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Research Article

Dynamic Changes the Content of Luteolin in Different Parts of Pennycress (*Thlaspi arvense* L.) During the Growth Period

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Abstract: Dynamic changes the content of luteolin were detected in different parts(roots, stems, leaves)of pennycress in growth stage(seedling stage, flowering stage, fruiting stage)by HPCL analysis. The results revealed that luteolin showed a good linear relationship at a range of 0.1~1.0μg/mL, r=0.9972.The average recovery rate was 98.6%,and RSD was 1.26%(n=5).In different growth stage, the content of luteolin was different in the same part of pennycress, seedling stage>flowering stage>fruiting stage, and had significant difference($P<0.05$).In the same growth stage, the content of luteolin was different in different parts(root, stem, leaf)of pennycress, leaf>stem>root, and had significant difference($P<0.05$).This conclusion provided valuable reference for pennycress reasonable planting and utilization, quality control.
Keywords: Pennycress (*Thlaspi arvense* L.), HPLC, Luteolin, Different Parts, Growth Period.

INTRODUCTION

Pennycress (*Thlaspi arvense* L.) was one of traditional Chinese medicines and belonged to Pennycress Cruciferae Capparales Dicotyledoneae. It distributed throughout the country. Its nature was acrid and warm, it had the efficiency of clearing away toxic material in liver and making the eyes bright, regulating the middle warmer and promoting diuresis to eliminate dampness, clearing away toxic material and relieving swelling. Chemical composition of pennycress were studied by other scientists[1-17], they found that pennycress contained luteolin, the new edition of Chinese Pharmacopoeia had the content of luteolin as one of the main indexes in evaluating efficacy of drugs. However, this issue that dynamic changes the content of luteolin in parts of pennycress during the growth period, few people had studied it systematically at home and abroad, so far, it was exploited and utilized as medicine mainly on seeds or whole plant, but pennycress roots had been regarded as non-medicinal parts for a long time, and did not get the full development and utilization. Dynamic changes the content of luteolin was detected in pennycress different parts in growth stage by HPCL analysis in this paper. And the separation effect of this method was good, simple operation, convenient, for reasonable planting and utilization and quality control to provide valuable reference.

MATERIALS AND METHODS

Instruments:

Agilent 1200 series high performance liquid chromatography(Agilent, USA),KQ-300VDE tri frequency of NC Ultrasonic Cleaner was purchased from Kunshan Ultrasonic Instrument Company Limited,BS-110s electronic balance was purchased from Beijing Sartorius instrument system company, super high speed grinder was purchased from Hong Kong Shanzhu Company Limited, centrifuge TG16W was purchased from Changsha Pingfan instrument limited company, UV visible light spectrophotometry meter(by uv-2550,Shimadzu,Japan).

Reagents:

Luteolin was purchased from Chengdu stegmann Biological Technology Company Limited, methanol and acetonitrile are identified to be chromatographically pure by Tianjin Siyou Company Limited, anhydrous ethanol is identified to be analytical pure by Tianjin Siyou Company Limited, pure water was purchased from Hangzhou Wahaha Company Limited.

Pennycress Materials

The full sample pennycress whole grass was used in the study. It was grown in 2014 in Shanxi, China. Root, stem and leaf samples were collected respectively when they grew to 30 days at seedling stage,60 days at flowering stage,90 days at fruiting stage. The harvested pennycress were dried to 10% moisture and then stored at room temperature until time of experiment.

Chromatographic Conditions

The pennycress extracts were filtered through a nylon syringe filter(0.45μm)(Filtrex Technology, Singapore)prior to HPLC analysis and analyzed in Agilent 1200 series HPLC chromatograph system equipped with Agilent Zorbax Eclipse XDB-C18 (150mmx4.6mm,5μm),using acetonitrile(A)-water(C),20%A:80%C as the mobile phase, The injection volume was 10μL,the flow rate was 1.0 mL/min at 30℃,while the monitored wavelength was 227nm.

Preparation of Standard Solution

Luteolin standard sample was weighed precisely 1.0mg, and was reconstituted with methanol to a volume of 10 mL, the concentration of solution was 0.10mg/mL. The solution was filtered through a nylon syringe filter(0.45μm),frozen and stored at refrigerator until used.

Preparation of Sample Solution

The harvested pennycress were dried to 10% moisture, root, stem and leaf samples were collected respectively when they grew to 30 days at seedling stage, 60 days at flowering stage, 90 days at fruiting stage, all samples were milled with a micro plant grinding machine set at a fine setting of 0.5mm,and were weighed precisely each 1g, were placed in 100 mL Erlenmeyer flask respectively, mixed 80% methanol with water, and poured a conical flask, the most suitable condition for the extraction is as follows: the frequency of supersonic wave was 45kHz and the temperature of extraction was 80℃and the time of extraction was 2h, and the liquid leaching ratio was 1:40.Extract liquid was poured into a centrifuge tube, and then the extract liquid were centrifuged at 4000r/min for 20 min in a centrifuge. Took supernatant, the solution was filtered through a nylon syringe filter (0.45μm), frozen and stored at refrigerator until used.

System Suitability Experiment

Standard solution and all sample solution were weighed precisely, filter the solution can be analyzed under chromatographic conditions, recorded the chromatograms. Identification of the luteolin was performed by comparisons to the retention time and UV spectra of authentic standards from Sigma.

Linear Relationship Study

Luteolin standard solution(0.1mg/mL)was weighed precisely 100, 200, 400, 600, 800μL and 1mL, were placed in 10mL volumetric flask respectively, dilute to a final volume of 10 mL. Get the concentrations of standard series were 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 μg/mL. The standard series were filtered through a nylon syringe filter (0.45μm), filter the solution can be analyzed under chromatographic conditions, recorded the peak area and retention time. Make the standard curve of the concentration of standard solution was used as abscissa(X) and the peak area was used as ordinate(Y).

Precision Test

Luteolin standard solution was weighed precisely 10μL, filter the solution can be analyzed 5 times repeatedly under chromatographic conditions, recorded the peak areas.

Repetitive Experimental

Samples of leaf at the same growth stage was weighed precisely, parallel sample solution (n=5) were prepared according to method of preparation of sample solution, filter the solution can be analyzed under chromatographic conditions, recorded the peak area.

Stability Experiment

Sample solution of leaf at the same growth stage was weighed precisely 10μL, filter the solution can be analyzed under chromatographic conditions in the 0, 4, 8, 10,12h respectively, recorded the peak area.

Sample Average Recovery Test

Sample solution of leaf at the same growth stage had been measured was weighed precisely (n=3),each about 1.00mL,then poured into luteolin standard solution 1mL respectively, filter the solution can be analyzed under chromatographic conditions.

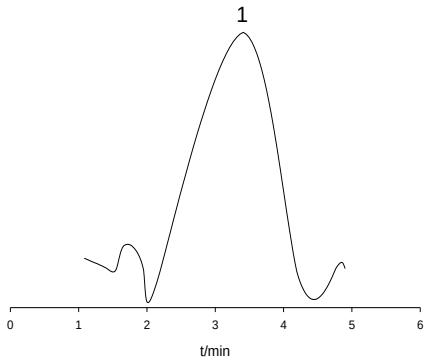
Determination of the Content of Luteolin in Sample

Sample solution had been prepared, each sample solution 3 portions, filter the solution can be analyzed under chromatographic conditions respectively, peak area of the content of luteolin in sample solution was measured. The content of luteolin in leaves, stems, roots of pennycress were calculated by external standard method.

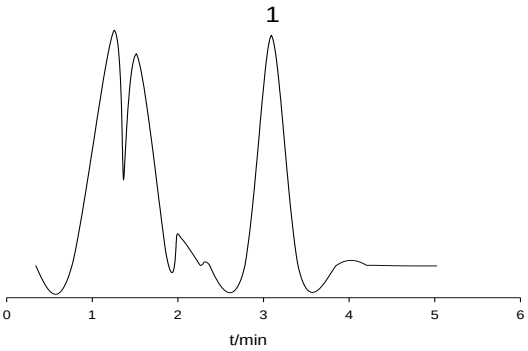
RESULTS AND DISCUSSION

Results of System Suitability Experiment

Figure1 showed that under the chromatographic conditions, the chromatographic peaks of luteolin and other components were completely separated in standard solution and all sample solution, the degree of separation exceeded 1.5,the theoretical plates of luteolin were not lower than 3000,with good separation effect. HPLC was the traditional technique for the analysis of compounds. In order to intuitively compare luteolin changes, HPLC chromatogram of standard solution and all sample solution were shown in Figure 1.


1
0 1 2 3 4 5 6
t/min

(A)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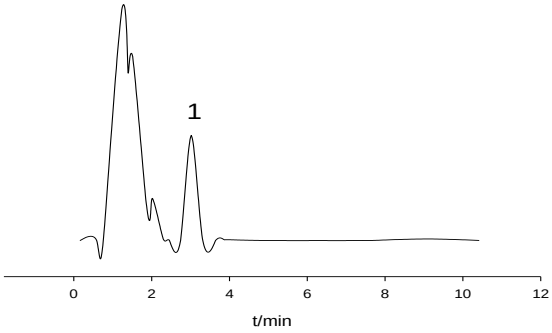
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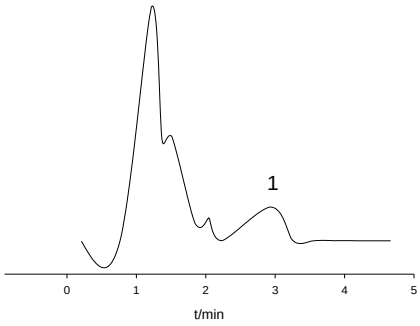
0 2 4 6 8 10 12
t/min

(C)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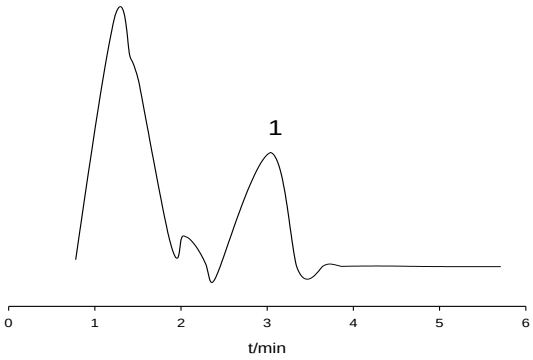
0 1 2 3 4 5
t/min

(D)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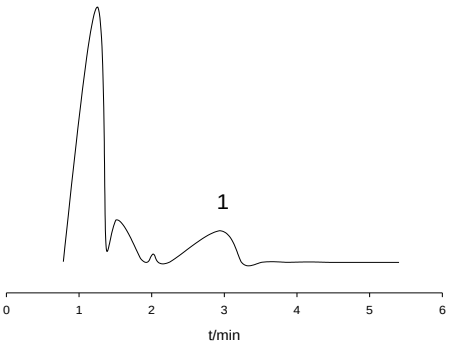
0 1 2 3 4 5 6
t/min

(E)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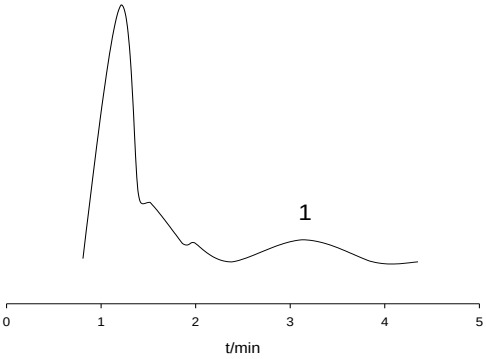
0 1 2 3 4 5 6
t/min

(F)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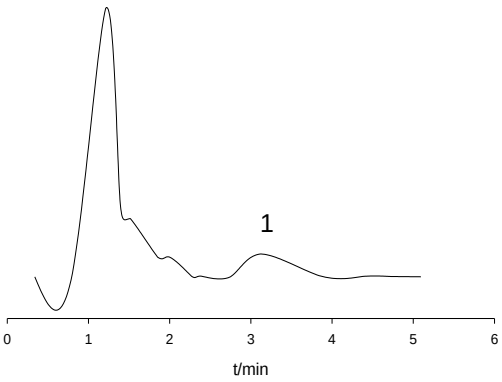
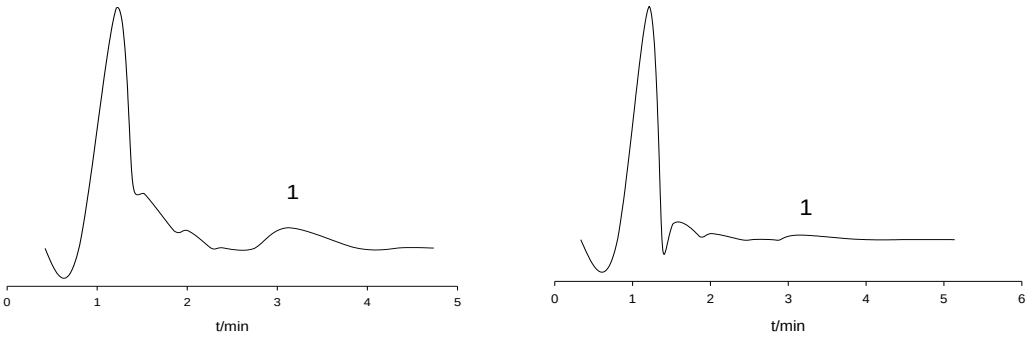
0 1 2 3 4 5
t/min

(G)


1
0 1 2 3 4 5 6
t/min

(H)



(I) (J)

1-luteolin

Figure 1 HPLC chromatogram of standard solution(A), HPLC chromatogram of sample solution of leaves of pennycress at seedling stage(B), HPLC chromatogram of sample solution of leaves of pennycress at flowering stage(C), HPLC chromatogram of sample solution of leaves of pennycress at fruiting stage(D), HPLC chromatogram of sample solution of stems of pennycress at seedling stage(E), HPLC chromatogram of sample solution of stems of pennycress at flowering stage (F), HPLC chromatogram of sample solution of stems of pennycress at fruiting stage(G), HPLC chromatogram of sample solution of roots of pennycress at seedling stage(H), HPLC chromatogram of sample solution of roots of pennycress at flowering stage(I), HPLC chromatogram of sample solution of roots of pennycress at fruiting stage(J).

Results of Linear Relationship Study

The linear equation was Y=37.059X+58.472 with the r=0.9972, showed that luteolin had a good linear relationship at a range of concentrations of 0.1~1.0μg/mL (Figure 2).

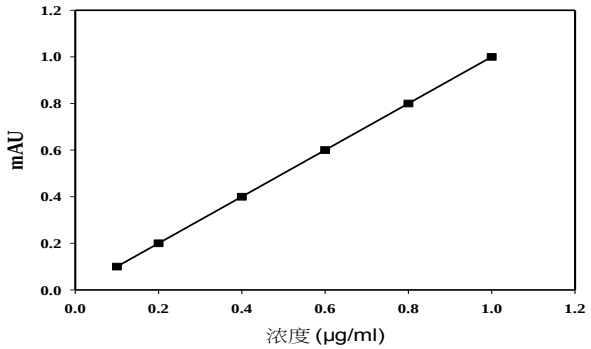


Fig-2 standard curve of luteolin

Results of Precision Test

The RSD of measurement precision test was 0.37 %(n=5).The results showed that the precision of instrument was very good.

Results of Repetitive Experimental

The RSD of the peak area of luteolin was 0.58%.The results showed that the repeatability of method was good.

Results of Stability Experiment

The RSD of the peak area of luteolin was 0.87% (n=5).The results showed that the stability of sample solution within 12 hours was good.

Results of Sample Average Recovery Test

The average recovery was 98.6%, the RSD was 1.26%.The results showed that the accuracy of this method was good.

Results of Determination of the Content of Luteolin in Sample

The results were showed in table 1-3

Table-1: The determination results of the content of luteolin in samples in seedling stage (n=3)

Sample Number	The Content of Luteolin (%)	Mean Value (%)
Root 1	0.0018	
2	0.0022	0.0021
3	0.0025	
Stem 1	0.0113	
2	0.0129	0.0115
3	0.0104	
Leaf 1	0.0879	
2	0.0987	0.0931
3	0.0928	

Table-2: The determination results of the content of luteolin in samples in flowering stage (n=3)

Sample Number	The Content of Luteolin (%)	Mean Value (%)
Root 1	0.0011	
2	0.0012	0.0011
3	0.0009	
Stem 1	0.0063	
2	0.0057	0.0063
3	0.0068	
Leaf 1	0.0587	
2	0.0614	0.0601
3	0.0602	

Table-3: The determination results of the content of luteolin in samples in fruiting stage (n=3)

Sample Number	The Content of Luteolin (%)	Mean Value (%)
Root 1	0.0008	
2	0.0005	0.0007
3	0.0009	
Stem 1	0.0045	
2	0.0045	0.0045
3	0.0045	
Leaf 1	0.0319	
2	0.0326	0.0319
3	0.0313	

Conclusively, pennycress had rich luteolin, and had great pharmacological activity, it was worth for people to develop and utilize. However, it was great waste for pennycress this excellent resource to study it rarely at home and abroad at present. This experiment aimed at this situation, dynamic changes the content of luteolin were detected in different parts(roots, stems, leaves)of pennycress in growth stage(seedling stage, flowering stage, fruiting stage)by HPCL analysis that was established at the first time. This method provided scientific basis for determining the effective medicinal parts of pennycress, and laid foundation for further research on its functions. Results of this experiment showed that luteolin existed in root, stem, leaf of pennycress; the content of luteolin was different in different parts of pennycress and in different growth stage. In different growth stage, the content of luteolin was different in the same part of pennycress, seedling stage>flowering stage>fruiting stage, and had significant difference($P<0.05$).In the same growth stage, the content of luteolin was different in different parts (root, stem, leaf) of pennycress, leaf>stem>root, and had significant difference($P<0.05$).The study of this experiment found that the content of luteolin was higher in leaf of pennycress, and in the seedling stage, leaf contained the content of luteolin was the most in whole growth stage. Therefore, it was suggested that pennycress should be picked at the seedling stage and should make full use of the leaf.

Acknowledgement

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