

Research on Effect on Quality of Two-year-old *Astragalus Membranaceus* by Seed-Coating Treatment

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ABSTRACT: The transformed situation of the content of astragaloside, flavonoids, saponins in two-year-old astragalus membranaceus (Fisch) Bge. has been studied through the treatment of astragalus seed-coating by different concentrations (2%, 4%, 6% and 8% of seed dry weight) and the double controls of seed coated with ND general seed-coating (8% of seed dry weight) and seed uncoated. The study on one-year-old astragalus has indicated that the content of the main active component-astragaloside IV and total flavonoids in radix astragali has been increased after treated by astragalus seed-coating, and the residue of seed-coating can be fully degraded with the grow of plants. Based on the above study, this paper conducted the contrast research on the transformed situation of the active content in two-year-old astragalus, in order to further prove that the quality of astragalus can be improved through astragalus seed-coating.

KEYWORD: *Astragalus membranaceus* (Fisch) Bge; seed-coating; quality

1 MATERIALS AND METHODS

1.1 Materials

Astragalus species: astragalus membranaceus (Fisch) Bge., buying from the medical herbs company. Astragalus Seed-Coating Formulation (AM): provide by Heilongjiang August First Land Reclamation University, Chemical Control Research Department. ND Seed-Coating Formulation (ND): provide by Heilongjiang August First Land Reclamation University seed-coating Formulation Company. Rutin and Astragaloside Control Article were purchased from the National Institute for The Control of Pharmaceutical and Biological Products. Me OH (chromatographic pure), 500ml. AN (chromatographic pure), 500ml. n-butanol (A.P), 500ml. aethyl-acerbity ethyl ester(A.P), 500ml. aether (A.P), 500ml, provided by Tianjin Kermel Chemical Reagent Co., Ltd. High Performance Liquid phase waters 600 UV detector waters-2487 manufactured by the U.S. Waters company; Spectrum 756 PC UV-Vis spectrophotometer.

1.2 Methods

1.2.1 The basic conditions of the testing land

Test was carried out on the soybean test base in Heilongjiang August First Land Reclamation University (east longitude125° 9', northern latitude46° 58'), the ground expanse, crop-

producing power uniformity, alkaline hydrolysis azotei : 178.50 (mg/kg), immediate effedt phos.: 25.40 (mg/kg), immediate effedt kalium: 257.40 (mg/kg), organic matter content is 3.08%.

1.2.2 The experiment design

The test was run on May, 20th, 2006. The concrete coating method is : firstly the choice Astragalus seeds are heterotherm disposed for 12h (41 °C constant temperature soak 4h, then 25 °C keep moist closed the seeds 8h , usually change the water), and use the absorbent paper to suction the surface water, Air-dry at room temperature for 30min, then coated through the treatment of astragalus seed-coating by different concentrations (2%, 4%, 6% and 8% of seed dry weight) and the double controls of seed coated with ND general seed-coating (8% of seed dry weight) and seed uncoated as double controls. then sowing. distance between rows: 45cm, distance between hills:10cm, areola areas8m², randomized blocks arrange, administered as farmland.

1.3 Sampling and Testing Methods

On half of May in the second year after planting, sampling for astragaloside-IV, the total saponins and flavonoids content. Meantime on Mid July sampling for measure the biological yield in unit area, select the typical 8 plants for measure the underground, the ground part growth level. The measured data of

mean value as the processing astragalus group character value. The test was three times repeated.

1.3.1 Assay method of Astragaloside-IV

Testing instruments, processing conditions as follows:

Chromatographic condition:

Chromatographic column: C18, 250×4.6mm, 5μ.

Tcol: 30.0°C. DET (λ): 202nm. F: 0.8mL/min.

RNAGE: 0.05AUFS. Vinj: 20ML.

Moving phase: ACN: water =32:68.

Standard preparation concentration:750mg/L.

Processing conditions: Take the Astragalus root powder 4g, put into the Soxhlet apparatus, add Me OH 40ml, cold dipped over the night. then add Me OH 40ml,heat 80°C and backstreaming 4h, the extraction Concentrated to dry. add n-butanol saturated water 20ml with ultrasound appearance to dissolve, water saturated N-butanol extract 4th, every time 40ml, merger N-butanol. With 1% Na OH washing 3 times, every time 40ml,abandon the Na OH layer. with N-butanol saturated water wash to neutral. N-butanol layer evaporate to dry, add water 5ml, through C18 solid phase column extractor, first wash with water 3ml,then wash with 20% Me OH 5ml, abandon the eluant. Finally, wash with 60% Me OH 20ml collect the eluant, evaporat to dry. Me OH volume to 5ml, prepared for testing.

1.3.2 Assay methods of total flavonoids and saponins

Determination wavelength of total flavonoids: 510nm. Determination wavelength of total saponin: 598nm.

Take Astragalus roots and leaves powder each 2g, put into the Soxhlet apparatus separately, Plus 40ml

aether to skim until colorless, the filter paper tube evaporate aether to dry, add Me OH 40ml, heat 90 °C backstreaming extract 2h, every time 2.5h. extraction Concentrated to dry, put water 20ml into the Residues then heat to solute, sucking filtration, take the water bath phase using water saturation aethyl-acerbity ethyl ester to extract 4 times, every time 20ml, the extraction reclaimed solvent. Residues plus methanol metered volume in the volume of 25ml measuring flask, get total flavonoids, to test; mother liquid plus water saturation N-butanol to extract 4 times, every time 20ml, the extraction reclaimed solvent, Residues plus methanol metered volume in the volume of 25ml measuring flask, get total saponin to test.

2 THE RESULTS AND ANALYSIS

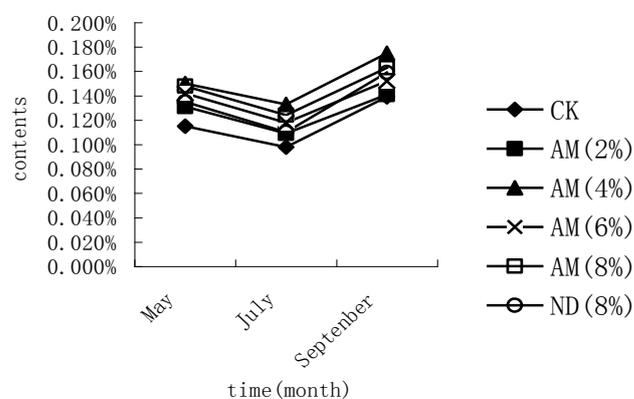


Figure1 Treatment on contents of astragalosid IV

Chart 1 Treatment on contents of total flavonoid

	May		July		September	
	leave	root	leave	root	leave	root
CK	0.387b	0.257c	0.469c	0.240b	0.518d	0.292b
AM(2%)	0.357c	0.245d	0.475bc	0.246b	0.515d	0.287b
AM(4%)	0.417a	0.293a	0.496a	0.270a	0.595a	0.310a
AM(6%)	0.393b	0.282b	0.471bc	0.247b	0.549bc	0.301ab
AM(8%)	0.410a	0.273b	0.479b	0.264a	0.554b	0.305ab
ND(8%)	0.392b	0.277b	0.464c	0.259a	0.536c	0.297b

* Letters a, b, c said that dealing with poor test new rehabilitation difference was significant, n=3

Chart2 Treatment on contents of total saponin

	May		July		September	
	leave	root	leave	root	leave	root
CK	2.061bc	1.134e	1.820f	0.714f	2.151c	0.887d
AM(2%)	2.034c	1.091f	1.834e	0.763d	2.157c	0.892d
AM(4%)	2.211a	1.206a	1.944b	1.040a	2.171ab	0.964a
AM(6%)	2.090b	1.159c	1.991a	0.903b	2.166b	0.912c
AM(8%)	2.162ab	1.177b	1.933c	0.865c	2.178a	0.943b
ND(8%)	2.112b	1.143d	1.865d	0.741e	2.162bc	0.925c

* Letters a, b, c said that dealing with poor test new rehabilitation difference was significant, n=3

2.1 The seed-coating effect on the astragalosid IV of agstragalas born in two years

Figure 1 indicates that the content of astragalosid IV can be increased after treated by Astragalus seed-coating Formulation . From each month of the second year after sowing to see, Astragaloside IV content along with the increase of Astragalus seed-coating concentration presents a dynamic changes - first increase then decline, the maximum increase group is AM (4%), compared with CK increase by 0.035%, 0.035% and 0.036%, the other coating groups have different degree raise than CK group; While compared to ND seed-coating of the same concentration, AstragalosideIV content which is processed by ND seed-coating to be lower than the Astragalus one, in each month after sowing is low by 0.013%, 0.014% and 0.004%.The above interclass empirical study has indicated that, AM (4%)treatment is the most suitable coating concentration . At the same time, Astragalus seed-coating because of its low toxicity and high-quality is better than ND seed-coating which is commonly used in the field to enhance the quality of Astragalus. Within the same group experimental research shows that: Astragaloside IV content in the germinating period in May and dormancy period in September has visibly raised to the growing period in July, which indicates that Astragalus is appropriate for spring and autumn to harvest.

2.2 The seed-coating effect on the total flavonoids and saponins of agstragalas born in two years

Treatment on content of total flavonoid as chart 1, we can see that, using astragalus seed-coating treated can improve the content of total flavonoid, AM (4%) treatment group is the max , next are AM (8%) and AM (6%) treatment. From each month after sowing to see , AM (4%) compared with CK increased by 0.036%, 0.030% and 0.018%; while compared to ND seed-coating of the same concentration , Astragaloside IV content which is processed by ND

seed-coating to be lower than the Astragalus one, in July and September after sowing are low by 0.005% and 0.008%.

The total flavonoids content of leaves also taking AM (4%) as the highest group, and over the same period which is in the roots ,show that the leaves have some use value.

Treatment on content of total saponin as chart 2, we can see that, using Astragalus seed-coating treated can improve the content of total saponin, as well as the same period the content of total saponin in the leaves obviously over which is in the roots. The two both at the highest of AM (4%) treatment. In each month after sowing, the content of AM (4%) treatment in the roots compared with CK is increased by 0.072%, 0.326% and 0.077%. the content of AM (4%) treatment in the leaves compared with CK increased by 0.15%, 0.124% and 0.02%; While compared with ND seed-coating of the same concentration, the total saponin content which is processed by ND seed-coating to be lower than the Astragalus one, in the roots each low by 0.034%, 0.124% and 0.018%, in the leaves each low by 0.05%, 0.068% and 0.016%.

3 CONCLUSION AND DISCUSSION

As the perennial legume herbaceous plant, astragalus root is one of the commonly used benefiting Chinese medicine in our motherland [1]. Pieces of astragalus are widely used in clinical prescriptions, which have many pharmacological effects. such as activating heart, anti-myocardial ischemia, improving immunity, reduce blood sugar, anti-aging, the protection of organs and so on [2-3].Seedling diseases of astragalus will directly affect the quality of medicines. The use of seed-coating formulation can increase seed germination rate and promote the healthy growth of seedlings. Thus the development of high efficiency and low toxicity of astragalus seed-coating formulation will play an important role in improving the quality of the medicine.

Astragalosides IV and total Saponin are the characteristic ingredient in traditional Chinese medicine. Astragaloside IV was included under the identification of astragalus item in Pharmacopoeia of Chinese. State the qualitative and quantitative determination of standards [4]. Japanese also makes it as a quantitative target for the evaluation of astragalus quality [5]. At present, astragalus medicinal herbs and the single and compound chinese drug preparations usually use the content of astragaloside IV and total saponin as the agents to evaluate the quality of traditional Chinese medicine [6]. In recent years, Zhang Guijuan et al. studies have shown that the total flavonoid in astragalus also has a wide range of pharmacological activities [7].

In this experiment, studies have shown that appropriate concentration of Astragalus seed-coating can increase the content of astragaloside IV, total flavonoid and total saponin. At the same time, on the content of active ingredient, in the same concentration, the treatment of Astragalus seed-coating is more effective than ND seed-coating which is commonly used in field crops.

In recent years, study has found that Astragalus stems and leaves contain the same Astragaloside IV, flavonoids and polysaccharides ingredients, which indicates that the stems and leaves as roots also have some important medicinal value [8,9].

The correlation studies have shown that dealing with the appropriate concentration of Astragalus seed-coating can increase the stem weight, and raise the total biological production, which is combined with experiments of this study showing that Astragalus seed-coating can significantly improve the quality of such medicinal herbs.

With the people all over the world more and more favoring the viridis Chinese medicine, the issue of pesticide residue in the drug gradually catches more and more peoples' attention [10]. The study on the one-year-old samples that are treated by Astragalus seed-coating have showed that the pesticide residue can be totally degraded in 150 days after the sowing.

By multiple comparison, we considered that between 4% and CK the difference was significant, so 4% was the best coating concentration.

Our test also shows that Astragalus IV content in the germinating period in May and dormancy period in September are higher than other periods, which indicates that Astragalus is appropriate for spring and autumn to harvest, which is coincide with the traditional harvest way.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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