ISSN NO: 2770-0003
Date of Publication: 30-12-2022

Antibiofilm formation activity of purified lectin from wheat seeds (*Triticum aestivum*) against *Candida albicans* causing oral candidiasis

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Abstract: Wheat seeds (*Triticum aestivum*) are among the grains with a high lectin concentration. The sort with the highest lectin content had the lectin removed and purified. It was purified via fractionation with 60% NH_3SO_4 saturation then loadeded on DEAE-Cellulose column followed by Sepharose-6B size exclusion chromatography, and a yield of 61.5% with specific activity of 853.3 U/mg. Oral candidiasis led to the isolation of seven different *Candida albicans* isolates, all of which produced variable quantities of biofilm. With percentages of biofilm inhibition ranging from 23 to 44% with 50 µg/ml. The isolated lectin demonstrated antibiofilm efficacy against all *Candida albicans* isolates. The inhibition grew with increasing doses, reaching 65-88% at 200 µg/ml. such that lectin can be used to treat an oral cavity that is infected with *Candida albicans*.

Keywords: Wheat seeds, lectin, oral candidiasis

Introduction

As opportunist pathogens and members of the natural flora, *Candida* species can impact the body's mucosa, skin, nails, and internal organs and can produce various clinical manifestations of candidiasis (1, 2). The organism invades the tissues of the mouth and throat, causing infections to appear on the tongue, gums, lips, roof of the mouth, and other areas. When the white patches are taken out of the mouth, there can be bleeding and an unsightly ulcerous region as well. The condition known as esophagitis, which results from esophageal thrush spreading to the esophagus, makes it unpleasant and difficult to swallow food and liquids (3). Because newborn babies have an underdeveloped immune system, the infection has historically been thought of as an acute illness that frequently affects them(4,5).

A subclass of proteins known as lectins can reversibly bind to specific carbohydrates without altering the covalent connections. Cereal grains, legumes, and fruits are examples of staple foods with relatively high lectin content (6). There are many different lectins found in high concentrations in beans (such as black and soybeans) in general, and grains in specifically (7). Most plants include lectins, which are proteins that bind to carbohydrates. They aid in defending plants from outside infections like fungus and other creatures. Some common food items, such legumes and cereal grains, have lectin concentrations that are comparatively high. Wheat germ agglutinin (WGA) is a term used to describe a portion of the proteins found in wheat germ (8). Natural lectins protect plants from external infections like fungus and other organisms by participating in biological recognition processes involving cells and proteins (9). As a result, lectin from wheat seeds was attempted to be extracted, purified, and its ability to destroy of *C. albicans* biofilms was examined.

Materials and methods

Preparation of seeds of wheat specimens

From supermarkets, six samples of wheat seed were collected. With the use of an electronic mill, these samples were reduced to powder.

Defatted ground wheat seeds process

The final powder was defatted by combining 10g of wheat seeds with 300 ml of aqueous acetone for 48 hours at 4°C, followed by centrifuging and drying the precipitate at room temperature.

Lectin extraction

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Five grams of the resulting powder were dissolved in 0.04 M saline phosphate buffer with a pH of 7.2 for 4 hours, and centrifuged for 10 minutes at 4°C with 3000 g, the clear extract was used as the crude extract. (10).

Hemagglutination activity

In crude extract, the hemagglutination activity was carried out by preparing serially two-fold dilutions of the sample in microtiter plates with 0.04 M saline phosphate buffer at pH of 7, then 25 µl of 2% red blood cell suspension. Following 30 minutes at room temperature, readings were recorded. Higher dilution that indicated agglutination is referred to as a hemagglutination titer (11).

Protein assay

The Bradford method (12) was used to conduct this test, utilizing bovine serum albumin as a reference, and assessing the absorbency at 595 nm.

Purification of lectin

At saturation levels of 60%, ammonium sulfate was used to fractionate the crude extract. The resulting pellets were dissolved in 0.01 M PBS with a pH of 7.2. After dialysis in special bags , the product was loaded on a column filled with DEAE-cellulose as stationary phase which previously washed with above buffer before being eluted with various salt concentrations ranging from 0.1 to 0.8 M. The hemagglutinating fractions were gathered, concentrated, and added to the sepharose-6B gel. In this column, the elution was carried out using 0.01 M PBS at a pH of 7.2. For subsequent trials, the active tubes with the higher hemagglutinating activity was collected.

Isolation and identification of oral cavity *Candida*

Sixteen samples of lesional tissue (the tongue and roof) from children with oral candidiasis were taken, and two portions of each sample were separated. The first component underwent direct inspection under a microscope right away, and the second part was routinely streaked on SDA medium. and CHROM agar Candida. Using API-Candida systems, the morphological characteristics of the culture media and germ tube production were used to identify *Candida*. *Candida* Spp was then used to provide the diagnosis. Vitek 2 system is used.

Biofilm formation by *Candida albicans*

The sabouraud dextrose broth was injected with each *Candida* isolate. Each isolate was first diluted 1:20 with new SD broth theng incubated at 37°C on a microtiter plate for 24 hours. The microtiter was then dried using an inversion after washing with distilled water. After that each well received 1% crystal violet with 200 µl and underwent two 15-minute washes in distilled water. An Elisa reader was used to measure the absorbance at 450 nm after each well received 200 µl of an 80:20 mixture of ethanol and acetone. For the control well, an optical density cut-off value was used using sterilized sabouraud dextrose broth. According to, positive samples were those whose absorption was higher than the cutoff value, and negative samples were those whose absorption was lower (13).

Inhibition of biofilm formation by lectin

To test the purified lectin's ability to prevent the growth of biofilm on microtiter plates, 100 µl of yeast suspension were combined with 50, 100, and 200 g/ml concentrations of the purified lectin in 0.04 M saline phosphate buffer. The biofilm test was performed as previously described after a 24-hour incubation period at 37^{o}C. In place of the purified lectin, 100 µl of phosphate buffered saline was added as the negative control. To calculate an inhibition percentage, the following formula was used(14):

Biofilm inhibition(%) =[O.D control-O.D treatment]/O.D control x100

Results and discussion

Lectin extraction from wheat seeds

Hexane was used to defatten the lectin from wheat seeds, and phosphate buffer saline was used for the extraction. All extracts were subjected to hemagglutination activity, and the findings revealed that harvested wheat seeds treated with erythrocytes varied in their lectin concentration. The sort 5 of the wheat seeds had the highest lectin content compared to the other sorts (figure-1). The hemagglutination activity is influenced by a variety of factors, including the temperature of the incubation medium, the RBC concentration, the kind of extracted lectin, and the testing technique (15). Using soak, degreasing, and homogenizing methods for lectin extraction, research described by (16) discovered that the declining strategy was better than the other approaches.

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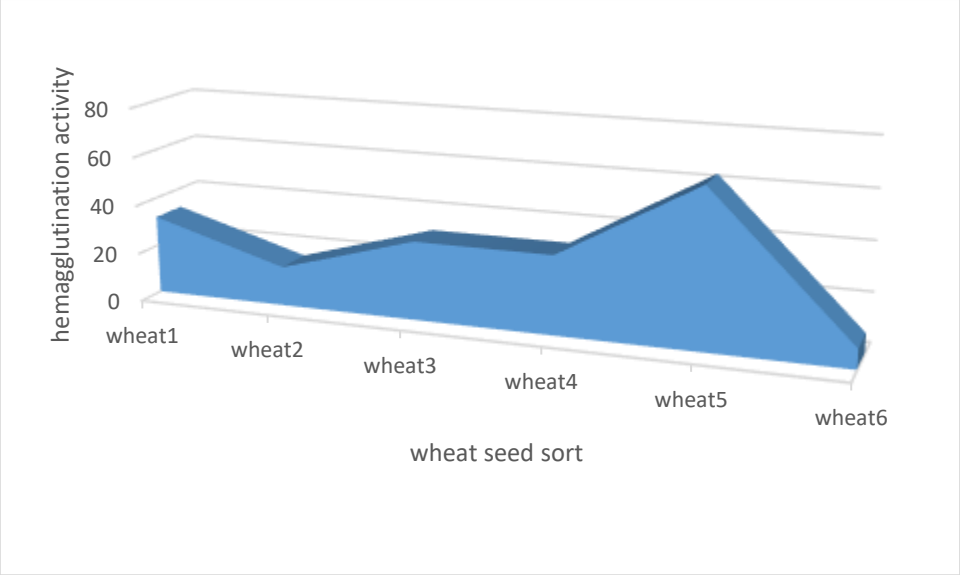

Figure(1): levels lectin contents in different wheat seeds samples

Purification of lectin

The purified wheat seed lectin was first precipitated with saturation at 60% ammonium salts with 55.6 U/mg as the highest specific activity. Following the precipitation process, the precipitated solution was applied to a DEAE-cellulose column, after which gradient concentrations of NaCl were used for elution. The given fractions showed that the hemagglutination activity first exhibited in the first peak (figure 2); the yield was 64%, and the purification efficiency increased by 8.46 times. According to figure (3) and table(1), the yield of the acquired lectin that was added to the gel filtering stage on the sepharose-6B column was 12 fold. Using chicken ovomucoid as the ligand, affinity chromatography was used to isolate and purify the wheat germ agglutinin (10). The lectin was purified 25 times via affinity absorption on porcine thyroglobulin-sepharose, yielding 74% (17). White Phaseolus vulgaris L. seeds were also used to extract the lectin by a number of procedures, including salts precipitation, ion exchange on DEAE cellulose followed by G-200 Sephadex chromatography(18).

Table(1): Purification diagram of *Triticum aestivum* lectin

Purification step	Size (ml)	Hemagglutin-ation activity(U/ml)	Protein conc. (mg/ml)	Specific activity (U/ mg)	Total activity	Purification fold	Yield (%)
Crude extract	65	64	2.9	22.06	4160	1	100
$(NH_4)_2SO_4$ precipitation	35	128	1.7	75.29	3200	3.12	76.9
DEAE- cellulose	12	256	0.8	204.8	3072	8.46	73.8
Sepharose-6B	5	512	0.6	853.3	2560	12	61.5

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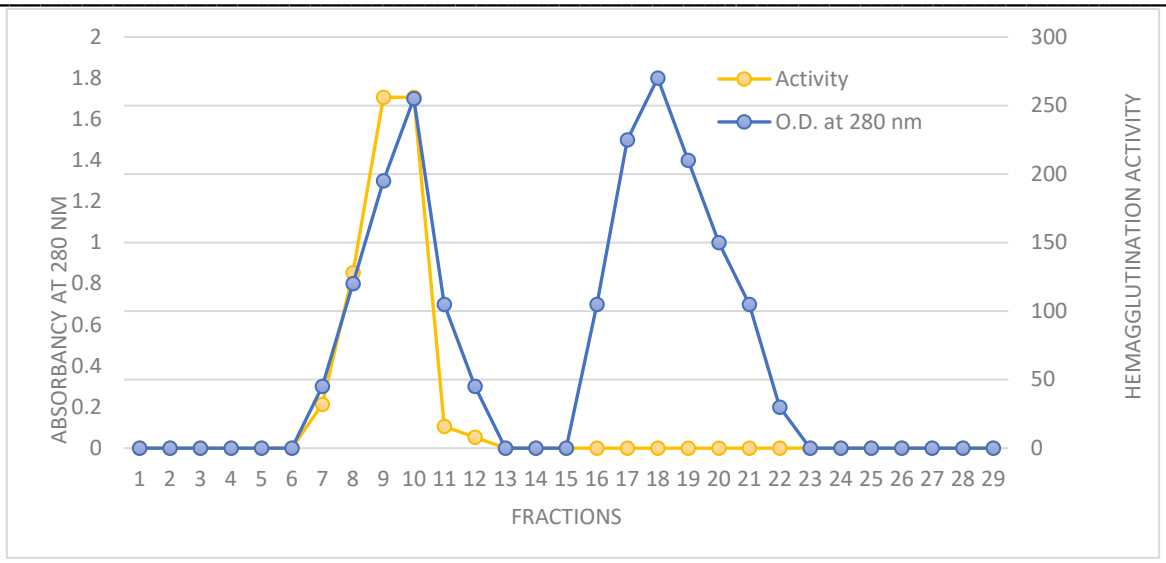
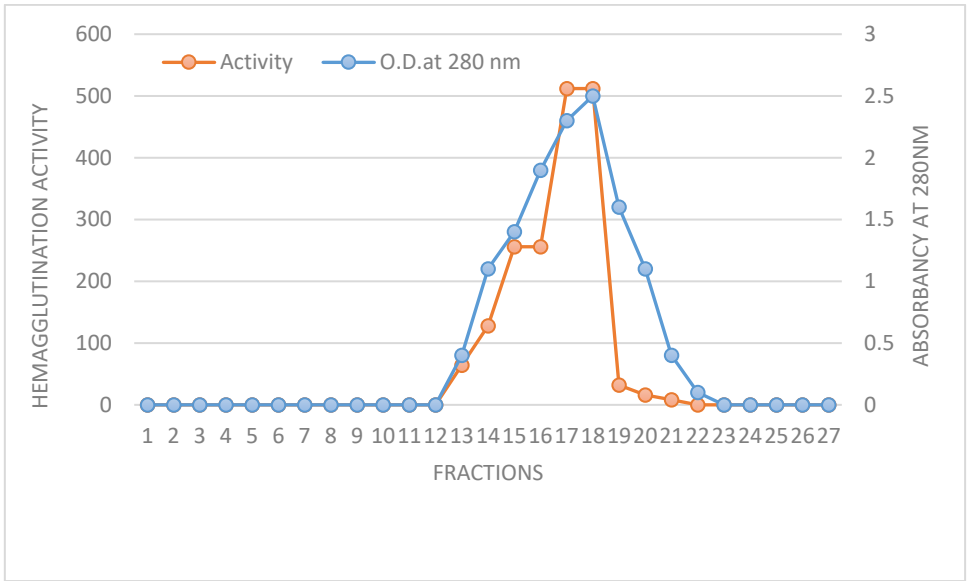


Figure(2): DEAE cellulose column for ion exchange chromatography to purification of lectin from *Triticum aestivum*



Figure(3): Sepharose-6B column for gel filtration chromatography to purification of lectin from *Triticum aestivum*

Presence of *Candida albicans* in oral candidiasis and biofilm formation

Seven out of the sixteen cotton swab samples taken from kids with oral candidiasis contained *Candida albicans*. They appeared oval to spherical under a microscope, were gram positive, and had colonies of a light greenish color on chromogenic media. When the ability of each isolate of *Candida albicans* to create biofilm was examined, it became clear that each isolate produced biofilm in variable degrees as shown in figure(4).

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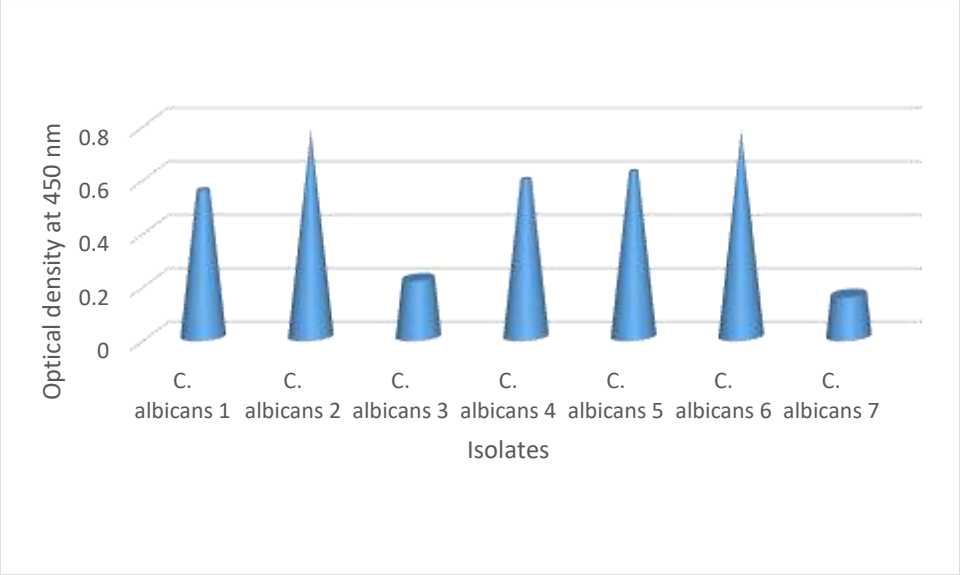

Figure(4): An ability of *Candida albicans* isolates to produce biofilm

Candida albicans microbiota are present in the mouth, but when they transform into pathogens and infect the host's tissues, they cause oral thrush (19,20). There are a lot of isolated *Candida albicans*, followed by *Candida tropicalis* and *Candida krusaei*, as the leading causes of dental caries in the mouth (20). According to (21) *Candida albicans* was the most prevalent infectious agent in oral infections in children. Also (22) found that 66% of the oral cavity's microbial population was *C. albicans*, followed by *C. dubliniensis*, *C. krusie*, and *C. glabrata*.

An introduction of new cells into oral cavity, where they can aid species in adjusting to their new environment and ensuring their own survival, may be possible with the help of biofilms like this one (23). *Candida albicans*, which populates the human oral cavity with a diverse microbiota, has the ability to produce biofilm on surfaces covered with saliva. Evidence suggests that *Streptococcus* and *Candida albicans* collaborate to encourage *Candida albicans*' adherence to dental tissues and the development of biofilms, which is compatible with its presence as a normal component in the human oral microbiota (24).

Inhibition of biofilm formation by lectin

All isolates of *Candida albicans* showed antibiofilm efficacy against the purified lectin. The percentage of biofilm inhibition was computed as shown in figure (5), which indicated that the percentage varied from 23 to 44% at 50 µg/ml, and that the inhibition grew with increasing concentrations, reaching 65-88% at 200µ g/ml.

The formation of biofilm and hyphae, the synthesis of enzymes, and the attachment to host tissues are all important in the pathogenesis of *Candida* (the illness it causes) (25). In order to avoid oral diseases such oral candidiasis, the lectin may be used in place of antibiotics and dangerous antibiofilm medications made chemically.

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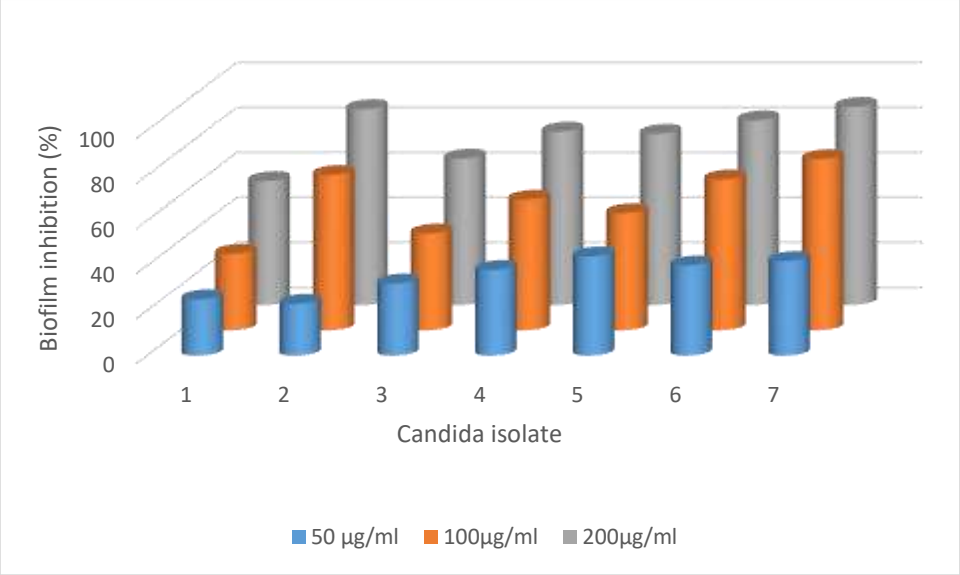

Figure(5): Effect of purified lectin on biofilm formation by *Candida albicans*

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