Antifungal Activity of Purified Lectin from Red Kidney Beans (Phaseolus Vulgaris) Against Vaginal Candidiasis Caused by Candida Albicans

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Abstract

The red kidney bean (Phaseolus vulgaris) considered from the cereals with high content of lectin. A Phaseolus vulgaris lectin capacity to hemagglutinate erythrocytes from humans and other animals lacks specificity. However, the highest levels of hemagglutinating activity were against sheep and rabbit erythrocytes. It purified by acetone aqueous extraction, 50% precipitation, with ammonium sulfate, ion exchange chromatography with DEAE-Cellulose followed by SephadexG-100 size exclusion chromatography with specific activity of 213.33U/mg and 64% a yield. Six Candida albicans isolates were isolated from vaginal candidiasis. The minimum inhibitory (MIC)concentrations of purified lectin ranged between 50-100 µg/ ml against all tested Candida albicans isolates, whilst the fungicidal (MFC) concentrations of lectin against Candida albicans isolates revealed between100-200 µg/ ml. thus the lectin has promising application as fungistatic and fungicidal agent against vaginal candidiasis caused by Candida.

Keywords: red kidney beans, lectin, vaginal candidiasis

1. Introduction

Lectins are widely distributed proteins or glycoproteins that can attach to certain carbohydrates while preserving their covalent structure [1]. Plants are the first and most frequently used source of lectin besides microorganisms, viruses and animals [2].

Plant lectin were classified to seven classes among them legume lectin class which become an excellent for studying their statures [3, 4]. There are different physiological uses for the proteins such as using them as antitumer, antimicrobial, antiviral and anti-insect activities [1].

Candidiasis is a fungal infection caused by yeasts that belonged to Candida genus. The healthy vagina has many beneficial bacteria and lower numbers of yeasts. Sometimes this balance lost and the yeast grow heavily and causes the symptoms [5], this losing to balances due to taking of antibiotics, diabetes, pregnancy and chemotherapy [6]. The changing of Candida from harmless commensalism to pathogenic state resulting in severe symptoms such as burning, white and thick vaginal discharge [7].Therefore was the benefit from this study to extract, purify of lectin from red kidney beans and investigation its activity as antifungal to treat of candidiasis.

2. Materials and Methods

Collection of red kidney beans specimens

Five sorts of from different origins were collected from supermarkets. These samples were grounded to a powder with an electrical mill.

Extraction of lectin

This powder defatted by mixing with acetone (200 ml of aqueous acetone: 10g seed) for 2 days at 4C after which the precipitate was centrifuged and dried at room temperature. 5 gm of the produced powder suspended at 0.2M NaCl with shaking for 4 h. The suspension was then centrifuged for five minutes, yielding a clear extract that was used as crude extract [8].

Preparation of Red blood cells

Two ml of rabbit, sheep, chicken, horse, goose and human group O+ blood were The pellet was twice rinsed and centrifuged for 10 minutes at 2000 rpm with 0.02 M PBS at pH 7.2, then diluted with concentration of 2% [9].

Hemagglutination activity

After preparation of serially two fold dilutions of 25µl the sample with0.02 M phosphate buffered saline (PBS) at pH 7.2. In microtiter plates, 25µl of 2% of red blood cells suspension was added to each well. Readings was recorded after 30 min at room temperature. Hemagglutination titer refer to higher dilution that revealed agglutination [9].

Protein assay

This test was performed by using bovine serum albumin as a standard in Bradford method [10] and reading the absorbency at 595 nm.

Screening blood affinity for red kidney bean lectins

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The affinity of various sorts of blood to red kidney bean lectins was screened as described above with 2% of erythrocytes obtained from different sources including rabbit, sheep, chicken, horse, goose and human group O+.

Purification of lectin

The crude extract was fractionated with ammonium sulfate at saturation levels of 40, 50, 60, and 70 percent, respectively. The obtained pellets were dissolved in 0.1 M NaCl in 0.02 M Tris-HCl at pH 8. Following dialysis, the sample was put onto a DEAE-cellulose column that had been previously equilibrated with the same buffer, and different salt concentrations ranging from 0.2 to 0.6M were used to elute the material. The fractions showing hemagglutinating activity were collected, concentrated and applied to sephadex G -100. In this column the elution performed by using 0.1 M NaCl in 0.02 M Tris-HCl at pH 8. The fraction with best hemagglutinating activity were chosen for another experiments.

Collection and reidentification of Candida albicans

25 vaginal samples from vaginal Candidiasis-infected women were obtained using cotton swabs, and they were cultured on sabouraud dextrose agar (SDA) and chromogenic Candida agar at 37ºC for 48C. The growing isolates were identified according to color, shape, texture, size and margin of the colonies. The identification was supported by using Vitek 2 system.

Effect of purified lectin against Candida albicans

The effect of purified lectin toward Candida albicans isolates was detected on the base of MIC value by microdilution method as follow: different concentrations ranging 400-12.5 µg/ml were prepared from the stock solution of purified lectin. 100 µl of sabouraud dextrose broth were distributed in the wells and 100µl of the stock added the first well before being serially diluted. After that 100 µl of Candida albicans culture were mixed with each well and incubated at 37C for 48 h [8]. The MIC was determined at a concentration which no visible growth could be observed with naked eye.

3. Results and Discussion

Red kidney bean lectins extraction

The red kidney bean lectin was defatted by hexane and the extraction was performed using phosphate buffer saline. To hemagglutination activity all extracts were subjected and according to the results there were variation in the lectin contents for collected samples of red kidney bean lectin with rabbit erythrocytes (figure-1). The hemagglutination activity affected with different factors as incubation temperature, RBCs concentration, nature of extracted lectin and method of agglitination assay [11]. In a research reported by [12] used soak, degreasing and homogenizing methods for extraction of lectin and found that the decreasing method was more suitable than other methods with specific activity 187U/mg.

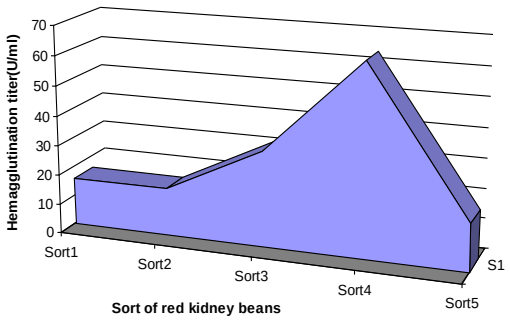

Figure (1): Hemagglutination titer of lectin for different sorts of red kidney bean

Screening blood affinity for red kidney bean lectins

The sample of red kidney bean with the highest lectin content was screened against different types of blood RBCs from various sources as rabbit, sheep,chicken, horse, goose and human group O+ blood. The lectin revealed capacity to hemagglutinate erythrocytes from humans and other animals lacks specificity. However, the highest levels of hemagglutinating activity were against sheep and rabbit erythrocytes, in contrast, the horse was the lowest as reported in figure(2). The rabbit erythrocytes showed more precise results than other types, but the blood from chicken was a cheaper and more available source for blood agglutination testes with lectins white beans on the large scale [11]. The chicken erythrocytes were used by Hou et al. [12] for detection the hemagglutination activity as a cheap source of blood with suitable activity.

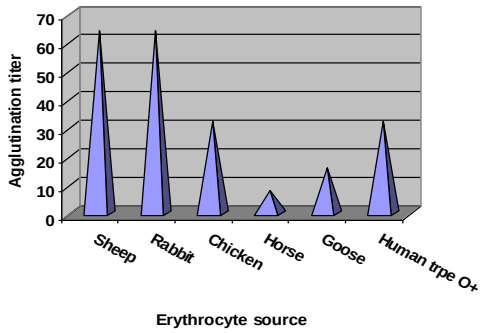

Figure (2): Hemagglutination activity of extracted lectin against human and animals erythrocytes

Purification of lectin

The extracted lectin from red kidney bean was purified firstly increased precipitation of ammonium sulfate at 50% saturation and greater specific activity 55.6 U/mg.located The precipitate pellet was suspended and applied to DEAE-cellulose column, After that the elution was applied with gradient concentrations of NaCl and the presented fractions showed hemagglutination activity located in the second peak (figure-3) with 64% a yield and with 8.46 fold of purification. The obtained lectin supplied to gel filtration step on sephadex G-100 column with 12 fold and a yield of 64% as recorded figure(4) and in table(1). Lectin was purified by affinity absorption on porcine thyroglobulin-sepharose with a yield 74% of the original erythroagglutinating activity and represented a

25-fold purification [13]. Also the lectin was purified n from Phaseolus vulgaris L. white seeds firstly with ammonium sulfate precipitation, DEAE cellulose for ion exchange chromatography and further purified by sieve chromatography on a Sephadex G-200 column [14].

Table (1): Sequenced steps of lectin purification from red kidney bean (Phaseolus vulgaris)

Purification step	Size(ml)	Hemagglutin-ation activity(U/ml)	Protein conc. (mg/ml)	Specific activity (U/mg)	Total activity	Purification fold	Yield (%)
Crude extract	50	64.0	3.60	17.770	3200	1.0	100.0
(NH4)2SO4 precipitation	20	128.0	2.30	55.60	2560	3.120	80.0
DEAE cellulose	8	256.0	1.70	150.50	2048	8.460	64.0
Sephadex G-100	8	256.0	1.20	213.330	2048	12.0	64.0

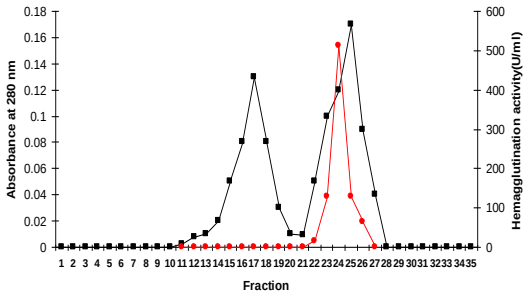

Figure (3): DEAE cellulose column for ion exchange chromatography to purification of lectin from Phaseolus vulgaris

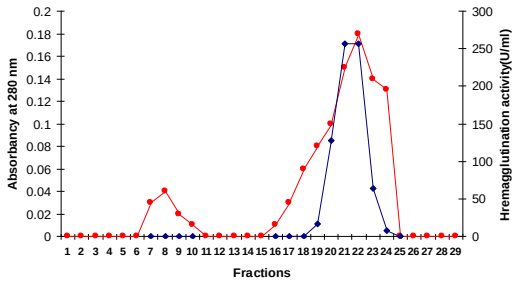

Figure (4): Sephadex G-100 column for gel filtration chromatography to purification of lectin from Phaseolus vulgaris

Collection and reidentification of Candida albicans

Six Candida albicans isolates were isolated from vaginal swabs and these isolates were able to grow on chromogenic Candida agar with light green color colonies and the result obtained by using Vitek 2 showed that all isolates belonged to Candida albicans. From vaginal candidiasis, different species of Candida spp. were isolated, and it was found that non-Albicans species occurred more frequently than Candida albicans [15]. The aberrant growth of Candida in the mucosa of the genital canal causes vaginal candidiasis, which has risen sharply in recent years.

Antifungal activity of purified lectin toward Candida albicans

The growth inhibition of six isolated Candida albicans isolates was observed when the pure lectin was present in minimum inhibitory (MIC) concentrations ranged between 50-100 µg and ml as shown in table (2).On the other hand, the fungicidal (MFC) concentrations of lectin against Candida albicans isolates revealed between100-200 µg/ ml.

This is important since many researches on plant lectins demonstrated their role asantifungal proteins in the host [8].The extracted lectin from S. iatifolia exhibited antifungal activity against Candida albicans, C. glabrata, C.rugosa and against Fusarium oxysporum and F. solani, and it plays a defense ability against the causes by binding to various fungi and inhibition of its growth and germination [16].

Table(2):MIC and MFC concentrations of purified lectin against Candida albicans isolates

Candidal isolate	MIC(µg/ml)	MFC(µg/ml)
Candida albicans V01	100	200
Candida albicansV02	50	200
Candida albicansV03	50	100
Candida albicansV04	100	100
Candida albicansV05	100	200
Candida albicansV06	50	100

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