İstanbul Ecz. Fak. Mec.
35-36 (1) (2002-2003)

J. Fac. Pharm. Istanbul
35-36 (1) (2002-2003)

FURTHER CHARACTERIZATION OF *ALOE VERA* LEAF PULP LECTINS

N. AKEV,* S. MALKOÇ* and A. CAN*

S U M M A R Y

Further characterization of Aloctin I and II, two isolectins of *Aloe vera* (L.) Burm. fil. leaves, isolated previously in our laboratory (1), was achieved. The absorption spectra of the isolectins purified by hydroxylapatite column chromatography were measured over a range of 230-303 nm and λ maximums of 295 nm and 272 nm were found for Aloctin I and II respectively. Further purification of Aloctin I, the major isolectin of *Aloe vera* L. leaves, was accomplished by Sephadex G-50 gel filtration. Purified Aloctin I was incubated with pronase at pH 7.4 for 72 hours at 37°C (enzyme/protein ratio: 1/8 w/w) and the digest was then subjected to gel filtration on Sephadex G-50. One major and two minor protein fractions resulting were analyzed for hemagglutinating activity, protein and carbohydrate contents. Native polyacrylamide disc gel electrophoresis (PAGE) gave a single band for each isolectin and showed that Aloctin I and II have the same molecular weight of 45 000. The fact that sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gave a subunit molecular weight of 15 000, tends to propose that both isolectins are composed of three subunits of equal molecular weight. On the other hand preparative PAGE demonstrated that hemagglutinating activity corresponds exactly to the protein band, showing that purification of the lectins was achieved to a great extent.

* Department of Biochemistry, Faculty of Pharmacy, University of Istanbul, 34116 Beyazıt, Istanbul, TURKEY

ÖZET

Daha önce laboratuvarımızda *Aloe vera* (L.) Burm.fil. yapraklarından saflaştırılan Aloctin I ve Aloctin II adlı iki izolektinin (1) özellikleri incelendi. Hidroksilapatit kromatografisi ile saflaştırılan izolektinlerin 230-303 nm arasındaki absorpsiyon spektrumları ölçüldü. Aloctin I ve II'nin λ maksimumlarının sırasıyla 295nm ve 272 nm olduğu saptandı. *Aloe vera* yapraklarının başlıca izolektini olan Aloctin I, Sephadex G-50 jel filtrasyonu ile daha ileri derecede saflaştırıldı. Saf Aloctin I, pronaz ile pH 7.4'te 37°C'de 72 saat süreyle inkübe edildi (enzim/protein oranı: 1/8 w/w) ve hidrolizat Sephadex G-50 jel filtrasyonuna uygulandı. Bu filtrasyon sonucunda elde edilen bir asıl ve iki küçük protein fraksiyonunun hemaglutinasyon aktiviteleri, protein ve karbohidrat miktarları saptandı. Natif poliakrilamid disk jel elektroforezinde (PAGE) her iki izolektinin de tek bir band içerdiği ve molekül ağırlıklarının da 45 000 olduğu belirlendi. Sodyum dodesil sülfatlı ortamda yapılan poliakrilamid jel elektroforezinde (SDS-PAGE), 15 000 molekül ağırlığına uyan tek bir band görülmesi, her iki izolektinin de eşit molekül ağırlığında 3 altbirimden oluştuğunu düşündürdü. Bunun yanında preparatif PAGE'de hemaglutinasyon aktivitesinin protein bandına tamamiyle uyduğunun görülmesi, lektinlerin ileri derecede saflaştırıldığını gösterdi.

Key words: Aloe; *Aloe vera*, lectin; phytohemagglutinin.

INTRODUCTION

The *Aloe* plant has been used for many years all over the world for various medical purposes as well as in cosmetic industry. The genus *Aloe* comprises about 600 species, most of which are indigeneous to North Africa (2). *Aloe vera* (L.) Burm. fil. (syn. *Aloe barbadensis* Miller) is one of the most common species of *Aloe* used as natural and folk medicine in Western countries.

Lectins are a class of proteins (generally glycoproteins) of animal or plant origin, which have the ability of binding to specific carbohydrate receptors on the cell surface, thus modifying the cell and inducing cell aggregation and clump formation (3-5). Hemagglutinating and mitogenic properties of lectins are used for their identification in tissues.

The occurence of lectins (phytohemagglutinins) in *Aloes* is known since 20 years (6). In the last decade some work has been done about the isolation and characterization of lectin and isolectins from *Aloe* species (7-11). The studies made on

Aloe lectins are especially concerned with the gel portion of the leaves or the whole leaf juice (8).

Two lectins were separated for the first time in our laboratory, from the pulp of *Aloe vera* leaves and called Aloctin I and II (1). In the present paper, we are presenting further characterization of these purified lectins.

MATERIALS AND METHODS

Plant material. Specimens of *Aloe vera* (L.) Burm. fil. (in Turkish "sarısabır") were collected from Kale (Demre) in Antalya, identified by Prof. Dr. N. Sütlüpınar and cultivated in the greenhouse of our Faculty. The fresh leaves of this cultivated plant were used.

Chemicals. Sephadex was purchased from Pharmacia Fine Chemicals and Pronase from Sigma (P-6911). Pharmacia Electrophoresis Calibration Kits for high and low molecular weight proteins were used as standart reference proteins in polyacrylamide gel electrophoresis. All other chemicals were analytical reagent grade.

Assay methods.

Absorption spectra. Absorption spectra of appropriately diluted Aloctin I and II solutions in phosphate buffer, were measured over a range of 230-303 nm. by means of Shimadzu UV 1208 spectrophotometer.

Hemagglutination tests were performed in microplates in a volume of 50 µl using a 4 % suspension of washed rabbit erythrocytes (1,12). Hemagglutination was estimated visually and hemagglutination titer was expressed as the reciprocal of the highest dilution showing detectable agglutination.

Protein contents of the samples were determined by $E_{280/260}$ method (3).

Carbohydrate was determined as neutral sugar by the method of Dubois *et al.* (14), using D-mannose as a standart.

Purification of the lectins. Aloctin I and Aloctin II were purified by hydroxylapatite column chromatography of the crude extract obtained by homogenization of *Aloe vera* leaf pulps in phosphate buffered saline (PBS) as described previously (1).

Gel filtration of Aloctin I. Aloctin I was concentrated to 3 ml (75 mg protein) by means of Millipore-CX-10 ultrafiltration membranes and then applied to a column of Sephadex G-50 (2.5 cm x 45 cm) equilibrated with 0.01 M Tris-HCl buffer (pH 8.5).

The elution was performed at 4°C, by washing the column with 3 column volumes of the same buffer. Fractions of 3 ml were collected at a flow rate of 45 ml/hour. The elution profile was obtained by measuring the absorbance of every tube at 280 nm. The fractions corresponding to each protein peak were pooled separately and examined for hemagglutinating activity.

Pronase digestion of purified Aloctin I. Purified Aloctin I obtained from gel filtration was concentrated to 5 ml (5.7 mg protein) and then digested with 0.75 mg pronase at pH 7.4, for 72 hours, at 37°C. The enzyme /protein ratio was 1/8; w/w. The digest (5ml) was subjected to gel filtration on a column (1.5cm. x 30 cm.) of Sephadex G-50, previously equilibrated with 0.01 M Tris-HCl buffer, pH 8.5. Elution was carried out with the same buffer and 2.5 ml fractions were collected at a rate of 21 ml/hour. The fractions were analyzed for absorbance at 280 nm., for neutral sugars and for hemagglutinating activity.

Polyacrylamide gel electrophoresis (PAGE). Non-denaturing PAGE was performed with the standart Tris-glycine system, essentially by the methods described by Ornstein (15) and Davis (16). 10 fold diluted solution of Tris-glycine buffer (3% Tris and 14.4% glycine) of pH 8.5 was used during electrophoresis. The electrophoresis was performed in 7.5% polyacrylamide gels of 6 x 0.6 cm. rods. Protein concentration was 0.152 µg/20µl/gel. The Pharmacia HMW calibration kit proteins were used as molecular mass standarts. This kit contained thyroglobulin (M_r 669 000), ferritin (M_r 440 000), catalase (M_r 232 000), lactate dehydrogenase (M_r 140 000) and bovine serum albumin (M_r 67 000). Electrophoresis was carried out initially at 120 V and 5 mA/gel for 20 min., then at 160 V at the same current for 10 min. Bromphenol blue was used as tracking dye. Gels were stained with 6% Amidoblack 10B. ***Preparative PAGE*** was undertaken in the same conditions. The samples were applied in duplicate to the gels. After electrophoresis one gel was stained for protein and the other gel was cutted by means of a slicer. Every piece of gel was placed in a test tube and 0.5 ml of PBS was added. The tubes were shaken by means of a Vortex mixer and were kept at 4°C overnight for the extraction of proteins. Hemagglutination test was performed by adding 25 µl of 4% erythrocyte suspension to 25 µl from each tube in microplates.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was done according to Weber and Osborn (17) in 10% gels containing 0.1% SDS. Electrophoresis buffer was 0.05 M sodium phosphate buffer of pH 7, containing 0.1% SDS. Appropriately concentrated Aloctin I and Aloctin II samples as well as molecular weight standarts, were treated with 2.5% SDS and 5% β-mercaptoethanol, and incubated at 100°C for 10 min. prior to electrophoresis. The Pharmacia HMW kit

contained as subunit molecular weight, thyroglobulin (M_r 330 000), ferritin (M_r 220 000), bovine serum albumin (M_r 67 000), catalase (M_r 60 000), lactate dehydrogenase (M_r 36 000) and ferritin (M_r 18 500). The Pharmacia LMW kit contained phosphorylase b (M_r 94 000), bovine serum albumin (M_r 67 000), ovalbumin (M_r 43 000), carbonic anhydrase (M_r 30 000), soybean trypsin inhibitor (M_r 30 000) and α-lactalbumin (M_r 14 400). Electrophoresis was carried at 200 V and 8 mA/gel for 4 hours. Bromphenol blue was used as tracking dye. Gels were stained 2 hours with 0.25% Coomassie brilliant blue.

RESULTS and DISCUSSION

Absorption spectra

The absorption spectra of *Aloe vera* isolectins, namely Aloctin I and Aloctin II resembles that of other proteins (18-20).

Aloctin I showed maximum absorbance at 295 nm and Aloctin II at 272 nm. (Fig 1).

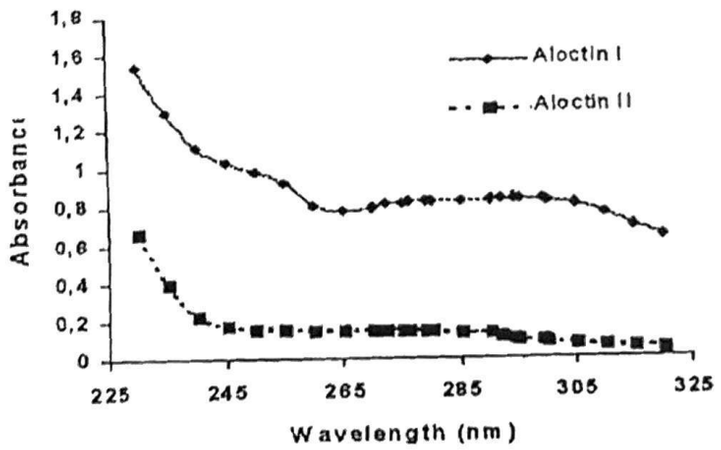


Figure 1 : Absorption spectra of Aloe vera leaf pulp lectins

Sephadex G-50 gel filtration of Aloctin I

Two protein peaks were separated by Sephadex G-50 gel fitration of Aloctin I. (Fig 2). The peak of hemagglutinating activity was coincident with the major peak. Aloctin I was thus further purified and this purified protein was used for digestion with pronase.

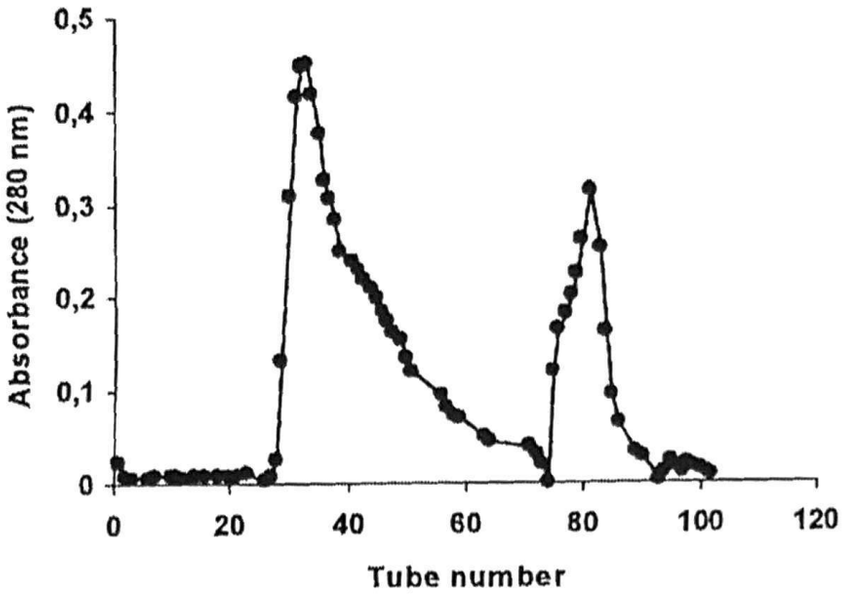


Figure 2 : Sephadex G-50 gel filtration elution profile of Aloctin I

Pronase digestion of purified Aloctin I

One major and two minor protein fractions resulted from gel filtration of pronase digested Aloctin I through Sephadex G-50 column (Fig. 3). The results of their analysis for protein, hemagglutinating activity and neutral sugar are shown in Table 1.

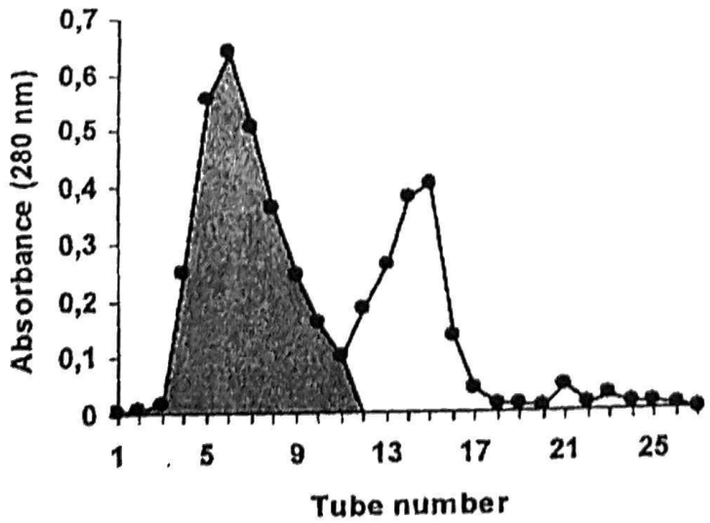


Figure 3 : Sephadex G-50 column chromatography elution profile of Aloctin I after digestion with pronase

Table 1. Hemagglutinating activity, protein and neutral sugar contents of the fractions obtained through Sephadex G-50 gel filtration of Aloctin I after digestion with pronase.

Fraction	*Hemagglutination (U/mg)*	*Total Protein (mg)*	*Total Carbohydrate (mg)*
Peak I	965.3	1.33	164.75
Peak II	0	0.24	81.34
Peak III	0	0.26	63.65

As a result of pronase digestion three glycoproteins were separated from Aloctin I. The carbohydrate content of the two peaks is lower than the major peak (I), which contained the totality of the hemagglutinating activity and 53% of the total carbohydrate (Table 1). This result is in accordance with Bourrillon *et al.* (20), who separated a glycopeptide from *Robinia pseudoaccacia* phytohemagglutinin containing 80% of the total sugar and all the hemagglutinating activity and with Lis *et al.* (21) who separated a glycopeptide containing 95% of the carbohydrates of soybean hemagglutinin. The lectin is thus cleaved without loss of activity and the coincidence of the major peak and the majority of the sugar demonstrated the covalent nature of the carbohydrate-peptide linkage. It is known that lectins in spite of their different origins and specificities, share many characteristics in common. Among them is their resistance to enzymatic digestion. As stated by Bouthet *et al.* (10), protease treatment of semi-purified hemagglutinin from *Aloe vera* leaf gel did not change its hemagglutination titer. Similary, in our study Aloctin I did not loose any of its activity by treatment with pronase.

Polyacrylamide gel electrophoresis (PAGE)

Non-denaturing PAGE gave a single band for each isolectin and showed a molecular weight of 45 000 for both isolectins (Fig. 4 and Graph 1).

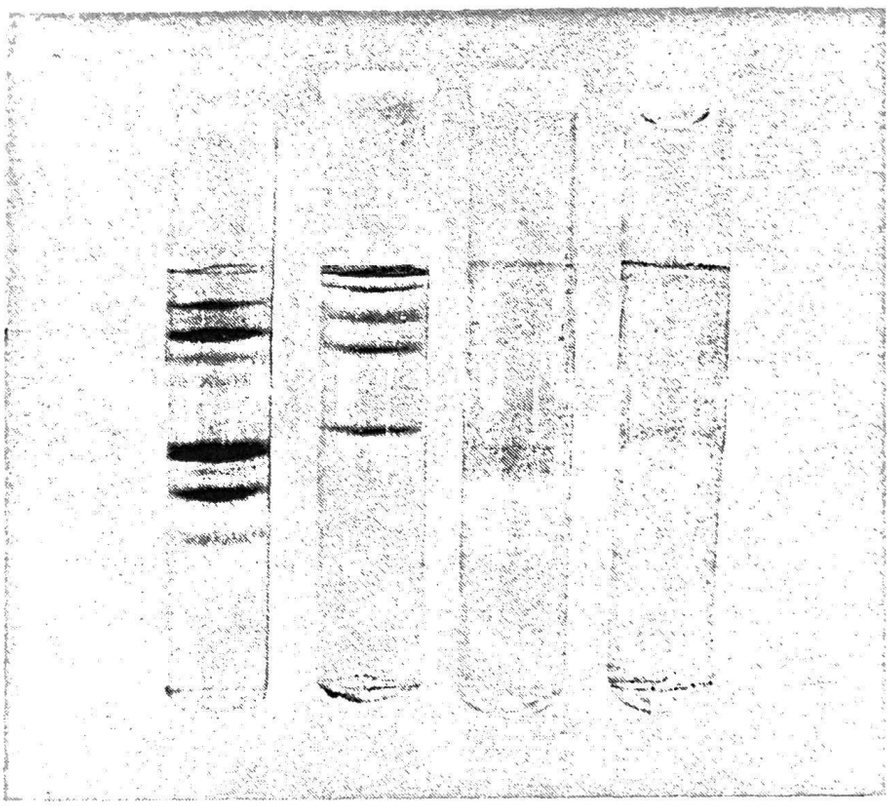
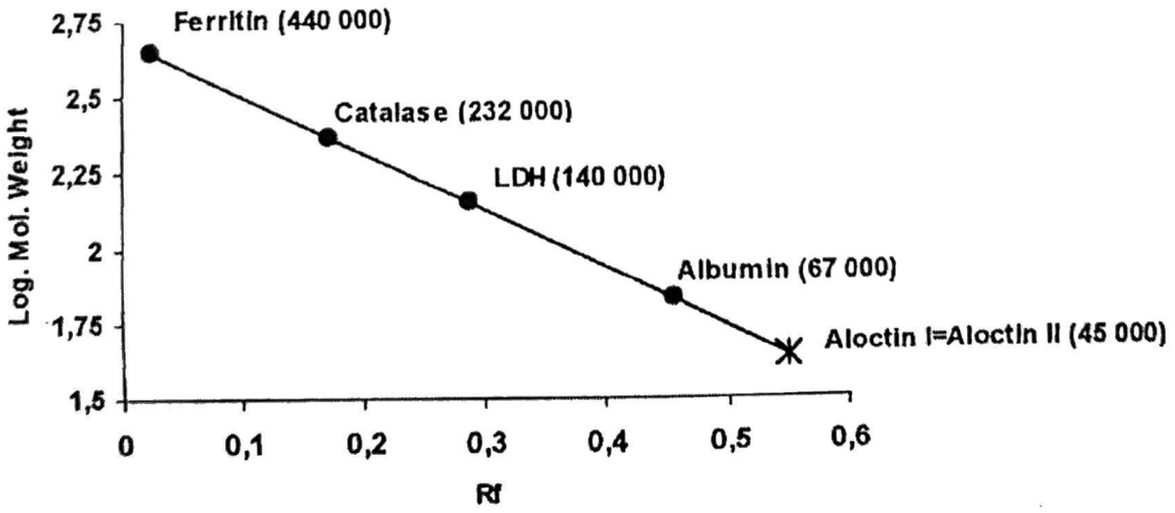


Figure 4 : Sephadex G-50 column chromatography elution profile of Aloctin I after digestion with pronase



Graph 1 : PAGE standart graph for HMW proteins

SDS-PAGE gave a single band for each isolectin, giving a subunit molecular weight of 15 000 (Fig. 5 and Graphs 2 and 3). These findings suggest that *Aloe vera* leaf

pulps contain two isolectins, Aloctin I and Aloctin II, each of them consisting of three subunits of equal molecular weight.

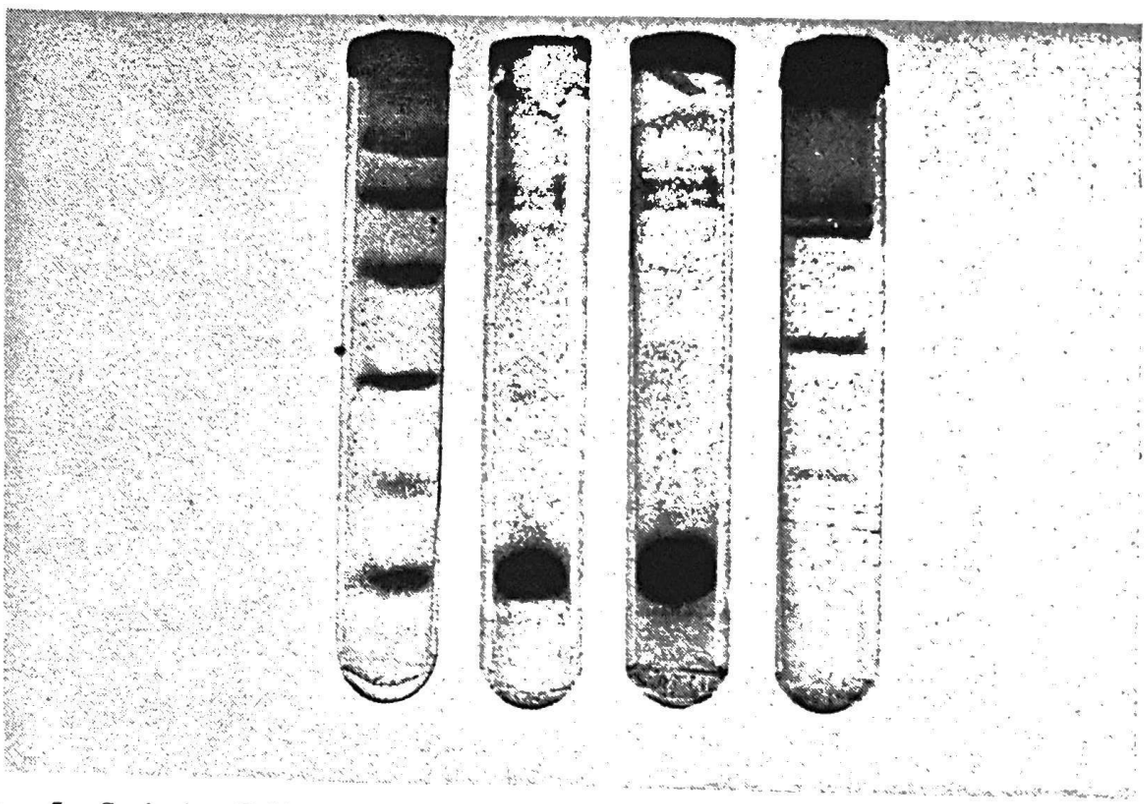
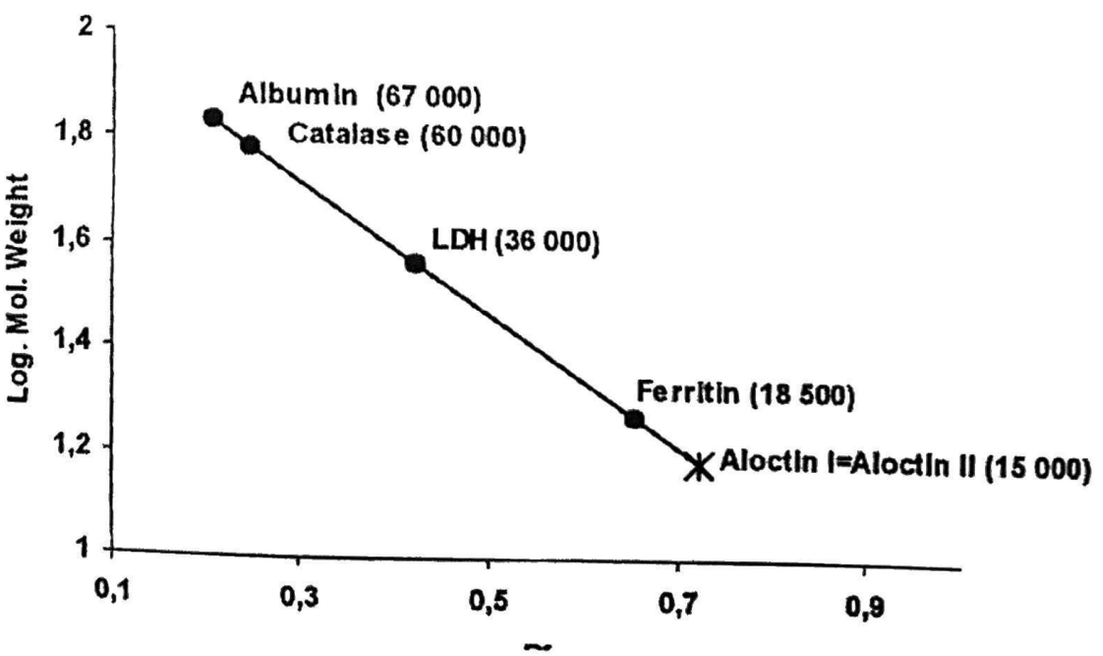
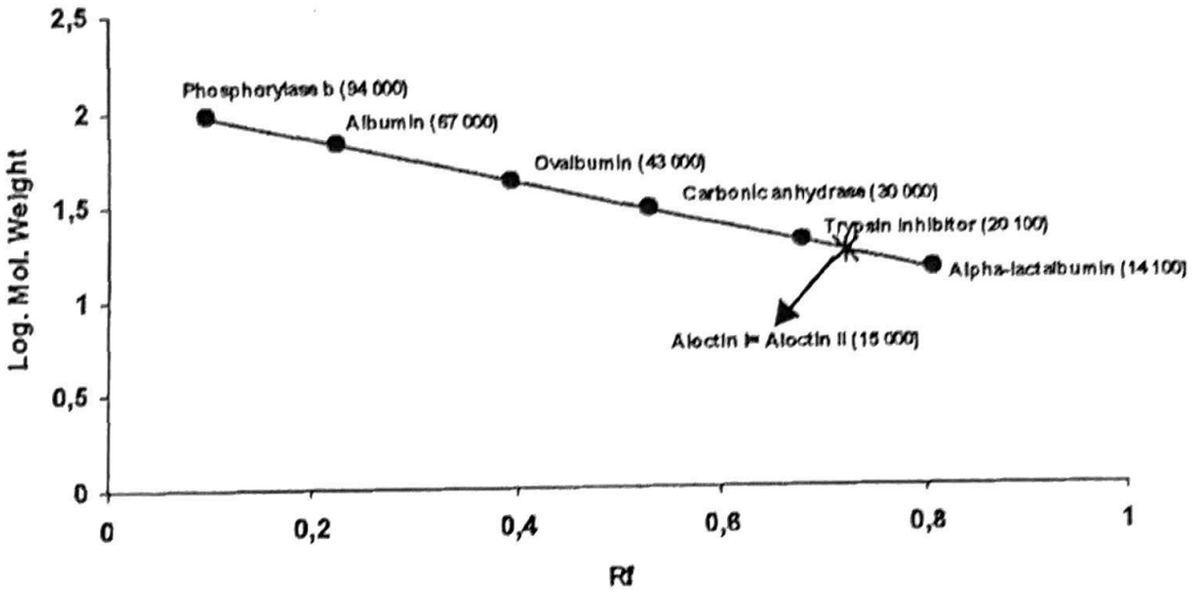


Figure 5 : Sephadex G-50 column chromatography elution profile of Aloctin I after digestion with pronase



Graph 2 : SDS-PAGE standart graph for HMW proteins



Graph 3 : SDS-PAGE standart graph for LMW proteins

Preparative PAGE demonstrated that hemagglutinating activity corresponds exactly to the protein band, showing that purification of the lectins was achieved to a great extent.

Acknowledgement: The authors wish to thank Prof.Dr . Nurhayat Sütlüpınar for providing and identifying the plant.

REFERENCES

1. Akev, N., Can, A., *Phytotherapy Research* **13**, 489-493 (1999).
2. Capasso, F., Borrelli, R., Capasso, R., Di Carlo, et al., *Phytotherapy Research* **12**, S124-S127 (1998).
3. Goldstein, I.J., Hughes, R.C., Monsigny, M. et al., *Nature* **285**, 66 (1980).
4. Sharon, N., Lis, H., *Science* **246**, 227-234 (1989).
5. Sharon, N., Lis, H., *FASEB J.* **4**, 3198-3207 (1990).
6. Suzuki, I., Saito, H., Inoue, S. et al., *J. Biochem.* **85**, 163-171 (1979).
7. Winters, W.D., *Phytotherapy Research* **7**, S23-S25 (1993).
8. Saito, H., *Phytotherapy Research* **7**, S14-S19 (1993).
9. Koike, T., Beppu, H., Kuzuya, H. et al., *J. Biochem.* (Tokyo) **118**, 1205-1210 (1995). Ref. Chem. Abstr. **124**, 168770d. (1996).
10. Bouthet, C.F., Schirf, V.R., Winters, W.D., *Phytotherapy Research* **10**, 54-57 (1996).

11. Yagi, A., Egusa, T., Arase, M. et al., *Planta Medica* **63**, 18-21 (1997).

12. Lis, H., Sharon, N., *Methods enzymol.* **28 Part B**, 366-368 (1972).

13. Warburg, O., Christian, W., *Biochem. Z.* **310**, 384-421 (1941).

14. Dubois, M., Gilles, K.A. et al., *Anal. Chem.* **28**, 350-356 (1956).

15. Ornstein, L., *Ann. N. Y. Acad. Sci.* **121**, 321-349 (1964).

16. Davis, B.J., *Ann. N.Y. Acad. Sci.* **121**, 404-427 (1964).

17. Weber, K., Osborn, M., *J. Biol. Chem.* **244**, 4406-4412 (1969).

18. Pallansch, M.J., Liener, I.E., *Arch. Biochem. Biophys.* **45**, 366-374 (1953).

19. Agrawal, B.B.L., Goldstein, I.J., *Arch. Biochem. Biophys.* **124**, 218-229 (1968).

20. Bourrillon, R., Font, J., *Biochim. Biophys. Acta* **154** , 28-39 (1968).

21. Lis, H., Sharon, N., Katchalski, E., *J. Biol. Chem.* **241**, 684-689 (1966).