica Makino resources become less and less, some wild species have been tamed to be cultivated ones. In this paper, we compared the content of polysaccharide in stem root between biennial and three-year old cultivated Dioscorea nipponica Makino. The results indicated there is no significant difference between these samples, while in different harvest stages (from the early of June to the late of September), the polysaccharide content displayed significant difference ($p < 0.01$). The polysaccharide content in the Epigean leaves also showed the same change rule in the growth stages (from the late of June to the late of July). Moreover, the pharmacologic activity analysis implied that the polysaccharide of cultivated Dioscorea nipponica Makino could reduce the hyperlipidemia mice serum cholesterol (TC), triglyceride (TG) low density lipoprotein cholesterol (LDL-C) level, while increase high density lipoprotein cholesterol (HDL-C) level. These results demonstrated that the polysaccharide accumulation in biennial cultivated Dioscorea nipponica Makino almost get the same standard as three-year old ones, and the polysaccharide from epigean leaves also have some therapeutic properties as the stem root.*

Keywords: *Dioscorea nipponica Makino, Polysaccharide, Pharmacological activity.*

Introduction

In recent years, with more naturally occurring plant products gaining attention for potential use in intervention against malignant invasive progression in late stage neoplastic diseases [1-2], the need for wild medical herb is growing. Previous study has also indicated that certain foods, as well as phytochemicals of diversified pharmacological efficacies, offer significant protection against various cancers [3-5]. There is increasing focus on providing a scientific basis for use of these agents as a preventive strategy for people with high risk of cancers. Medical herb has recently become attractive as health beneficial foods and as a source material for drug development for their fewer side effects [6].

Traditionally, many species of *Dioscorea nipponica* Makino have been used as folk medicine for syndromes related to metabolic disorders. The extracts of *Dioscorea* species have been reported to have a hypoglycemic effect [7], immunostimulatory effect [8], anti-inflammatory effect [9], anti-tumor activity [10], and anti-osteoporotic activity [11]. *Dioscorea* has high saponin content and is used in folk medicine as a treatment for coronary heart disease, asthma,

rheumatoid arthritis, and hyperlipidema, as well as source of hormonal sterol synthesis precursors [12]. Recently, studies revealed that *Dioscorea* plants extracts exert anti-obesity effects [13]. Due to these therapeutic properties, wild *Dioscorea nipponica* Makino resources become less and less, some species have become endangered. In order to meet the medical demands for medicine, some wild species have been tamed to be cultivated species. Previous studies indicated that in order to get high yield and content of saponin, the best growth years for cultivated species of *Dioscorea nipponica* Makino is three-year old and optimum harvest stages is in the late of September [14]. While fewer studies focus on non saponin especially on polysaccharide.

Based on our previous studies on the saponin in cultivated *Dioscorea nipponica* Makino researches, in this paper, we compare the content of polysaccharide in stem root between biennial and three-year old and in different harvest stages of cultivated *Dioscorea nipponica* Makino, the polysaccharide accumulation in epigean leaves also elucidated, the present study aimed to expand the utilization of cultivated *Dioscorea nipponica* Makino for medical resources.

Material and methods

Samples collecting and processing

All the cultivated *Dioscorea nipponica* Makino samples collected from the experimental field in Tonghua Horticultural Institute, for the stem root, we collected the samples every 15 days from May 18th to October 15th, while for the epigean leaves, we collect samples every 6 days from June 12th to July 23rd. All the samples be dried on natural shade, and then smashed by Chinese medicine grinder (Q-500B2). Separated the samples in bag after over 100 mesh.

Polysaccharide extraction

Polysaccharide from cultivated *Dioscorea nipponica* Makino samples was prepared by the method of Wang et al. [15], the meshed samples (100 g) were put in 1.5 liter of boiling water and decocted for 2 h by a traditional method for Chinese medicinal herbs. The decoction was left to cool at room temperature, filtered and then freeze-dried to obtain crude polysaccharides were refluxed three times to remove lipids with 150 ml of chloroform: methanol solvent (2:1) (v/v). After filtering the residues were air-dried. The result product was extracted three times in 300 ml of hot water (100°C) and then filtered. The combined filtrate was precipitated using 150 ml of 95% ethanol, 100% ethanol and acetone, respectively. After filtering and centrifuging, the precipitate was collected and vacuum-dried, giving desire polysaccharide (13 g).

Polysaccharide content was measured by phenol sulfuric method [16]. Results showed that polysaccharide content may reach 98.87%.

Mouse model of hyperlipidemia lipids establishment

According to Wan et al [17], total cholesterol TC kit bought from RUIQI biotechnology limited company. Seventy kuming mice were randomly arranged into 5 treatments (10 mice per treatment) to conduct the anti-hyperlipidenmia test based on the body weight. One treatment was fed on the common diet, while the other treatments were fed on high-fat (3.0% cholesterol + 0.2% propylthioracil + 10.0% lard + 86.8% common feed) feed. After the success of hyperlipidenmia model, four treatments feed on hith-fat feed were lavaged with polysaccharide form stem root and epigean leaves of *Dioscorea nipponica* Makino at the levels of 400 mg/kg and 200 mg/kg respectively. After one-week treatment, extract blood from eyeball, after centrifuge for 10 min at 3000 r/min, keep the serum and be ready to test the level of TC, TG, HDL-C and LDL-C.

Statistical analyses

Statistical analyses in tables expressed as mean significant difference (SD) and differences between groups were assessed by analysis of variance (ANOVA). Differences were considered to be statistically significant if $p < 0.05$. All statistical analyses were carried out using SPSS for Windows, Version 11.5 (SPSS, Chicago, IL).

Results

Polysaccharides accumulation in stem root of biennial cultivated Dioscorea nipponica Makino

During the growth stages, the polysaccharides content in the stem root showed significant difference ($p < 0.01$) (Table 1), the results of variance analysis showed in different harvest stages, the content of polysaccharide displayed different change level (Table 2), compared to other harvest stages, there is no significant difference when harvest between June 17th (polysaccharide content is 8.0567%) and August 31st (polysaccharide content is 7.290%), the same results also appeared among August 16th (polysaccharide content is 7.1367%), July 17th (polysaccharide content is 7.0367%), July 2nd (polysaccharide content is 6.9367%) and August 31st (polysaccharide content is 7.290%), and compared to September 15th (polysaccharide content is 5.8667%), September 30th (polysaccharide content is 4.4967%), June 2nd (polysaccharide content is 3.7700%) and October 15th (polysaccharide content is 3.5933%), July 17th and August 1st showed significant difference.

Table 1. Variance analysis of polysaccharide content from stem root of biennial *Dioscorea nipponica* Makino during the growth stage

Variance resources	SS	df	Ms	*F*-value	*p*-value
Treatment between group	78.826	10	7.8827	78.472	0.0001
Treatment in group	2.2099	22	0.1005		
Total variance	81.0367	32			

Note: SS is Stdev freedom, df is Degree square, Ms is Mean square

Table 2. Significant difference testing of polysaccharide content from stem root of biennial *Dioscorea nipponica* Makino during the growth stage

Harvesting stages	Polysaccharide content, (%)	5% significant level	1% significant level
June 17th	8.0567	a*	A**
August 31st	7.2900	ab	AB
August 16th	7.1367	bc	B
July 17th	7.0367	c	B
July 2nd	6.9367	c	B
August 1st	6.9200	c	B
September 15th	5.8667	d	C
September 30th	4.4967	e	D
May 18th	4.2833	ef	DE
June 2nd	3.7700	fg	E
October 15th	3.5933	g	E

Note: * means with the same letter in a row are not significantly different at $p < 0.05$ level by Fisher's least significant difference tests; ** means with the same letter in a row are not significantly different at $p < 0.01$ level by Fisher's least significant difference tests

Polysaccharides accumulation in stem root of three-year cultivated Dioscorea nipponica Makino

The results of polysaccharide accumulation in stem root of three-year cultivated *Dioscorea nipponica* Makino is same as those for biennial ones. During the growth stages, the polysaccharides content in the stem root showed significant difference ($p < 0.01$) (Table 3), the results of variance analysis showed in different harvest stages, the content of polysaccharide displayed different change level (Table 4), besides harvest in the following stages July 2nd (polysaccharide content is 10.9014%) and September 30th (polysaccharide content is 11.1445%), October 15th (polysaccharide content is 9.147%) and June 2nd (polysaccharide content is 8.2446%), June 2nd (polysaccharide content is 8.2446%), July 17th (8.0305%) and August 1st (7.7841%), July 17th , August 1st and September 15th, September 15th and August 16th（6.6419%）, August 16th, June 17th and August 31st, there is no significant difference, but when harvest in other stages , there is significant difference.

Table 3. Variance analysis of polysaccharide content from stem root of three-year *Dioscorea nipponica* Makino during the growth stage

Variance resources	SS	df	Ms	*F*-value	*p*-value
Treatment between group	119.2503	10	11.925	40.387	0.0001
Treatment in group	6.49590	22	0.2953		
Total variance	125.7462	32			

Table 4. Significant difference testing of polysaccharide content from stem root of three-year *Dioscorea nipponica* Makino during the growth stage

Harvesting stages	Polysaccharide content, (%)	5% significant level	1% significant level
September 30th	11.1445	a	A
July 2nd	10.9014	a	A
October 15th	9.1417	b	B
July 2nd	8.2446	bc	BC
July 17th	8.0305	cd	BC
August 1st	7.7841	cd	BC
September 15th	7.2416	de	CDE
August 16th	6.6419	ef	DEF
June 17th	6.1524	f	EF
August 31st	5.7938	fg	FG
May 18th	4.8838	g	G

Polysaccharide content comparison between same harvesting stages and different years

According to Chinese Pharmacopoeia standard, saponin content of biennial cultivated *Dioscorea nipponica* Makino already gets medical standard. In order to explore the optimum harvest time of cultivated *Dioscorea nipponica* Makino for polysaccharide, we compared polysaccharide content for biennial and three year cultivated *Dioscorea nipponica* Makino, the results indicated that there is no significant difference (Fig. 1). for biennial ones, the maximum appeared in June 17th (polysaccharide content is 8.0567%), then the polysaccharide content almost remain the same level until August 1st, then decreased, on October 15th, the polysaccharide content gets the lowest, only 3.59%.

For three-year cultivated *Dioscorea nipponica* Makino, the first peak of polysaccharide appeared on July 2nd, then decreased, the maximum appeared in September 30th (polysaccharide content is 11.1445%).

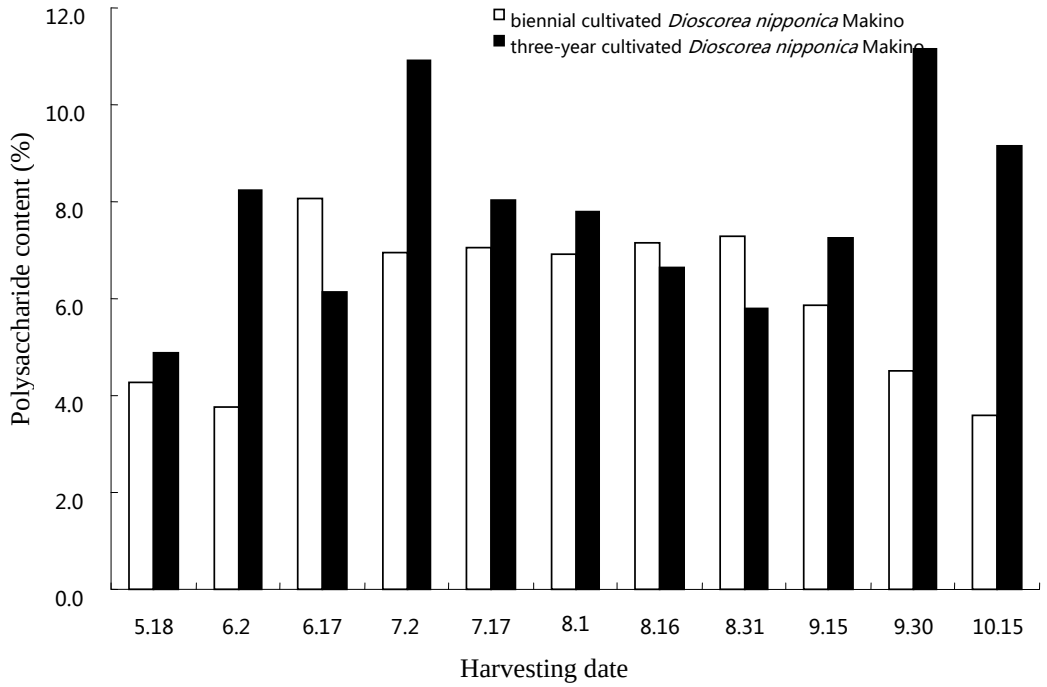

Fig. 1 Polysaccharide content in *Dioscorea nipponica* Makino stem root

Polysaccharides accumulation in epigean leaves of three-year cultivated Dioscorea nipponica Makino

From June 12th to July 24th , the polysaccharide accumulation in epigean leaves of three-year cultivated *Dioscorea nipponica* Makino displayed different significant difference (Table 5), compared to other stages, there is no significant difference when harvest in June 24th (polysaccharide content is 6.7023%) and July 18th (polysaccharide content is 5.8616%), July 24th (polysaccharide content is 4.1312%) and June 18th (polysaccharide content is 3.6006%), June 30th (polysaccharide content is 3.2139%) and July 6th (polysaccharide content is 3.2739%), the highest polysaccharide content is 5.86% when harvest on July 17th.

Table 5. Variance analysis of polysaccharide content from epigean leaves of three-year *Dioscorea nipponica* Makino during the growth stage

Variance resources	SS	df	Ms	*F*-value	*p*-value
Treatment between group	63.4319	7	9.0617	22.432	0.0001
Treatment in group	6.4633	16	0.404		
Total variance	69.8952	23			

Table 6. Significant difference testing of polysaccharide content from epigean leaves of three-year *Dioscorea nipponica* Makino during the growth stage

Harvesting stages	Polysaccharide content, (%)	5% significant level	1% significant level
June 24th	6.7023	a	A
July 18th	5.8616	a	A
July 24th	4.1312	b	B
June 18th	3.6006	b	BC
July 6th	3.2739	bc	BC
June 30th	3.2139	bc	BC
July 12th	2.4881	c	CD
June 12th	1.3533	d	D

Pharmacologic activity of polysaccharide in stem root and epigean leaves of Dioscorea nipponica Makino

Mouse model of hyperlipidemia lipids establishment

According to Wan et al [17] method, after one week treatment, total cholesterol was measured by TC kit, the results indicated that mouse model of hyperlipidemia lipids has been successfully established (Table 7).

Table 7. Total cholesterol (TC) content of mouse before and after treatment with high-fat food feeding

Groups	Mouse number	TC content (mmol/L)
Control	10	3.21
High-fat food fed	10	5.31

The effect of polysaccharide in stem root and epigean leaves of Dioscorea nipponica Makino on blood lipids reducing

After two weeks treatment of lavaging with different concentration of polysaccharide from stem root and epigean leaves of *Dioscorea nipponica* Makino on mouse, total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) extracting from serum were measured, the results showed that weather epigean leaves polysaccharide or stem root polysaccharide could decrease level of TC, TG LDL-C, while enhance level of HDL-C of hyperlipidemia lipids in serum of mouse, implied that the polysaccharide has the effect on reducing blood lipids.

Discussion and conclusion

Polysaccharide accumulation in stem root in different harvest stages of biennial cultivated Dioscorea nipponica Makino

During the growth stage, from the middle of May, polysaccharide content rose, the highest content of polysaccharide was in the middle of June, then from this stage until the end of August, polysaccharide accumulation almost remain the same level, then reduced, at the end of October, the polysaccharide content is lowest. And compared to three-year old ones, there is no significant difference. According to medical standard, if polysaccharide is considered to be the main active component, biennial cultivated *Dioscorea nipponica* Makino could be harvested for medicine.

Polysaccharide accumulation in stem root in different harvest stages of three-year cultivated Dioscorea nipponica Makino

For three-year cultivated *Dioscorea nipponica* Makino, polysaccharide accumulation has two-peaks during the growth stages, one is in the early of July (polysaccharide content is 10.9%), the other is at the end of September (polysaccharide content is 11.14%). The reason for this is that during the early of July, with vegetative growth exuberant, accumulation of photosynthetic product increase, polysaccharide content increase, then with the reproductive growth coming, most of photosynthetic product required for flower and fruit growth, polysaccharide decreased. During the end of September, with dormant comes, photosynthetic product transferred to storage organs-stem root, so accumulated plenty of polysaccharide. Form this result, we suggest that the optimum harvesting stage for three-year cultivated *Dioscorea nipponica* Makino in Changbai Mountain is at the end of September, and this conclusion is consistent with Qin's [14] research.

Polysaccharide accumulation in epigean leaves in different harvest stages of three-year cultivated Dioscorea nipponica Makino

The first highest peak of polysaccharide content in epigean leaves of three-year cultivated *Dioscorea nipponica* Makino is at the late of June, and at this stage, the synthesis of polysaccharide is more than decomposition. Considered the growth period of *Dioscorea nipponica* Makino, in the middle of June, products of photosynthesis accumulated rapidly with nutrition exuberant growth of epigean leaves, then with the coming of reproductive growth stage, the content of polysaccharide reduced. In the middle of July, the second highest peak of polysaccharide content presented with the end of florescence.

Pharmacologic activity of polysaccharide of Dioscorea nipponica Makino

Dioscin and diosgenin, the saponins present in *Dioscorea nipponica* Makino, showed a strong inhibitory effect on pancreatic lipase and suppressed the time-dependent increase of plasma TG concentration in mice [18], Han and co-workers have found that saponins prevented the high-fat diet-induced increase in parametrial adipose tissue weight by inhibiting the intestinal absorption dietary fat via inhibition of the pancreatic lipase activity. Considering relative abundance as well as diosgenin, we continued to evaluated the effect of polysaccharide on blood lipids in mice induced by feeding a high-fat feed, we do not exclude the possibility that polysaccharide in the herbal extract might have contributed to the lipase inhibition as it showed the strong lipase inhibitory activity among the polysaccharide tested (Table 8).

Table 8. The effect of polysaccharide on TC, TG, HDL-C and LDL-C level in serum of mouse model

Groups	Mouse number	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Negative control	10	3.24	1.29	1.31	0.88
Positive control	10	5.13	2.07	1.06	1.12
Polysaccharide from epigean leaves (400mg/kg)	10	3.56	1.65	1.23	0.9
Polysaccharide from epigean leaves (200mg/kg)	10	3.82	1.58	1.19	1.01
Polysaccharide from stem root (400mg/kg)	10	3.94	1.44	1.22	0.98
Polysaccharide from Stem root (200mg/kg)	10	3.68	1.72	1.27	0.94

In the present study, we found that as diosgenin accumulated, polysaccharide both in stem root and in epigean leaves of cultivated *Dioscorea nipponica* Makino could also accumulated abundance after two-year growth. However, the effective compounds of this extracted compound still need to be further clarified.

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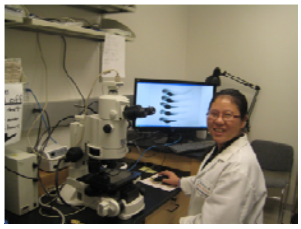

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