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Research Article

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Purification and partial characterization of a novel lectin from *Ruta montana* roots

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ABSTRACT

Lectins are proteins or glycoproteins, which participate in various biological processes. The purpose of this study was to purify new lectin from *Ruta montana* roots. Lectin of *Ruta montana* has been purified from roots using ultrafiltration and precipitation with ammonium sulfate followed by gel filtration chromatography whereas protein estimation was done by Bradford's method. Within sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) analysis, the extract exhibited three bands and one band after purification. The molecular weight of lectin was determined by SDS–PAGE and gel filtration chromatography, which was found to be a monomeric protein of approximately 28.8 kDa. The agglutination activity of *Ruta montana* lectins was stable within a temperature range from 4 to 50° C for 30 min and the pH range from 4.2 to 9. This study presents a natural source of lectins that can be used in several other studies due to its different biological activities.

KEYWORDS: Lectins, *Ruta montana*, Rutaceae, FPLC

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INTRODUCTION

Lectins are proteins of non-immune origin, which possess at least one non catalytic domain having the capability to bind specific sugars (a mono- or oligosaccharide) (Peumans & Van Damme, 1995). They typically agglutinate cells or precipitate polysaccharides and glycoconjugates (Moreira *et al.*, 1991). Plant lectins are mainly involved in defense mechanisms against pathogens (Van Holle & Van Damme, 2018), they have important roles in reserving proteins and some lectins mediate the symbiotic relationship (Van Holle & Van Damme, 2019). They have attracted great interest because of their various biological activities, such as cell agglutination, antifungal, HIV-1 reverse transcriptase inhibitors, immunomodulatory, anti-insect activities, and antitumor (Lam & Ng, 2011), detection, isolation and characterization of glycoconjugates, histochemistry of cells and tissues, tumor cell recognition (Mishra *et al.*, 2019). Plant lectins were grouped into different families (Amaranthin, legume lectins, Chitin- binding lectins, monocot mannose-binding lectins, type II ribosome-inactivating proteins, and other lectins) (Santos *et al.*, 2014; Mishra *et al.*, 2019). In Algeria, the *Ruta montana* (*R. montana*), known as Fijel belongs to the Rutaceae family (Khabbach *et al.*, 2012). Ruta presents mainly shrubby plants, the main habitat of this genus is in the Mediterranean region (Pollio *et al.*, 2008). *R.montana* contains alkaloids, sterols and triterpenes, combined anthracenes, and reducing compounds (Daoudi *et al.*, 2016) and flavonoids (Benali *et al.*, 2020). In traditional medicine, *R.montana* is used for the treatment of abscesses, emetic in pediatric (Khabbach *et al.*, 2012), menstrual problems, sore eyes and as an antispasmodic, sedative, abortifacient (Hammami *et al.*, 2015). Several studies indicate the activities of *R.montana* such as antioxidant, antimicrobial activities (Benali *et al.*, 2020) and antiacetylcholinesterase activity (Khadhria *et al.*, 2017). The aim of this study was to purify lectin from *R.montana* roots.

MATERIALS AND METHODS

Extraction

The roots of *Ruta montana* were collected from Khenchela (East of Algeria). During September and ground by blinder high speed. Lectin of *Ruta montana* was extracted by stirring a suspension of flour (10% w/v in water adjusted to pH 4) for 1 hour at room temperature (Megias *et al.*, 2013). Supernatant resulting from centrifugation at 14.000 rpm for 20 min was concentrated to half of its volume using an Amicon cell filtration

unit (Millipore, MA, USA) equipped with a 3 KDa ultrafiltration membrane (Regenerated Cellulose, MA, USA).

Ammonium Sulfate Precipitation

The ultraretenate was fractionally precipitated with ammonium sulfate at 50% - 100% saturation; the pellet resulting from centrifugation (12.000 rpm for 5 min, 4°C) was dissolved in a minimal volume of distilled water, and dialyzed against distilled water for a period of 48 h at 4°C.

Purification

The ultraretentate and dialysate were loaded onto a gel filtration chromatography using an FPLC AKTA purifier system equipped with a Superose 12 HR 10/30 column. The injection volume was 1 mL. Fractionation was carried out at a steady flow rate of 0.5 mL/min at room temperature, then, the proteins were eluted with phosphate 50 mM, NaCl 0.5 M and pH 7. The different fractions of dialysate were pooled and concentrated to test its agglutinating activity.

Proteins Concentration

The protein content was estimated by the Bradford's method, using bovine serum albumin (BSA) as the standard (Bradford, 1976).

SDS-PAGE

The ultraretenate and the fraction which showed agglutinating activity were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), According to the reported method by Schägger and von Jagow (1987) at a constant voltage for stacking gel and separation gel were 60 V and 120 V respectively with 25 mM Tris, 190 mM Gly pH 8 as running buffer. The gel was stained with a solution containing Coomassie blue R-250 (0.25 %) in 45 % of methanol, 10% acetic acid and 45 % H_2O for 1 h at room temperature, and de-stained until the protein bands were visible. The molecular weight of the proteins was determined in comparison with the protein molecular mass marker from Pharmacia LKB Biotechnology.

Agglutinating Activity

Fresh rat erythrocytes were separated from plasma by centrifugation at 500 g for 10 min and repeatedly washed with PBS. The resulting erythrocytes were resuspended 1/10 (v/v) in PBS containing 0.5 % (v/v) glutaraldehyde after incubation in a shaker for 1 hour at room temperature. The erythrocytes were centrifuged and washed three times with PBS, and were resuspended in PBS (20 % v/v) containing sodium azide (0.1% w/v). The fixed erythrocytes (2 mL) were incubated with trypsin (2 mg) for 30 min at 37 °C and washed three times with PBS to remove the enzyme. To determine the agglutinating activity, rat erythrocytes were incubated with increasing concentrations of lectins (40 µL, 4 % w/v in PBS buffer) in 96 U shape well microplates for 1 hour at 4°C.

pH and Temperature Effect

The effect of pH was determined by incubating the samples at room temperature with different pH buffers ranging from 2 to 10.6 for 1 h and the agglutination activity was detected against rat erythrocyte suspension.

For the thermal stability of lectin, the sample was incubated for 30min at a range of 4 °C to 100 °C, then placed immediately on ice for 5 min, the agglutination activity was determined using the rat erythrocyte.

RESULTS AND DISCUSSION

Lectins have attracted the attention of scientists owing to their biomedical applications and their many bioactivities including anti-tumor, antiviral and immunomodulatory properties. In the present investigation, the lectin of the root of *Ruta montana* was extracted in water at pH = 4. The extract was ultrafiltered using 3 kDa ultrafiltration membrane to eliminate the low molecular weight compounds, the resulting ultraretenate was then subjected to precipitation by ammonium sulfate followed by gel filtration seperose 12 chromatography.

Agglutinating Activity

Agglutination assays were performed in 96 well microtiter plates with intact and trypsinized rat erythrocytes. The extract of the plant was showing strong agglutination with rat erythrocyte, this result indicated the presence of the lectins in the extract. The activity of the lectin was greatly increased with trypsinised red blood cells of rats (Table 1) which explains the capacity of trypsin to digest the proteins, which mask the membrane carbohydrates, this leads to the improvement of the reactivity of the treated red blood cells.

Purification

Gel filtration chromatography of 50-100 % ammonium sulfate precipitate of *Ruta montana* roots using a Superose 12 column revealed two peaks peak I (fractions 14–18) and peak II (fractions 20–23) (Figure 1b). The fractions corresponding to the first peak (16-18ml) showed agglutinating activity with a molecular weight of approximately 28.8 kDa. While gel filtration chromatography of ultraretenate showed two non- complete major resolved peaks (Figure 1a).

SDS-PAGE of *Ruta montana* extract demonstrated the existence of three bands with an approximate molecular weight of 53.9; 46.08; 28.26 kDa (Figure 2a). While the fraction that showed

Table 1: Minimum concentration of protein of *Ruta montana* required to agglutinate the erythrocyte

	Crude extract with intact rat erythrocytes (mg/mL)	Crude extract with trypsinised rat erythrocytes (mg/mL)
Minimum concentration of protein	0,564	0,376

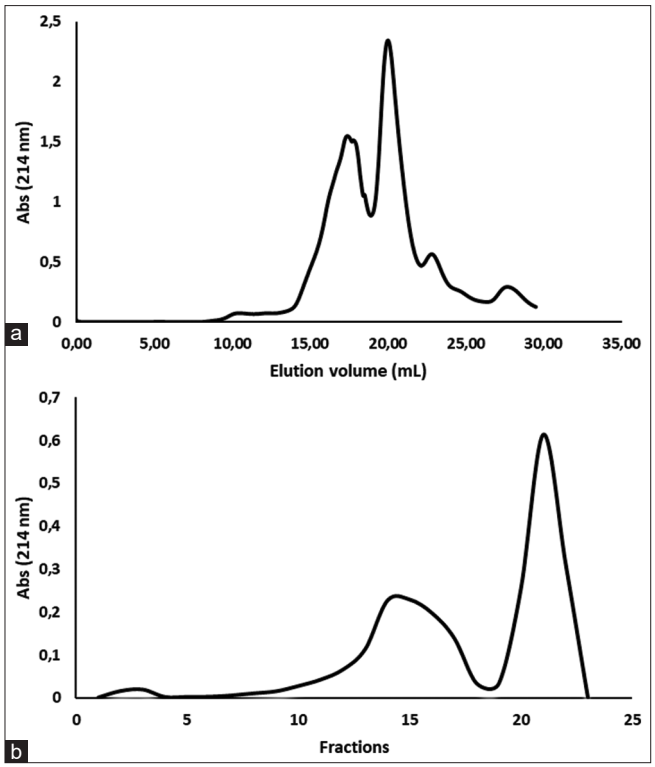

Figure 1: Superose 12 gel filtration chromatography of *R. montana* extract. (a) Extract after concentration by ultrafiltration (3 kDa cut-off), (b) Partially purified lectin (50-100% ammonium sulfate precipitate).

agglutinating activity exhibited one band with a molecular mass of 28.26 kDa (Figure 2b).

Reducing SDS-PAGE of *Ruta montana* showed a single band with a molecular mass of approximately 28.26 kDa, and about 28.8 kDa in the gel filtration method. These results indicate that it may be possible that the purified lectin is a monomeric protein with a molecular weight of approximately 28.8 kDa. This value is close to the molecular weight reported for the monomeric lectin which is purified from *Astragalus membranaceus* and *Bauhinia forficata* seeds with a molecular mass of 31.5 kDa (Yan *et al*., 2010) 27,85 kDa, (Silva *et al*., 2012) respectively.

pH and Temperature Effect

To study the thermal stability of lectin, *Ruta montana* lectin was incubated for 30 min at various temperatures of 4 °C to 100 °C. The lectin was completely maintained agglutinating activity at temperatures ranging from 4 to 50 °C for 30 min, its activity was sharply dropped and lost its activity at 70 °C for 30 min (Figure 3a). The lectin was remarkably less thermostable as compared to the lectin that Silva *et al*., (2012); Li *et al*, (2012); Yan *et al*. (2010) and Correia and Coelho (1995) purified. The optimum pH for maximum agglutinating activity of the lectin of *Ruta montana* was exhibited at pH 4.2 - 9 and lost the whole activity below pH 3, at pH 10 its activity was decreased to ~ 50% (Figure 3b), Similar result had been observed in lectin purified from *Ruditapes philippinarum* (Bulgakov *et al*., 2004).

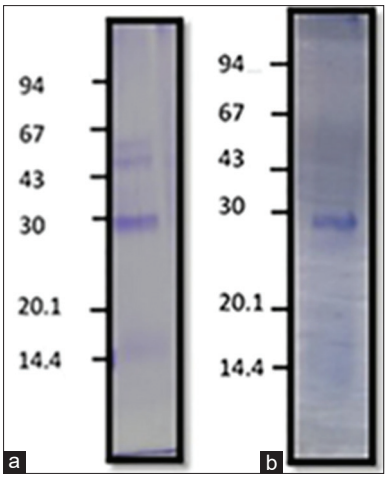

Figure 2: SDS–PAGE of crude extract of *Ruta montana*. Molecular weight standards are shown on the side (kDa). (a) Extract after concentration by ultrafiltration (3 kDa cut-off), (b) purified lectin.

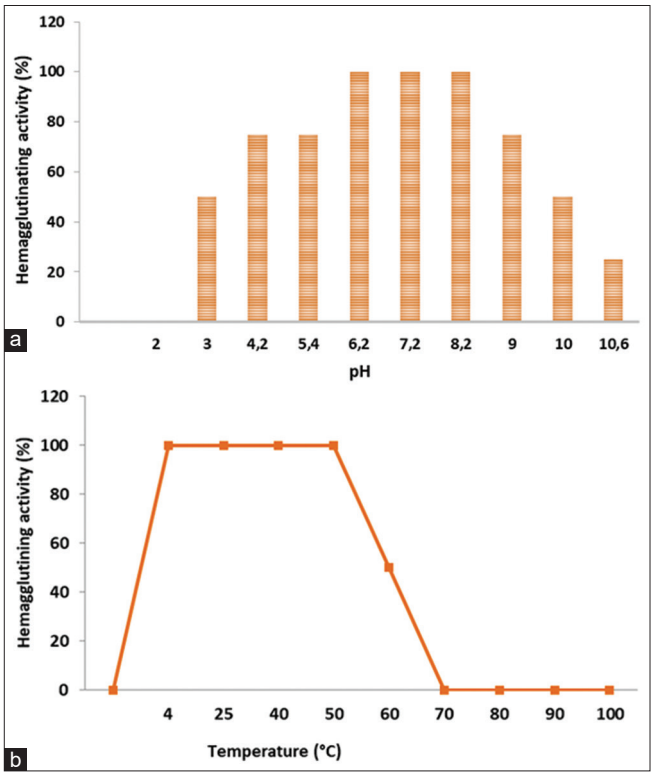

Figure 3: pH stability (a) and Thermal stability (b) of *R.montana* lectins.

CONCLUSIONS

For the first time, lectin was extracted from *Ruta montana*, and then purified by ammonium sulfate precipitation and gel filtration chromatography. The molecular weight of purified lectin from *Ruta montana* was determined by SDS-PAGE and gel filtration chromatography, it was found to be a monomer lectin with a molecular weight of approximately 28.8 kDa. Further studies are necessary to study the structure of this lectin and its biological activities.

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