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Extraction and Purification of a Lectins from Iraqi Truffle *(Terfezia sp.)*

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Abstract
Lectins are a glycoprotein that plays a significant role in plant and fungi resistance mechanism against insect and microorganism attacks. This paper describes for the first time the extraction and purification of a lectin protein found in the tubers of Truffle (*Terfezia sp*.) by a series of steps. Initially, precipitated by ammonium sulfate and accompanied by chromatographic techniques include ion-exchange chromatography and gel permeation chromatography. Lectin proteins were showed purification fold about 20.851 in the final step of the purification and specific activity about 377.581 unit/mg protein. Also, the hemagglutination activity assay of the Truffle lectin was showed activity to agglutinate for each step with the blood types A and O more than B group at room temp, under moderate pH 7.0 and also show high stability temperature extend from 0°to 40°C based on the hemagglutination activity in repeated experiments under various temperatures. The carbohydrates specificity assay results showed that the lectins have complex specificities for many sugars, but not have specific for another group in this test.

Keywords: Truffle, *Terfezia sp.*, lectins, chromatographic separation, hemagglutination action, carbohydrates specificity assay

Introduction

Lectins are binding haemagglutinating glycoproteins that could attach specifically and reversibly with certain carbohydrate compounds (e.g. glycoproteins, polysaccharides, and glycolipids). Lectins are noticed in wide ranges of organisms from microbes, fungi to complex animals and plants [1]. They can be found as a bound or free in cell membranes and mediate many biological activities such as bacteria and fungi adhesion to host cells, cell-cell interactions and immune responses [2–4]. Another characteristic key of lectins is their capability to agglutinate with erythrocytes, which can provide an unambiguous indicator for lectins presence. Several physiological roles have been reported for the lectin proteins, including antifungal [5], mitogenic [6-7], antiviral [8-9], and antitumor [10-13], anti-insect [14-15] activities.

Truffles are obligate hypogynous ascomycetes of ectomycorrhizal fungi that grow in relationship with host roots of *Helianthemum sp.* plants [16]. Truffles are socioeconomically important fungi That emerge during the rainy season in the deserts usually in the period between February to April and naturally found in higher amounts in the middle east (Iran, Iraq and Kuwait) during the autumn rainy and thunderstorm season [17]. *Terfezia sp*. tubers are a source for many proteins, carbohydrates, vitamins and minerals [18].

Thus, the present study was focused on the detection, extraction, and purification of lectin proteins from Truffle (*Terfezia sp*.), Hemagglutinating activity for every human blood type group (A, B, O) with test the sugar specificity for the lectins and study some lectins activity conditions.

Experimental Technique

Extracts preparation

Tuber bodies of the Truffle *Terfezia sp*. purchased from the local market that obtained from the Anbar desert in Iraq. Truffle *Terfezia sp*. samples (100gm) were cleaning and cutting into smaller pieces then extracted using a high-speed blender with 250 ml extraction buffer (0.025M Tris-hydrochloric acid and 0.15 M Sodium chloride with pH 7.5). The homogenate was filtered through gauze and centrifuged (10,000 g, 20 min, 4 ºC) to obtain the supernatant (crude extract) which was used in haemagglutination assays and protein purification methods [19]. The suspension was partially deposited by ammonium sulfate at 60%, 80%, and 100 saturated percent. After centrifugation, the three

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pellets were mixed and diluted with a minimum buffer amount (0.025M Tris-hydrochloric acid with pH 7.5) and dialyzed for 24 hours by using distilled water at 4 °C.

Hemagglutinating activity test

Haemagglutination assay was performed using two-fold serial dilutions of lectins solution in micro-titer v-plates (50µL) and mixed with 25 µL of 5% suspension of the human red blood cell (obtain from the blood bank in Al-Ramadi city) in 50 µL buffer (0.025M Tris-hydrochloric acid and 0.15 M Sodium chloride with pH 7.0). Results were monitored and recorded after 30 minutes at room temperature. The last tube shows a visible agglutination was considered as the cross for the highest dilution offer hemagglutination, that was observed at hemagglutination-unit. The calculation of the particular protein activity was indicated as the amount of hemagglutination units per mg of protein [20].

Sugar specificity test

The lectins specificity test for sugars was evaluated in a similar to the hemagglutination study. The following sugars were taken: D-galactose, D-glucose, D-fructose, D-mannose, sucrose, arabinose, xylose, maltose and lactose. The sugar tested solution was prepared by it dissolving with distilled water to a certain concentration (0.5 M). The same amounts (50 µL) of the lectins sample and (50 µL) of the sugar sample were combined in micro-titer v-plates and stored at room temperature for 30 minutes. The solution then combined with (25 µL) for 2 percent human RBC suspension to detect and monitor the inhibitory effect of the hemagglutinating activity of the lectins by various sugar classes.

Lectins extraction and purification

Crude extract after dialyzed was loaded on a strong anion-exchanger (quaternary ammonium (high Q)) column (1.5 cm ×10 cm) that had been equilibrated with the buffer (0.15M Sodium chloride with 0.2M Tris- hydrochloric acid (pH 8.0)). Initially, the column was cleaned with the same balancing buffer to eliminate all proteins that not have uniquely attached to the resin, after that, applied linear salt series concentration elution. Fractions exhibiting hemagglutinating behavior in a solution (0.15 M Sodium chloride with 0.2 M Tris-hydrochloric acid in pH 8.0) were further processed by gel filtration chromatography on a Sephacryl 300HR column (2.5 cm ×50 cm).

Protein Concentration Determination

Bradford Assay calculated the protein concentration using bovine serum albumin (BSA) as a standard protein solutions prepared using 0.1, 0.2, 0.3, 0.4 to 0.9 mg. ml of BSA. Absorption readings of these standard solutions at 595 nm were used to make a calibration curve to determine the concentration of the protein sample [21].

Results and Discussion

The present study shows successfully detected of lectins in the Truffle *Terfezia sp.* Extraction by using Hemagglutinating activity assay for each step was used to observe the activity of this protein and determined the activity for each type of blood groups (A, B, O) that show activity to agglutinate with the groups A and O more than B group at pH 7.0 in room temperature (Figure.1). Based on the lectin's tendency to associate with carbohydrate compounds, this protein had been used in the structural characterization and isolation of glycoconjugates, studies of the composition of the cellular surface and blood typing [22-23].

Sugar specificity for the crude extracts was tested by inhibition of hemagglutination. Glucose, mannose, maltose, sucrose and fructose showed inhibition. D-Galactose, arabinose, xylose and lactose were all have non-inhibitory effect (Figure. 1). These result shows that lectin's specificity from Truffle *Terfezia sp.* is similar to Cajanus cajan root and seed lectins results [24].

(a) (b)

Fig. 1. (a) agglutination activity assay in the presence of the crud extracted Truffle *Terfezia sp.* for each blood group (A, B, O). (b) Sugar specificity assay of the crude extract of Truffle *Terfezia sp.* lectins showed the inhibition of hemagglutination for the sugars that have specificity for the lectins and non-inhibition of hemagglutination for the sugars that not have specificity or the lectins.

Methods that used for purifying of lectins are differing one another based on the source of the lectins which were obtained, but traditional methods that used in protein purification, such as chromatographic methods and salting-out [7, 25-27] remains the basic methods that used in lectins purify.
Purification results by strong anion-exchanger show three adsorbed fractions F1, F2 and F3 by this type of chromatography. Only one fraction showed hemagglutinating activity which was fraction F2 with the larger peak (Figure. 2). Furthermore, results of Gel filtration chromatography show a smaller peak S1 and a larger peak S2 (Figure. 2) and only the S2 peak show hemagglutinating activity.

In comparison with the methods that were used for lectins purification from mushroom published by Zhang *et al.* [25], that include also two columns step for the separation, ion exchange containing DEAE-cellulose and gel filtration Superdex type 75-HR 10/30 column have now been replaced with strong anion-exchanger (quaternary ammonium (high Q)) and Sephacryl 300-HR column. Furthermore, the buffer (10 mM NH_4HCO_3 buffer with (pH 9.4)) for ion exchange and alter pH to 8.5 in the mobile phase for gel filtration in the original study [25] has been also replaced by buffer (0.15M Sodium chloride with 0.2M Tris-hydrochloric acid with pH 8.0) for each technique of ion exchange and gel permeation for the present study considered a good purification results under change of the media and buffer conditions.

The regression equation of the standard curve for standard protein BSA was y=0.0082x+0.0043 (R2=0.9864). for each extract, and the protein concentration was calculated based on this regression equation. The results of the lectins purification showed an increase in the specific activity for the protein that begins from 18.109 (unit/mg) for the crude extraction to reach 377.581 (unit/mg) in the final step after purification (Table 1). The activity referred to the highest dilution exhibiting hemagglutination, which was considered as one hemagglutination-unit. The lectins still stables and maintains its hemagglutinating activity in the pH range from 2.0 to 10.0 [28]. Also, the lectins shows high stability and activity at a temperature from 0°to 40°C that observed by hemagglutinating assay under this rang of temperature.

Conclusion

A lectins found in Truffle *Terfezia sp*. has been successfully extracted and purified by using traditional column chromatography and precipitated by ammonium sulfate, this protein provides stability through purification steps under room temperature and wide range of pH conditions. Hemagglutinating activity assay shows activity to agglutinate with both A and O blood groups more than B group at PH 7.0 in the room temperature, high activity and stability at a wide range of temperature and maximum specificity for both mannose and glucose in contrast with the other simple saccharides. During the analysis it is apparent and important to given special attention to the initial sample preparation because the disruptive suspension has a high viscosity, and also to prevent the contaminating proteins

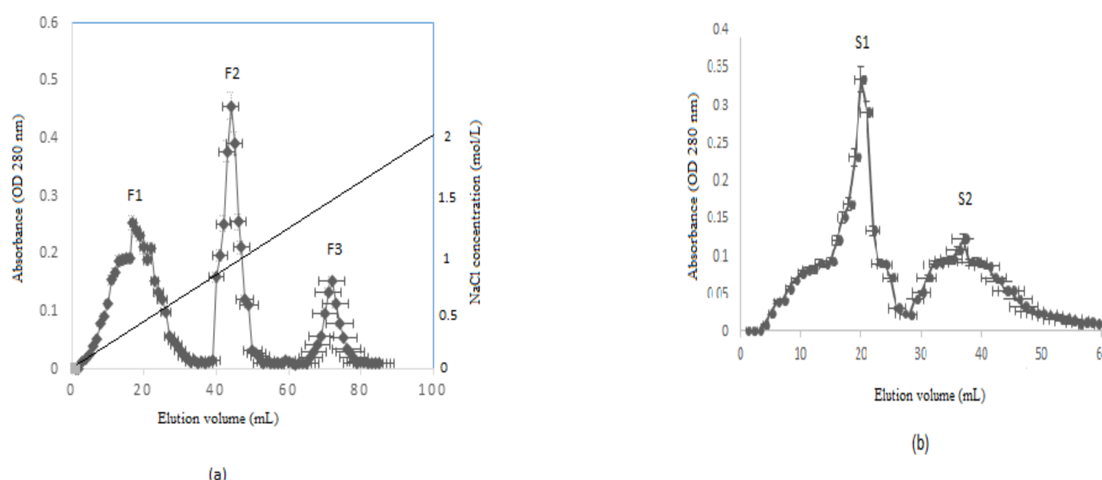

Fig. 2. (a) Fractionations of the supernatant solution of Truffle Terfezia sp. by strong anion-exchanger (quaternary ammonium (high Q)). Three peaks obtained as a result, among which peak-F2 only showed hemagglutination action. (b) Fractionations of peak-F2 at a Sephacryl 300-HR media. Alone the top S1 showed hemagglutination action.

Table 1. Summary of purification of Truffle *Terfezia sp*. lectins.

Purification step	Volume (ml)	Activity (unit/ml)	Protein (mg/ml)	Specific activity (unit/mg)	Total activity (unit)	fold	Yield (%)
Crud extraction	100	32	1.767	18.109	3200	1	100
Precipitation protein by ammonium sulfate	15	64	1.252	51.118	960	2.823	30
Ion-exchange chromatography	8	128	0.463	276.458	1024	15.266	32
Gel filtration chromatography	7	128	0.339	377.581	896	20.851	28

References

[1] Lis H., Sharon N. Lectins: carbohydrate-specific proteins that mediate cellular recognition. *Chem. Rev.*, 98 (2): 637–674 (1998).

[2] Pusztai A. *Plant lectins*. Cambridge University Press, (1991).

[3] Sharon N., Lis H. Lectins as cell recognition molecules. *Science,* 246 (4927): 227–234 (1989).

[4] Imberty A., Mitchell E. P., Wimmerová M. Structural basis of high-affinity glycan recognition by bacterial and fungal lectins. *Curr. Opin. Struct. Biol.*, 15 (5): 525-534 (2005).

[5] Barrientos L. G., Gronenborn A. M. The highly specific carbohydrate-binding protein cyanovirin-N: structure, anti-HIV/Ebola activity and possibilities for therapy. *Mini Rev. Med. Chem.*, 5 (1): 21–31 (2005).

[6] Nagre N. N. *et al.* A potent mitogenic lectin from the mycelia of a phytopathogenic fungus, Rhizoctonia bataticola, with complex sugar specificity and cytotoxic effect on human ovarian cancer cells. *Glycoconj. J.* 27 (3): 375-386 (2010).

[7] Sharma A., Ng T. B., Wong J. H., P. Lin P. Purification and characterization of a lectin from Phaseolus vulgaris cv.(Anasazi beans). *Biomed Res. Int.*, 2009: 2009.

[8] Van Asbeck E. C., Hoepelman A. I. M., Scharringa J., Herpers B. L., Verhoef J. Mannose binding lectin plays a crucial role in innate immunity against yeast by enhanced complement activation and enhanced uptake of polymorphonuclear cells. *BMC Microbiol.*, 8 (1): 229 (2008).

[9] Luo Y. *et al.* A novel mannose-binding tuber lectin from Typhonium divaricatum (L.) Decne (family Araceae) with antiviral activity against HSV-II and anti-proliferative effect on human cancer cell lines. *BMB Rep.*, 40 (3): 358–367 (2007).

[10] Feng K. *et al.* Isolation and characterization of a novel lectin from the mushroom Armillaria luteo-virens. *Biochem. Biophys. Res. Commun.*, 345 (4): 1573–1578 (2006).

[11] Bies C., Lehr C.-M., Woodley J. F. Lectin-mediated drug targeting: history and applications. *Adv. Drug Deliv. Rev.*, 56 (4): 425–435 (2004).

[12] Li *et al.* J. Odorranalectin is a small peptide lectin with potential for drug delivery and targeting. *PLoS One*, 3 (6) (2008).

[13] De Mejía E. G., Prisecaru V. I. Lectins as bioactive plant proteins: a potential in cancer treatment. *Crit. Rev. Food Sci. Nutr.*, 45 (6): 425–445 (2005).

[14] Rubinstein N., Ilarregui J. M., Toscano M. A., Rabinovich G. A. The role of galectins in the initiation, amplification and resolution of the inflammatory response. *Tissue Antigens*. 64 (1): 1–12 (2004).

[15] Singh *et al.* T. Carbohydrate specificity of an insecticidal lectin isolated from the leaves of Glechoma hederacea (ground ivy) towards mammalian glycoconjugates. *Biochem. J.*, 393 (1): 331–341 (2006).

[16] Dundar A., Yesil O. F., Acay H., Okumus V., Ozdemir S., Yildiz A. Antioxidant properties, chemical composition and nutritional value of Terfezia boudieri (Chatin) from Turkey. *Food Sci. Technol. Int.*, 18 (4): 317–328 (2012).

[17] Al-Laith A. A. A. Antioxidant components and antioxidant/antiradical activities of desert truffle (Tirmania nivea) from various Middle Eastern origins. *J. Food Compos. Anal.*,23(1): 15–22 (2010).

[18] Bokhary H. A., Parvez S. Chemical composition of desert truffles Terfezia claveryi. *J. Food Compos. Anal.*, 6 (3): 285–293 (1993).

[19] Sampaio A. H., Rogers D. J., Barwell C. J. A galactose-specific lectin from the red marine alga Ptilota filicina. *Phytochemistry*, 48 (5): 765–769 (1998).

[20] Wang H., Gao J., Ng T. B. A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom Pleurotus ostreatus. *Biochem. Biophys. Res. Commun.*, 275 (3): 810–816 (2000).

[21] Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72 (1–2): 248–254 (1976).

[22] Díaz A. O., García A. M., Goldemberg A. L. Glycoconjugates in the mucosa of the digestive tract of Cynoscion guatucupa: a histochemical study. *Acta Histochem.*, 110 (1) 76–85 (2008).

[23] Parillo F., Verini Supplizi A. Characterization of glycoconjugates in the secretory epithelium of the equine ampulla ductus deferentis. *Histol. Histopathol.*, (2008).

[24] Naeem A., Khan R. H., Vikram H., Akif M. Purification of Cajanus cajan root lectin and its interaction with rhizobial lipopolysaccharide as studied by different spectroscopic techniques. *Arch. Biochem. Biophys.*, 396 (1): 99–105 (2001).

[25] Zhang G. Q., Sun J., Wang H. X., Ng T. B. A novel lectin with antiproliferative activity from the

medicinal mushroom Pholiota adiposa. *Acta Biochim. Pol.*, 56 (3). (2009).
[26] Shi J., Xue S. J., Kakuda Y., Ilic S., Kim D. Isolation and characterization of lectins from kidney beans (Phaseolus vulgaris). *Process Biochem.*, 42 (10): 1436–1442 (2007).
[27] Silva *et al.* J. A. Isolation and biochemical characterization of a galactoside binding lectin from Bauhinia variegata candida (BvcL) seeds. *Protein J.*, 26 (3): 193–201 (2007).
[28] Jiang *et al.* B. Two-step isolation, purification, and characterization of lectin from zihua snap bean (phaseolus vulgaris) seeds', *Polymers (Basel).* 11 (5): 785 (2019).

استخلاص وتنقية بروتين اللكتين من الكمأ العراقي (*Terfezia sp.*)

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الخلاصة

يعتبر اللكتين من البروتينات السكرية التي تلعب دوراً رئيسياً في آلية مقاومة النباتات والفطريات ضد هجوم الحشرات والأحياء المجهرية. تناولت هذه الورقة البحثية ولأول مرة استخلاص وتنقية بروتين اللكتين من الأجسام الدرنية للكمأ العراقي (*Terfezia sp.*) بواسطة سلسلة من الخطوات التي بدأت بعملية ترسيب البروتينات باستخدام كبريتات الأمونيوم تبعها استخدام تقنيتي فصل حيوي شملت كروموتوغرافيا التبادل الأيوني وكروموتوغرافيا الترشيح الهلامي. عملية تنقية بروتين اللكتين أظهرت حصيلة قدرها 20.851 في الخطوة النهائية لعملية التنقية وكذلك أظهرت فعالية نوعية قدرها 377.581 وحدة تلازنية/ ملغم. كذلك اظهر اختبار فعالية التلازن مع كريات الدم الحمراء ان بروتين اللكتين ذو الفة عالية على التلازن مع فصائل الدم A و O اكثر من فصيلة الدم B بدرجة حرارة الغرفة واس هيدروجيني متعادل pH 7.0 كذلك اظهر ثباتيه عالية تحت درجات حرارة امتدت من 0 درجة مئوية وحتى 40 درجة مئوية اعتماداً على الفعالية التلازنية مع كريات الدم الحمراء وذلك بتكرار هذه التجربة بدرجات حرارة مختلفة. أظهرت نتائج اختبار التخصص للكاربوهيدرات على قدرة بروتين اللكتين على الارتباط مع مجموعة مختلفة من السكريات وبالوقت نفسه عدم قدرته على الارتباط مع مجموعة أخرى من هذه السكريات.