Research article

DISTRIBUTION OF ISOFLAVONE AND PINITOL CONTENT IN DIFFEENT PARTS OF SERICA LESPEDEZA

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ABSTRACT

Isoflavone is an important functional component that is associated with human health benefits such as the decreased risk of heart disease, menopausal symptoms, cardiovascular disease, and bone resorption as well as breast, prostate, and colon cancers. Pinitol (also referred to as D-Pinitol) is a type of sugar and classified as a chiro-inositol (sugar alcohol), has been demonstrated to exert insulin-like, anti-inflammatory and a hypoglycemic effect (to reduce blood sugar level). Sericea lespedeza (*Lespedeza cuneata*) species were collected from the different parts of Korea in the autumn season of 2011 and analyzed the isoflavone and pinitol content of leaves and stems by High-Performance Liquid Chromatography (HPLC). A large variation of isoflavone and pinitol content in the leaves and stems of lespedeza species was observed. The average isoflavone content of leaves and stems were 1,612 and 873 µg/g and ranged from 44 ~ 6,536 and 25 ~ 3,666 µg/g, respectively. Similarly, the average pinitol content of leaves and stems were 15,195 and 9,104 µg/g and ranged from 5,049 ~ 35,289 and 1284 ~ 27,185 µg/g, respectively. Both isoflavone and pinitol content in the leaves were significantly higher than in the stems. Leaves of sericea lespedeza showed unexpectedly higher isoflavone and pinitol. The average isoflavone content in leaves and stems among nine provinces were also significantly different. The highest isoflavone content in leaves (6,536 µg/g) and stems (3,666 µg/g) were measured in the samples collected from Chungcheongnam and Gangwon provinces, respectively. Similarly, the highest pinitol content in the leaves (35,289 µg/g) and stems (27,185 µg/g) were measured in the samples collected from Gyeonggi and Gangwon provinces, respectively. The genotypes with high isoflavone and pinitol content especially in the leaves of this medicinal herb could be used as elite genetic resources for food industries to make quality functional food products as well as medicines especially for diabetes.

Keywords: Sericea lespedeza, isoflavone, pinitol

INTRODUCTION

Isoflavones are secondary metabolites synthesized by 2-hydroxyisoccuring group of isoflavone and found almost exclusively in legumes (Heller & Forkmann, 1994; Liggins et al., 2000). They are a naturally occurring group of phytoestrogens categorized chemically according to their functional groups. Functional four subgroups are aglycones (genistein, daidzein, and glycitein), glycosides (genistin, daidzin, and glycitin), malonyl glycoside (malonyl genistin, malonyl daidzin, and malonyl glycitin), and acetyl glycosides (acetyl genistin, acetyl daidzin, and acetyl glycitin) (Eldridge, 1982; Kudou et al., 1991). The physiological function of isoflavone is mediated by a variety of mechanisms including estrogenic activity as well as inhibition of topoisomerase and protein kinases (Omoni & Aluko, 2005; Ososki & Kennelly, 2003). Consumption of isoflavone is positively associated with human as well as animal health benefits such as the decreased risk of heart disease, osteoporosis, menopausal symptoms, cardiovascular disease, and bone resorption and reduce the risk of breast, prostate and colon cancers (Adlercreutz et al., 1992; Allred et al., 2005; Anderson & Gardner, 1997; Anthony et al., 1996; Cassidy et al., 1994; Kim et al., 2005; Kennedy, 1995; Messina, 2000; Munro et al., 2003). Soy isoflavones alone and along with soy proteins lower serum total and LDL cholesterol in humans (Taku et al., 2007).

Pinitol is the 3-O-methyl ether of D-chiro-inositol with both enantiomers occurring in various plant sources, positively associated with health benefits like antidiabetic, anti-inflammatory antioxidant and immunosuppressive potential (Davis et al., 2000; Singha et al., 2001; Orthen et al., 1994; Singh et al., 2011) and used in the treatment of hypertension, rheumatism, cardiovascular diseases, AIDS and neurological disorders (Ostlund & Sherman, 1996; Kim et al., 2005). D-pinitol (3-O-methyl-chiro-inositol) is claimed to exert insulin-like effects and lowering blood glucose with no side effects and toxicity (Bates et. al, 2000; Sripathi et al., 2011).

Sericea lespedeza is a warm-season perennial wild legume belonging to the Fabaceae family which can grow well on soils of low fertility in an environment with relatively high humidity and high temperature. Plants belonging to the genus lespedeza are widely distributed both in Eastern North America and in Eastern Asia, and about 40 species have been reported (Han, et. al., 2010). It is recognized for its tolerance of drought and acidity and for its ability to grow in shallow soils of low fertility. Some species are used in soil conservation, forage production and in wildlife habitat improvement. Some perennial lespedeza species can be used as ornamentals also. It is native to parts of temperate and tropical Asia and Australasia (Harden, 2001), from Georgia and Afghanistan in the west through South Asia, to China, Japan and the Philippines and south throughout Southeast Asia to Papua New Guinea and Australia. It is also reported to be a common native in Korea. In the pharmaceutical field, the aerial parts of this plant have been used to protect the function of liver, kidneys and lungs in traditional Asian medicine (Kwon, et.al., 2007). Lespedeza species are known to contain flavonoids, pinitol, tannins and β-sitosterol (Matsuura, et. al., 2007), used as medicinal plants such as treating kidney disease in France, Germany and Poland (Halpern & Trolliet, 1953; Obrowsky, 1958; Kiczak & Wichert, 1964; Mertz & Keine, 1964), used in Chinese herbal medicine for treating coughs, fevers, and diarrhea (Yao et al., 2002) and used for the treatment and prevention of diabetes in South Korea since ancient times (Zhou et al., 2011). Similarly, Lespedeza species have been used as antipyretic, anti-inflammatory, and diuretic agents (Im, 1998). Several bioactive compounds including flavonoids, D-fructose, D-pinitol, sterols, and catechins, have been isolated from different species of *lespedeza* (Deng et al., 2007) which have been reported to have antidiabetic activities (Sharma et al., 2014). Flavonoids have been identified in leaves and roots of lespedeza species (Wagner et al. 1972; Linard et al. 1982; Wang et al. 1987; Li et al. 1992). Antiallergic and antioxidative compounds (lespedezols, stilbenoids, and prenylated isoflavonones) have been isolated from different lespedeza species (Miyase et al., 1999a, 1999b; Maximov et al., 2004). The highest pinitol content was observed in sericea lespedeza among seventeen different wild legumes collected inside South Korea. Among different parts of sericea lespedeza, pinitol content was highest in leaves followed by the stems, pod shells, seeds and roots (Seo et al., 2011). Overall, lespedeza plants contain many useful compounds which can contribute to human and animal health as medicinal herbs. Major arial parts of sericea lespedeza are leaves and stems and the distribution of both isoflavone and pinitol in different germplasm is not investigated. So, this study was designed to investigate isoflavone and pinitol content in different parts (leaves and stems) of lespedeza species collected from different provinces of South Korea.

MATERIALS AND METHODS

Eighty-nine Sericea Lespedeza samples were collected from nine provinces of South Korea in the autumn season of 2011. Collected plant samples were allowed to dry in natural conditions (room temperature). Seeds, leaves and stems were separated and leaves and stems were completely dried in an oven for isoflavone and pinitol extraction.

Analysis of Isoflavone

For isoflavone extraction, 0.2 g (200 mesh) of dry leaves and stems powder was added with 10 ml of 80% Ethyl alcohol (EtOH) and incubated in an ultrasonic bath at 50°C for 1 hour. The samples were then placed in a shaking incubator set at a temperature of 50°C and 150rpm for 15 hours and were filtered by 0.45μm syringe filter for isoflavone analysis using high-performance liquid chromatography (HPLC). HPLC analysis of isoflavone was based on the work of Wang & Murphy (1994). The HPLC system consisted of a TOTALCHROM V6.2.0.0.1 with LC Instrument control (PerkinElmer series 200, USA) and a COL-CHOICE C18 column 4.6 mm × 150 mm (5 μm) packed. A linear HPLC gradient used acetonitrile (solvent A) and 0.1% of acetic acid in water (solvent B). After injection of a 10 μL sample volume, solvent A was increased from 0 to 45% over 10.2 min. It was further increased from 45% to 90% over 6 min, remained constant for 3.6 min, and then was reduced from 90 to 0% over 15 min. The solvent flow rate was 1.0 mL/min. The elution was monitored by UV-absorption (series 200 UV/vis detector) at 260 nm.

Analysis of Pinitol

For pinitol extraction, dry leaves or stems powder 0.2g (50 mesh) of each sample was added with 5 ml of 50% EtOH and incubated in an ultrasonic bath at 50°C for 1 hour. The samples were then placed in a shaking incubator set at a temperature of 60ºC and 150rpm for 24 hours and were filtered using a 0.45µm syringe filter for pinitol analysis using HPLC. The HPLC system consisted of Waters 1525 instrument control (Waters Co., USA) and Waters high-performance carbo-hydrate column (Waters Co., WAT044355, 4.6 mm × 250 mm, USA) with 35ºC column temperature. Waters 410 Differential Refractometer was used as a detector. A linear HPLC gradient used 85% acetonitrile solvent. The solvent flow rate was 0.8 mL/min.

Statistical Analysis

Analysis of variance (ANOVA) and multiple mean comparisons were performed using the general linear model (GLM) by Statistical Analysis System (SAS 9.1) to identify significant treatment effects. Differences among mean values were determined using Least Significant Difference at $P \leq 0.05$. Isoflavone and pinitol content in the leaves and stems of each sample were replicated three times for its accuracy. The term isoflavone and pinitol content throughout the article stands total isoflavone and total pinitol. Data were analyzed in a completely randomized design.

RESULTS AND DISCUSSION

Isoflavone and pinitol content in leaves and stems of sericea lespedeza collected from the different regions (provinces) of South Korea are shown in Tables 1 and 2, respectively. A large variation of isoflavone and pinitol content in leaves and stems were observed among collected samples of sericea lespedeza. The mean isoflavone content of eighty-nine leaves and stems samples were 1612 and 873 µg/g and ranged from 44 ~ 6536 and 25 ~ 3666 µg/g, respectively. The highest isoflavone content in leaves (6536 µg/g) and stems (3666 µg/g) were observed in the samples collected from Chungcheongnam and Gangwon provinces, respectively (table 3). The lowest isoflavone content in leaves (44 µg/g) and stems (25 µg/g) were observed in the samples collected from Gyeonggi and Chungcheongnam provinces, respectively. This represents 148.5 and 146.6-fold differences in isoflavone content between the highest and lowest genotypes for leaves and stems, respectively. The average pinitol content of sixty-four leaves and sixty- nine stems samples were 15195 and 9104 µg/g and ranged from 5049 ~ 35289 and 1284 ~ 27185 µg/g, respectively. The highest pinitol content in leaves (35289 µg/g) and stems (27185 µg/g) were measured in the samples collected from Gyeonggi and Gangwon provinces, respectively (table 4). The lowest pinitol content in leaves (5049 µg/g) and stems (1284 µg/g) were observed in the samples collected from Jeollabuk and Gyeongsanbuk provinces, respectively. This represents 7 and 21-fold differences in pinitol content between the highest and lowest samples for leaves and stems, respectively. The distribution of isoflavone and pinitol content in both leaves and stems showed normal distribution (Figures 1 and 2). The isoflavone and pinitol content for the leaves and stems in most of the collected genotypes concentrated around the mean. Both isoflavone (1612 µg/g) and pinitol (15195 µg/g) content in the leaves were significantly higher than isoflavone (873 µg/g) and pinitol (9104 µg/g) content in the stems, respectively. Leaves of sericea lespedeza showed unexpectedly higher isoflavone and pinitol as compared to stems. This represents almost 2 and 1.5-fold differences in isoflavone and pinitol content in leaves as compared to stems, respectively. The result of higher pinitol content in leaves was consistent with the result of Seo et al., 2011. Isoflavones contents are controlled by both genetic and environmental factors (Lee et al., 2003; Prikomo et al., 2005) and pinitol content is controlled by environmental factors like temperature (Guo & Oosterhuis, 1995). Large variation in isoflavone and pinitol content in the collected samples were due to genotype of plants from where sample were collected itself and variation of the environment of collected places.

Table 1. Average isoflavone (µg/g) content in different parts of Sericea lespedeza collected from the different regions of South Korea

Parts	No of samples	Mean ± SD	Range
Leaves	89	1612 ± 1111	44 ~ 6536
Stems	89	873 ± 586	25 ~ 3666
LSD at 5%		263	

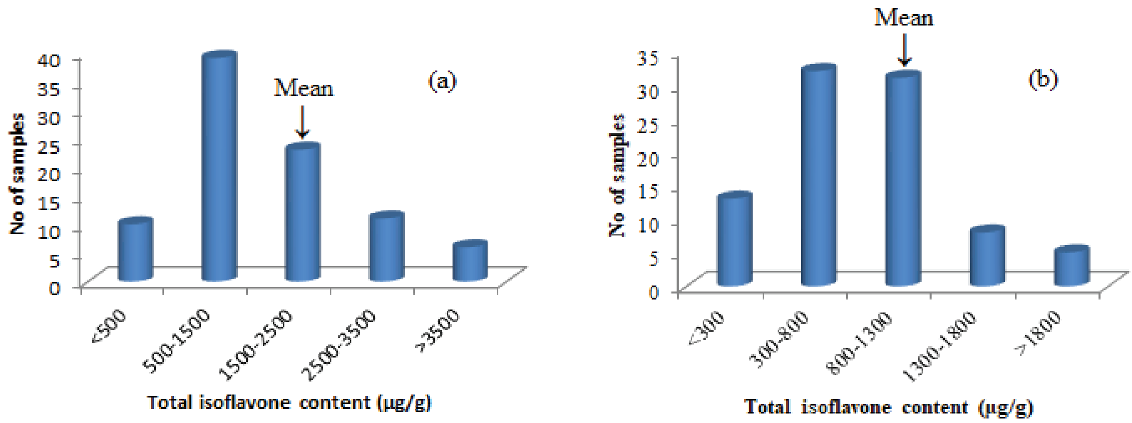

Figure 1. Distribution of isoflavone (µg/g) content in eighty-nine leaves (a) and stems (b) samples of Sericea lespedeza collected from the different regions of South Korea

Table 2. Average pinitol content (µg/g) in different parts of Sericea lespedeza collected from the different regions of South Korea

Parts	No of samples	Mean ± SD	Range
Leaves	64	15195 ± 7581	5049 ~ 35289
Stems	69	9104 ± 5649	1284 ~ 27185
LSD at 5%		2283	

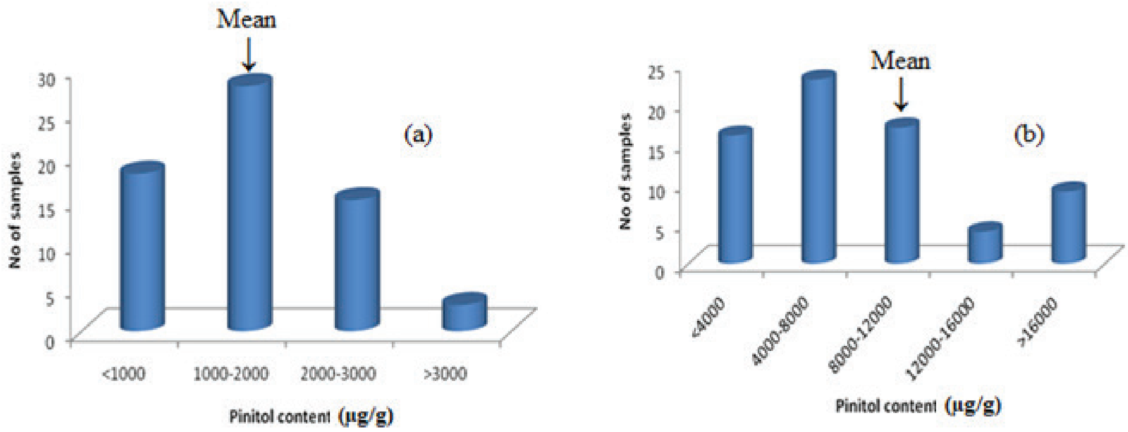

Figure 2. Distribution of pinitol (µg/g) content in sixty-four leaves (a) and sixty-nine stems (b) samples of Sericea lespedeza collected from the different regions of South Korea

Province-wise average isoflavone and pinitol content of different parts of sericea lespedeza are shown in Tables 3 and 4, respectively. The highest isoflavone content (2196 µg/g) with the range of 413 ~ 6536 µg/g in leaves was observed in samples collected from Chungcheongnam province but was not significantly different from the other eight provinces. But, significantly highest isoflavone content (1484 µg/g) with the range of 471~3666 µg/g in the stems was found in samples collected from Gangwon province which was statistically at par with isoflavone content in samples collected from Gyeongsangnam province. Similarly, the significantly highest pinitol content (21887 µg/g) with the range of 7889~35289 µg/g in leaves was observed in samples collected from Gyeonggi, followed by Gangwon province. No significant differences were observed in the pinitol content of stems among nine provinces but samples collected in Gangwon provinces showed the highest value (10549 µg/g) with the range of 4405~27185 µg/g.

Table 3. Average isoflavone content (μg/g) in different parts of Sericea lespedeza, collected from difference provinces of Korea

Province	No of Sample	Leaves		Stems	
		Mean ± SD	Range	Mean ± SD	Range
Gangwon	9	1540±921	103~817	1484±1001	471~3666
Gyeonggi	8	1762±1841	44~4676	806±561	143~1708
Gyeongsangnam	12	1576±940	574~3686	1008±375	114~1641
Gyeongsangbuk	17	1465±908	318~3626	913±612	113~2619
Jeollanam	9	1403±888	87~2592	754±548	75~1546
Jeollabuk	10	1291±832	543~3046	810±481	25~1702
Jeju	5	1408±264	1148~1769	696±180	443~944
Chungcheongnam	10	2196±827	413~6536	681±502	216~1861
Chungcheongbuk	9	1903±866	792~2932	568±218	220~919
LSD at 5%		1148		509	

Table 4. Average pinitol content (μg/g) in different parts of Sericea lespedeza, collected from difference provinces of Korea

Province	Sample size	Leaves		Stems	
		Mean ± SD	Range	Mean ± SD	Range
Gangwon	2	18946±3090	16761~21131	10549±9198	4405~27185
Gyeonggi	8	21887±11022	7889~35289	7192±7113	3162~19850
Gyeongsangnam	11	15442±6480	7945~26978	7580±4437	3939~10335
Gyeongsangbuk	13	13279±7195	6053~28946	10058±7260	1248~25110
Jeollanam	9	11298±4165	6344~17550	9178±3349	4666~16723
Jeollabuk	5	14308±8476	5049~27066	10052±5720	3816~18766
Jeju	5	-	-	8086±2491	6404~10948
Chungcheongnam	7	16354±7165	7578~25973	8765±5124	4720~19623
Chungcheongbuk	9	14366±7023	9759~30033	7912 ± 5592	1689~20271
LSD at 5%		5518		4157	

Selected five samples with the highest isoflavone and pinitol content in leaves and stems are shown in Tables 5 and 6, respectively. Among eighty-nine collected samples of seicea lespedeza, the highest isoflavone (6536 μg/g) content in leaves was found in the sample collected in Chungcheongnam province followed by Gyeonggi (4676 μg/g) province but the highest isoflavone (3666 μg/g) content in the stems was found in samples collected from Gangwon province followed by Gyeongsangbuk (2619 μg/g). Among sixty-four collected sericea lespedeza samples, the highest pinitol (both 35289 and 31823 μg/g) content in the leaves were found in samples collected from Gyeonggi followed by Chungcheongbuk (30033 μg/g) province. But, among sixty-nine samples, the highest pinitol (27185 μg/g) content in the stems was found in sample collected from Gangwon province followed by Gyeongsangbuk (25110 μg/g).

Table 5. Sericea lespedeza lines showed high isoflavone content (µg/g) in leaves and stems.

Plant parts	Area collected	Isoflavone content
Leaves	Chungcheongnam	6536
	Gyeonggi	4676
	Chungcheongnam	3835
	Gyeonggi	3714
	Gyeongsangnam	3686
Stems	Gangwon	3666
	Gyeongsangbuk	2619
	Gangwon	2563
	Gyeongsangbuk	1999
	Chungcheongnam	1861

Table 6. Selected sericea lespedeza lines showed high pinitol content (µg/g) in leaves and stems

Plant parts	Area collected	Pinitol content
Leaves	Gyeonggi	35289
	Gyeonggi	31823
	Chungcheongbuk	30033
	Gyeonggi	29994
	Gyeongsangbuk	28946
Stems	Gangwon	27185
	Gyeongsangbuk	25110
	Gyeongsangbuk	23722
	Chungcheongbuk	20271
	Gyeonggi	19850

CONCLUSION

Isoflavone and pinitol are functional components that are positively associated with human health. The isoflavone and pinitol content in the leaves and stems of collected sericea lespedeza showed significant variation. Leaves of sericea lespedeza showed significantly higher isoflavone and pinitol content as compared to the stems which indicates that leaves are more desirable in food and medicine industries. Province wise isoflavone and pinitol content was also significant. This information on genetic variability of isoflavone and pinitol content in leaves and stems of sericea lespedeza will be useful in food and medicine industries to make quality food products.

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