ISSN: 0067-2904

Antibacterial and Antibiofilm of Purified β-glucan from *Saccharomyces cerevisiae* against Wound Infections Causative Bacteria

Alyaa A Khadam, Jehan AS. Salman*
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

Received: 16/1/2023 Accepted: 13/5/2023 Published: 30/5/2024

Abstract

Twenty species were isolated from wounds' swabs. The most common bacterial species were *Pseudomonas aeruginosa* (8), *Staphylococcus aureus* (5), *Escherichia coli* (5) and *S. epidermidis* (2). These isolates underwent tests for biofilm formation and susceptibility to ten different antibiotic discs. Most isolates exhibited resistance to amikacin, aztreonam, and carbenicillin. However, they showed some sensitivity to ciprofloxacin, norfloxacin and imipenem. This study found out that all isolates were different in biofilm formation (weak, moderate, strong), except two isolates of *E. coli*. The determination of β-glucan effects produced by *S. cerevisiae* against multi-antibiotic resistant wound infection bacteria, were conducted through the Minimum Inhibitory Concentration (MIC). The antibacterial activity of β-glucan against wound infection causative bacteria was evaluated using the (MIC) ranging from 6.25 to 400 mg/mL. The antibiofilm effects of purified β-glucan (at sub-MIC) demonstrated highest inhibition percentage (92%) for biofilm formation after 72 h against multidrug-resistant (MDR) *P. aeruginosa* $_{(2)}$, while *P. aeruginosa* $_{(5)}$ had the lowest (32%). β-glucan activity on biofilm degradation was detected in all isolates. The highest percentage of biofilm degradation was 90% for MDR *P. aeruginosa* $_{(1)}$.

Keywords: Wounds infections, *Pseudomonas aeruginosa*, β-glucan, Antibacterial, Antibiofilm.

فعالية ضد البكتيريا والغشاء الحيوي للبيتاكلوكان المنقى من ضد *Saccharomyces cerevisiae* البكتريا المسببة لاصابات الجروح

علياء عبد الحسين كاظم و جيهان عبد الستار سلمان*
قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق

الخلاصة

جمعت 20 عزلة بكتريا من مسحات الجروح . اخضعت هذه العزلات للفحوصات الزرعية و المجهرية و الكيموحيوية فضلا عن استعمال نظام Vitek2 لتأكيد التشخيص. كانت 8 عزلات تعود إلى *Pseudomonas aeruginosa* و 5 عزلات الى *Escherichia coli* و *Staphylococcus aureus*5 و 2 عزلات من *S. epidermidis*

تم اختبار حساسية هذه العزلات لعشرة من المضادات الحيوية ، وقدرتها على تكوين الغشاء الحيوي. أظهرت جميع العزلات التي خضعت لهذه الدراسة مقاومة متفاوتة للمضادات الحيوية المستخدمة ، حيث كانت جميع

*Email: alyaaabdulhussein.ph.d.mic.2020@uomustansiriyah.edu.iq

العزلات مقاومة لـ carbencillin،amikacin, aztreonam ، في حين أظهرت جميع العزلات حساسية لـciprofloxacin, norfloxacin, imipenem فيما امتلكت جميع العزلات القدرة على تكوين الغشاء الحيوي ماعدا عزلتين من *Escherichia coli*. تم تحديد فعالية البيتا كلوكان المنتج من خميرة*S. Cerevisiae* ضد البكتيريا المسببة لاصابات الجروح المتعددة المقاومة للمضادات من خلال تحديد التركيز المثبط الادنى MIC، أظهرت النتائج أن التركيز المثبط الادنى للبيتا كلوكان النقي قد تراوح بين 6,25−400 ملغم/ مل. أظهرت النتائج تأثيراً مثبطاً على تكوين الغشاء الحيوي لمعظم العزلات، اعلى نسبة تثبيط لتكوين الأغشية الحيوية بعد 72 ساعة بلغت 92% الزائفة الزنجارية المتعددة المقاومة للمضادات الحيوية (2) *P. aeruginosa* (MDR) بينما أقل نسبة تثبيط (32 ٪) للزائفة الزنجارية (5) *P. aeruginosa*، كما ظهر أن البيتا كلوكان له تأثيراً على تحلل الغشاء الحيوي لجميع العزلات ، وكانت أعلى نسبة تحلل للغشاء الحيوي 90٪ لبكتريا الزائفة الزنجارية (1) *P. aeruginosa* MDR

1. Introduction

Yeast glucans are polysaccharides that form structurally distinct D-glucose polymers and are classified as α -D-glucans, β -D-glucans, and α, β-D-glucans, based on the anomeric structure of glucose [1]. β -D-glucan biopolymer can be found in the cell walls of yeast, fungi, bacteria, algae and plants. The cell wall of yeast *S. cerevisiae* is one of the most glucan organisms producing and is composed of β-glucan about 55-65% of the yeast, (1-3)-β-D-glucan backbone gives the cell walls strength and links to the chitin, mannoprotein and (1-6)-β-D-glucan side chains [2]. Due to its role as an immuno-stimulator, yeast -glucan has been found to be advantageous for the immune systems of both humans and animals [3]. (1-3)-β-glucan has the capacity to modify body's natural healing processes by promoting epithelial hyperplasia, inflammatory cell activity, angiogenesis and fibroblast proliferation [4]. *Pseudomonas aeruginosa* and *S. aureus* are the two bacterial species that cause wound infections most frequently. Gram-positive bacteria, especially *S. aureus*, seem to colonize most frequently during the first week of an infection, in particular [5]. Gram-negative bacteria, such as *P. aeruginosa*, began to colonize the wound at the start of the second week, and if they get into the lymphatic system or blood vessels, they can even cause sepsis [6].

Over the past ten years, the relationship between chronic wounds and the formation of microbial biofilms has been studied [7]. Microbial biofilms are collections of bacterial populations that are encased in an extracellular matrix made of exopolysaccharides (EPS), nucleic acid and proteins produced by themselves [8]. Biofilms allow bacteria to attach to various inanimate and *in vivo* environments and protect from stress environmental conditions, noxious agents and antibiotics biofilm formation which may lead to chronic local inflammation, infection delaying wound healing [9]. Common bacteria that form biofilms include *P. aeruginosa*, *S. aureus, S. epidermidis,* and *E. coli* is an essential factor in the persistence of infections [10]. *P. aeruginosa* is a nosocomial bacterium that causes wound injuries [11]. Alginate, Pel and Psl, as well as proteins and extracellular DNA, make up the *P. aeruginosa* biofilm matrix [12]. In *P. aeruginosa*, biofilm is a significant element that confers resistance to environmental stressors, phagocytic defenses, antimicrobial drugs and xenobiotics [13], and because of this bacterium's rising intrinsic resistance to many antibiotics, treatment of its infections is limited. [14]. Polysaccharide intercellular adhesion (PIA) which is the biofilm matrix of *S. aureus*, and *S. epidermidis*, works on adhesion and architectural framework [15]. *S. epidermidis* is the most typical opportunistic biofilm forming and the most frequent cause of implant-associated infections. Some strains of *S. epidermidis* may be involved in the pathogenesis of common skin illnesses [16]. The *E. coli* biofilm is made up of a bacterial colony encased in an extracellular polymeric substance (EPS) matrix that shields the microorganisms from harmful environmental factors and causes infection. Additionally, infectivity associated with indwelling medical devices is caused by *E. coli*

biofilm [17]. Song *et al*. [18] exhibited that β-glucan acts as an antibacterial agent by damaging the cell integrity of *S. aureus* and changing the cell permeability. Therefore, the purpose of this study was to determine the ability of β-glucan purified from *S. cerevisiae* to inhibit the growth and biofilm of wound infection causative bacteria.

2. Materials and Methods

2.1 Saccharomyces Cerevisiae

Commercial Baker's yeast was collected from the commercial market and was activated and re-identified using cultural, microscopical, biochemical, and Vitek 2 systems [19].

2.2 Wounds Infections Causative Bacteria

Twenty isolates were collected from different hospitals in Baghdad. Wound swabs were obtained from patients of various ages with specific clinical signs of skin infections. All isolates were examined using cultural, microscopic and biochemical tests, as well as the Vitek 2 technique.

2.2 Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method was used to conduct the antibiotic susceptibility test for 10 antibiotic discs, comprising imipenem, tetracycline, doxycycline, ceftriaxone, ceftazidime, gentamicin, amikacin, cefepime, tobramycin and aztreonam (Mastdiscs (UK)

2.3 Biofilm Formation

Microtiter plate technique was used to test the biofilm-forming capabilities of bacterial isolates from wounds, as reported by [20]. According to the absorbance values, the biofilm formation of each isolate was classified into the following [21]: OD ≤ ODc (None), ODc < OD ≤ 2 ODc (Weak), 2ODc < OD ≤ 4 ODc (Moderate), 4ODc < OD (High).

2.4 β-glucan Extraction from S. cerevisiae by Autolysis of Yeast Cell

S. cerevisiae suspension was made and incubated for 48 hours at 30°C with 200 rpm shaking. A 15% w/v suspension was mixed with distilled water with a pH of 5.0 and was then incubated at 50°C for 48 hours at 120 rpm in a shaker incubator. Yeast cells were then recovered by centrifugation at 4°C for 10 min. Later they were placed in a water bath and heated to 80°C for 15 minutes. The obtained yeast cells were centrifuged at 5,000 rpm for 10 minutes at 4°C. After being treated with 5-fold 1.0 M NaOH, the autolyzed yeast cells were incubated in a stirrer at 80°C for two hours. Centrifugation at 6000 g for 25 minutes at 4°C collected the cell pellet which was then dissolved in three times distilled water. Cells were thoroughly mixed before being centrifuged at 6000 g for 25 min at 4°C. The cell pellet was then dissolved in 5-fold 1.0 CH_3COOH and stirred at 80°C for two hours. The separation of the pellet was performed by centrifugation at 6000 g for 25 min at 4°C. The resulting pellet was dried in a hot air oven at 60°C after being washed three times with distilled water [22].

2.5 Purification of β-glucan

This process was carried out in accordance with the procedure described by [23], with minor modifications. The crude glucan was dissolved in 0.3% distilled water and heated for one hour at 90°C to ensure that whole substance had dissolved. After the ammonium sulfate had completely dissolved, the crude glucan was precipitated using ammonium sulfate at a saturation rate of 30% by stirring and then placed it in the refrigerator for 24 hours. Centrifugation was performed, and the precipitate was taken and re-dissolved in water and re-heated to the boiling point for an hour, then cooled down and precipitated using cold acetone and placed in the refrigerator for 24 hours, followed by centrifugation. The precipitate was re-

dissolved with a quantity of water, followed by dialysis (MWCO 6,000-7000 Da) with distilled water for 24 h, and then the content was taken and the pH 7.5 was adjusted. The glucan content was lyophilized for 96 hours.

2.6 Antibacterial Activity of Purified B-glucan against Wounds Infections Causative Bacteria

Antibacterial activity of β-glucan purified from *S. cerevisiae* against *E. coli, P. aeruginosa, S. aureus, S. epidermidis* was tested by the flat-bottomed 96-well microdilution technique microplate titer based on minimum inhibitory concentration (MIC) values. The experiment was carried out in accordance with the guidelines provided by [24]. A stock solution of purified β-glucan was diluted to 400 to 0.78 mg/ml concentration. Briefly, 100 µl of sterile Muller Hinton broth medium was added to the microplate first column and then 100 µl of the β-glucan solution was added and mixed in the first column with the medium. Serially, 100 µl were transferred to subsequent wells and 100 µl of the mixture was discarded in the last column resulting in a final volume of 200 µl for each well. Control well contained Muller Hinton Broth only, without β-glucan. The standardized wound infections causative bacteria suspension was then diluted by 1:100 in the broth and 50 µl of the bacterial suspension was added to all wells containing β-glucan and to the control well. Microplates were incubated for 24 h at 37°C. All wells were added 30 µl of resazurin dye (0.015%) which was then incubated for 2-4 hours to observe any color changes. After the incubation, column with no resazurin (blue) color change was scored as MIC value.

2.7 Antibiofilm Effects for Purified β-glucan

β-glucan effects on biofilm formation of wounds causative bacteria were tested in microtiter plate according to the procedure described by [25]. All isolates were grown on 96 flat-bottom well microtiter plates for 24, 48 and 72 hours at 37^{o}C in the presence and absence of glucan at the sub-MIC concentration. Twenty µl of bacterial suspension compared to 0.5 MacFarland, was added in to each well which contained 80µl sterilized Brain Heart Infusion Broth with 2% sucrose and then mixed with 100 µl of β-glucan, while control contained only 180µl and 20µl of bacterial suspension. After incubation, the medium was removed from the wells and washed three times with sterile PBS to remove the unattached bacteria cells and was then left to dry for 15 min at room temperature. Next the wells were filled with 200 µl of crystal violet (0.1%) and were left for 20 minutes. The stained wells were rinsed three times with PBS (PH 7.2) to remove unbound dye and were then left to dry at room temperature for 15 minutes. Finally, 200 µl of 95% ethanol was added to each well and the optical density was read at 630 nm by ELISA reader. The inhibition of biofilm formation percentage was calculated according to equation described by [21].

$$\% \textbf{ Inhibition of biofilm formation } = \frac{\textbf{OD control} - \textbf{OD treatment}}{\textbf{OD control}} \times \mathbf{100} \quad \textbf{Equation 1}$$

2.8 Degradation of Biofilm by Purified β-glucan

The degradation of biofilm by purified β-glucan was tested in a microtiter plate using an appropriate medium, brain heart infusion supplemented by 2% sucrose depending on the procedure mentioned by [26]. The plate was inoculated with wounds causative bacteria suspension compared to 0.5 MacFarland. The final volume of liquid in each well was 200 µl which contained 180 µl of sterilized Brain Heart Infusion broth with 2% sucrose and 20 µl of bacteria suspension. After incubation at 72 h, the broth was removed and 200 µl of β-glucan at MIC concentration was added. Whereas the control contained only 200 µl Brain Heart Infusion broth. After 24h incubation, the β-glucan was removed from the wells and washed 3 times with sterile PBS and was then left to dry for 15 min at room temperature. The wells were later filled with 200 µl of crystal violet (0.1%) and left aside for 20 minutes. The stained wells were rinsed 3 times with PBS (PH 7.2) to remove unattached stain and were then left to

dry at room temperature for 15 min. Finally, 200 µl of 95% ethanol was added to each well and the optical density was read at 630 nm by ELISA reader. The degradation of biofilm formation percentage was calculated according to the following equation:

$$\textbf{Degradation of biofilm } \% = \frac{\textbf{OD control} - \textbf{OD treatment}}{\textbf{OD control}} \times \mathbf{100} \quad \textbf{Equation 2}$$

3. Results and Discussion

3.1 Collection and Identification of Isolates

Twenty isolates included (8) isolates of *P. aeruginosa*, (5) *S. aureus*, (2) *S. epidermidis* and (5) *E. coli* which were initially diagnosed in hospitals before wounds sources were collected. To ensure this diagnosis, the bacterial isolates were re-identified using cultural characteristic, biochemical tests and Vitek 2 system.

3.1.1 Cultural Examination

On blood agar, the colonies of *Pseudomonas spp.* isolates produced a clear zone due to hemolysis production and gave grape–like or tortilla-like odor. On MacConkey agar and Pseudomonas ceteramid agar, the growing colonies appeared as pale yellow (non-fermenter lactose) smooth and in round colonies with green pigment production that diffused in agar. *Staphylococcus spp.* Diagnostics as mentioned earlier, *S. aureus* colonies looked golden yellow around with a wide yellow margin, spherical, smooth, elevated and mucoid on mannitol salt agar which is a selective and differentiating media. By fermenting mannitol and creating acids, the indicator phenol red turned from pink to yellow, giving the yellow color [27]. *S. epidermidis* isolates appearance cleared hemolysis around their colonies in blood agar, on mannitol salt agar colonies appeared colorless, round, smooth, raised because they do not ferment mannitol. The colonies of *Escherichia spp*. on MacConkey agar appeared as large, pink colonies on an agar (lactose fermenter). The colonies of *E. coli* on Eosin-methylene blue agar appeared as metallic sheen with a dark center indicating lactose fermentation and this agrees with what was reported by Tom *et al.* [28].

3.1.2 Biochemical Test

During biochemical testing on bacterial isolates, *P. aeruginosa* showed positive results for the oxidase and catalase tests. Whereas *S. aureus* showed negative results for the oxidase and positive results for the catalase tests. This result agrees with that of Al-Naqshbandi *et al.* [29].

3.1.3 Identification Using Vitek-2 System

Vitek-2GN, GP card results were used as the basis for the final isolate identification. According to the results above, gram-negative bacteria (65%) were found to be more prevalent than gram-positive bacteria (35%). *P. aeruginosa* (40%) and *E. coli* (25%) were the most common gram-negative microbes, whereas *S. aureus* was the most common gram-positive bacterium in 25% of cases followed by *S. epidermidis* (10%) (Table 1).

Table 1: percentage of pathogenic bacteria isolated from wounds.

Isolation Source	***P. aeruginosa***	***E. coli***	***S. aureus***	***S. epidermidis***
	No. of isolates%	No. of isolates%	No. of isolates%	No. of isolates%
Wounds	8(40%)	5(25%)	5(25%)	2(12%)
Total	20	20	20	20
Chi-square (P-value)	4.061 * (0.0477).			
	* (P≤0.05).			

Wounds are a sensitive area for the opportunistic colonization of organisms from both endogenous and exogenous origin. These results agree with Puca *et al*. [27] who revealed in their research that the most common wound swabs were *P. aeruginosa* (40.2%), *E. coli* (20.7%) and *S. aureus* 36.6%. Whereas Sisay *et al*. [30] showed that in the bacterial isolates from wounds, *S. aureus* was 36%. Jindal *et al.* [31] revealed in their research the most prevalent bacteria in wounds swab were *E. coli* followed by *P. aeruginosa*. *S. aureus* and *S. epidermidis*. Zhu *et al*. [32] demonstrated that the most predominant species found in wounds MDR *E. coli* were 20.7% resistant to azatreonam, ceftazidime and ciprofloxacin. Whereas, *S. aureus* were 28.7% resistant to carbenicillin, cefotaxime and amikacin. The mechanism of azatreonam and carbenicillin inhibits synthesis of the bacterial cell wall, while ceftazidime impact is mediated through binding to essential penicillin-binding proteins. Ciprofloxacin inhibits DNA replication, amikacin binds to the 30 S bacterial ribosome subunit, resulting in inhibition of protein synthesis.

3.2 Antibiotic Susceptibility Test

The disc diffusion test used for antibiotic susceptibility test was carried out on all isolates of *P. aeruginosa, E. coli, S. epidermidis* and *S. aureus.* The results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI). All of the bacterial isolates in this investigation showed varying resistance to the ten antibiotics (ceftazidime, cefotaxime, aztreonam, carbenicillin, amikacin, tetracycline oxytetracycline, imipenem, ciprofloxacin, and norfloxacin) that were put to the test. All *P. aeruginosa* isolates were resistant to carbenicillin, although their sensitivity to other antibiotics in various dosages varied. The 7(8) *P. aeruginosa* isolates from 8 were found to be resistant to tetracycline and ciprofloxacin. Whereas the 5(8) *P. aeruginosa* isolates were found to be resistant to azatreonam and ceftazidime. The findings revealed that all *E. coli* isolates were resistant to aztreonam, although sensitivity to other antibiotics in varying concentrations varied.

On the other hand, gram positive bacteria *S. aureus* isolates showed resistance to amikacin, aztreonam, and carbenicillin. Whereas *S. epidermidis* was resistant to carbenicillin and its sensitivity to other antibiotics varied depending on the quantities used (Table 2).

Antimicrobial resistance (AMR) is defined as the resistance of microorganisms to an antimicrobial agent to which they were initially sensitive. This resistance may be generated and transmitted in many different ways, making clinical management of infections a real challenge, especially in patients with comorbidity. The World Health Organization (WHO) has considered antimicrobial resistance as one of the top ten threats to global health because of MDR bacteria presence in the wound [33]. In this study, results showed that all isolates were multi-resistant to antibiotics with the highest percentage of 72% for *P. aeruginosa*, followed *S. aureus, E. coli, S. epidermidis* 60, 58, 55% respectively. This made the onset of multi-drug resistant strains, about 50% of infections associated with *E. coli*, *S. aureus*, and *P. aeruginosa* showed resistance against the most effective antimicrobials such as third-generation cephalosporin [34]. These results agreed with study by Zhu *et al.* [32] who found out that MDR *S. aureus* and *E. coli* were found to be resistant against oxytetracycline. Alharbi *et al*. [35] reported that the *E. coli* isolates from wound infections were resistant to ciprofloxacin, piperacillin and tetracycline. According to the results by Zainulabdeen *et al*. [36], *P. aeruginosa* isolates were found to be resistant to tetracycline, imipenem, and doxycycline but more sensitive to amikacin, tobramycin and aztreonam.

Skin and soft tissue infections (SSTIs) caused by *S. aureus* can range in severity from benign to lethal right away. Omololu [37] demonstrated that all isolates were sensitive to ciprofloxacin. However, these findings disagree with the finding of the current study that *S.*

aureus showed partial resistance (50%) towards of ciprofloxacin. Alabi *et al*. conducted yet another investigation [38] Where he found that *S. aureus* and *S. epidermidis* had ciprofloxacin resistance, whereas *S. epidermidis* had cefotaxime resistance [31].

Table 2: Antibiotic susceptibility test for pathogenic bacteria isolated from wound infections.

Bacterial Isolates	CAZ	CIP	T	AM	ATM	CTX	PY	OT	NOR	IMP
P. aeruginosa$_{(1)}$	S	S	R	R	R	R	R	R	R	R
P. aeruginosa$_{(2)}$	S	R	R	R	R	R	R	R	R	R
P. aeruginosa$_{(3)}$	R	R	R	S	R	R	R	R	S	S
P. aeruginosa$_{(4)}$	R	R	S	S	S	S	R	S	S	R
P. aeruginosa$_{(5)}$	R	R	R	R	S	S	R	R	S	R
P. aeruginosa$_{(6)}$	R	R	R	R	R	S	R	R	R	R
P. aeruginosa$_{(7)}$	R	R	R	S	R	R	R	R	R	S
P. aeruginosa$_{(8)}$	S	S	R	R	S	R	R	S	S	R
E. coli$_{(1)}$	S	R	R	R	S	R	S	R	S	S
E. coli$_{(2)}$	R	R	R	R	R	S	R	S	R	R
E. coli$_{(3)}$	R	R	S	S	S	R	R	R	S	R
E. coli$_{(4)}$	R	R	S	S	R	S	S	R	S	R
E. coli$_{(5)}$	S	R	S	R	S	S	R	R	R	S
S. aureus$_{(1)}$	R	R	S	S	R	S	S	R	S	R
S. aureus$_{(2)}$	R	S	R	R	R	S	R	S	S	R
S. aureus$_{(3)}$	S	R	S	R	R	S	R	R	R	S
S. aureus$_{(4)}$	S	S	R	R	S	R	R	S	S	S
S. aureus$_{(5)}$	S	R	R	R	R	R	R	R	R	R
S. epidermidis$_{(1)}$	R	S	S	S	R	S	R	R	R	S
S. epidermidis$_{(2)}$	S	R	R	R	S	R	R	S	S	R

AM: Amikacin, ATM: Aztreonam, CTX: Cefotaxime, CIP: Ciprofloxacin, PY: Carbenicillin, CAZ: Ceftazidime, T: Tetracycline, OT: Oxytetracycline, NOR: Norfloxacin, IMP: Imipenem, R: resistance, S: sensitivity.

3.3 Biofilm Formation

Results showed that 18 isolates from 20 isolates of wound infections causative bacteria, had ability to form biofilm. However, 2 isolates of *E. coli* had no ability to form biofilm (Table 3). Biofilm formation is one of the common strategies in order to survive in harsh environmental conditions. Bacteria frequently form biofilms on a variety of biotic surfaces, including those found in water systems and in natural aquatic habitats [14].

Biofilms, intricate structures made up of a significant bacterial population interacting with one another and generating an extracellular matrix that is diverse and protective, are the primary source of wound infections. Due to this structure's high tolerance for chemical and physical eradicators as well as biofilm growth within the wound, treatment problems often arise. Furthermore, a systemic infection that poses a serious risk of death can arise from a biofilm-based wound infection [39].

Biofilms can be created by a wide variety of microorganisms, whether they are present in the environment or on living hosts. The strategies available to control biofilm formation include focusing on the enzymes and proteins specific to the microorganism, as well as those involved in the adhesion pathways leading to the formation of resistant biofilms. These

include pathogenic bacteria that can act as a reservoir for persistent infections [40]. Dydak *et al*. [41] in their research revealed that all *S. aureus* isolates, 1 *S. epidermidis*, 2 *E. coli* and 4 *P. aeruginosa* isolates had ability to form biofilm. Murugan *et al*. [42] observed that *S. aureus* and *P. aeruginosa* isolates were very high biofilm formers. The formation of biofilms by *S. epidermidis* was studied using microtiter plate method [43].

Table 3: Detection of biofilm formation of pathogenic bacteria isolated from wound infections.

Bacterial Isolates	Biofilm Formation	Bacterial Isolates	Biofilm Formation
P. aeruginosa (P1)	Weak	*E. coli (3)*	Moderate
P. aeruginosa (P2)	Strong	*E. coli (4)*	not biofilm formation
P. aeruginosa (P3)	Weak	*E. coli (5)*	not biofilm formation
P. aeruginosa (P4)	Weak	*S. aureus (S1)*	Weak
P. aeruginosa (P5)	Weak	*S. aureus (S)*	Weak
P. aeruginosa (P6)	Weak	*S. aureus (S3)*	Moderate
P. aeruginosa (P7)	Moderate	*S. aureus (S4)*	Weak
P. aeruginosa (P8)	Moderate	*S. aureus (S5)*	Strong
E. coli (1)	Strong	*S. epidermidis (1)*	Strong
E. coli (2)	Week	*S. epidermidis (2)*	Strong

3.4 Extraction of β-glucan from S. cerevisiae

Glucan can be produced from baker's yeast with varying degrees of purity and with great variation in its physical and chemical properties and its biological activity, depending on the extraction method used. In this study autolysis method, the most suitable procedure, was adopted to obtain high concentration glucan extracts from yeast. Crude β-glucan was dried and weighed as 6gm/L. This study agrees with Al-Rabie [44] that the autolysis method was good in obtaining high purity glucan extracts.

3.5 Purification of β-glucan

The crude β-glucan was purified by many steps. Ammonium sulphate (30%) was used as the first step to precipitate β-glucans. It was used in the purification and separation of β-glucan from other compounds followed by dialysis and treatment with protease enzyme. The results showed that the dry weight of β-glucan was 2.2 g/L and high purity β-glucan was obtained compared with the standard glucan. Wang *et al*. [45] reported that after precipitation by using ammonium sulphate, he followed the dialysis step that led to reduce other contents in crude β-glucan by dialysis bag (cut-off 14 kDa). Allaith *et al*. [46] studied the β-glucan precipitation by adding cold acetone (96%).

3.6 Antibacterial Activity of Purified B-glucan from S. cerevisiae Against Skin Infection Causative Bacteria Isolates

The antibacterial activity of purified β-glucan was examined depending on MIC values by using the resazurin-based assay. The wells which appeared blue color after 24 h and 4 h incubation with resazurin, showed no growth of bacterial isolates. Whereas all wells appeared pink or pale pink from the original blue color, thus indicating the growth of bacterial isolates compared with control (Table 4). MIC of β-glucan at 400 - 0.78 mg /mL concentration was determined for all of wounds causative bacteria isolates, except two isolates of *E. coli* that were not biofilm-forming. The results showed that the MIC for all isolates was between 6.25 – 400 mg/mL. The results also showed that the MIC for *P. aeruginosa, E. coli, S. aureus, S.*

epidermidis isolates was 400 mg/ml while that for *P. aeruginosa*$_{(4)}$, *P. aeruginosa*$_{(7)}$ and *S. aureus*$_{(5)}$ was 12.5, 25, 6.25 mg/ml respectively.

Inhibiting the growth of bacteria isolated from wound infections by β-glucan was conducted by using the resazurin-based assay. β-glucan had antimicrobial activity against fungi, yeast and multidrug-resistant bacteria with MIC of 0.39 and 0.19 mg/mL in the case of resistant *S. aureus* (MRSA) and *P. aeruginosa* respectively [47]. It was confirmed in another study that the antibacterial activity of the β-glucan against *S. aureus*, *E. coli* and *P. aeruginosa* was evaluated by determining its MIC, which was < 60 μg/ml for all isolates [48]. Saravana Kumar *et al*. [49] proved β-glucan antibacterial activity against *S. aureus*. A study by Juyi *et al*. [18] exhibited that β-glucan acts as antibacterial agent by damaging the cell integrity of *S. aureus* and changed the cell permeability. Sivignona *et al*. [50] demonstrated that β-glucan extracted from yeast cell walls was shown to inhibit 95% of *E. coli*.

Table 4: Minimum Inhibitory Concentration (MIC) of β-glucan against bacteria isolated from wound infections.

Bacterial Isolates	MIC β-glucan Concentration (Mg/Ml)	Bacterial Isolates	MIC β-glucan Concentration (Mg/Ml)
P. aeruginosa$_{(1)}$	400	*E. coli*$_{(1)}$	400
P. aeruginosa$_{(2)}$	400	*E. coli*$_{(2)}$	400
P. aeruginosa$_{(3)}$	400	*E. coli*$_{(3)}$	400
P. aeruginosa$_{(4)}$	12.5	*S. aureus*$_{(1)}$	400
P. aeruginosa$_{(5)}$	400	*S. aureus*$_{(2)}$	400
P. aeruginosa$_{(6)}$	400	*S. aureus*$_{(3)}$	400
P. aeruginosa$_{(7)}$	25	*S. aureus*$_{(4)}$	400
P. aeruginosa$_{(8)}$	100	*S. aureus*$_{(5)}$	6.25
S. epidermidis$_{(1)}$	400	*S. epidermidis*$_{(2)}$	400

3.7 Inhibition of biofilm formation by Purified β-glucan

In this study, β-glucan was demonstrated to have preventative biofilm formation of all wounds causative bacteria. The biofilm formation reduced in all of the isolates, except two isolates of *E. coli* that were not biofilm-forming at different incubation times of 24, 48, 72 hours compared to the control. The highest inhibition percentage for biofilm formation after 72h was 92, 86 and 82% for MDR *P. aeruginosa* $_{(2)}$, *S. aureus* $_{(2)}$ and *S. epidermidis*$_{(2)}$ respectively. While low inhibition percentage of 32% was recorded for *P. aeruginosa* $_{(5)}$. And after 48h the highest percentage recorded was 89, 80 and 79% for MDR *P. aeruginosa*$_{(2)}$, *S. aureus*$_{(2)}$, *S. epidermidis*$_{(2)}$ respectively. Low inhibition percentage (26 & 28%) was recorded for *P. aeruginosa* $_{(5)}$ (Table 5). Various methods and developments have recently been used to identify antibiofilm drugs and their mechanisms of biofilm suppression, such as adhesion inhibitors, quorum sensing inhibitors, cyclic diguanylate inhibitors and polymer surface modification [40]. Other effective combating strategies with potential anti-biofilm agents, including plant extracts, peptides, enzymes, lantibiotics, biosurfactants, metal nanoparticles, and polysaccharides such as polymer β-glucan has antibiofilm activity against gram-positive and -negative bacteria at 100 μg/ml [50]. Iswarya *et al*. [51] proved that β-glucan prevented the biofilm inhibition of gram-positive and -negative bacteria at 25 μg/ml concentration.

Table 5: Purified β-glucan effect of inhibition of biofilm formation at 24, 48 and 72h

Bacterial Isolates	Biofilm Inhibition %		
	24h	48 h	72h
P. aeruginosa$_{(1)}$	63	71	78
P. aeruginosa$_{(2)}$	87	89	92
P. aeruginosa$_{(3)}$	61	83	88
P. aeruginosa$_{(4)}$	50	64	77
P. aeruginosa$_{(5)}$	19	28	30
P. aeruginosa$_{(6)}$	50	51	54
P. aeruginosa$_{(7)}$	61	80	86
P. aeruginosa$_{(8)}$	18	26	32
E. coli$_{(1)}$	63	67	71
E. coli$_{(2)}$	53	59	62
E. coli$_{(3)}$	50	55	61
S. aureus$_{(1)}$	44	68	71
S. aureus$_{(2)}$	66	80	86
S. aureus$_{(3)}$	24	26	30
S. aureus$_{(4)}$	63	71	74
S. aureus$_{(5)}$	66	69	72
S. epidermidis$_{(1)}$	65	75	78
S. epidermidis$_{(2)}$	75	79	82

3.8 Degradation of Biofilm by Purified β-glucan

Degradation of biofilm for *P. aeruginosa, E. coli, S. epidermidis* and *S. aureus* by purified β-glucan was also investigated in this study And the results showed that β-glucan had the degradation effects on biofilm of isolates. The highest degradation percentage for biofilm was 90, 85, 80 and 74% for MDR *P. aeruginosa*$_{(1)}$, *S. epidermidis*$_{(2)}$, *E.coli*$_{(1)}$, *S. aureus*$_{(4)}$ respectively. Whereas, low degradation percentage (41%) was recorded for *S. aureus*$_{(2)}$ (Table 6) . This result agrees with the study by Divya *et al*. [50] who exhibited that β-glucan caused 100% of the disintegration of the biofilm. Vincenzo *et al*. [52] showed that the crude β-glucan has the ability to biofilm degradation at 400 mg/ ml concentration and biofilm inhibition percentage of 59 & 54% for *S. aureus, P. aeruginosa* respectively.

Table 6: Degradation of biofilm by purified β-glucan.

Bacterial Isolates	Degradation of Biofilm %	Bacterial Isolates	Degradation of Biofilm %
P. aeruginosa$_{(1)}$	90	*E. coli*$_{(1)}$	73
P. aeruginosa$_{(2)}$	80	*E. coli*$_{(2)}$	80
P. aeruginosa$_{(3)}$	81	*E. coli*$_{(3)}$	50
P. aeruginosa$_{(4)}$	55	*S. aureus*$_{(1)}$	48
P. aeruginosa$_{(5)}$	46	*S. aureus*$_{(2)}$	65
P. aeruginosa$_{(6)}$	63	*S. aureus*$_{(3)}$	41
P. aeruginosa$_{(7)}$	70	*S. aureus*$_{(4)}$	74
P .aeruginosa$_{(8)}$	61	*S. aureus*$_{(5)}$	66
S. epidermidis$_{(1)}$	66	*S. epidermidis*$_{(2)}$	85

4. Conclusion

It can be concluded that the purified β-glucan from *S. cerevisiae* had an inhibitory effect on growth of wounds infections causative bacteria and decreased biofilm formation and could also degrade biofilm of these isolates.

5. Ethical Clearance

The ethical clearance was obtained from the committee at scientific research by the approval of both environmental and health and higher education and scientific research ministries in Iraq.

6. Conflict Of Interest

The authors declare that they had no conflict of interest.

7. Acknowledgments

My profound thanks to the Department of the Biology/College of Science, Mustansiriyah University and hospitals for providing the necessary facilities during this study

References

[1] A. Synytsya and M. Novák, "Structural diversity of fungal glucans," *Carbohydr. Polym.*, vol. 92, no. 1, pp. 792–809, 2013.

[2] M. A. A. da Cunha, S. L. Albornoz, V. A. Q. Santos, W. N. Sánchez, A. M. Barbosa-Dekker, and R. F. H. Dekker, "Structure and biological functions of d-glucans and their applications," *Stud. Nat. Prod. Chem.*, vol. 53, pp. 309–337, 2017.

[3] E. J. Murphy, E. Rezoagli, I. Major, N. J. Rowan, and J. G. Laffey, "β-glucan metabolic and immunomodulatory properties and potential for clinical application," *J. Fungi*, vol. 6, no. 4, p. 356, 2020.

[4] S. D. V. Medeiros *et al.*, "Effects of purified Saccharomyces cerevisiae (1→ 3)-β-glucan on venous ulcer healing," *Int. J. Mol. Sci.*, vol. 13, no. 7, pp. 8142–8158, 2012.

[5] J. Glik, M. Kawecki, T. Gaździk, and M. Nowak, "The impact of the types of microorganisms isolated from blood and wounds on the results of treatment in burn patients with sepsis," *Polish J. Surg.*, vol. 84, no. 1, pp. 6–16, 2012.

[6] S. M. Huszczynski, J. S. Lam, and C. M. Khursigara, "The role of Pseudomonas aeruginosa lipopolysaccharide in bacterial pathogenesis and physiology," *Pathogens*, vol. 9, no. 1, p. 6, 2019.

[7] J.-Y. Maillard, G. Kampf, and R. Cooper, "Antimicrobial stewardship of antiseptics that are pertinent to wounds: The need for a united approach," *JAC-antimicrobial Resist.*, vol. 3, no. 1, p. dlab027, 2021.

[8] H.-C. Flemming and S. Wuertz, "Bacteria and archaea on Earth and their abundance in biofilms," *Nat. Rev. Microbiol.*, vol. 17, no. 4, pp. 247–260, 2019.

[9] R. C. Costa *et al.*, "Polymicrobial biofilms related to dental implant diseases: unravelling the critical role of extracellular biofilm matrix," *Crit. Rev. Microbiol.*, pp. 1–21, 2022.

[10] Baker, P. Hill, P.J. Snarr, B.D. Alnabelseya, N, Pestrak MJ, Lee MJ, Jennings LK, 4Tam J, Melnyk RA, Parsek MR (2016). Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent Pseudomonas aeruginosa biofilms. Sci Adv.; vol. 2, no.5, e1501632.

[11] J. B. Lyczak, C. L. Cannon, and G. B. Pier, "Lung infections associated with cystic fibrosis," Clin. Microbiol. Rev., vol. 15, no. 2, pp. 194–222, 2002.

[12] C. Ryder, M. Byrd, and D. J. Wozniak, "Role of polysaccharides in Pseudomonas aeruginosa biofilm development," *Curr. Opin. Microbiol.*, vol. 10, no. 6, pp. 644–648, 2007.

[13] P. Bhatt, K. R. Rathi, S. Hazra, A. Sharma, and V. Shete, "Prevalence of multidrug resistant Pseudomonas aeruginosa infection in burn patients at a tertiary care center" *Indian J. Burn.*, vol. 23, no. 1, p. 56, 2015.

[14] H. Rodulfo *et al.*, "Virulence factors and integrons are associated with MDR and XDR phenotypes in nosocomial strains of Pseudomonas aeruginosa in a Venezuelan university hospital," *Rev. Inst. Med. Trop. Sao Paulo*, vol. 61, 2019.

[15] S. A. Gholami, H. R. Goli, M. R. Haghshenas, and B. Mirzaei, "Evaluation of polysaccharide intercellular adhesion (PIA) and glycerol teichoic acid (Gly-TA) arisen antibodies to prevention of biofilm formation in Staphylococcus aureus and Staphylococcus epidermidis strains," *BMC Res. Notes*, vol. 12, no. 1, pp. 1–6, 2019.

[16] M. M. Severn and A. R. Horswill, "Staphylococcus epidermidis and its dual lifestyle in skin health and infection," *Nat. Rev. Microbiol.*, pp. 1–15, 2022.

[17] G. Sharma *et al.*, "Escherichia coli biofilm: development and therapeutic strategies," *J. Appl. Microbiol.*, vol. 121, no. 2, pp. 309–319, 2016.

[18] J. Song, H. Chen, Y. Wei, and J. Liu, "Synthesis of carboxymethylated β-glucan from naked barley bran and its antibacterial activity and mechanism against Staphylococcus aureus," *Carbohydr. Polym.*, vol. 242, p. 116418, 2020.

[19] T. Mathur, S. Singhal, S. Khan, D. J. Upadhyay, T. Fatma, and A. Rattan, "Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods," *Indian J. Med. Microbiol.*, vol. 24, no. 1, pp. 25–29, 2006.

[20] S. K. R. Namasivayam, M. Preethi, A. Bharani, G. Robin, and B. Latha, "Biofilm inhibitory effect of silver nanoparticles coated catheter against Staphylococcus aureus and evaluation of its synergistic effects with antibiotics," *Int J Biol Pharm Res*, vol. 3, no. 2, pp. 259–265, 2012.

[21] C. Chaiyasut *et al.*, "Extraction of β-glucan of Hericium erinaceus, Avena sativa L., and Saccharomyces cerevisiae and in vivo evaluation of their immunomodulatory effects," *Food Sci. Technol.*, vol. 38, pp. 138–146, 2018.

[22] H. S. Karim, M. W. Zainulabidden, And D. A. Sabir, "Comparison of The Responsibility To Extract And Purify B-Glucan Of Some Cereal Grains For Specific Method," *Iraqi J. Agric. Sci.*, Vol. 52, No. 5, Pp. 1238–1247, 2021.

[23] J. A. S. Salman, A. A. Kadhim, and A. J. Haider, "Biosynthesis, characterization and antibacterial effect of ZnO nanoparticles synthesized by Lactobacillus Spp," *J Glob. Pharma Technol*, vol. 10, no. 03, pp. 348–355, 2018.

[24] O. A. U. Ali, "Prevention of Proteus mirabilis biofilm by surfactant solution," *Egypt. Acad. J. Biol. Sci. G. Microbiol.*, vol. 4, no. 1, pp. 1–8, 2012.

[25] N. Jaffar, Y. Ishikawa, K. Mizuno, T. Okinaga, and T. Maeda, "Mature biofilm degradation by potential probiotics: Aggregatibacter actinomycetemcomitans versus Lactobacillus spp.," *PLoS One*, vol. 11, no. 7, p. e0159466, 2016.

[26] S. M. Hayes *et al.*, "Staphylococcus aureus internalization in mast cells in nasal polyps: Characterization of interactions and potential mechanisms," *J. Allergy Clin. Immunol.*, vol. 145, no. 1, pp. 147–159, 2020.

[27] V. Puca *et al.*, "Microbial species isolated from infected wounds and antimicrobial resistance analysis: Data emerging from a three-years retrospective study," *Antibiotics*, vol. 10, no. 10, p. 1162, 2021.

[28] I. M. Tom et al., "Infection of wounds by potential bacterial pathogens and their resistogram," Open Access Libr. J., vol. 6, no. 7, pp. 1–13, 2019.

[29] A. A. Al-Naqshbandi, H. A. Hassan, M. A. Chawsheen, and H. H. Abdul Qader, "Categorization of bacterial pathogens present in infected wounds and their antibiotic resistance profile recovered from patients attending rizgary hospital-erbil," ARO-The Sci. J. Koya Univ., vol. 9, no. 2, pp. 64–70, 2021.

[30] M. Sisay, T. Worku, and D. Edessa, "Microbial epidemiology and antimicrobial resistance patterns of wound infection in Ethiopia: a meta-analysis of laboratory-based cross-sectional studies," *BMC Pharmacol. Toxicol.*, vol. 20, no. 1, pp. 1–19, 2019.

[31] A. K. Jindal, K. Pandya, and I. D. Khan, "Antimicrobial resistance: A public health challenge," *Med. J. armed forces India*, vol. 71, no. 2, pp. 178–181, 2015.

[32] G. Zhu, Z. Sun, P. Hui, W. Chen, and X. Jiang, "Composite film with antibacterial gold nanoparticles and silk fibroin for treating multidrug-resistant E. coli-infected wounds," *ACS Biomater. Sci. Eng.*, vol. 7, no. 5, pp. 1827–1835, 2020.

[33] P. Behzadi, E. Urbán, and M. Gajdács, "Association between biofilm-production and antibiotic resistance in uropathogenic Escherichia coli (UPEC): an in vitro study," *Diseases*, vol. 8, no. 2, p. 17, 2020.

[34] P. Behzadi, E. Urbán, and M. Gajdács, "Association between Biofilm-Production and Antibiotic Resistance in Uropathogenic Escherichia coli (UPEC): An In Vitro Study," *Diseases*, vol. 8, no. 2, p. 17, 2020, doi: 10.3390/diseases8020017.

[35] N. S. Alharbi *et al.*, "Prevalence of Escherichia coli strains resistance to antibiotics in wound infections and raw milk," *Saudi J. Biol. Sci.*, vol. 26, no. 7, pp. 1557–1562, 2019.

[36] S. M. S. Zainulabdeen, K. J. Khalaf, and J. A. S. Salman, "Detection of some virulence factors of Pseudomonas aeruginosa isolated from burns and wounds"2020.

[37] J. Omololu *Staphylococcus aureus* Surface Colonization of Medical Equipment and Environment, Implication in Hospital-Community Epidemiology. *J H M M*., vol. 7, no. 2, 2017.

[38] M. Alabi, M. Bayode, I. Aina, J. Adesanya, and O. Areo, "Prevalence, Sensitivity Profile and Resistance of Gram-Positive Bacteria in Wounds to Conventional Antibiotics," *Prevalence*, vol. 7, no. 2, 2021.

[39] G. Han and R. Ceilley, "Chronic wound healing: a review of current management and treatments," *Adv. Ther*., vol. 34, no. 3, pp. 599–610, 2017.

[40] S. Nadar, T. Khan, S. G. Patching, and A. Omri, "Development of antibiofilm therapeutics strategies to overcome antimicrobial drug resistance," *Microorganisms*, vol. 10, no. 2, p. 303, 2022.

[41] K. Dydak et al., "In vitro efficacy of bacterial cellulose dressings chemisorbed with antiseptics against biofilm formed by pathogens isolated from chronic wounds," *Int. J. Mol. Sci*., vol. 22, no. 8, p. 3996, 2021.

[42] K. Murugan, K. Selvanayaki, and S. Al-Sohaibani, "Urinary catheter indwelling clinical pathogen biofilm formation, exopolysaccharide characterization and their growth influencing parameters," *Saudi J. Biol. Sci*., vol. 23, no. 1, pp. 150–159, 2016.

[43] C. Takahashi, M. Sato, and C. Sato, "Biofilm formation of staphylococcus epidermidis imaged using atmospheric scanning electron microscopy," *Anal. Bioanal. Chem*., vol. 413, no. 30, pp. 7549–7558, 2021.

[44] A.A.W. Al-Rabie A. Production and extraction of glucans from Saccharomyces cerevisiae and study of its effect on stimulating macrophages ex vivo. (2009)

[45] Y.-J. Wang, L. Yang, and T. Sontag-Strohm, "Co-migration of phytate with cereal β-glucan and its role in starch hydrolysis in-vitro," *J. Cereal Sci*., vol. 93, p. 102933, 2020.

[46] S. A. Allaith et al., "Screening and Molecular Identification of Lactic Acid Bacteria Producing β-Glucan in Boza and Cider," *Fermentation*, vol. 8, no. 8, p. 350, 2022.

[47] E. Mahmoud Amer et al., "Enhancement of β-glucan biological activity using a modified acid-base extraction method from Saccharomyces cerevisiae," *Molecules*, vol. 26, no. 8, p. 2113, 2021.

[48] M. Divya *et al.*, "Phenoloxidase activation, antimicrobial, and antibiofilm properties of β-glucan binding protein from Scylla serrata crab hemolymph," *Int. J. Biol. Macromol.*, vol. 114, pp. 864–873, 2018.

[49] K. Saravana Kumar, E. Jeevithan, X. Hu, R. Chelliah, D.-H. Oh, and M.-H. Wang, "Enhanced anti-lung carcinoma and anti-biofilm activity of fungal molecules mediated biogenic zinc oxide nanoparticles conjugated with β-D-glucan from barley," *J. Photochem. Photobiol. B Biol.*, vol. 203, p. 111728, 2020.

[50] A. Sivignon, S.-Y. Yu, N. Ballet, P. Vandekerckove, N. Barnich, and Y. Guerardel, "Heteropolysaccharides from S. cerevisiae show anti-adhesive properties against E. coli associated with Crohn's disease," *Carbohydr. Polym.*, vol. 271, p. 118415, 2021.

[51] M. Divya *et al.*, "Isolation of β-glucan from Eleusine coracana and its antibiofilm, antidiabetic, antioxidant, and biocompatible activities," *Microb. Pathog.*, vol. 140, p. 103955, 2020.

[52] A. Iswarya, M. Anjugam, and B. Vaseeharan, "Role of purified β-1, 3 glucan binding protein (β-GBP) from Paratelphusa hydrodromus and their anti-inflammatory, antioxidant and antibiofilm properties," *Fish Shellfish Immunol.*, vol. 68, pp. 54–64, 2017.