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Development of an efficient method for protein isolation from Reynoutria × bohemica Chrtek et Chrtkov

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Reynoutria × bohemica Chrtek et Chrtkova is a perennial herbaceous plant of the genus Reynoutria of the family Polygonaceae, the hybrid of Reynoutria japonica Houtt. × Reynoutria sachalinensis (F. Schmidt) Nakai of central European origin, first described in 1983. It is introduced to many regions of the world, where it breeds very aggressively, and is an invasive species for the central Russia, mentioned in the "Black Book of Flora of Middle Russia". Nevertheless, plants of the genus Reynoutria are used in official medicine in Japan, China, Korea, since preparations from leaves, shoots and rhizomes of Reynoutria demonstrate a significant pharmacological effect.

Various parts of the plant are rich in vitamin C, flavonoids such as rutin and quercetin, compounds with antioxidant activity from the groups of stilbenes and anthraquinones, and protein. Plant biomass is used for food and as fodder for cattle. It is relevant and promising to study Reynoutria bohemica, growing in the Russian Federation, from the standpoint of its application in medicine, and from the position of a source of nutrients in food and agricultural industries.

The aim of the study is to develop a simple and efficient method for protein isolation from Reynoutria bohemica.

Objectives of the study:

- To select the optimal conditions for protein extraction from leaves of Reynoutria bohemica.
- To determine the amount of protein extracted at each stage by Bradford method.
- Separate the extracted protein mixture using electrophoresis.
- Perform purification by dialysis and HPLC.

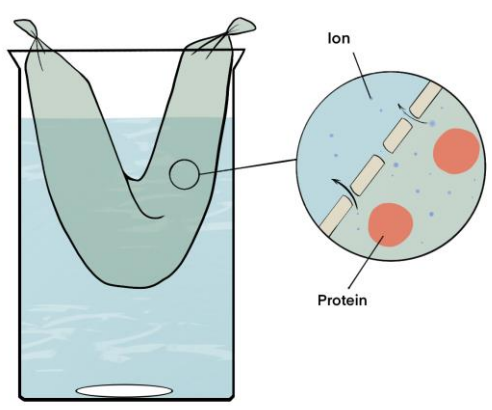
The objects of the study are the leaves of Reynoutria bohemica, harvested in the Botanical Garden of the I. M. Sechenov First Moscow State Medicine University (Sechenov University)

Homogenization of fresh leaves was carried out in a mortar with silica sand and 10 mM phosphate buffer (pH 7.4) in a 1:10 ratio followed by ultrasound treatment.

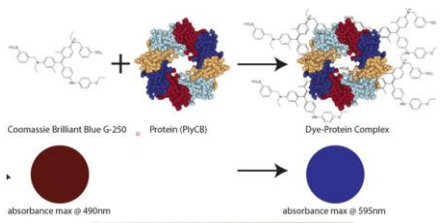
https://browin.ru/magazin/produkt/312008/granitnaya-kuhonnaya-stupka-s-pestikom

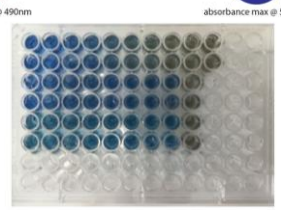
http://xn--m1aacrs.xn--p1ai/en/oborudovanie/vspomogatelnoe-oborudovanie/245-ultrazvukovoj-gomogenizator-vibra-cell-vcx-750

The homogenate was centrifuged at 3,500 g for 10 min, protein was precipitated from the supernatant with ammonium sulfate (95% saturation) for 12 hours, the precipitate was separated by centrifugation and dialyzed against phosphate buffer.



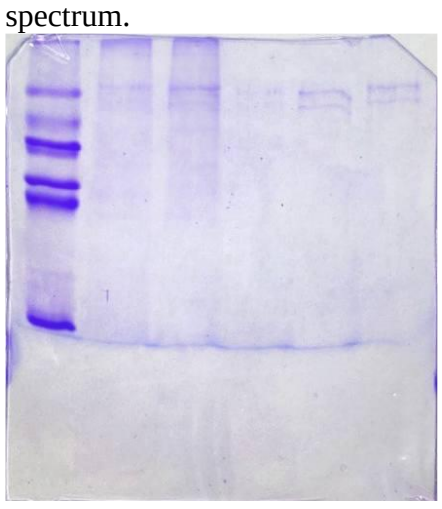
The amount of protein in the sample was determined by the Bradford method.



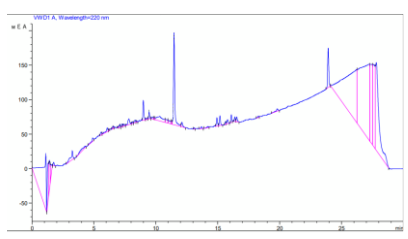


https://github.com/har1eyk/Bradford-Assay-Protein-Quant-with-One-Phase-Assoc

Analysis by SDS-electrophoresis in 12% polyacrylamide gel showed that protein in the molecular mass range of 45 to 90 kDa dominated in the total spectrum.



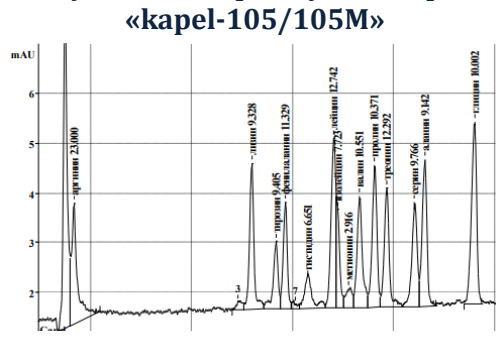
HPLC on a C18 column with gradient elution in a water-acetonitrile system with 0.1% TFA was used as the final purification step. The proteins were characterized by retention time and peaks were collected for further identification.



The total content of amino acids (free and bound amino acids)

Ama	%	Ama	%
Arg	0,81	His	<0,01
Lys	1,21	Leu+Ile	1,74
Tyr	<0,01	Met	<0,01
Phe	1,33	Val	0,43
Pro	0,76	Gly	0,42
Thr	0,44	Glu	1,02
Ser	0,52	Cys	0,09
Ala	0,54	Asp	0,53

Electrophoregram of amino acids obtained on the system for capillary electrophoresis «kapel-105/105M»



Conclusion. Thus a simple efficient method for protein extraction from Bohemian Reinutria has been developed which allows obtaining 45 mg of protein from 1 g of biomass.

References:

1. Виноградова Ю. К., Майоров С. Р., Хорун Л. В. Черная книга флоры Средней России: чужеродные виды растений в экосистемах Средней России. – Общество с ограниченной ответственностью" Издательство ГЕОС", 2010. – С. 512-512.
2. Kuklina A. G., Tsybulko N. S. Phytochemical analysis of the vegetative organs of hybridogenous complex Reynoutria Houtt //VIII International Scientific and Practical Conference on Biotechnology as an Instrument for Plant Biodiversity Conservation 1324. – 2018. – С. 381-388.