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Extraction, Purification And Antifungal Activity Of Lectin From Rice (Oryza Satival.)

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Abstract

The lectins was extracted from the rice gain by hexane and possessed the ability to agglutinate rabbit erythrocytes indifferent levels with higher titer 128U/ml of hemagglutination activity. The lectin was extracted by hexane and purified using the fractionation method withammonium salts followed by loading on DEAE -celluloseion exchangerafter that applied to sephadex G-50 in size exclusivetechnology with 19.33fold of purification and a yield of 46%. Cladosporium sp., Fusarium sp., Aspergillus sp.and Trichoderma spwere the most fungal isolates that isolated from pepper plants. The purified lectin showed inhibitory activity against fungal isolates Aspergillus sp.with higher inhibitory activity toward followed by Fusarium sp.The percent of inhibition for Aspergillus sp. was 40% while 32% for Fusarium sp.In contrast, lower inhibition for Cladosporium sp. and this lead to increase the benefic with using the lectin as biological control agent for phytopathogenic fungi.

Key words: Oryza sativa, lectin andphytopathogenic fungi.

Introduction

Rice(Oryza sativa) considered one of the great important cereal plants which consumeby human that belong to the family of Poaceae(1).Rice is a major source of carbohydrate such as starch that is founf in the endosperm of the rice grain. The rice lectin differs from other classical plant lectins thatenhanced by stress of salts salt stress, drying and by hormones of plant such as absorbic acid(2)

Lectins are glycoproteins that revealed reversible binding to mono or polymericcarbohydrate due to the presence of specific dominant in their structure(3). They can agglutinate red blood cells evenchanging the characteristics of the carbohydrate(4).The lectins were purified from different organisms like plants, animals, (17-34) viruses and bacteria, but the highest content of lectin found in the plants(3).

Plant lectins produced commercially and have potential applications in medical field as antimicrobial, antiviral, anticancer and anti-insects and promoting the growth of plants by associative with nitrogen fixation(3,4), also in agriculture and industrial applications.

The lectins were purified from various parts of legumeplants such as tubers, roots and seeds, in contrast, in the case of non- legume plants there were a low content of lectin. So that the purpose from this research to extract the lectin from rice samples and detect of its activity as antifungal agent.

Materials and Methods

Collection of plant samples

Ten sorts of local rice samples were collected from different markets. These samples were grounded by mortar and pestle and deffated by suspension the produced powder in hexane at ratio(1:2)(w/v).After centrifugation the precipitate was overnight dried at room temperature.0.4ml of the presented powder was mixed with 4 ml phosphate buffersaline (0.02M, pH 7.2)then shaked for 5 h. The produced suspension was centrifugated for 5 min and the clear extract was kept for next uses(5).

Preparation of untreated erythrocytes

Two ml of rabbit blood weresubjected to centrifugation for 10 min with2000 rpm and the formed pellet rinsed twice with phosphate buffersaline (0.02M, pH 7.2).After washing of erythrocytes wasdiluted to at concentration of 3% and used for detection of hemagglutination activity(HA)(6).

Hemagglutination assay in microplate

The quantitative screening in microtiter plate described by(6). 50 µlof the crude extract of each sample were serially diluted 2- fold by mixing with the buffer that prepared previouslythen 50 µ l from 3% of prepared rabbit erythrocytes and at 30°C with incubated period for 2 hour.The hemagglutination activity was observed with naked eye and measured the titer by representing of higher dilution which cause full aggluination.Hemagglutination was represented with (H.U.)that defined with taking the inverse of the highest dilution can result the agglutination.

Total protein content

A standard Bradford method Was used to estimate the concentration of protein with serum albuminbovineto prepare the standard curve at 595 nm (7).

Purification of lectin

The purification for lectin was performed by modification the method of (8).The crude extract was assayed for precipitation with different saturation ratios of ammonium sulfate from 40% to 80%. The precipitated pellet was dissolved in the buffer that mentioned above then the HU activity and with proteinwere determined and the fraction with best saturation rate dialyzed overnight with the buffer and then loadedon DEAE-cellulose column that pre-washed with the buffer that mentioned above and theelution were completed with fifferent concentrations of NaCl(0.1-0.5M). The obtained fractions were tested for hemagglutination assay. The active fractions concentrated and loaded to

Sephadex G-50 column that and the recovery performed with the above buffer. Reading of Protein content at 280 nm was determined besides the hemagglutination activity.

Isolation and identification of fungal pathogens

Pepper plant leaves were cut to different pieces after washing several times with distilled water. They placed in CZAPEK-dox yeast extract agar(CDYE)and incubated at 28C.The fungal colonies were identified morphologically depending on their texture,color, spore formation type besides and other fungal properties (9).

Antifungal activity of lectin

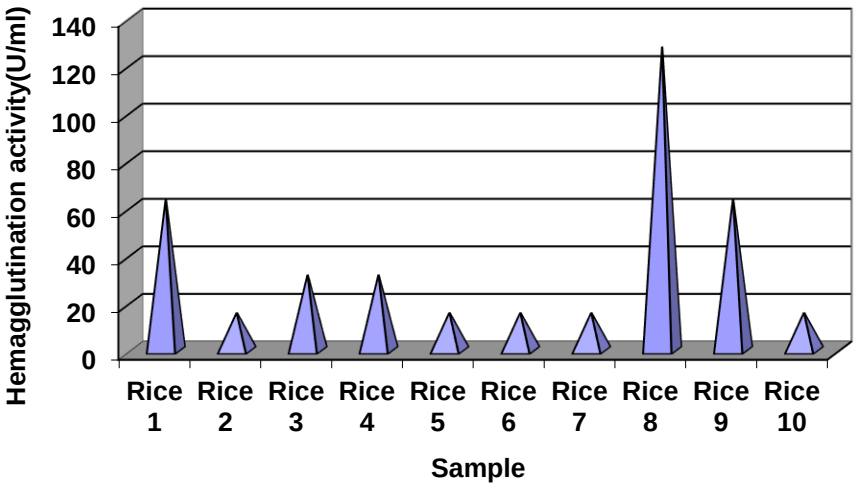
Potato dextrose agar (PDA) plates were prepared with 10% volume fraction of purifiedlectin or with distilled water for negative control.The prepared discs of mycelium of chose fungi cultured on these plates in the medium and incubated for 5 days at 28°. After incubation time the straight diameter of the myceliumwas measured. Theinhibition percent determinedas follow: inhibition percentage(%)= C-T/C*100%

*where C: mycelium diameter before treatment; T: mycelium diameter after treatment

Results and discussion

Rice lectins extraction

The rice lectin was defatted by aqueous acid extraction and the extraction was done by phosphate buffer saline. After the extraction the all rice samples revealed different values of hemagglutination activity with rabbit erythrocytes as demonstrated in figure(1).The hemagglutination activity ranged between 16-128U/ml.The concentration of erythrocytes, nature of agglutinin, the incubation timeand the method of hemagglutination assay had influence in the titer of hemagglutination activity and the result was considered positive if the settling was fast and non-agglutining if the settling was slowly(5) .



Figure(1): Titers of hemagglutination activity for lectin from Oryza sativa

Lectin purification program

After extraction of lectinfrom rice plant with ammonium sulfate precipitation at 60% saturation with higher specific activity 142.2U/mg . The obtained precipitate firstly loaded on ion exchange DEAE-cellulose column and recovered with NaCl concentration at 0.4M. The hemagglutination titer was concentrated in the first protein peak (figure-2). This step led to purify of lectin with 12.88fold and final yield of about 53%. The steps of lectin purification sequenced by providing the sample to sephadex G-50 column with 19.33 fold and a yield of 46% as reported in table(1) and figure(3).

Table(1): stages of lectin purification from Oryza sativa

Purification step	Size(ml)	Hemagglutin-ation activity(U/ ml)	Protein conc. (mg/ml)	Specific activity (U/ mg)	Total activity	Purification fold	Yield (%)
Crude extract	60	128	2.9	44.13	7680	1	100
$(NH_4)_2SO_4$ precipitation	20	256	1.8	142.2	5120	3.22	66
DEAE cellulose	8	512	0.9	568.8	4096	12.88	53
Sephadex G-50	7	512	0.6	853.3	3584	19.33	46

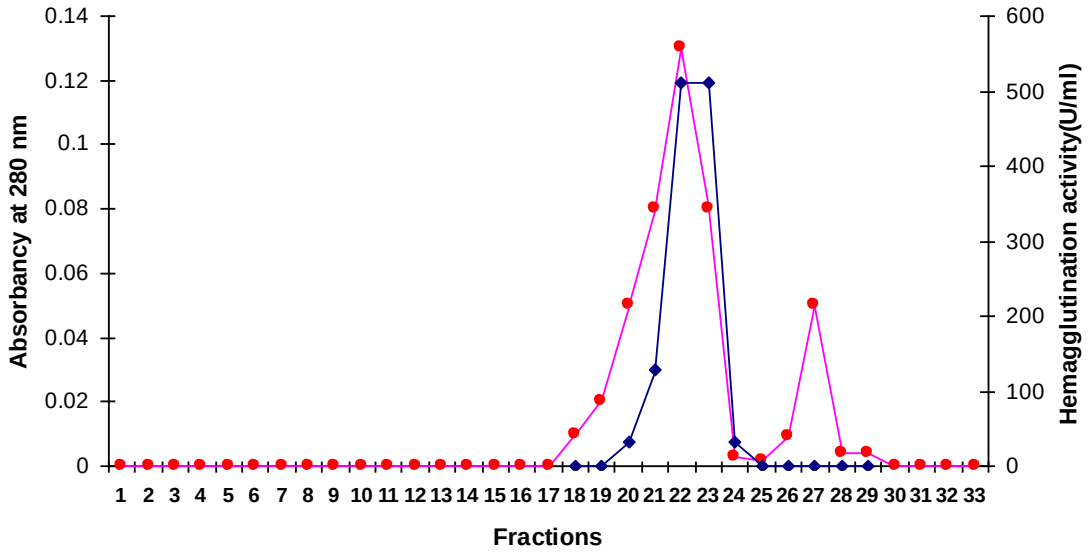
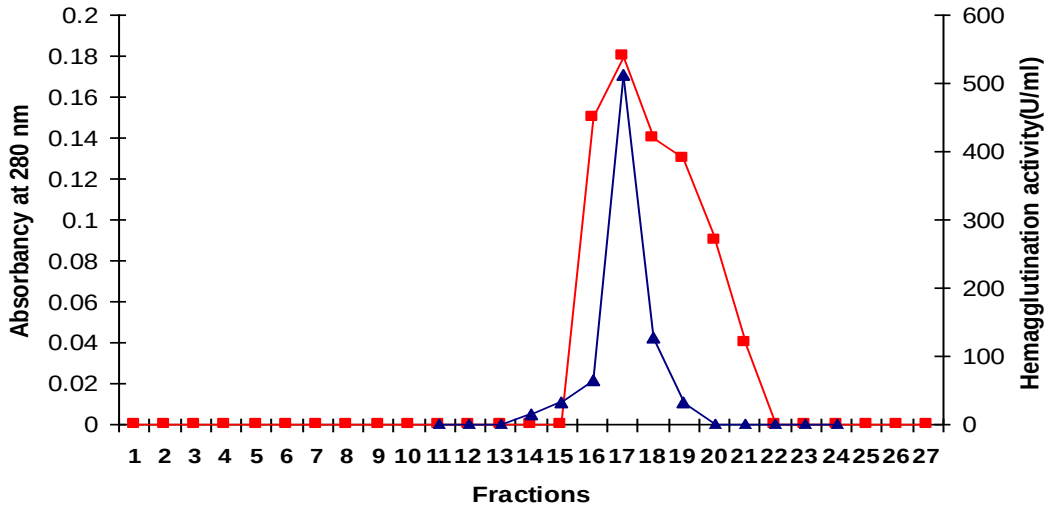


Figure (2): Ion exchange chromatography of lectin purified from Oryza sativa by using DEAE cellulose column



Figure(3):Gel filtration chromatography of lectin purified from Oryza sativaby using Sephadex G-50 column

A previous study reported by(10)purified lectin fromOryza sativain two chromatography steps included affinity chromatography followed by cation-exchange chromatography. Another study reported by found that there were two forms of lectins that extracted from rice bran with 90% saturation of ammoninm sulfate and loaded sephadex G-50 column then DEAE- cellulose on ion exchange chromatography(5). In contrast, (11) revealed that The lectin was purified fromseeds of Indigofera heterantha by ion exchange step with Deae- cellulose column then filtration on sephadex G-100gel.

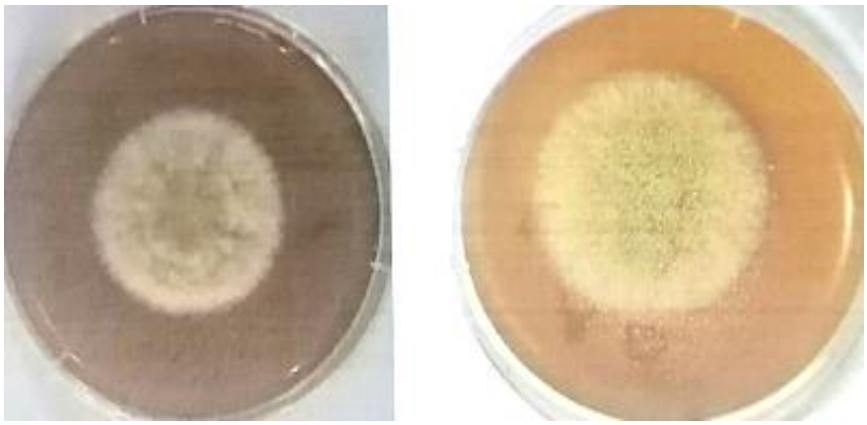
Isolation and identification of fungal pathogens

Four fungal isolates were isolated from pepper plants included Cladosporiumsp., Fusariumsp., Aspergillus sp.and Trichodermasp.. These fungal genera were found the most common resident fungi that isolated from medical plants(12). Fusarium sp.and Trichodermasp. were recognized as saprophytic fungi besides being revealed as parasitic on other fungi(13).

Antifungal activity of lectin

The purified lectin showed inhibitory activity against fungal isolates Aspergillus sp.with higher inhibitory activity (figure-4)followed by Fusarium sp.The percent of inhibition for Aspergillus sp. was 40% while 32% for Fusarium sp.In contrast, lower inhibition for Cladosporium sp. as shown in table(2). This attempt increased the benefit by using of lectin as biological agent against phytopathogenic fungi. (14) found that the purified lectinfrom seeds of Indigofera heteranthahas shown antifungal activity againstphytopathogenic fungi such asAspergillus oryzae, Aspergillus niger and Fusarium oxysporum.The extracted lectins from plants can inhibit the germination process of produced hypha or spores of the fungus(15). In addition, The extracted lectins from Phaseoulus vulgariesshowed a high level of inhibition

toward Fusariumoxysporum(16). The mechanism of inhibition by lectins return to binding of lectin with hypha causing poorly of nutrients adsorption and the influence on the spore germination.



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Figure(4): Effect of purified lectin on Aspergillus sp.a- Control(without lectin) and b- With lectin

Table(2): Antifungal activity of purified lectin from Oryza sativa

Fungus	Diameter of colony(mm) after treatment	Percent of inhibition(%)
Aspergillus sp.	**18**	**40**
Fusarium sp.	**21**	**32**
Cladosporium sp.	**23**	**1l4**
Trichoderma sp.	**24**	**10**

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