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재배 황기의 Phenolic Compounds 함량의 변이

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Variation of Phenolic Compounds Contents in Cultivated *Astragalus membranaceus*

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ABSTRACT : This study was conducted to determine the contents of phenolic compounds and astragalosides in *Astragalus membranaceus*. Between the phenolic compound groups, flavonoids compounds (51.13%) had higher average concentrations than the phenolic acids groups. Among the 30 phenolic compounds, quercetin (353.11 $\mu g\ g^{-1}$) provided the highest concentrations. According to different cultivation year, 3-year-cultivated *Astragalus* (2612.57 $\mu g\ g^{-1}$) showed the highest concentrations of phenolic compounds. According to different harvest days, 6/5 harvesting *Astragalus* (2615.80 $\mu g\ g^{-1}$) showed the highest concentrations of phenolic acids. Comparison between the top and subterranean parts of harvested *Astragalus* plants cultivated for 2 years, 3 years, and 5 years showed big difference of total phenolic compounds in concentrations. Further, the top part had higher amounts of the total phenolic compounds than the subterranean part among all *Astragalus*. This tendency was similar to those of the top root and lateral root. The concentration of the phenolic compounds in the top root was higher than that of the lateral root.

Key Words **:** *Astragaluds membrabaceus*, Phenolic Compounds, Variation

INTRODUCTION

Astragalus is considered to be one of the largest genera, containing about 2,500-3,000 species (annual, perennials, and shrubs) distributed throughout the northern temperate zones (Podlech, 1986). The greatest number of *Astragalus* species is found in the arid, continental regions of western North America (400 species) and central Asia (2,000 - 2,500 species). An additional 150 species are present in temperate South Africa (Liston and Wheeler, 1994).

Especially, the roots of *A. membranaceus*, called Huangqi, which is a herb native to northern China and the elevated regions of the Chinese provinces, Yunnan and Sichuan, are commonly used in traditional Chinese medicine (TCM). This drug has been used in the treatment of night sweats, deficiency of qi (e.g., fatigue, weakness, and loss of appetite) and diarrhea (Foster and Yue, 1992). *A. membranaceus* is often harvested in autumn and dried use in decorations, powder, and tinctures. It also possesses hepatoprotective, antioxidative, antiviral, antihypertensive, and immuno-stimulant properties (Rios and Waterman, 1997). *A. membranaceus* roots have been reported to contain triterpene saponins, phenolic compounds, and polysaccharides (Hirotami *et al.,* 1994; Subarnas *et al.,* 1991).

A. membranaceus is an important ingredient in many traditional Chinese formulas. It is treatment of cold sensitivity, poor circulation, and low energy as well as with *Atractylodes macrocephala* and *Ledebouriella seseloides* for

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treatment of allergies, frequentcolds, diabetes, kidney problems, prolapsed organs, anemia, and slow-healing skin eruptions. It also improves recovery and longevity in cancer patients undergoing chemotherapy or radiation treatment.

Phenolic compounds are widely distributed in plant constituents. They have traditionally thought to play an important role in plant-herbivore interaction (Feeny, 1976; Swain, 1979). Indeed, phenolic compounds play a role in virtually any interaction that a plant has with its environment, either biotic or abiotic (Niknam and Ebrahimzadeh, 2002). It is well-known that diets rich in fruits and vegetables offer protective effects against cardiovascular disease, certain forms of cancer, and perhaps other diseases as well. These protective effects have been attributed, in large part, to the presence of antioxidants, including vitamin C and β-carotene, but also carotenoids and plant phenolics such as flavonoids and phenylpropanoids (Catherine *et al.,* 1995). Flavonoids constitute a large class of ubiquitous compounds in plants, and they contain a number of phenolic hydroxyl groups attached to their ring structure, conferring antioxidant activity (Harborne *et al.,* 1986). Flavonoids and other plant phenolics have been reported to have multiple biological activities (Ho *et al.,* 1992; Kinsella *et al.,* 1993) in addition to their free radical scavenging activities (Kandaswami and Middleton, 1994), including vasodilatory (Duarte *et al.,* 1993), anticarcinogenic, antiinflammatory, antibacterial, immuno-stimulating, antiallergic, antiviral, and estrogenic effects, as well as being inhibitors of phospholipase A2, cyclooxygenase, and lipoxygenase (Brown, 1980).

As mentioned above, herbal medicines possess biologically functional substances. For this reason, more research is needed to determine whether or not *Astragalus* is safe and has few side effects. Therefore, this study focused on the concentrations of phenolic compounds in *Astragalus* plants raised for different time periods and harvested at different dates.

MATERIALS AND METHODS

1. Plant materials

Twenty-seven astragalus (*Astragalus membranaceus*) plants were obtained from the Department of Herbal Medicine Resource, Kangwon National University (Dogye Campus, Hwangjori 3, Dogye-up, Samcheok, Kangwon-Do, Korea) and Jeongseon Agricultural Technology and Extension Center (Jeongseongun, Kangwon-Do, Korea) in 2010. They were divided based on sample types as shown in Table 1.

2. Analysis of phenolic compounds by HPLC

1) Sample preparetion

Each of the *Astragalus* were stored in a vacuum drier and then ground. The extraction of *Astragalus* samples were followed a method of Kim *et al.* (2006). The ground samples (2 g) were extracted in 10 ㎖ of acetonitrile and 2 ㎖ of 0.1 N hydrochloric acid, and stirred for 2 h at room temperature. The extract was filtered through No. 42 Whatman filter paper and concentrated by a vacuum evaporator. The residues were redissolved with 10 ㎖ of aqueous methanol (HPLC grade, J. T. Baker, USA), and filtered through a 0.2 ㎛ syringe filter (TITAN, Nylon) and analyzed by HPLC equipment.

2) Analysis of Phenolic compounds

The high performance liquid chromatography (HPLC) analysis was conducted using an Agilent 1100 (Palo Alto, CA, USA) series system with photodiode array (PDA) detector (Agilent, Germany). Separation was primarily achieved by a YMC-Pack ODS AM-303 (5 ㎛, 250 ㎜ × 4.6 ㎜ I.D.) column. The absorbance was measured at 280 nm. The mobile phase was 0.1% glacial acetic acid in distilled water (solvent A) and 0.1% glacial acetic acid in acetonitrile (solvent B). The injection volume was 20 ㎕ and the gradient was followed: 0 min, 92% A: 8% B; 0~2 min 90% A: 10% B; 2 ~ 27 min, 70% A: 30% B; 27 ~ 50 min, 10% A: 90% B; 50 ~ 51 min, 0% A: 100% B; 51 ~ 60 min, 0% A: 100% B; 60 ~ 63 min, 92% A: 8% B. Run time was 63 min and flow rate was 1 ㎖ min^{-1}. The 30 kinds of phenolic compounds standards were bought from Sigma-Aldrich (USA), and used to establish calibration curves. All of the solvents were used as HPLC grade (J. T. Baker, USA). The standard stock solutions were made with dimethyl sulfoxide (DMSO). Phenolic compounds in *Astragalus* were determined based on their retention times of standards and the plotting standard concentration was obtained at three concentrations, 1, 50, 100 ppm also, a high linearity of $r^2 > 0.996$ was obtained from each curve.

3. Statistical analysis

Statistical analyses were conducted by the general linear model procedure (GLM) of the statistical analysis program

Table 1. List of *Astragalus* used in this study.

Sample No.	Cultivated location	Harvest Date	Cultivation year	Plant part	Classification
1	Taebaeg	6/5	2	top part	improved variety
2	Taebaeg	6/5	2	subterranean part - main root	improved variety
3	Taebaeg	6/5	2	subterranean part - lateral root	improved variety
4	Taebaeg	6/5	3	top part	improved variety
5	Taebaeg	6/5	3	subterranean part - main root	improved variety
6	Taebaeg	6/5	3	subterranean part - lateral root	improved variety
7	Taebaeg	6/5	5	top part	improved variety
8	Taebaeg	6/5	5	subterranean part - main root	improved variety
9	Taebaeg	6/5	5	subterranean part - lateral root	improved variety
10	Taebaeg	7/8	2	top part	improved variety
11	Taebaeg	7/8	2	subterranean part - main root	improved variety
12	Taebaeg	7/8	2	subterranean part - lateral root	improved variety
13	Taebaeg	7/8	3	top part	improved variety
14	Taebaeg	7/8	3	subterranean part - main root	improved variety
15	Taebaeg	7/8	3	subterranean part - lateral root	improved variety
16	Taebaeg	7/8	5	top part	improved variety
17	Taebaeg	7/8	5	subterranean part - main root	improved variety
18	Taebaeg	7/8	5	subterranean part - lateral root	improved variety
19	Taebaeg	9/21	2	top part	improved variety
20	Taebaeg	9/21	2	subterranean part - main root	improved variety
21	Taebaeg	9/21	2	subterranean part - lateral root	improved variety
22	Taebaeg	9/21	3	top part	improved variety
23	Taebaeg	9/21	3	subterranean part - main root	improved variety
24	Taebaeg	9/21	3	subterranean part - lateral root	improved variety
25	Taebaeg	9/21	5	top part	improved variety
26	Taebaeg	9/21	5	subterranean part - main root	improved variety
27	Taebaeg	9/21	5	subterranean part - lateral root	improved variety

(SAS, 2000). The experimental design was a completely randomized design with three replications. The least significant different (LSD) test was based on a 0.05 probability level.

RESULTS AND DISCUSSION

1. Distribution of phenolic compounds concentrations in *Astragalus*

Among 30 phenolic compounds, quercetin (353.11 $\mu g\ g^{-1}$) showed the highest average concentrations while, gallic acid (0.30 $\mu g\ g^{-1}$) revealed the lowest average concentrations. Pyrogallol, gentisic acid, chlorogenic acid, rutin and ferulic acid showed 181.00 $\mu g\ g^{-1}$, 100.47 $\mu g\ g^{-1}$, 145.50 $\mu g\ g^{-1}$, 112.95 $\mu g\ g^{-1}$ and 151.25 $\mu g\ g^{-1}$, respectively (Table 2).

Phenolic compounds can be divided into three groups. There are phenolic acids, flavonoids, and a miscellaneous group. First, the phenolic acids present were gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *ρ*-hydroxybenzoic acid, *β*-resorcylic acid, vanillic acid, caffeic acid, syringic acid, *ρ*-coumaric acid, ferulic acid, veratric acid, *m*-coumaric acid, *o*-coumaric acid, and *t*-cinnamic acid. Second, the flavonoids consisted of (+)catechin, rutin, naringin, hesperedin, myricetin, quercetin, naringenin, kaempferol, hesperetin, formononetin, and biochanin A. Third, the other compounds included pyrogallol, homogentisic acid, vanillin, and resveratrol.

In the present study, due to the diversity and complexity of the natural mixture of phenolic compounds in the *Astragalus* extracts, it was difficult to characterize every compound and assess or compare their antioxidant activities. Each *Astragalus* sample generally contained different phenolic compounds, and each of these compounds possessed different degrees of antioxidant activity. Thus, in this experiment, we can assume that the phenolic compounds in *Astragalus*, as shown in the figure, had antioxidant activity. Based on a report by Zheng and Shiow (2001), there was a positive

Table 2. Comparison of 30 phenolic compounds in *Astragalus*.

No.	Phenolic compounds name	Average concentrations ($\mu g\ g^{-1}$)	CV (%)	$LSD_{(0.05)}$
1	gallic acid	0.30	32.63	0.20
2	pyrogallol	181.00	4.97	18.49
3	homogentisic acid	67.50	1.74	2.42
4	protocatechuic acid	14.55	57.68	17.25
5	gentisic acid	100.47	19.99	41.29
6	chlorogenic acid	145.50	64.90	194.10
7	(+)catechin	72.28	49.42	73.42
8	*p*-hydroxybenzoic acid	28.45	27.27	15.95
9	*β*-resorcylic acid	26.52	19.31	10.53
10	vanillic acid	27.96	22.27	12.80
11	caffeic acid	66.91	24.13	33.19
12	syringic acid	14.06	32.09	9.27
13	vanillin	21.67	8.47	3.77
14	*p*-coumaric acid	24.64	12.52	6.34
15	rutin	112.95	1.52	3.54
16	ferulic acid	151.25	112.81	350.73
17	veratric acid	29.68	33.40	20.38
18	*m*-coumaric acid	4.42	118.98	10.82
19	naringin	140.71	29.84	86.32
20	hesperedin	143.73	4.97	14.68
21	*o*-coumaric acid	20.72	10.04	4.28
22	myricetin	136.09	49.35	138.06
23	resveratrol	155.76	16.54	52.95
24	quercetin	353.11	16.46	119.49
25	*t*-cinnamic acid	31.95	28.54	18.74
26	naringenin	49.00	20.45	20.60
27	kaempferol	46.51	8.94	8.55
28	hesperetin	38.17	81.86	64.23
29	formononetin	51.53	2.57	2.72
30	biochanin A	20.49	8.53	3.59
*phenolic acids		687.38	14.25	201.35
**flavonoids		1164.57	11.13	266.31
***others compounds		425.93	6.01	52.59
total		2277.89	9.13	427.42

*Phenolic acids; gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxybenzoic acid, *β*-resorcylic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, veratric acid, *m*-coumaric acid *o*-coumaric acid and *t*-cinnamic acid,
**Flavonoids; (+)catechin, rutin, naringin, hesperedin, myricetin, quercetin, naringenin, kaempferol, hesperetin, formononetin and biochanin A,
***other compounds; pyrogallol, homogentisic acid, vanillin and resveratrol.

linear correlation between the phenolic contents and antioxidant capacities of herbs.

Based on the data, significant variation in the phenolic content of a specific *Astragalus* was observed. Studies on the distribution of phenolic content in *Astragalus* showed that the flavonoids (51.13%) had a higher concentration compared to the phenolic acids and other groups (Fig. 1). The average value of total phenolic compounds was 75.93 $\mu g\ g^{-1}$.

Typical phenolics that possess antioxidant activity are mainly phenolic acids or flavonoids (Khknen *et al.,* 1999). Phenolic acids had been repeatedly implicated as natural antioxidants in fruits, vegetables, and other plants. For example, vanillic acid, caffeic acid, and ferulic acid are widely distributed in plants. Caffeic acid has high antioxidant activity comparable to that of quercetin (Larson, 1988). Ferulic acid has been shown to inhibit the photoperoxidation of linoleic acid at somewhat high concentrations of 10^{-3}M (Larson, 1988). The most widespread and diverse phenolic compounds are flavonoids that share the same C_{15} (C_6-C_3-C_6) skeleton and possess antioxidant activities toward a variety of

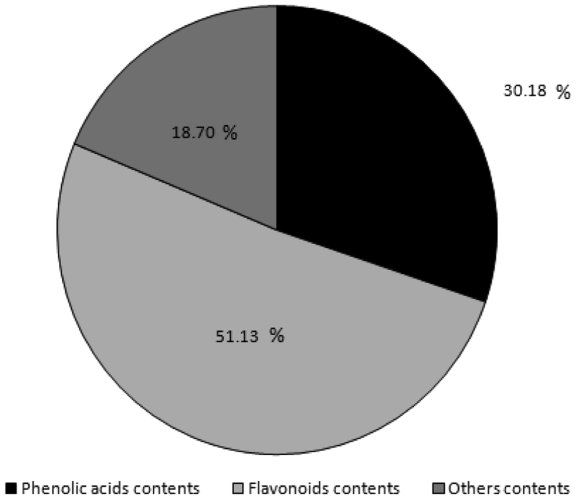

Fig. 1. The percent contents of phenolic compounds in the *Astragalus*.

oxidizable compounds (Robards *et al.,* 1999). In many herbs, the main flavonoid constituents are flavonol aglycones such as quercetin, mycicetin, kaempferol, and their glycosides (Khknen *et al.,* 1999). In general, flavonoids containing multiple hydroxy groups have high antioxidant activities against peroxylradicals than do phenolic acids. However, flavonoid glycosides, including rutin, naringin, and hesperidin, usually have low oxygen radical absorbance capacity (ORAC) values (Robards *et al.,* 1999).

In conclusion, *Astragalus* contains a high concentration of phenolic compounds and thus might have good effects as a medical plant.

2. Distribution of phenolic contents in *Astragalus* according to harvest time and cultivation year

According to different harvest time, total phenolic concentrations in *Astragalus* ranged from 2052.01 μg g^{-1} to 2615.80 μg g^{-1}. 6/5 harvesting *Astragalus* had the highest contents of total phenolic compounds (2615.80 μg g^{-1}). In contrast, 9/24 harvesting *Astragalus* had the lowest total concentrations of phenolics (2052.01 μg g^{-1}). Thus, it can be inferred that we predict a good harvest. The harvest season of *Astragalus*, June is the appropriated time of harvest in Taebaeg area. In this study, we compared phenolic compounds concentrations between different cultivation years. The total phenolic compounds according to cultivation year ranged from 1986.39 μg g^{-1} to 2612.57 μg g^{-1} in *Astragalus*. 3 years-cultivated *Astragalus* had the highest content (2612.57 μg g^{-1}) among the *Astragalus* plants

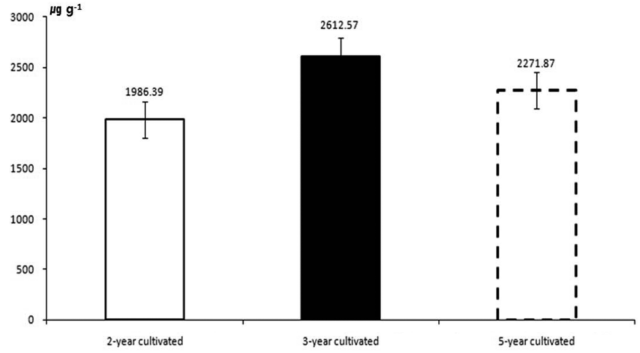

Fig. 2. The total average value of total phenolic compounds according to cultivated-year in *Astragalus*.

cultivated for different years. By contrast, 2-year-cultivated *Astragalus* had the lowest concentration (1986.39 μg g^{-1}). The average value of total phenolic concentrations was 2290.28 μg g^{-1} (Fig. 2). There was a significant pattern in the contents of phenolic compounds among the variously cultivated *Astragalus* plants. From the 2nd to 3rd year, the phenolic compound contents increased, whereas from the 3rd to 5th year, the phenolic compounds contents decreased. Thus, it can be inferred that 3-year-cultivated *Astragalus* may be of no commercial value.

Based on this data, significant variation in the contents of phenolics among specific varieties of *Astragalus* plants cultivated for different time periods was observed. The *Astragalus* cultivated for different time periods were significantly influenced by the surrounding environment. Variation in the content of secondary metabolites was due to many factors, for example a genetic component (Bowers and Stamp, 1992). However, the genotype can be modified by a variety of biotic and abiotic features. For example, seasonal changes in biochemistry were caused by shifting patterns of resource allocation, which reflect the different physiological demands associated with growth, defense, and reproduction. At the same time, environmental stresses contribute to spatial variation within and among populations (Waterman and Mole, 1994; Dudt and Shure, 1994). In order to determine factors that contribute to variations in phenolic contents between species, field or greenhouse experiments are necessary.

3. Comparison of total phenolic contents between the top and subterranean parts of *Astragalus* according to harvest time and cultivation year

Comparison of the total phenolic contents between the

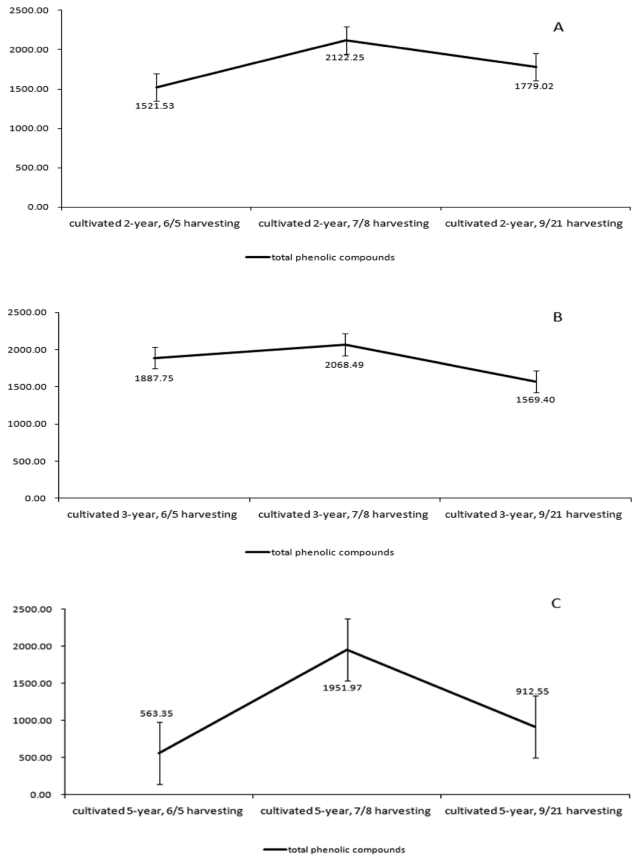

Fig. 3. The comparison of phenolic compounds contents between top part and subterranean part according to the cultivated 5-year and different harvesting-time in the *Astragalus*. A; cultivated 2-year, B; cultivated 3-year, C; cultivated 5-year.

top and subterranean parts of 2-, 3- and 5-year-cultivated *Astragalus* is shown in Fig. 3. The total phenolic contents in 2 years-cultivated *Astragalus* ranged from 1521.53 μg g^{-1} to 2122.25 μg g^{-1}, and this value reflected the phenolic content in the top part minus that in the subterranean part. 7/8 harvesting *Astragalus* cultivated for 2 years had the difference of total phenolic concentrations (2122.25 μg g^{-1}) between the top part and subterranean part. In contrast, 6/5 harvesting *Astragalus* cultivated for 2 years had the smallest gap in total phenolic content (1521.53 μg g^{-1}) between the top part and subterranean part.

The total phenolic contents in the 3-year-cultivated *Astragalus* ranged from 1569.40 μg g^{-1} to 2068.49 μg g^{-1}. 7/8 harvesting *Astragalus* cultivated for 3 years showed big difference of total phenolic concentrations (2068.49 μg g^{-1}) between the top part and subterranean part. In contrast, 9/21 harvesting *Astragalus* cultivated for 3 years revealed little difference of total phenolic content (1569.40 μg g^{-1})

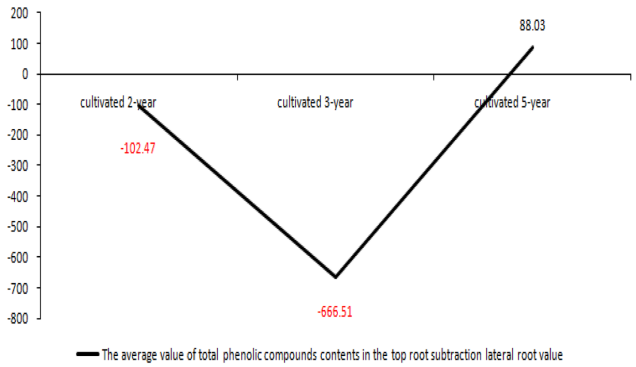

Fig. 4. The comparison of phenolic compounds contents between top root and lateral root according to the cultivated-year in the *Astragalus*.

between the top part and subterranean part. The total phenolic compounds contents in the 5-years-cultivated *Astragalus* ranged from 563.35 μg g^{-1} to 1951.97 μg g^{-1}. 7/8 harvesting *Astragalus* cultivated for 5 years had the largest gap in total phenolic content (1951.97 μg g^{-1}) between the top part and subterranean part. On the other hand, 6/5 harvesting *Astragalus* cultivated for 3 years showed small difference in total phenolic concentrations (563.35 μg g^{-1}) between the top part and subterranean part.

Significantly, the total phenolic content of the top part minus that of the subterranean part was a positive number in *Astragalus*. The higher amount of phenolics in the top part might have been attributed to the presence or absence of light. There is a well established positive relationship between the intensity of solar radiation and the quantity of phenolics produced by plants. Generally, there is a rise in total phenolic content in plants grown in sunny environments relative to those grown in shady ones. This can be seen at the intra-individual level by comparing plants exposed to different amounts of light (Mole *et al.*, 1988). An adaptive interpretation of this response in terms of plant physiological needs is that phenolics are produced as a way of reducing the photo destruction of exposed tissues. This is seen as being particularly likely whenever UV light is absorbed (Del, 1972).

4. Comparison of phenolic contents between the top and lateral roots of *Astragalus*

Comparison between the total phenolic contents of the top and lateral roots of 2, 3- and 5-year-cultivated *Astragalus* (Fig. 4). Total phenolic content in 2 year-cultivated *Astragalus* was -102.47 μg g^{-1}, and this value

was the phenolic content of the top root minus that of the lateral root. Total phenolic content in 3 year-cultivated *Astragalus* was -666.51 μg g^{-1}, and this value was the phenolic content of the top root minus that of the lateral root. Total phenolic content in 5 year-cultivated *Astragalus* was 88.03 μg g^{-1}, and this value was the phenolic content of the top root minus that of the lateral root.

So, it could infer a result from a above-mentioned shown data. Total phenolic content in lateral root of *Astragalus* plants increased upon cultivation from 2 years to 3 years. On the contrary, that of the lateral root decreased upon cultivation from 3 years to 5 years. In conclusion, total phenolic content of lateral root decreased from 3 years of cultivation.

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