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Full Length Research Paper

Bioactivity of lectin from Egyptian *Jatropha curcas* seeds and its potentiality as antifungal agent

Mahmoud A. Al-Saman[1,3], Safinaz A. Farfour[2,3], Ahmed A. Tayel[1,3], Nashwa M. Rizk[2,3]

[1]Industrial Biotechnology Department, [2]Environmental Biotechnology Department, [3]Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt

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Lectins are non-immune, carbohydrate-binding proteins, which are very specific for sugar moieties and agglutinate cells or precipitate polysaccharides. Lectin from Egyptian *Jatropha curcas* seeds was isolated and purified using ammonium sulphate fractionation and gel filtration columns chromatography. The purified lectin has a specific activity of 351.5 hemagglutination unit/mg protein and a molecular weight of 28.00 kDa. The hemagglutination activity of lectin was suppressed by D-glucose, D-mannose and D-galactose. The amino acid composition of the purified lectin contained high ratio of acidic amino acids (38.42%), the hydroxy amino acids (11.10%), with minor amounts of sulfur containing amino acids and cysteine. The purified lectin from *J. curcas* seeds was heat - stable up to 60 ºC with trypsinized cattle erythrocytes and the hemagglutination activity decreased with increasing temperature. The purified lectin was insensitive from acidic to neutral conditions but was markedly affected by basic pH and the hemagglutination activity was encouraged by Mg^{2+}, Ca^{2+} and Mn^{2+} ions. The purified lectin showed an antifungal activity against *Fusarium oxysporum* with a minimal inhibitory concentration of 70 µg / ml. However, lectin extraction from *J. curcas* could be recommended as a source for that could be applied as antifungal agent against phytopathogenic fungi.

Keywords: Agglutinin; Hemagglutination activity; Purification; Antifungal; Phytopathogens

INTRODUCTION

Many types of proteins have been separated from the plants, which have an important role as defense proteins against fungi; including thaumatin-like proteins, chitinases, b-1.3 glucanases, thionins, plant defensins, ribosome-inactivating proteins, protease inhibitor-like proteins and lectins (Vaz *et al*., 2010).

The “Lectin” term is derived from the Latin word *legere*, which means to select, pick and choose; also known earlier as agglutinin, haemagglutinin or phytohaemagglutinin (Peumans et al., 1995). Lectins are carbohydrate-binding protein of non-immune origins that are very specific for sugar moieties and agglutinate cells or precipitate polysaccharides or glycoconjugates (Goldstein et al., 1980; Dixon 1981). In general, the structure of the lectin subunits contains four major types, e.g. merolectins, hololectins, chimerolectins and superlectins (Van et al., 1996). Since each lectin binds to a specific kind of sugar, and sugar chains on the cell surface differ according to cell type and

*Corresponding Author’s Email: farfour.safinaz@yahoo.com; FAX: +2-048-2601266-8.

animals species, lectins can distinguish the types of cells. Moreover, lectins not only recognize specific cell types, but also affect cell physiology (Jimbo et al., 2012).

Lectins can be found almost in all kingdoms from bacteria to animals (Loris, 2002), and in particular, lectins are most abundant in the seeds of plants, mainly in the leguminoseae and graminaceae seeds (Pusztai 1991). Lectins in leguminous plants are sited mainly in the cotyledons; smaller amounts are sometimes found in embryos and even in seed coats (Howard et al., 1972).

Plant lectins may not only play a role in the plant itself, as a store of nitrogen or as a specific recognition factor, but also found that lectin concentrations in plant seeds decreases with growth, which may indicates a role in plant germination and in the seed's permanence itself. Some plant lectins have been found to recognize non-carbohydrate substances like phytohormones including adenine, auxins, cytokinin and indole acetic acid, it has been suggested that these interactions may be physiologically significant (Komath et al., 2006). Further role of the lectins is their interaction with glycoconjugates of other organisms and interference with the normal functioning of those organisms. Lectins are the only plant proteins that have the ability to recognize glycoconjugates present on the surface of microorganisms (i.e. bacteria and fungi) or exposed along the intestinal tract of insect or mammalian herbivores (Peumans et al., 1995).

Some lectins have antifungal activities; for instance a chitinase-free lectin from stinging nettle (*Urtica dioica*) could inhibit the growth of *Botrytis cinerea*, *Trichoderma hamatum* and *Phycomyces blakesleeanus* (Broekaert et al., 1989). Also, the lectin from the rubber tree *(Hevea brasiliensis)* was characterized as a chitin-binding protein that can also inhibit fungal growth (Van Parijs et al., 1991). Plant lectins have affinity for the unusual carbohydrates (e.g. muramic acid, *N*-acetylmuramic acid and muranyl dipeptides) present on bacterial pathogens. Muramic acid is similar in structure to Glc and GlcNAc, especially of the Viciaceae such as soyabean lectin (Ayouba et al., 1994).

Jatropha curcas seeds are the nut belonging to the *Euphorbiaceae* family. It is cultivated in Central and South America, South East Asia, India and Africa (Gubitz et al., 1999). It is commonly known as the "Physic nut", "Curcas bean", "Barbados nut" or "Black vomit nut" (Everist 1981). The seeds contain ~27-40% oil (Achten et al., 2007; Achten et al., 2008), that can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine.

The seeds are also a source of the highly poisonous toxalbumin curcin or jatrophin, like ricin from beans of the castor tree, *Ricinus communis* and abrin from *Abrus precatorius* (Stirpe et al., 1976).

Curcin is a lectin which has the ability to bind with carbohydrate and lead to hemagglutination of blood cells (Jaffe 1969). The curcin has a powerful inhibitory action toward protein synthesis in reticulocyte lysate; it has, also, an obvious antitumor effect and its mechanisms are related to the N-glycosidase activity (Juan et al., 2003).

Fusarium spp. are a widespread group of fungi and commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders (Nelson et al., 1983). All diseases caused by *Fusarium*, probably the most important are the vascular wilt diseases caused by *Fusarium oxysporum*. These fungi attack a different group of plants including crops, ornamentals and trees (Nelson et al., 1981). Classical taxonomy of *Fusarium* spp., depended on its morphological characteristics and pathogenicity (Abd El-Salam et al., 2003). *Fusarium* spp. could produce some hazardous mycotoxins in cereal grains, e.g. fumonisins, which are considered as the second serious toxin group, after aflatoxins, to cause crops loss and human illnesses (Munkvold 1997).

However, the present study was designed for the isolation, purification and characterization of a lectin from Egyptian *Jatropha curcas* seeds, and to evaluate the antifungal activity of purified lectin against the pathogenic fungus *Fusarium oxysporum*.

MATERIALS AND METHODS

The steps overview, to obtain the purified lectin from *J. curcas* seeds, is illustrated in Fig. 1 and detailed descriptions, of the applied methods, are given below:

Preparation of the seed meals

The fruits of Egyptian physic nut (*Jatropha curcas* L.) were obtained from the Central Administration for Afforestation, Ministry of Agriculture, Giza, Egypt, at their optimal harvesting time in June. Selected mature fruits were individually washed, sun dried for 3-4 days and divided manually into three parts (Pericarps, Seed coats, Embryos). The mature embryos of seeds were ground finely with a coffee grinder and defatted with two volumes of petroleum ether, at 60-70 °C for 24 h using a Soxhlet apparatus (Ajiwe et al., 1996), the resulting powder was left to dry in a desiccator at room temperature.

Extraction of the lectin

Extraction of *J. curcas* lectin was carried out according to the reference procedures (Stirpe et al., 1976; Juan et al., 2003). 1976 as follows: 500 g of mature embryos defatted powder were extracted with 5 l of 5 mM-sodium phosphate buffer (SPB), pH 7.2, containing 0.2 M NaCl. The mixture was stirred on a magnetic stirrer for 2-3 h, and left overnight at 4 °C. The clear supernatant was collected by centrifugation at 3000 rpm for 20 min. Ammonium sulphate, $(NH_4)_2SO_4$, was gradually added to the collected supernatant up to final amount of 166 g /l, with constant stirring for 30-60 min, until reaching a semi saturation state. The precipitate was

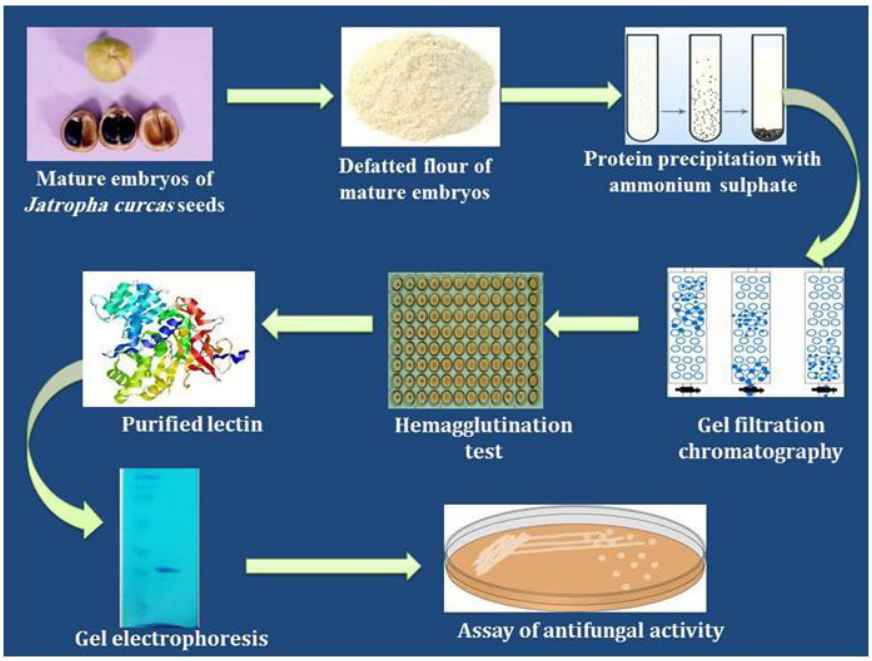

Figure 1: Overview of the extraction, purification and characterization of lectin isolated from Egyptian *Jatropha curcas* seeds.

left to settle overnight at 4 °C and the precipitated protein was collected by centrifugation at 12.000 rpm for 20 min at 7 °C. An additional quantity (184 g/l) of $(NH_4)_2SO_4$ was added to the remaining supernatant to reach full saturation. The precipitant was recollected and suspended in a 25 ml of SPB.

The suspension was dialyzed for 24-48 h at 4 °C against a continuous flow of the SPB buffer. Any insoluble material which was present after dialysis was removed by centrifugation at 3000 rpm for 20 min. The resulting brownish supernatant was referred to as crude lectin (curcin).

Purification of the lectin

According to the method of Stirpe *et al.*, the crude lectin fraction was loaded on a column (37×3.2 cm) of Sephadex G-100, previously equilibrated with 5 mM SPB, pH 7.2 containing 0.2 M NaCl. The column was eluted with the same buffer at a flow rate of 100 ml/h, and 150 fractions were collected using fraction collector (Gilson® FC-203, Gilson, Middleton, WI, USA), the absorbance of 3 ml fraction was recorded by using spectrophotometer at 280 nm. Fractions containing hemagglutination activity were dialyzed and applied to a Sephacryl S-200, column (17x1.2 cm) previously equilibrated with 0.1 M Tris-HCl buffer, pH 7.0, containing 0.4 M NaCl. Elution was done by the same buffer at a flow rate of 40 ml/h and 2 ml fractions were collected and the protein concentrations were estimated. The protein content in all fractions were determined as described by Bradford (Bradford MM (1976), using a spectrophotometer at absorbance of 280 nm. Bovine serum albumin (BSA) was used as a standard for comparison.

Hemagglutination activity of Jatropha curcas lectin

The animal blood samples (whole cattle blood) were obtained from the University of Sadat City farm, Sadat City, Egypt. The hemagglutination activity was carried out by measuring the absorbance of unsedimented layer of trypsinized erythrocytes and examining the agglutination microscopically (Gordon and Marquardt 1974). The hemagglutination assays were performed at 25 ºC, using 2-fold serial dilutions of purified lectin in SPB; 1 ml was incubated with an equal volume of the standard erythrocytes suspension, and the content of each tube was placed in a rack that holds them in an exactly vertical position, after 2.5 h the absorbance of tubes suspensions were read at 620 nm.

Calculation of hemagglutination activity

Colorimetery

The hemagglutination unit (HU) was defined as the reciprocal of dilution of purified lectin (fractions) which was required to cause a decrease of 50 % in the absorbance of the erythrocytes suspension in 2.5 h.

Microscopically

The content of the tubes were examined microscopically after 1 h for agglutination. Hemagglutination activity was

expressed as inverse of minimum amount of the sample in mg/ml of the assay which formed agglutination.

Sugar specificity test

The sugar specificity test was performed according to Cano Asseleih *et al*., In brief, 1 ml from different concentrations (10, 30, and 50 mM), of the following sugars (D-glucose, D-galactose, D-mannose, D-lactose and sucrose) was added to 1 ml of each fraction and allowed to stand for 30 min at 25 °C. An erythrocyte suspension (1ml) was then added and the mixture was left for 30 min with a non-sugar containing solution (blank). The hemagglutination activity was observed microscopically.

Gel electrophoresis and molecular weight determination

Sodium dodecyl sulfate polyacrylamid gel electrophoresis (SDS-PAGE) of the lectin fractions were carried out using a discontinuous buffer system; 10 % separating and 5 % stacking gels (Laemmli et al., 1970). 100 µl of fraction and an equal volume of sample buffer (0.5 M Tris-HCl pH 6.8) were added. The protein was denatured by heating in a boiling water bath for 5 min, and immediately cooled on ice. After electrophoresis, the gel was stained in 50 ml of staining solution using Coomassie blue R-250 for 30-45 min with shaking at 25 °C and destained in destaining solution. The molecular weights were determined by comparison with a molecular weight protein marker (14-116 kDa).

Effect of pH, temperature and metal ions

The effect of pH on hemagglutination activity was determined by incubating lectin samples at various pH values at 25 °C for 1 h in selected buffers (0.1 M acetate buffer, pH 4-5, 0.1 M phosphate buffer, pH 6-7 and 0.1 M tris-HCl buffer, pH 8-11). The residual hemagglutination activity was assayed after adjusting the mixture to pH 7.0. Thermal stability was determined by incubation of lectin samples at various temperatures (30-90 ºC) for 30 min and the residual hemagglutination activity was then assayed. The effect of metal ions were determined by incubation of lectin samples (1 mg/ml) with 5, 10 and 20 mM concentrations of Mg^{2+}, Ca^{2+}, Mn^{2+} and EDTA ions in 150 mM NaCl, with continuous shaking. The lectin samples were dialyzed exhaustively against 150 mM NaCl, and the hemagglutination activity was assessed before and after addition of metal ions.

Amino acid analysis

Amino acid analysis was carried out using the amino acid analyzer (LC3000, eppendorf, Germany) (Anderson et al., 1977).

Antifungal activity of purified lectins

The purified lectin was tested against the pathogenic plant fungus *Fusarium orysporum*, using the filter paper disc diffusion method (Amare 2002). In brief, a disc with 5 mm diameter was taken from the margin of fungal culture and put in the center of a Petri dish contained potato dextrose agar (PDA) medium. Four filter paper discs (Whatman No. 1, 6 mm diameter) were loaded with 25µL from different concentrations of lectin. The inoculated plates were incubated at 25 ˚C for 6 days. Controls were implemented with sterilized distilled water instead of lectin.

Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) using the statistical analysis system. Means were calculated according to least significant difference (LSD) (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Purification of lectin from the seeds of Jatropha curcas

The extraction and purification of lectin from mature embryos of *J.curcas* seeds involves two steps: The first step is the precipitation of the lectin fraction from the saline extract using ammonium sulphate followed by purification by gel-filtration column chromatography using Sephadex G-100 and Sephacryl S-200 matrixes.

Crude lectin solution obtained from defatted mature embryos was used for chromatographic purification of *J. curcas* lectin on Sephadex G-100 column equilibrated with the saline phosphate buffer (SPB). Two peaks of proteins were detected; the first peak from fractions No. 30 to 76 and the second peak from fractions No. 77 to 130. Hemagglutination activity (HA) was found only in the first peak. The hemagglutination activity was positively correlated with protein concentration. The maximum activity was seen in fraction number 48 (Fig. 2).

The bold fractions (peak 1) were then purified on Sephacryl S-200 column; peak 1 was obtained from fractions No. 5 to 19 and the maximum hemagglutination activity was recorded from fraction number 13 (Fig. 3).

The purification procedure of lectin from *J. curcas* seed extract is summarized in Table 1. The lectin fractions from defatted mature embryos of *J. curcas* seeds; saline solution, $(NH_4)_2SO_4$ and purified lectin obtained from Sephadex G-100 and Sephacryl S-200 columns were used for determined and observed the agglutination activity using trypsinized cattle erythrocytes. The higher agglutination activity was observed in purified lectin from

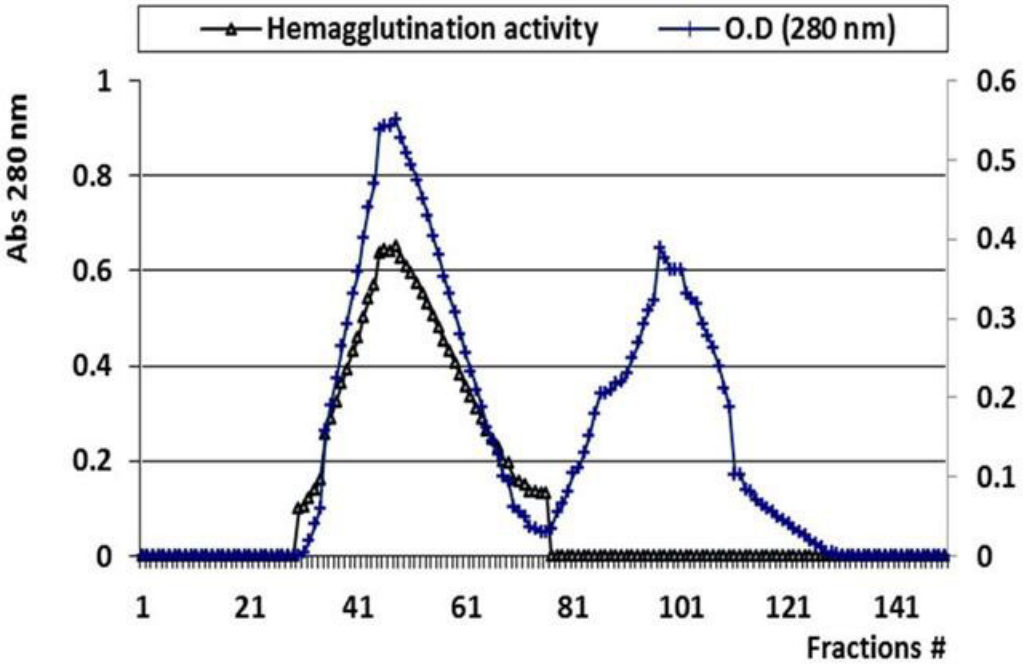

Figure 2: Fractionation of ammonium sulfate partially purified lectin of *Jatropha curcas* seeds after applying on Sephadex G-100. + represents spectrophotometric measurement of fractions, while Δ represents hemagglutination activity.

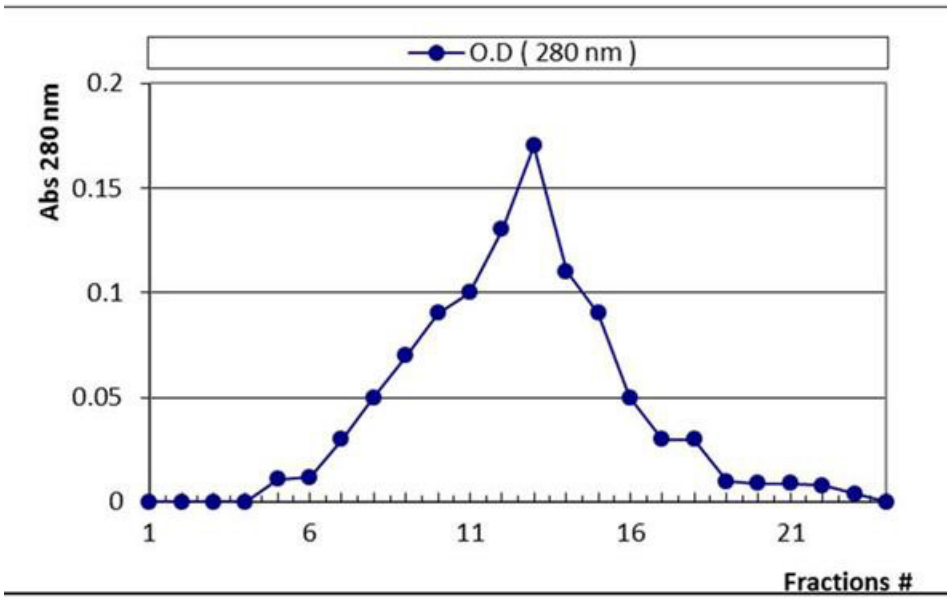

Figure 3: Gel filtration chromatography on Sephacryl S-200 of mature embryos lectin of *Jatropha curcas* seeds obtained from Sephadex G-100 column.

Table 1: Summary of the purification steps of the lectin from *Jatropha curcas* seeds

Fraction	Defatted mature embryos (100 g/l)			
	protein (mg/ml)	HA (titre^{-1})*	Specific activity (HU/mg)	Purification (fold)
Saline solution	9.320	16.0	1.72	1.00
$(NH_4)_2SO_4$	3.620	17.7	4.89	2.84
Sephadex G-100	1.270	71.7	56.46	32.82
Sephacryl S-200	0.330	116.0	351.51	204.37

Hemagglutinating activity (HA) was determined with cattle erythrocytes. Specific HA corresponds to the ratio between HA (titre^{-1}) and protein concentration (mg/ml). Purification corresponds to the ratio between specific HA of the step and specific HA of the saline solution step.

* Initial concentration from saline solution, $(NH_4)_2SO_4$, Sephadex G-100, Sephacryl S-200 fractions were 1.0, 0.9, 0.9, and 1.1 mg/ml respectively.

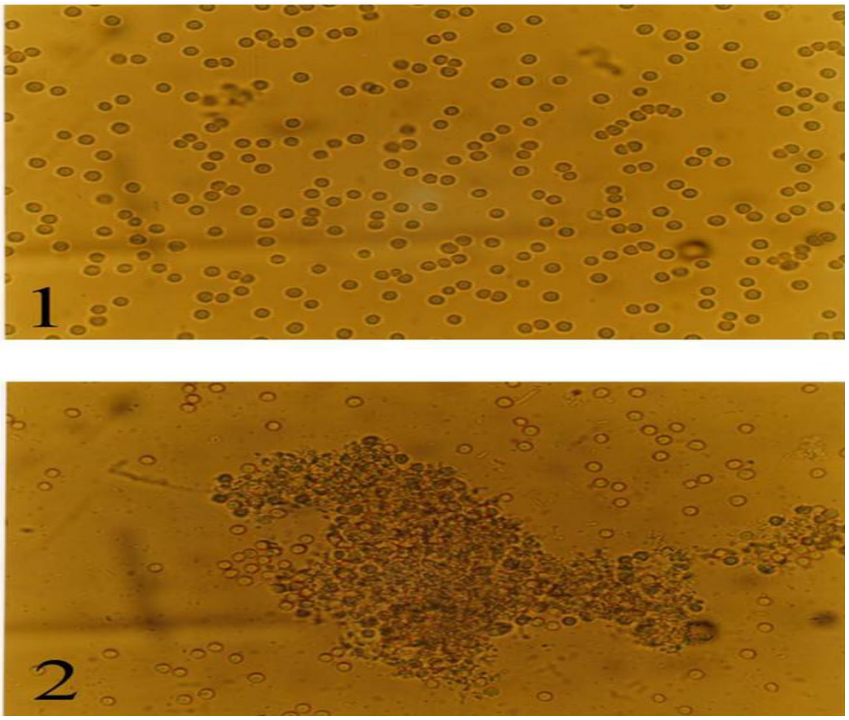

Figure 4: Effect of lectin treatment on the hemagglutination of trypsinized erythrocytes
1, Trypsinized erythrocytes from cattle blood
2, Pattern for hemagglutination of trypsinized erythrocytes by the purified lectins

Sephacry S-200 column (Fig. 4) with hemagglutinating activity of 351.51; a 204.37 fold activity increasing.

It was reported (Absar et al., 2005), that affinity chromatography is widely used for purification of lectin due to the comparatively high specificity and good yields obtained. Galactose-specific lectin from *J. curcas* seeds was purified by an affinity chromatography column (Juan et al., 2003).

The inhibition of agglutination by sugars was studied, *J. curcas* lectin-induced agglutination of trypsinized cattle erythrocytes. From Fig. 4, no agglutination in the trypsinized erythrocytes could be observed, in the absence of lectin (Fig. 4-1). On the other hand, trypsinized erythrocytes, treated with purified lectin, were remarkable coagulated and combined in clusters (Fig. 4-2). The agglutination activity was inhibited by sugars "which are known to inhibit action of lectins" and the carbohydrate binding specificity of the lectin was investigated by incubating the lectin with different sugars. If the lectin has an affinity to the added sugar, hemagglutination is inhibited because the lectin already binds to the sugar and vice versa (Jimbo et al., 2012).

The assay for different sugars' inhibitory concentration, on hemagglutination activity of purified lectin of *J. curcas*,

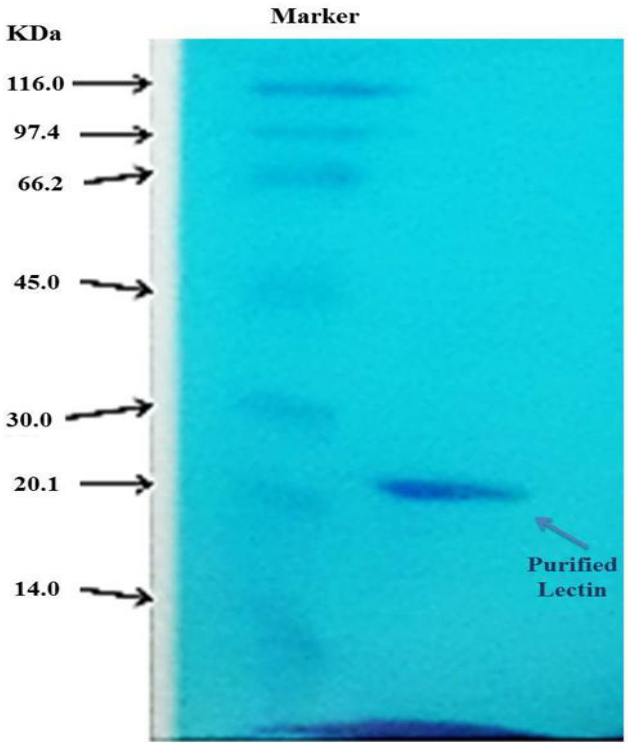

Figure 5: SDS-PAGE analysis of the purified lectin
Left lane: molecular weight markers Right lane: purified lectin.

indicated that the agglutination of trypsinized cattle erythrocytes by *J. curcas* lectin was entirely inhibited by D-glucose, D-mannose and D-galactose. On the other hand, lactose and sucrose did not exhibit any inhibitory activity even at a concentration of 50 mM. The highest inhibitory activities were observed with D-glucose and D-galactose at concentrations of 30 and 10 mM, respectively, and lowest inhibitory activities were observed with D-glucose and D-mannose at a concentration of 50 mM.

Only D-galactose and aminogalactoses were reported to bind to the lectin and do not preferentially bind to blood group A (Cano et al., 1989); this showed that the lectin from *J. curcas* possessed galactose binding groups. Also, it was reported that D-galactose inhibited the hemagglutination activity of BmoLL, a galactose-specific lectin (Coelho and Silva 2000).

Polyacrylamide gel electrophoresis

Electrophoretic analysis was carried out using SDS-PAGE for various intermediate fractions from the purification procedure of mature embryos lectin. The SDS-electrophoretic pattern of the ammonium sulfate precipitated which represents the crude lectin (after dialysis) showed many protein bands, with characterized molecular weights (MW) ranged from ~24 to 43 KDa. After the sephacryl S-200 purification step, a significant sole band with a MW of 28.0 KDa in SDS-PAGE was separated (Fig. 5), which indicated to the purified lectin.

These results are in agreement with earlier investigation (Juan et al., 2003), which reported that the cloned and expressed curcin, from *J. curcas,* had a molecular weight of about 28.2 KD by SDS-PAGE. Cano Asseleih *et al*., studied the SDS-PAGE analysis of the lectin obtained in fraction the second gel filtration on Sephacryl S-200 yielded; from the main protein band and two other faint bands, only one band with isoelectric point of 5.75 was obtained and the hemagglutination activity was corresponding to this main protein band. Also, these results were in agreement with previous study (Charungchitrak et al., 2011), which reported that MW of other plant lectins range from 30 to 50 KDa.

Amino acid analysis

The analysis of the amino acid composition of purified lectin of *J. curcas* seeds is illustrated in Table (2), it is evidenced that the purified lectin contained high ratio of acidic amino acids (38.42 %), included glutamic acid (30.94 %) and aspartic acid (7.48 %). The reported hydroxy amino acids ratio was 11.10 % and includes serine (5.03 %), threonine (2.82 %) and tyrosine (3.25 %). High ratio of aliphatic amino acids was observed (29.62 %), with

Table 2: Amino acid composition of the purified lectin of *Jatropha curcas* seeds

Peak No.	Retention time (min)	Amino acid	Conc. (mg/g)	Conc. (%)
1	10.38	Asp	66.96	7.48
2	13.57	Thr	25.20	2.82
3	14.85	Ser	45.00	5.03
4	16.12	Glu	276.84	30.94
5	18.67	Pro	124.38	13.90
6	23.73	Gly	28.98	3.24
7	24.70	Ala	88.08	9.85
8	29.68	Val	31.44	3.51
9	32.25	Cys	nd*	nd*
10	34.77	Leu	18.12	2.03
11	35.63	Isoleu	98.34	10.99
12	38.70	Phe	15.36	1.72
13	40.62	Tyr	29.10	3.25
14	50.00	His	21.84	2.44
15	52.60	Lys	12.78	1.43
16	60.92	Arg	012.24	01.37

glycine (3.24 %) alanine (9.85 %), valine (3.51 %), leucine (2.03 %) and isoleucine (10.99 %), as main acids.

The purified lectin appears to be poor in sulfur containing amino acids and cysteine was not detected under the conditions of the analysis. There was a relatively high ratio in total hydrophilic amino acids (54.75 %), while the total hydrophobic amino acids ratio is (45.25 %).

The content of amino acids in lectin was recorded earlier (Cano et al., 1989); this study could not detect cysteine but reported that very low levels of sulfur-containing amino acids as well as methionine content in *J. curcas*, although being rich in acidic amino acids. The presence of high ratio from acidic amino acids in the lectin structure indicates that the carbohydrate moiety in the glycoprotein may attach to these amino acids by a covalent link. Additionally, the presence of hydrophobic amino acids (alanine, leucine, isoleucine, phenylalanine, tyrosine, valine and praline) suggests the involvement of hydrophobic bonding in the maintenance of the molecular structure, which could give the *J. curcas* lectin more functions in the life cycle of the plant.

Assay of antifungal activity

The evaluation of lectin antifungal activity, against *Fusarium oxysporum*, revealed that purified lectin had a stronger effect than crude lectin, for the inhibition of fungal growth. *F. oxysporum* was able to grow at concentrations of crude lectin up to 90 µg/ml, whereas 100 µg/ml was the inhibitory concentration from the crude protein. On the other hand, the concentration of 70 µg/ml from purified lectin, and above, was able to inhibit the fungal growth on Potato Dextrose Agar (PDA) medium (Fig. 6). *F. oxysporum* had a normal hyphal development in the control assay, after six days of growth, in contrary with the assays with 100 µg/ml of crude lectin or with the inhibitory concentrations from pure lectin (Fig. 6).

The antifungal activity of purified lectin could be attributed to its binding with some fungal wall components and altering their function. Chitin could be from these components, which constitute from a long *N*-acetyl glucosamine polymer chain and is a distinctive component of the fungal cell walls. The *N*-acetyl glucosamine compound is derived primarily from glucose; which is from the sugars that have the ability to bind with the purified *J. curcas* lectin.

It was reported found that the inhibitory effects of *Talisia esculenta* lectin on the growth of the fungi could be due to the interaction with chitin or other fungal structures; mannan, chitin and other saccharides are major components of most fungal cell walls, and lectin may inhibit fungal growth by interacting with these carbohydrates (Freire et al., 2002).

Also, it was suggested that chitin-binding proteins could form cross-linkages with chitin and preventing cell expansion at the tip of the growth hyphae. This binding could slow hyphal growth, as the first line of an integrated defense mechanism (Chrispeels and Raikhel 1991).

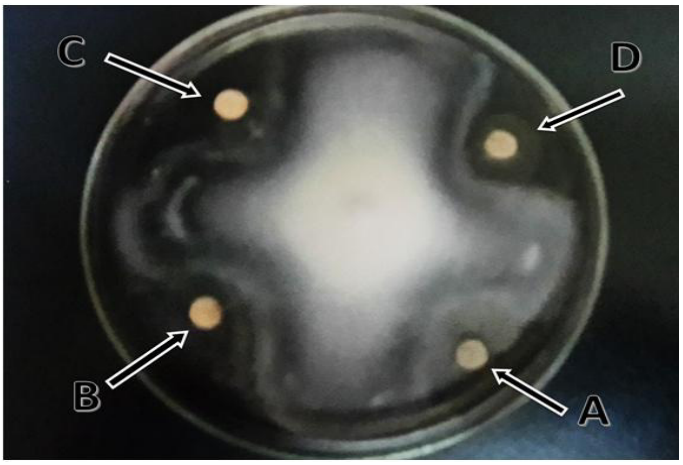

Figure 6: Effect of the lectin on the inhibition of *Fusarium oxysporum* mycelial growth
Disks were loaded with 25µL from:
(A) Crude lectin, 90 µg/ml
(B) Crude lectin, 100 µg/ml
(C) Purified lectin, 80 µg/ml
(D) Purified lectin, 70 µg/ml

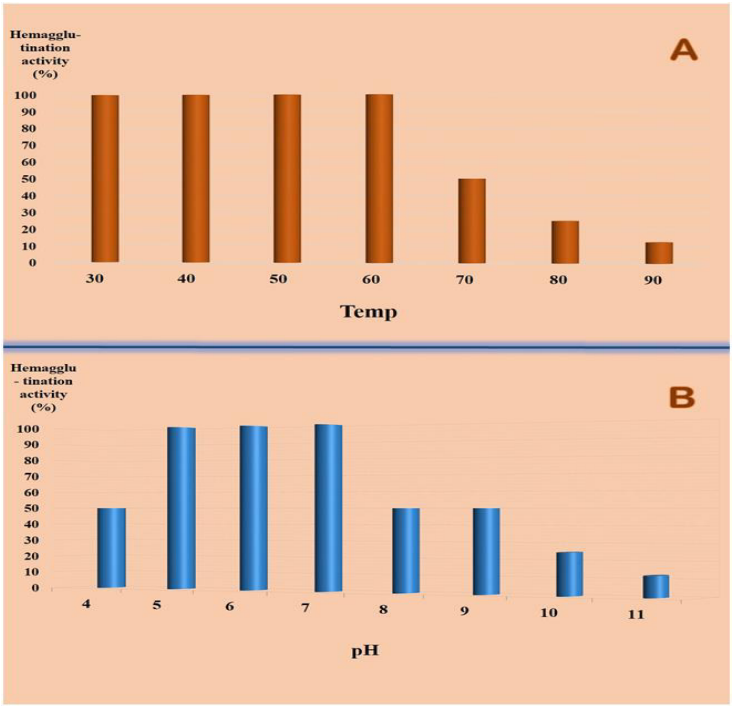

Figure 7: Hemagglutination activity of *Jatropha curcas* lectin as influenced by different incubation temperatures (A) and pH values (B).

Effects of temperature, pH and metal ions on hemagglutination activity of lectin

Thermal stability was determined by incubation of lectin at various temperatures for 30 min. The purified lectin of *J. curcas* seeds proved to be heat - stable up to 60 ºC, where it maintained 100% of its hemagglutination activity. Increasing the temperature above 60 ºC lead to the decrease in hemagglutination activity with 50, 25 and 12.5% at incubation temperatures of 70, 80 and 90 ºC, respectively (Fig. 7A). Reduction the hemagglutination activity, with high incubation temperatures, could be due to a change in the chemical structure and partial denaturation of lectin with increasing temperatures.

The effect of pH on hemagglutination activity was determined by incubating lectin samples at various pH values at 25 ºC for 1 h in selected buffers. Hemagglutination activity was at the optimum at pH 5.0, 6.0 and 7.0, e.g. stable at acidic to neutral conditions, but was markedly affected by extra acidic and basic conditions. i.e. reduced hemagglutination of 50% at pH values of 4, 8 and 9. The maximum decrease in hemagglutination were recorded at pH values of 10 and 11, where the activity reduced to 25 and 12.5%, respectively (Fig. 7B). These results could be explained with the suggestion that the relative reduction in hemagglutination activity of plant lectin, at the basic pH values, may be due to some degree of base induced denaturation (Vega and Pérez 2006).

Effect of metal ions

The effect of metal ions on hemagglutination activity was determined by incubation of *J. curcas* seed lectin with different concentrations of divalent metal ions; the agglutination of trypsinized cattle erythrocytes by lectin was completely inhibited by EDTA at concentrations of 5, 10 and 20 mM, whereas the hemagglutination activity was effective with Mg^{2+}, Ca^{2+} and Mn^{2+} ions at all of the above concentrations. It was reported that Mg^{2+}, Ca^{2+} and Mn^{2+} ions are essential for the hemagglutination activity (Moreira et al., 1997).

CONCLUSIONS

Egyptian *Jatropha curcas* seeds could be considered as a rich source for lectin extraction. The hemagglutination activity, of purified lectin, was stable at high temperature (up to 60 ºC), pH (from acidic to neutral conditions) and presence metal ions (Mg^{2+}, Ca^{2+} and Mn^{2+} even at a concentration of 20 mM). The molecular mass of purified lectin was 28.0 KDa and contained high ratio of acidic amino acids with minor amounts of sulfur containing amino acids and cysteine. The purified Lectin from *Jatropha curcas* seeds is more efficient than the crude lectin in the *in vitro* growth inhibition of the plant pathogenic fungus *Fusarium oxysporum*.

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