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ADVANCED RESEARCH IN LIFE SCIENCES
8, 2024, 34 – 38

www.degruyter.com/view/j/arls

DOI: 10.2478/arls-2024-0004
Research Article

An Efficient Protein Extraction Method from *Astragalus Armatus* Willd. Roots for Proteomic Analysis

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Received November, 2023; Revised December, 2023; Accepted January, 2024

Abstract
The first step of the proteomic study is the extraction and the success of this technique was based mainly on the choice of the best extraction. The purpose of this study was to determine the simplest and lowest-cost method of total protein extraction. Initially, various extraction methods were performed for protein extraction from roots of *Astragalus armatus* Willd. The methods employed were extraction by RIPA buffer, hypotonic buffer and distilled water. After determination of protein concentration by Bradford method and SDS PAGE electrophoretic analysis, the quantity and quality of extracted proteins using different protocols from *A. armatus* were determined and compared. The protein yield of RIPA buffer method was higher than the yields of hypotonic buffer method and distilled water method. The best protein patterns were produced by RIPA buffer method. The extract obtained by RIPA buffer was the optimal protocol for protein extraction.

Keywords: RIPA buffer, Hypotonic buffer, distilled water, SDS PAGE.

Introduction

Astragalus armatus Willd. is an endemic shrub of the family of fabaceae, found in the Northern Africa [1]. Genus Astragalus consists of about 3000 species worldwide [2]. Studies carried out on species of this genus (around 100 species) have shown that they contain several bioactive compounds, and are rich in phenolic compounds and saponins, which contribute to various biological activities [2-5]. Moreover, the seeds of Astragalus species are good sources of carbohydrates, polyunsaturated fatty acids, microelements, vitamins, and protein, which are important nutrients for humans [6].

In folk medicine, *A. armatus* has been used as a tonic, a stimulant, and for the treatment of Anaemia [3]. Many studies indicated that *A. armatus* possessed various activities such as antioxydante [3-5], antimicrobial [3], anticholinesterase, phagocytic [5], and anticomplementary activities [7].

Protein is considered one of the most important macromolecules found in living organisms. They are composed of elements such as carbon, nitrogen, oxygen and hydrogen [8]. Plant proteins are primarily used to ensure good health or nutrition and are recommended in various diseases, including heart disease, diabetes, obesity and cancer [9] This led consumers to trend toward the use of plant proteins as an alternative protein to animal proteins. Furthermore, research should be realised to discover the suitable extraction techniques for these proteins [10].

Protein extraction is the most crucial step in proteomic analysis. Proteomic studies need to standardize extraction protocols for different plant samples because the method of extraction from these different plant species has varied depending on the quantity and types of interfering non-protein compounds. Additionally, proteomic studies are influenced by the conditions used

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during the extraction step, as the latter leads to changes in the quantity and quality of total proteins [11, 12].

Several studies were reported that protein have various biological activities, such as, antimicrobial [13], antioxydant [14], immunomodulatory [15], antiproliferative [16], antihypertensive activities [17]. Many research studies have been achieved to identify secondary metabolites of *A. armatus* [2, 5], however, there is no proteomic study carried out on this plant. This may be due to the difficulty and complexity of plant protein extraction, there is no common protein extraction protocol for all types of plants. The effectiveness of the protein extraction technique depends on the chemical characteristics of the protein and their structure [18]. The objective of the study was a selection of the most suitable protocol for efficient extraction of proteins from *A. armatus* roots.

Materials and methods

Plant material

The roots of *Astragalus armatus* Willd. were collected from Khenchela (Mountain of Babar), located in Est of Algeria (35° 14' 38.63" N 7° 12' 33.30" E, at 1320 m a.s.l.). *A. armatus* roots were dried in the air and ground to a powder using an electric mill.

Protein extraction methods

Powdered roots (Five grams) were extracted with three different extraction methods: RIPA buffer (1 mL): 10 mM Tris/HCl, 150 mM NaCl, 1 mM EDTA, 1% v/v triton × 100, 0.5% w/v sodium deoxcholate, 0.1% w/v SDS and1 % protease inhibitor, pH7.4 [19], distilled water (1 mL) and hypotonic buffer (1 mL): 10 mM Tris/HCl, 10 mM NaCl, 15 mM 2-mercaptoethanol, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and protease inhibitor, pH 7.2 [20]. The suspensions were centrifuged at 20000*g* for 30 min at 4°C. Then, the resulting supernatant was analyzed.

Determination of Protein Concentration

The protein content was determined using Bradford's method [21], with bovine serum albumin (BSA) as the standard.

SDS–polyacrylamide gel electrophoresis

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on a 10 % gel according to Laemmli [22] using 4% polyacrylamide as the stacking gel and 10% polyacrylamide as the separating gel. Each extracted protein (30 *µ*g) was mixed with in sample buffer, and heated at 100°C for 10 min. The electrophoresis was run at 25 mA. At the end of the electrophoresis, the gel was removed from the plates and stained with a solution containing coomassie blue R-250 (0.25 %) in 50 % of methanol, 10% acetic acid and 40 % H_2O for 2 h. In order to visualize the protein bands, the excess dye was removed with 20 % of methanol, 10% acetic acid.

Results and discussion

Protein extraction is a preliminary and important step in studying proteins (purification, separation and mass spectrometry). However, the extraction of the majority of vegetable proteins is generally very complex due to the presence of other different molecules (polysaccharide, lipids, and cell wall) and large amounts of secondary compounds that interfere with protein extraction [11]. Therefore, this extraction step is considered a challenge to achieve the best extraction. In this study, we determined and compared the quantity and quality of extracted proteins from *A. armatus* using different methods.

Indeed, the difficulty in protein extraction from the roots of *A. armatus* is caused by the presence of large quantities of other compounds such as, triterpenes, Carbohydrates, Flavonoids, Saponins, Alkaloids/nitrogenous bases (trace amoun), Tannins/phenolic compounds [2] as well as to the significant quantity of Saponin [4].

Recently several scientists have been interested in studying protein extraction protocols for a wide diversity of organisms [23-25]. Although the authors have reported the extraction of proteins from different plant species [26-28], nevertheless there is no report for *A. armatus*. In this study, we assessed the efficiency of three extraction methods of total proteins from *A. armatus* roots

Protein Concentration

Firstly, the quantity of proteins in each extract was analyzed using the Bradford method. The results show that protein extraction using RIPA buffer had the largest protein concentrations (6,47mg/mL) as compared with the hypotonic buffer and water extraction methods, while distilled water extraction method had the smallest protein concentrations (3,79 mg/mL) (Fig.1). According to the results obtained, RIPA buffer extraction method produced the best total protein yield when compared to hypotonic buffer and distilled water extraction methods. This result is explained by the fact that the extraction with RIPA buffer involves the use of detergents. SDS is a detergent that has a high efficiency for protein extraction [29]. In addition, triton x 100 is a non-ionic detergent that facilitates protein solubilization. Agents of EDTA, b-mercaptoethanol, and protease inhibitor that found in the extraction buffers can inhibit activities of proteolytic enzymes [30].

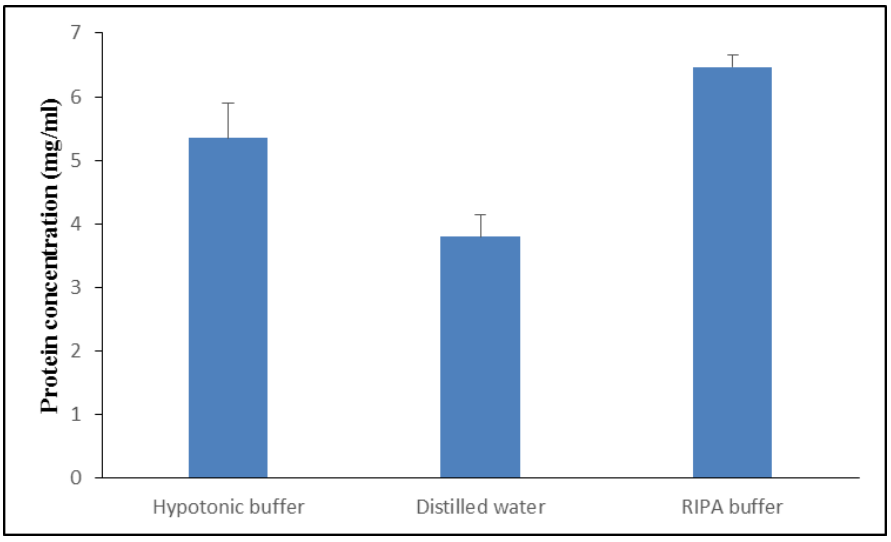

Figure 1. Mean (± SD) protein concentration of *Astragalus armatus* Willd. Roots protein extracts obtained by different methods

Protein Analysis

The effect of different extraction methods on aspects of protein extraction, (patterns, interfering substances, and range of protein molecular weight) was evaluated using SDS–PAGE gels. Equal amounts of protein from each extraction were analyzed by SDS-PAGE.

The patterns obtained by each extraction method are shown in Fig.2. Extraction with RIPA and hypotonic buffers showed similar patterns with high resolution. This result is due to the presence of EDTA, b-mercaptoethanol, and protease inhibitors that remove interfering compounds [30], this helps to improve the patterns. Besides, extracted protein using RIPA buffer extraction method also showed less smearing as compared with hypotonic buffer extraction method. There were much more bands in protein extracts by the RIPA and hypotonic buffer extraction methods than those by distilled water extraction method.

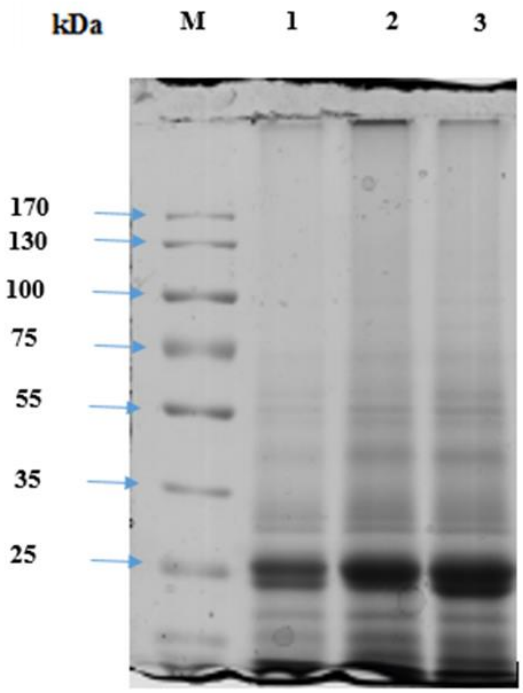

Figure 2. SDS–PAGE of protein extracts of *Astragalus armatus* Willd. Roots obtained by different methods. Molecular weight markers in kDa (Lane M), distilled water (lane 1), RIPA buffer (lane2), hypotonic buffer (lane3).

In the SDS-gel of distilled water extract, a preferential loss of almost all high-molecular weight proteins was observed. This result was due to the absence of the protease inhibitors that prevent degradation of proteins. These results showed that the RIPA buffer extraction method was more efficient for protein extraction from *A. armatus* roots. There was no smearing of the protein bands when proteins were extracted with SDS-containing buffer.

Conclusions

In this work, three distinctive protein extraction protocols from roots of *Astragalus armatus* Willd. were employed (RIPA buffer, hyptonic buffer and distilled water). The most optimal extraction of total protein was RIPA buffer extraction; this method showed a higher protein concentration. The extraction of proteins using RIPA buffer was suitable for SDS-PAGE with less smearing and vertical streaking. This method is rapid, simple, and economical, and it provides useful information that may be used starting point for proteomic studies of other related plant species. Moreover, this study is the first report on the extraction of proteins from roots of *A. armatus*. According to the results obtained, this plant can be considered a good source of proteins; therefore, further studies will be necessary in order to investigate the structure of these proteins and their biological activities.

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