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Characterization of *Lentinula edodes* Lectin: Effects of pH, Temperature, and Molecular Weight on Antifungal Activity

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Abstract:

Lectin are characterized according to different methods like pH , temperature, molecular weight , different PH was taken in the range from (4_9) and the activity of lectin was determined by measuring the hemagglutination and different temperatures was taken to determine the lectin activity in the range (22,27,32,37 and 42 °C) in different durations (10,20,30) minutes and the molecular weight determination was done by gel filtration chromatography and the effects of different PH and temperatures on *Candida* spp. growth and lectin production by measuring the inhibition zones. The results of temperatures characteristics was shown have varying degrees of thermal stability the lectin activity peaked at 32(60.3)and the hemagglutination activity decrease with rising temperature and in PH the lectin activity peaked at PH 5(50.6) and the activity was decreased in PH (7,8,9) and the molecular weight of lectin was 100000.And the antifungal effectiveness of purified lectin against several spp. of *Candida* no inhibition was observed at very alkaline or extremely acidic PH values , lectin activity increase gradually from 22 to peak between 32 and37 and declined at 42 °C.

Keywords: Lectin , characterization , edible mushroom, PH , temperature , molecular weight ,*Candida* spp.

Note: The research is based on a PhD dissertation .

Introduction:

Lectins are a broad group of carbohydrate-binding proteins that have attracted significant attention due to their therapeutic potential. four major types of lectins: plant, animal, fungal, and marine, and examines their unique properties and promising applications in medicine (Radhakrishnan *et al*.,2022). Plant lectins are known for their ability to modulate immune

responses and exhibit a range of biological activities, including anticancer, antiviral, and antibacterial effects. They are excellent candidates for the creation of new medicinal agents because of these qualities. Animal lectins, which are present in both insects and mammals, are essential for innate immunity and have been extensively studied for their potential to cure inflammatory illnesses and infections (Raposo *et al*., 2021). Marine lectins are a promising but little-studied source of bioactive chemicals with distinct binding specificities that are generated from marine creatures.Their potent biological activities suggest they may serve as effective tools for future drug development. Fungal lectins, which are less studied but no less important, also show potential in therapeutic applications, particularly in immune modulation and antimicrobial activity (Shaikh&Uzgare ,2025). Lectins are nonimmune proteins and glycoproteins that selectively bind to carbohydrates on the cell surface, leading to cell 3 agglutination. Lectins, which have been identified from various sources, including plants, animals, microbes, vegetables, fruits, beans, and mushrooms, are essential for the host's defensive mechanism against fungus and insects. They also serve as intermediaries in the metastasis process. Because of their nutritional and medical benefits, mushrooms have been used as food and medicine since ancient times. Today, they are a rich source of lectins (Zurgany, & Shafiq,2025). Mushroom lectins usually comprise two to four identical or non-identical subunits held together by non-covalent interactions. However, at least two examples exist of mushroom lectin subunits being linked by disulphide bridges (*Lactarius lignyotus* and *Phallus impudicus*). The molecular mass and oligomeric state of isolated mushroom lectins vary greatly, ranging from 10 to 190 kDa, the characterization of lectin is an essential step to determine its biochemical and biophysical properties, as well as its functional specificity. After extraction and purification, lectins are characterized to identify their molecular weight, carbohydrate specificity, structural stability, and biological activities (Hassan *et al*., 2015). *Candida* infection may be localized, impacting the mucosa, or systemic, affecting the heart, lungs, liver, and brain (Batista *et al*., 1999). Infections caused by *Candida* spp. fungi are classified into two categories: superficial and systemic (Talapko *et al*., 2021). Infections produced by *C. albicans* necessitating medical intervention are categorized as either mucosal or systemic. C. albicans predominantly infects vaginal (vulvovaginal candidiasis [VVC]), oral (oropharyngeal candidiasis [OPC]), esophageal (esophageal candidiasis [EPC]), and, to a lesser extent, nail mucocutaneous surfaces (onychomycosis). A limited minority of individuals with particular hereditary

immune weaknesses ever manifests cutaneous candidiasis. Systemic candidiasis refers to *Candida* overgrowth in the liver, heart, central nervous system (CNS), spleen, and/or kidneys, impacting typically sterile regions of the body, such as the bloodstream. The abdominal cavity may be affected, with or without systemic dissemination (Pappas *et al*., 2018).

The main job for this research to characterize the lectin and to evaluate the effect of PH and temperature on *Candida* spp. and growth

Materials and Methods

Characterizations of lectin from mushrooms

The characterization of lectin according to PH temperature and molecular weight was performed as follows:

Characterizations of lectin from mushrooms According to PH

Using appropriate buffer systems, purified lectin samples were made in buffer solutions with PH values regulated from acidic to alkaline conditions, precisely spanning from PH 4 to 9. To aid in equilibration, each sample was incubated at room temperature for a set amount of time, usually 30 minutes. The impact of PH on lectin functioning was then determined by measuring the lectin activity using hemagglutination.

Characterizations of lectin from mushrooms According to temperature

Aliquots of lectin were incubated at different temperatures (22°C, 27°C, 32°C, 37°C, and 42°C) for varying durations (usually 10, 20, and 30 minutes) in order to perform temperature characterization. Following incubation, samples were cooled and centrifuged to remove precipitates, and their activity was then measured using a hemagglutination assay.

Determination of the Hemagglutinating Activity

Microtiter plates were used to measure the hemagglutinating activity (HA). A solution containing 0.5 mg/mL of lyophilized lectin powder was made. 50 µL of 2% rabbit red blood cell suspension was then added after 50 µL of lectin solution had been serially diluted twice with stroke-physiological saline. After 45 minutes at ambient temperature, when the negative control had completely sedimented, the results were examined (Jiang *et al*., 2019).

Characterizations of lectin extracted from mushrooms according Molecular weight determination by gel filtration chromatography

Gel filtration chromatography was used to determine the protein's molecular weight. Elution was carried out using a Sephadex G-150 column (2x40cm) that had been equilibrated with 0.05M phosphate buffer (pH 7). Alcohol-dehydrogenase (mol.wt of 150,000), albumin (mol.wt. 66,000), carbonic anhydrase (mol.wt. 29,000), and lysozyme (mol.wt. 14300) were the crystalline proteins used as molecular weight markers. Blue Dextran was

used to estimate the void volume at 600 nm, and a UV-Vis Bio-Rad spectrophotometer was used to measure the elution volume for each standard protein at 280 nm. The molecular weight of known standard proteins was used to calculate the molecular weight of the enzyme based on its elution volume.

Determination of protein concentration

Protein concentration was determined according to the method of Bradford (1979) using bovine serum albumin standard curve as follow:

Different concentrations (0, 0.1, 0.2, -------to 1 mg/mL) were prepared from BSA stock solution (1mg/1ml) as shown in Table (1). Then 2.5 mL of Coomassie brilliant blue G-250 dye prepared in (B) was added, mixed and left to stand for 2 min at room temperature. Absorbance at 595 nm was measured; the blank was prepared from 0.45 mL of 0.05M phosphate buffer pH 7 and 2.5 mL of the dye reagent. A standard curve was plotted between the BSA concentrations against the corresponding absorbance of bovine serum albumin at 595 nm Protein concentration was estimated by mixing 0.05mL of the test sample,0.45mL of phosphate buffer and 2.5 mL of Comassie brilliant blue G- 250, left to stand for 2 min at room temperature before measuring the absorbance at 595 nm. Fig 1

Table (1): Preparation of Bovine serum albumin concentrations:

Tube number	Volume of stock solution (BSA) (mL)	Volume of distilled water (mL)	Protein (BSA) concentration (mg/mL)
1	0.0	1	0.0
2	0.1	0.9	0.1
3	0.2	0.8	0.2
4	0.3	0.7	0.3
5	0.4	0.6	0.4
6	0.5	0.5	0.5
7	0.6	0.4	0.6
8	0.7	0.3	0.7
9	0.8	0.2	0.8
10	0.9	0.1	0.9
11	1	0.0	1

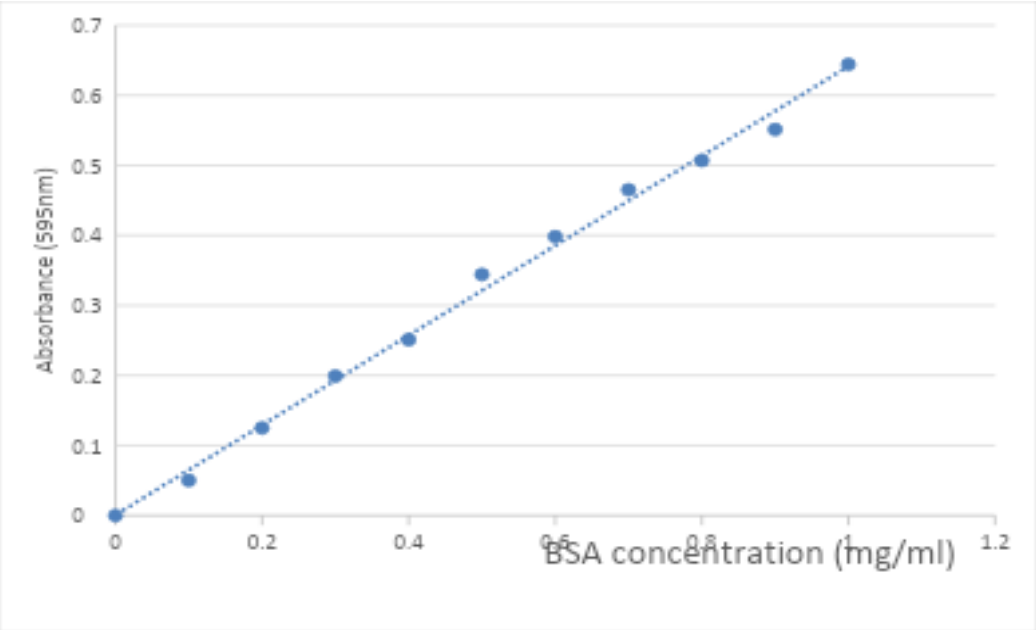

Figure (1): Standard curve of BSA

A.Bovine serum albumin stock solution (1mg/ml)

It was prepared by dissolving 0.01g bovine serum albumin in 10 mL of distilled water.

B. Comassie brilliant blue dye (G-250)

This dye was prepared by dissolving 0.1 g of comassie brilliant blue G-250 in 50 mL of 95% ethyl alcohol, then 100 mL of 85% phosphoricacid was added, mixed well and filtered using filter paper (Whatman No.1) and the volume was completed to 1 litter by distilled water then saved in dark bottle.

The effect of different ph on *candida* spp. growth and lectin production

Each PH has a unique substance. PH is prepared in the range (3–8) using a specialized buffer, changed by adding acid or base, and measured by a PH meter. The fraction of enzyme is selected, and two tubes contain PbS and another tubes contain ph. Take 100 microns and 100 from the enzyme mixed well in a tube and incubate for 15 minutes at 37 or 30 degrees Celsius. The tube is cooled by adding to wells. Glycine–HCl (PH 1–3), acetate (PH 4–5), citrate–phosphate (PH 5), 2-(N-morpholine)-ethane sulfonic acid (PH 6), phosphate (PH 7), Tris–HCl (PH 8–9), and glycine–NaOH (pH 10–11) were the buffers utilized. (Thakur *et al*., 2007).And checking the culture by McFarland put it in the pores incubated in incubator for 24hrs at 37°C zones are appear measure it .

The effect of different temprature on *candida* spp. growth and lectin production

After incubating the enzyme (lectin) for 15 minutes at various temperatures (22, 27, 32, 37, and 42) and cooling it in cold water, take a petri dish filled with sabourod dextrose agar and culture on it by loop after checking the culture by McFarland. Create pores in the culture using corkpore, then insert the pippet from the enzyme into the pores. Place the culture in an incubator for 24 hours in 37 and measure the zones using a rupper.

Statistical Analysis

One way and 2-way variance analysis (ANOVA) was done using Excel software, with a significance level of 0.05. Mean ± SE was the way the data was presented. Every experiment was performed three times.

Result and discussion

Characterizations of lectin from mushrooms according to temperature

Purified lectin characterization was carried out in accordance with various temperatures, and the lectin activity was determined by measuring hemagglutination and remaining activity. According to the data in Figure (2), lectin activity peaked at 32°C (60.3), and it decreased as the temperature increased because of thermal sensitivity. The decline is caused by protein denaturation, which destroys the protein's three-dimensional structure and reduces its affinity for binding carbs. High temperatures may also encourage precipitation or aggregation, which would reduce the amount of active lectin molecules. The findings demonstrate that 32 °C is the optimal temperature for preserving lectin activity, underscoring the need of regulating heat conditions throughout the extraction, purification, and application processes. This study's finding that lectin activity declines with temperature is consistent with other findings. It was discovered that a lectin from *Wollemia nobilis* was active at temperatures as high as 65 °C (de Oliveira *et al*., 2023). However, a lectin made from *Cucumis melo* seeds was found to be most active at 55 °C by El-Maradny *et al*. (2021). It has been demonstrated that *Phaseolus vulgaris* lectins remain stable at temperatures as high as 60 °C (Konozy & Osman 2022). The findings demonstrate that different lectins have different levels of thermal stability, suggesting that the observed decrease in activity with rising temperature may be caused by the specific characteristics of the lectin being studied. The hemagglutination activity of CPL was assessed at temperatures between 30 and 100°C for 30 minutes. The lectin remained completely active until the temperature reached 60 °C. After that, the hemagglutination activity began to decline; at temperatures above 80 °C, it reached 75%.At 90 °C, CPL showed complete inactivity. (Islam *et al*., 2024).

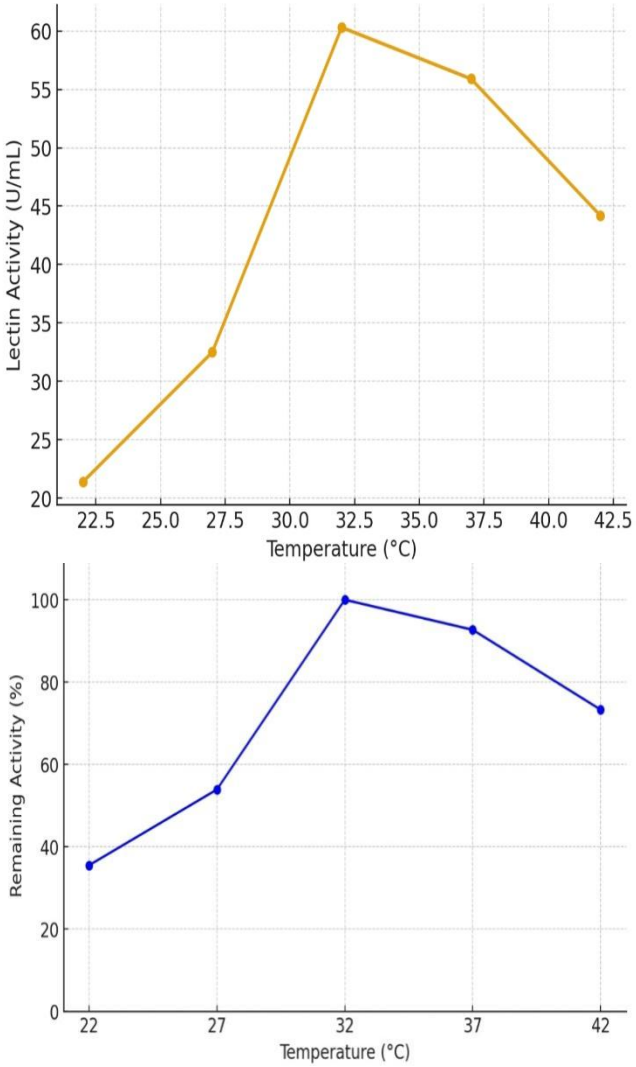

figure (2): Characterizations of lectin from mushrooms according to temperature

Characterizations of lectin from mushrooms according to PH

Purified lectin characterization is carried out in accordance with various PH levels, and the lectin activity is determined by measuring hemagglutination and residual activity. According to the findings, lectin activity peaked at pH 5 (50.6) and remained at 100%. Activity started to decline in pH 6 and reached its lowest point in pH 9. Figure (3) showed that lectin activity varied significantly with pH changes, peaking at pH 5 (50.6 U/mL) and pH 6 (49.8 U/mL). This implies that the lectin is most active in an environment that is moderately acidic. The higher activity in this pH range indicates that the lectin's carbohydrate-binding sites and structural shape remain stable and correctly folded in moderately acidic environments. Lectin activity was shown to progressively diminish after pH 6, with a significant drop at pH 7

(28.9 U/mL) and minimal activity at pH 8 (11.7 U/mL) and pH 9 (9.3 U/mL). This decrease could be attributed to partial denaturation of the protein at high pH levels or to modifications in the ionization state of a mino acid residue at the binding sites. Hemagglutination activity is consequently decreased when the lectin's affinity for erythrocyte surface carbohydrates declines. At pH 11.0 or above, the protein lost 80–100% of its activity (Islam *et al*., 2024). The present findings are consistent with previous research on a number of plant and fungal lectins, which frequently exhibit optimal activity in conditions that are moderately acidic to neutral. According to (Li *et al*., 2023), the lectin produced from *Phellodon melaleucus* was rendered inactive at more extreme pH values but retained substantial activity within the pH range of 6 to 9. According to (Resendiz *et al*., 2024), the activity of Amaranthus hypochondriacus lectin peaked at pH 7 and significantly decreased in extremely acidic or alkaline environments. (Elmubarak *et al*., 2025) found that while the lectin from remained active in the pH range of 5.5–9.5, it's stability decreased outside of this range. Together, these findings confirm that pH is critical for maintaining lectin structural integrity and carbohydrate-binding effectiveness, and that the optimal activity at pH 5–6 found in this work is consistent with the normal properties of the majority of lectins reported in the literature.

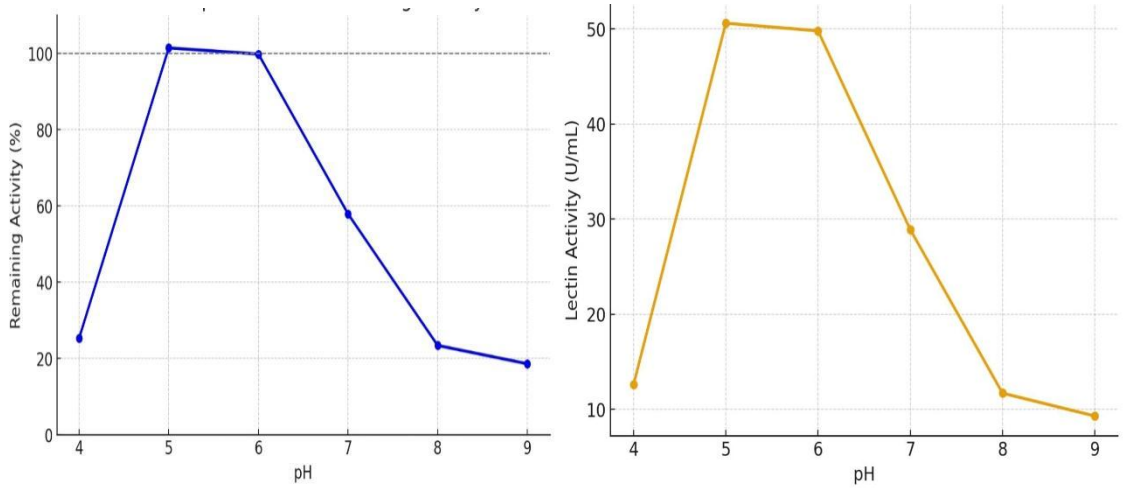

figure (3):Characterizations of lectin from mushrooms according to temperature.

characterization of lectin according to molecular weight

The molecular weight of the purified lectin was determined using the Sephadex-G150 column. Plotting the linear relationship between the molecular weight of each standard protein and recovery volume in relation to

the void volume (Ve / Vo) (Fig. 4) showed that the lectin molecular weight was 100,000 Dalton. Mushroom lectins are composed of two or more subunits that may be homologous (homo) or heterologous (hetero). The lectin from *Pholiota squarrosa* has the smallest molecular mass, at about 4.5 kDa (Singh et al., 2020). These results demonstrate the structural variety of lectins found in mushrooms. Their biological activity, thermostability, and sugar-binding selectivity are all significantly impacted by changes in molecular weight and oligomeric state. The significance of molecular characterisation as an initial step in comprehending their possible medicinal and biotechnological applications is shown by this diversity. Typically, mushroom lectins have a molecular weight between 10 and 190 kDa and are made up of two or more subunits that may or may not be similar. Similar to lectins from *Bjerkandera adusta* (24 kDa), *Hypsizygus marmoreus* (19 kDa), and *Pleurotus citrinopileatus* (32.4 kDa), PML is a homodimeric lectin with a low molecular weight (Li *et al*., 2023).

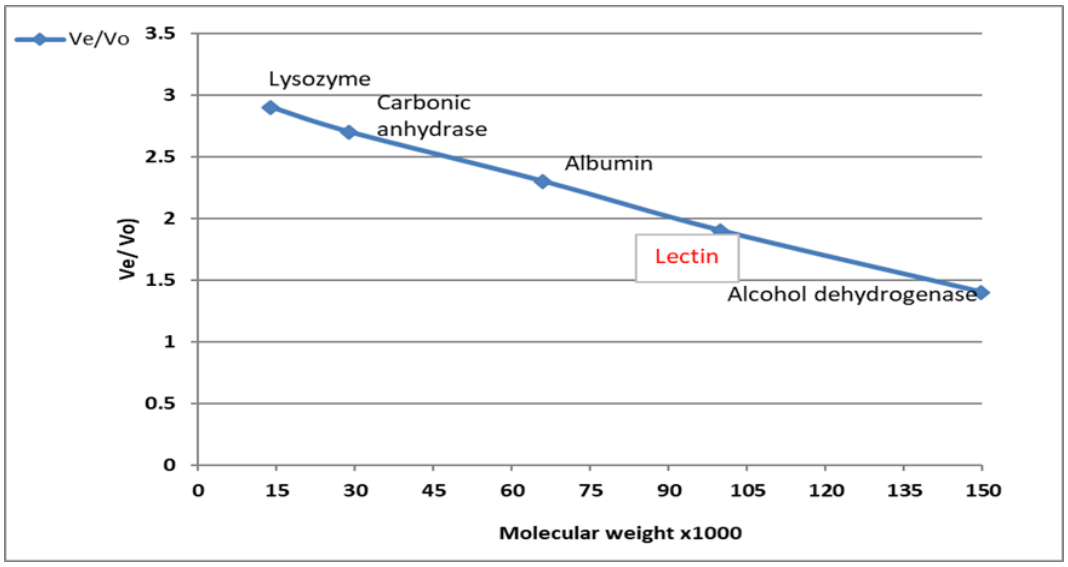

Figure (4) molecular weight of the purified lectin

The effect of different ph on *candida* spp. growth and lectin production

The antifungal effectiveness of purified lectin against several species of *Candida* was evaluated across a pH range of 4 to 9. The lectin appears to be pH-dependent because only certain pH values displayed inhibitory zones, with pH 5 and pH 6 displaying the highest activity.This demonstrates that the lectin maintains its structural integrity and ability to bind carbohydrates in relatively acidic settings, which enhances its interaction with fungal cell wall components. A. *Candida albicans* (C015), and *Candida glabrata* (C018) were the isolates with the highest lectin sensitivity at these pH levels. (Table

2) However, at very alkaline or extremely acidic pH values, no inhibition was seen. This is probably because the lectin's biological function is compromised by structural denaturation or conformational changes. These results are in line with recent research (Sharon & Lis, 2004) that demonstrated how variations in protein folding and binding affinity caused by pH have a substantial impact on lectin activity. The high pH permanently changed the chemical and/or physical characteristics of the lectin, which affected the hemagglutinating activity. (Li *et al*., 2023).

Table(2): The effect of different PH on *candida* spp. growth and lectin production

***Candida* spp .**	**PH5**	**PH6**
C.tropicalis (CO1)	12.67bcdef	17.00b
C.albicans (CO2)	12.00def	14.00def
C.albicans(CO3)	12.33cdef	12.33f
*C.albicans(*CO4)	13.67bcde	15.67bcd
C.Kruzei (CO5)	12.67bcdef	15.67bcd
C.albicans(CO6)	12.00def	16.33bc
C.albicans(CO7)	11.00f	14.00def
C.tropicalis(CO8)	11.67ef	15.67bcd
C.albicans(CO9)	14.00bcd	14.33cdef
C.albicans(CO10)	11.67ef	15.67bcd
C.tropicalis(CO11)	12.00def	13.00ef
C.tropicalis(CO12)	12.00def	16.33bc
C.glabrata (CO13)	15.33a	21.00a
C.tropicalis(CO14)	14.33bc	16.00bcd
C.albicans(CO15)	14.00bcd	17.33b
C.glabrata(CO16)	13.00bcdef	17.67b
C.tropicalis(CO17)	14.67b	16.33bc
C.glabrata (CO18)	14.33bc	17.67b
C. cifferic (CO19)	13.33bcde	14.00def
C.dublensis(CO20)	13.00bcdef	16.00bcd
Control	17.00a	17.00b
LSD 0.05	2.01	2.15

The effect of different temperature on *candida* spp. growth and lectin production

The study evaluated lectin-like activity in 20 isolates of *Candida* spp. (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. dubliniensis*, and *C. cifferii*) across a temperature range (22 °C, 27 °C, 32 °C, 37 °C, and 42 °C). The findings in Table 3 demonstrate that temperature has a substantial effect on lectin activity. Lectin activity increased gradually in the majority of species from 22°C to a peak between 32°C and 37°C before sharply declining at 42°C. With respective values of 21.33 and 20.33 mm, *Candida albicans* and *Candida tropicalis* were the isolates with the highest lectin activity at 37C°. These temperatures appear to be optimal for lectin stability and activity since protein structure is maintained within the physiological temperature range. At 42C°, lectin activity was significantly reduced in almost all isolates. 12.67 mm instead of 20.33 mm.Lectin activity significantly decreased in almost all isolations. For example, *C. albicans* activity dropped from 21.00 mm at 37C° to 14.00 mm at 42C°, and *C. tropicalis* activity dropped from 20.33 mm to 12.67 mm. Heat-induced denaturation or structural changes in the lectin protein could be the cause of this decrease. However, lectin activity was moderate to low at lower temperatures, such as 22C°, suggesting that lower temperatures do not promote optimal lectin production or function. Significant variations between species and temperature points were found by statistical analysis (LSD at $p < 0.05$). For comparison, the control values didn't change. According to the research, lectin activity and synthesis are temperature-dependent, with the highest bioactivity taking place between 32 and 37C°. Future therapeutic applications of fungal lectins seem promising in this temperature range, especially those targeting antioxidant, antifungal, or anticancer characteristics. The lectin that was extracted from the *Ganoderma capense* fungus was stable in the pH range of 3.0–12.0 and was fully active for an hour at 100C° (Ngai & Ng, 2004). *Aspergillus nidulans* lectin completely destroyed at temperatures above 40C°, which may be due to the high carbohydrate content. Additionally, the lectins of *Aspergillus terricola*, *Lentinus squarrosulus*, and *Rhizopus stolonifer* have been found to have good thermostability.(Singh *et al*.,2010).

Table (3) : The effect of different temperature on *candida* spp. growth and lectin production

Candida spp .	**22Ċ**	**27 Ċ**	**32 Ċ**	**37 Ċ**	**42 Ċ**
C.tropicalis	13.33defg	15.00cde	18.33bcde	19.33bcde	12.00def
C.albicans	14.33bcdef	14.67de	18.00cde	20.33bc	12.33cde
C.albicans	13.33defg	13.67e	17.00cdef	21.33b	11.00efg
C.albicans	11.33g	13.67e	18.00cde	17.33e	11.00efg
C.Kruzei	13.33defg	15.00cde	18.00cde	19.00cde	13.00bcd
C.albicans	12.67fg	14.67de	17.67cde	18.00de	14.33b
C.albicans	11.67fg	15.67bcde	19.00bcd	18.00de	12.67bcde
C.tropicalis	14.67bcde	16.00bcde	20.67b	20.33bc	12.67bcde
C.albicans	13.67cdefg	17.33bc	19.33bc	20.00bcd	13.00bcd
C.albicans	16.33bc	16.33bcd	19.00bcd	21.00bc	14.00bc
C.tropicalis	14.00bcdefg	16.33bcd	17.00cdef	21.00bc	11.00efg
C.tropicalis	12.33fg	14.33de	14.67f	18.00de	10.00g
C.glabrata	13.33defg	16.00bcde	17.67cde	20.00bcd	12.00def
C.tropicalis	13.00efg	15.67bcde	19.33bc	17.67e	12.33cde
C.albicans	16.00bcd	15.00cde	19.00bcd	18.00de	10.33fg
C.glabrata	12.67fg	15.00cde	17.00cdef	19.00cde	12.33cde
C.tropicalis	14.00bcdefg	15.67bcde	17.67cde	21.00bc	10.00g
C.glabrata	16.67b	16.33bcd	16.67def	20.00bcd	11.67defg
C. cifferii	16.67b	17.67b	16.00ef	17.67e	12.00def
C.dublensis	13.00efg	16.33bcd	19.00bcd	17.67e	11.00efg
Control	25.00a	25.00a	25.00a	25.00a	25.00a
LSD 0.05	2.80	2.50	2.66	2.03	1.92

Conclusion:

The mushroom lectin showed a molecular weight of about 100,000 Da and was most stable near neutral pH but lost activity under extreme pH or high temperatures. Its antifungal effect against *Candida* was also reduced by heat and extreme pH. Overall, it is a heat-labile, pH-sensitive lectin with promising antifungal potential.

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تشخيص اللكتين من العراهين القابله للأكل (*lentinula edodes*)

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مستخلص البحث:
تم تشخيص الليكتينات وفقا لطرق مختلفة مثل الرقم الهيدروجيني ودرجة الحرارة والوزن الجزيئي وتم أخذ قيم مختلفة من الرقم الهيدروجيني في المدى من (4_9) وتم تحديد نشاط الليكتين عن طريق قياس التراص الدموي وتم أخذ درجات حرارة مختلفة لتحديد نشاط الليكتين في المدى (37،32،27،22 و 42 درجة مئوية) في مدد زمنية مختلفة (30،20،10) دقيقة وتم تحديد الوزن الجزيئي بواسطة تقنية الترشيح الهلامي وتم دراسة تأثير درجات الحرارة المختلفة والرقم الهيدروجيني على نمو فطريات المبيضات وإنتاج الليكتين عن طريق قياس مناطق التثبيط. أظهرت نتائج خصائص درجات الحرارة أن هناك درجات متفاوتة من الاستقرار الحراري حيث بلغ نشاط الليكتين ذروته عند 32 (60.3) وانخفض نشاط التراص الدموي مع ارتفاع درجة الحرارة وفي درجة الحموضة بلغ نشاط الليكتين ذروته عند 5 (50.6) وانخفض النشاط في درجة الحموضة (9،8،7) وكان الوزن الجزيئي لليكتين 100000. ولم يلاحظ أي تثبيط للفعالية المضادة للفطريات لليكتين المنقى ضد عدة أنواع من المبيضات عند قيم درجة الحموضة القلوية للغاية أو الحمضية للغاية، ويزداد نشاط الليكتين تدريجيًا من 22 إلى ذروته بين 32 و37 وينخفض عند 42 درجة مئوية.

الكلمات المفتاحية : اللكتين، العراهين القابلة للاكل ، درجة الحرارة ، درجة الحموضة ، التراص الدموي ،فطريات الكانديدا
ملاحظة : هل البحث مستل من رسالة ماجستير او اطروحة دكتوراه ؟ نعم :