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BIOLOGICAL ACTIVITY OF PURIFIED MANNOPROTEIN FROM BAKER'S YEAST AGAINST PSEUDOMONAS AERUGINOSA

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ABSTRACT

This study aimed to evaluate the biological activity of mannoprotein purified from baker's yeast Saccharomyces cerevisiae available in local markets. The mannoprotein was extracted, purified and the emulsifying activity was detected at each step. The emulsifying activity of crude mannoprotein was 67.85%. The crude mannoprotein was partially purified by precipitation using cold ethanol and the emulsifying efficiency reached to 75%. Then it was completely purified using gel filtration chromatography with Sephadex G-100 gel, the purified mannoprotein had 37.61 mg/ml protein and 32.93 mg/ml carbohydrate with emulsifying activity 87.55%. Mannoprotein was characterized using FTIR and amino acid analysis which showed that the mannoprotein was glycoprotein. The antibacterial and antibiofilm activity of mannoprotein against Pseudomonas aeruginosa isolates from different clinical sources was tested and show very high effect in the reduction of bacterial growth with rate reached to 98.4%. Also, the mannoprotein inhibited the biofilm formation for bacterial isolates at different rates, the highest rate was 60.09%, while the rates of biofilm degradation were lower and reached to 21.12%. FE-SEM examination showed a reduction in biofilm formation.

Key words: **amino acids, antibiofilm activity, FE-SEM, FTIR, mannoproteins.**

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INTRODUCTION

Saccharomyces cerevisiae, the model yeast, is a popular subject to both fundamental and applied research, such as biosensor development and industrial production of pharmaceutical compounds (Drozdova *et al*., 2024). It is distinguished by its small, oval to round, multilateral budding yeast cells. More precisely, it belongs to the kingdom of fungi (Rasheed and Haydar, 2023). *S. cerevisiae* is regarded as a generally recognized as safe (GRAS) organism because it produces recombinant proteins and development processes that are immediately used without additional thought (Le *et al*., 2024). As *S. cerevisiae* is a food-grade yeast that is extensively utilized in the food industry, so as a result, the emulsifier is believed to be non-toxic (Elsaygh *et al*., 2023). The cell wall is extremely thin-less than 100 nm-and is made up of a dense network of β-1,3- and β-1,6-glucans, mannoproteins and chitin (Bakir *et al*., 2024). Mannoproteins constitute non-filamentous glycoproteins found in the outermost layer of the yeast cell wall (Ghanegolmohammadi *et al*., 2021). This family of glycoproteins is the second most prevalent part of the cell wall in *S. cerevisiae*, behind *β*-glucan (Snyman *et al*., 2023). Mannoproteins make up 30-50% of the dry mass of the *S. cerevisiae* cell wall, which accounts for 20-25% of the total dry weight of the yeast cell (Assunção Bicca *et al*., 2022). Mannoproteins linked to polysaccharides comprise the cell wall, which is constantly changing as a result of cell division, mating,

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gametogenesis or stress adaptation (Novačić *et al*., 2020). Significant structural differences have also been observed between mannoproteins with respect to their mannose/glucose ratios, the presence of other monosaccharides such galactose, the percentage of carbohydrates to protein and degree of glycan branching, molecular weight, and charge distribution (Snyman *et al*., 2023). Yeasts, fungi, and bacteria frequently generate surface-active chemicals. The biological surfactants are eco-friendly since they are safe, biodegradable, have high specificity, good surface activity, and work well in stressed situations. Yeast biomass and cell wall products boost animal nutrition by binding enteropathogenic bacteria while also benefiting lactobacilli and bifidobacteria (Bzducha Wróbel *et al*., 2022). *Pseudomonas aeruginosa* is one of the most prevalent pathogens, rod-shaped, gram-negative, and able to grow both aerobically and anaerobically; it can adapt to a variety of environmental circumstances and is found in soil, water, and moist environments. Antibiotic resistance and the presence of several pathogenicity factors during infections are linked to *P. aeruginosa*'s capacity to produce serious infections. Numerous virulence factors, including flagella, pili, proteases, elastase, exopolysaccharides, iron chelators, lipases, and a range of toxins, including exotoxin A and the Type III Secretion System toxins, in addition to chemical compounds and pyocyanin, make these infections extremely difficult to eradicate. Furthermore, *P. aeruginosa*'s outer membrane contains lectins and lipopolysaccharide, a complex glycolipid that contributes to the pathogen's pathogenicity (Ibrahim, 2022). Additionally, the most common bacterial pathogen among the microorganisms that cause respiratory tract infections is *P. aeruginosa*. Diseases brought on by microorganisms that are resistant to multiple drugs (MDRs) are a significant issue facing people today. The goal of studies on this topic is to identify potential solutions and extracts have been employed in several disciplines. The World Health Organization promotes studies on disease prevention and treatment with non-antibiotic medicines. Where the health and safety of both humans and animals puts at risk because of the emergence and spread of antibiotic resistance in harmful microorganisms (Bzducha Wróbel *et al*., 2022). The development of bioemulsifiers with high stability and emulsifying qualities that are acceptable to the environment has garnered more attention on several occasions. In sectors like food, medicine, and the bioremediation of oil-contaminated waste, these bioemulsifiers could take the place of synthetic surfactants, promoting sustainable growth (Elsaygh *et al*., 2023). The present study aimed to evaluate the biological activity of mannoprotein purified from baker's yeast *Saccharomyces cerevisiae* available in local markets.

MATERIALS AND METHODS

Microorganisms :*Saccharomyces cerevisiae*:

Baker's yeast *Saccharomyces cerevisiae* Angel type of Russian origin was obtained from the local market and confirmed by VITEK.

Pseudomonas aeruginosa

It included 5 isolates from different clinical sources that were multi-drug resistance and form biofilm and diagnosed by cultural, microscopic, biochemical and VITEK, and used in antibacterial and antibiofilm experiments (Salman *et al*., 2024).

Extraction of mannoproteins

Baker`s yeast was activated by culture in Sabouraud dextrose broth and incubated at 30°C for 24 h. YPG medium was then inoculated with yeast isolate at 2% and incubated in a shaking incubator at 200 rpm for 48 h at 30°C. The obtained cells were separated by centrifugation at 10000 rpm for 20 min at 4C. The filtrate was discarded and the cells that settled at the bottom were kept. The suspended cells with phosphate buffer saline were then boiled in a water bath at 100°C for 5 min. Then they were left to cool and centrifuged at 3000 rpm for 10 min. Then the filtrate represents crude mannoprotein (Alcantara *et al*., 2010).

Emulsification activity (E24%)

The emulsifying activity of mannoprotein was investigated based on what was stated in Dhivya *et al*. (2014) by mixing 2 ml of engine oil with 2 ml of mannoprotein in test tubes and mixing it well using a vortex for 5 minutes and

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leaving it for 24 hours without stirring. Then the emulsifying activity was determined using the following equation:

Emulsion Index (E24) % = Height of emulsion formed / Total height of solution x 100

The emulsification index (E24) is used to characterize the biosurfactant/bioemusifier in emulsifying the hydrophobic phase in the hydrophilic phase (Kadhum and Haydar, 2020).

Partial Purification of mannoprotein by ethanol precipitation: Partial purification was carried out by precipitation with three volume of cold ethanol. Then the mixture was centrifuged at 4000 rpm for 30 min. Then, the filtrate was discarded and the precipitate was taken.

Protein and carbohydrate concentration measurement: Protein and carbohydrate content of mannoprotein were determined. Protein was estimated according to the Bradford method (Bradford, 1976). While Carbohydrates were estimated according to the method described by Dubois *et al*. (1956).

Purification of mannoprotein using gel filtration chromatography with Sephadex G-100: The Sephadex gel packed in a column of dimensions (1.5 * 60) cm, then washed the column by passing 0.1 M phosphate buffer with pH 7.2 in an amount twice the volume of the column, then the flow rate was adjusted to be 3 ml / 10 min, then 2 ml of the partially purified extract was passed on the inner sides of the column near the surface of the gel, then the recovery process was carried out with the same buffer solution and the separated fractions were collected from the column using test tubes in an amount of 3 ml / 10 min for each. The protein concentration in the separated fractions was determined by reading the absorbance at a wavelength of 280 nm and carbohydrate concentration at 490 nm by using phenol sulphuric acid.

Characterization of mannoproteins:

Fourier Transform Infrared (FT-IR) Spectrometry: In order to determine the functional groups and distinctive features of mannoprotein, an ALPHA II Fourier transform infrared (FT-IR) spectrometer was utilized. The FT-IR analysis was carried out in the 400–4000 cm^{-1} wavelength range. The result obtained in graphic chart, where the X-axis represented the wave number while the Y-axis represents the percentage of transmittance.

Amino Acid Analysis

Mannoprotein's amino acid content was determined using a AAA-OPA automatic amino acid analyzer (Clarity), with separation using ion exchange chromatography. The methodology was based on the procedure suggested by Restek.

Determination of antibacterial activity of mannoprotein: The inhibitory effect of mannoprotein against *P. aeruginosa* was evaluated using Co-culture technique where bacterial isolates were grown in nutrient broth with mannoprotein (1:1) as treatment medium and in control medium which contained nutrient broth only. For 24 h., the co-culture and control were incubated at 37°C. After the incubation period, a series of dilutions were carried out for the cultures, then transferred and spread on nutrient agar plates and the plates were incubated for 24 h at 37°C (Salman, 2013). The following formula, as stated by Gosh *et al*. (2010) was employed to calculate the bacterial reduction percentage:

$R(\%)=[A-B]/A \times 100$

A represents the number of bacterial colonies from the control medium, B represents the number of bacterial colonies from the mannoprotein treatment and R is the reduction rate.

Inhibition of biofilm: Inhibition of biofilm formation by *P. aeruginosa* by using mannoprotein was examined in a microtiter plate using the method described by Salman and Kareem (2021). Every isolate was cultured for 24 hours at 37 °C on 96 flat-bottom well microtiter plates. The treatment wells contained 80 µl of Brain Heart Infusion broth (BHI) with 2% sucrose and were mixed with 100 µl of Mannoprotein, while the control wells contained only 180 µl of BHI with 2% sucrose and all were inoculated with 20 µl of bacterial suspension compared with 0.5 MacFarland. Following incubation at 37 °C for 24 h., the medium was taken out of the wells, rinsed three times with PBS to get rid of any unattached bacterial cells, and allowed to dry at room temperature for 15 minutes. Next, 200 µl of 0.1% crystal violet was added to the

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wells, and they were left for 20 minutes. To get rid of the unbound dye, the stained wells were rinsed three times with PBS (PH 7.2) and allowed to dry for 15 minutes at room temperature. Ultimately, 200 µl of 95% ethanol were added to each well, and an ELIZA reader was used to measure the optical density at 630 nm. The following equation was used to determine the inhibition percentage of biofilm formation.

Inhibition of biofilm formation % = O.D control – O.D treatment / O.D control x 100

Examination the antibiofilm of purified mannoprotein by Field Emission Scanning Electron Microscopy (FE-SEM): Purified mannoprotein was tested for antibiofilm activity using Field Emission Scanning Electron Microscopy (FE-SEM). Bacterial biofilm of *P. aeruginosa* was grown on cover slips with and without purified mannoprotein. Cover slips without mannoprotein as controls. Then the two slides incubated at 37°C for 24 h. (Chopra *et al*., 2015). After incubation harvested, and rinsed three times with PBS before being fixed with 2.5% glutaraldehyde. Finally, the samples had been examined by FE-SEM (Wang *et al*., 2022).

Determination of biofilm degradation activity of mannoprotein: The degradation effect of purified mannoprotein from *S. cerevisiae* on the biofilm of *P. aeruginosa* that have formed completely after 72 hours was evaluated using flat micrototer plate using brain heart infusion broth medium supplemented with 2% sucrose depending on the procedure mentioned by Jaffar *et al*. (2016), after comparison with 0.5 MacFarland the plate was inoculated with the bacterial suspension of *P. aeruginosa*. The final volume of the liquid in each well was 200 µl where each well contained 180 µl of medium and was inoculated with 20 µl of the bacterial suspension. After incubation for 72 h at 37 °C, the wells were emptied and 200 µl of mannoprotein was added and considered as treatment wells and 200 µl of brain heart infusion broth medium supplemented with 2% sucrose was added and considered as control wells and the plate was incubated for 24 h at 37 °C. After the incubation period was over, the wells were emptied and washed three times with BPS and then left to dry for 15 min at room temperature. Then the wells were filled with crystal violet (0.1%) and left for 20 min. The stained wells were washed three times with PBS to remove the unbound dye and left for 15 min to dry at room temperature. Finally, 200 µl of 95% ethanol was added to each well and the optical density was determined by Eliza reader at a wavelength of 630 nm. The percentage of biofilm degradation was calculated according to the equation below:

Degradation biofilm % = O.D control – O.D treatment / O.D control x 100

RESULTS AND DISCUSSION

Extraction of mannoproteins: S. cerevisiae strains vary in the amount of mannoprotein they produce depending on their quality (Gonzalez-Ramos and Gonzalez, 2006). A study conducted by Perez-Traves *et al*. (2015) showed that the reason for the difference in the production of this bioemulsion is due to the difference in gene expression, which affects the structural composition of the cell wall. Giovani *et al*. (2010) concluded that the difference in mannoprotein production may be attributed to differences in agricultural conditions such as temperature and sugar concentration. According to *S. cerevisiae* growth kinetics, the bioemulsifier's synthesis was growth-associated and maximum during the stationary phase (Elsaygh *et al*., 2023). In addition to that the source of nitrogen used also effect on the production rate for example yeast extract with peptone show high efficient rate in the production of dextranase as state by Abbood and Awda (2020).

Purification of mannoprotein

Mannoprotein was precipitated with 99% ethanol and purified by fractionation based on molecular weight using Sephadex G-100. The most common organic solvents chilled ethanol used in the purification of mannoprotein and showed high emulsifying efficiency E24 which is 75% as shown in Figure (1). It can be noted that the emulsifying efficiency of the partially purified extract with cold ethanol increased compared to the emulsifying efficiency of the crude extract, which was 67.85%. In contrast, Virgie *et al*. (2010) used acetone for extraction of mannnoprotein and obtain high emulsification index, this may because the

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differences in composition between extracts, as state by Bzducha Wróbel *et al*. (2022) that the biological characteristics of the compounds under discussion are determined by the significant variation in the chemical structure and composition of the components of the yeast cell wall among strains, species, and growth circumstances. The reason may also be due to the solvent used, which may vary depending on the manufacturer.

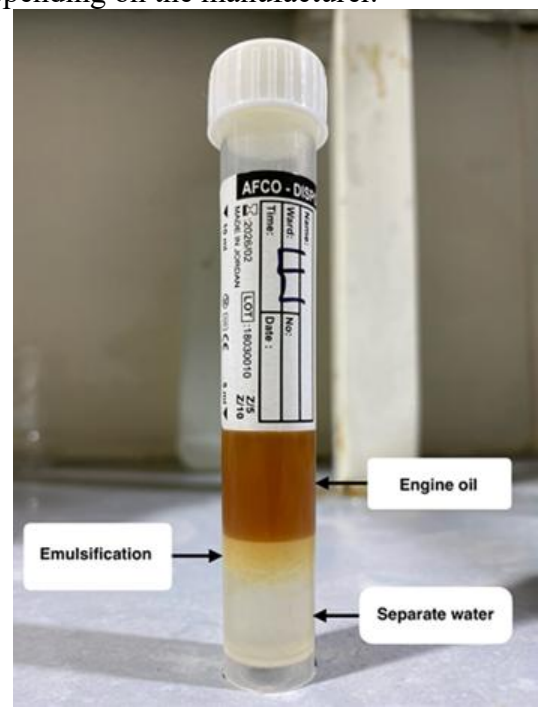

Figure 1. Emulsifying activity of partially purified mannoprotein by ethanol

Purification of mannoproteins according to the principle of precipitation with organic solvents is one of the most common methods, as it was used by Alcantara *et al*. (2010), and Dhivya *et al*. (2014) in the partial purification of mannoproteins extracted from baker's yeast *S. cerevisiae*. Organic solvents are a diverse group of chemicals and precipitation with organic solvents is a common method of protein concentration and its primary goal is to reduce the amount of water from the crude extract (Bonner, 2019). When a solvent like ethanol or acetone is added to an aqueous extract of proteins, a number of events occur that collectively cause the extract to precipitate. The main effect is a decrease in the water activity, which is the result of an average decrease in the dielectric constant, thus leading to a decrease in the water-solubilizing power of the charged aqueous protein molecule, which in turn reduces the solubility of the protein and causes precipitation (Hutti-Kaul, and Mattiasson, 2003). Mannoprotein was also purified using gel filtration chromatography with Sephadex G-100 gel and the separated fractions were read at 280 nm for protein determination and at 490 nm for carbohydrate determination. Two separate peaks of protein and one peak of carbohydrate were obtained. The separated fractions were combined to increase their concentration. The protein concentration was 37.61 mg/ml while the carbohydrate concentration was 32.93 mg/ml and the emulsifying activity was 87.55%, as shown in Table (1) and the relationship between the separated fractions and the absorbance concentration was plotted as in Figure (2). The results consistent with what was reached by Saleh (2021), as he obtained two peaks of protein and one peak of carbohydrates, with an emulsifying efficiency of 86.4%. Therefore, after purifying the mannoprotein by gel filtiration, a high emulsifying efficiency emerged, which reflects its elimination of impurities.

Table 1. Concentration of protein and carbohydrate of mannoprotein purification steps and emulsion index E24%

Sample	Volume (ml)	Protein concentration (mg/ml)	Carbohydrate concentration (mg/ml)	Emulsion index (E24) %
Crude extract	500	44.97	127.78	67.85 %
Ethanol precipitated	29	118.7	97.8	75 %
Mannoprotein purified by gel filtration chromatography	18	37.61	32.93	87.55 %

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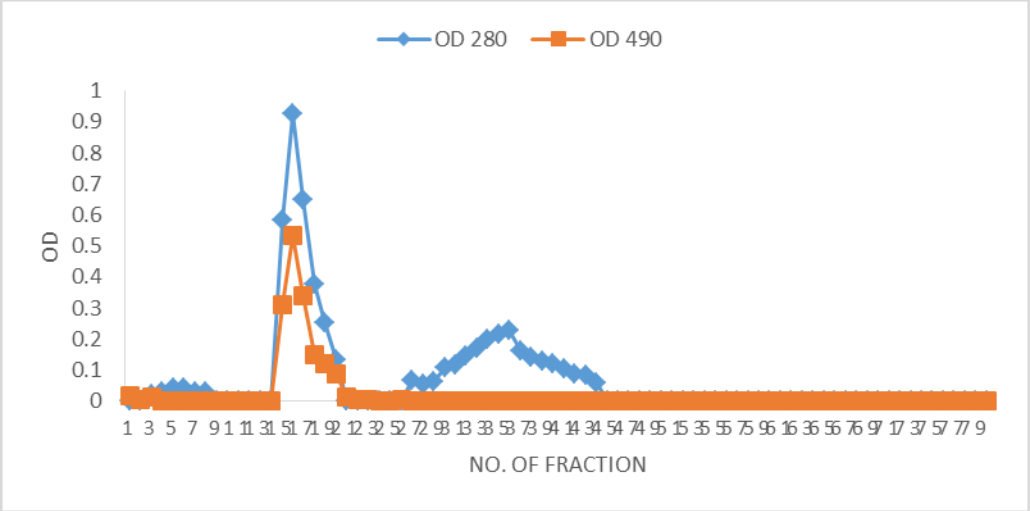

Figure 2. Gel filtration chromatography for purification of mannoprotein from *S. cerevisiae* using Sephadex G-100 with dimensions (1.5 * 60) cm, balanced with phosphate buffer at a concentration of 0.1 M, pH 7.2, and a flow rate of 3 ml/10 min

The inhibitory impact of the partially and fully purified mannoprotein fraction obtained from *S. cerevisiae* on specific bacterial strains was assessed by Saleh (2021) where he observes that the preparation's inhibitory efficacy against eleven of the bacterial isolates under study was enhanced following the mannoprotein purification procedure using gel filtration.

Characterization of mannoprotein

Fourier Transform Infrared (FTIR) Spectrometry: The functional groups in mannoprrptein were detected using FTIR. The frequency range and functional groups of purified mannoprotein showed that the stretching vibrations of OH and CH were responsible for the absorption peaks at 3439.76 cm^{-1} and 2959 cm^{-1}, whereas the typical acylamino absorption peaks were found in the band at 1638.82 cm^{-1} which indicate the presence of amino acids. Where the peak 1384.80 cm^{-1} refer to tyrosine, 1271.40 cm^{-1} refer to cystine and 1086.98 cm^{-1} refer to therionine. As De Meutter and Goormaghtigh (2021) pointed out, the range 1673, 1633 cm^{-1} indicates the presence of amino acids. Simultaneously, the distinctive absorption peaks of the pyranose ring were also detected using the CH deformation vibrations (1466 cm^{-1}) and the distinctive absorption peaks of the pyranose ring of saccharide was observed on the C=O vibration (1086.98 cm^{-1}). While 990.43 cm^{-1} which is finger print region of carbohydrate showed that mannoprotein was glycoprotein. Figure (3) showed the FTIR spectra for mannoprrptein purified from *S. cerevisiae* that present the frequency range and functional groups.

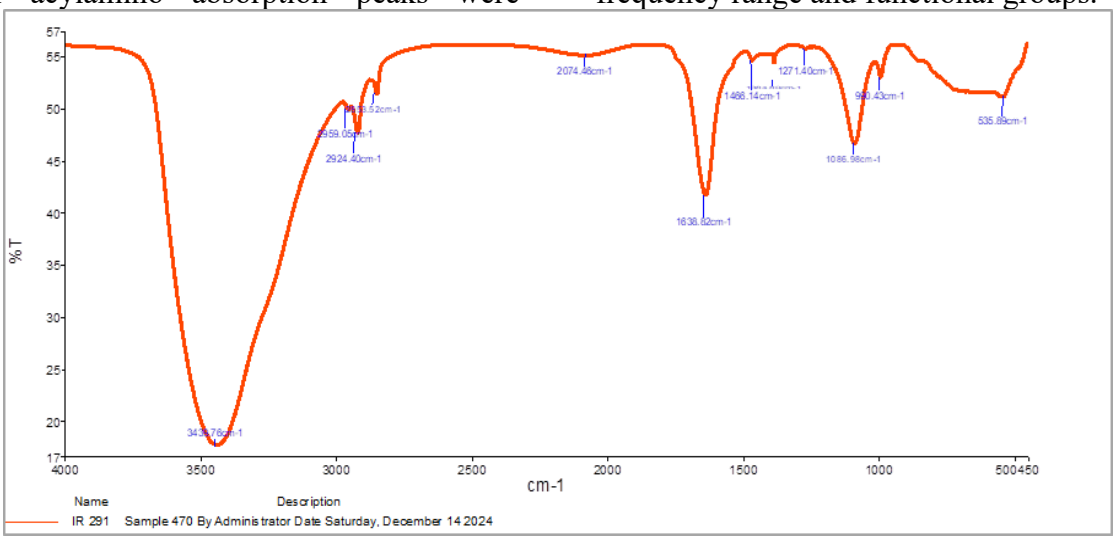

Figure 3. FTIR spectra for mannoprrptein purified from *Saccharomyces cerevisiae*

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This result come consistent with Rasheed and Haydar (2023) that found comparable results in their research. While Bzducha Wróbel *et al*. (2022) found somewhat similar results in their research. Side chains of amino acids are essential for both stabilizing protein structures and catalysing enzymatic reactions. Infrared spectroscopy is being used more and more to study these areas at the molecular level. When studying the mechanism of protein reactions, amino acid side chain absorption offers extremely useful information. This is due to the fact that side chains frequently form the core of the mechanism underlying chemical reactions. The fate of the several individual groups participating in the reaction can be tracked in a single experiment using infrared spectroscopy. Infrared spectroscopy has the benefit of allowing for the simultaneous observation of the protein's side chains and backbone. This allows for a comparison between the kinetics of backbone structural changes and amino acid side chain signals (Barth, 2000). The efficiency of bioemulsifiers is determined by their chemical composition and the quantity of reactive groups exposed in the structure (Elsaygh *et al*., 2023). Functional groups are significant since they predict the compound's future chemical reactions (Su *et al*., 2021). With absorption peaks that match the frequency of vibrations between the bonds of the atoms that comprise the material, the technique generates an infrared spectrum that functions as a distinct fingerprint of a sample. No two compounds create the same infrared spectrum since every material has a unique atom combination (Karaca *et al*., 2022). The hydroxyl group is a distinguishing feature of carbohydrates (Magengelele *et al*., 2023). The polysaccharide has a high amount of hydroxyl groups due to hydrogen bonds created between them, as shown in normal O-H stretching patterns (Karaca *et al*., 2022).

Amino acid analysis

Since the composition analysis showed that mannoprotein was a glycoprotein, this study used amino acid analysis. The amino acid composition analysis of the protein component of Mannoprotein show that it contains 16 kinds which is Aspartic acid, Glutamic acid, Serine, Histidine, Glysine, Therionine, Arginine, Alanine, Tyrosine, Cystine, Valine, Methionine, Phenylalanine, Isoleucine, Leucine and Lysine. This was consistent somewhat with the findings of Liu *et al*. (2014). The highest weight of the amino acid was 985,995 mg, represented by the amino acid tyrosine, and the lowest weight of the acid was 71,844 mg, represented by the amino acid aspartic acid as showen in Figure (4).

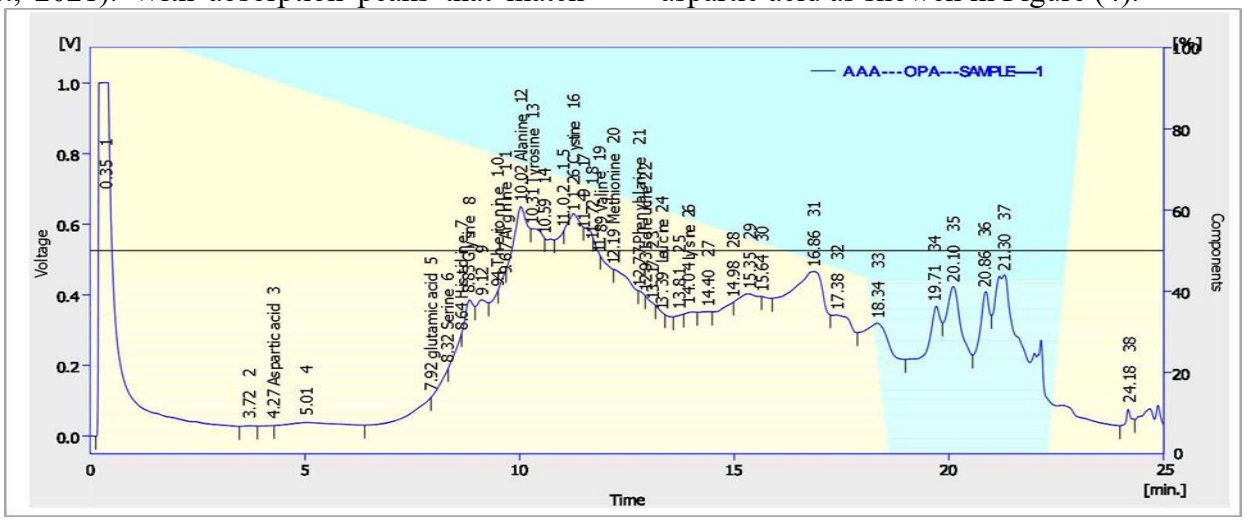

Figure 4. Composition of amino acid in the mannoprotein

Therefore, mannoprotein contains amino acids, which gives it the effectiveness in inhibiting the biofilm. As state by Idrees *et al.* (2020), amino acids' function as anti-biofilm agents is one of its significant uses. Amino acids are efficient molecules with a wide range of applications, including anti-biofilm agents, drug excipients, drug solubility enhancers, and drug adjuvants (Khaleel and Haydar. 2024). Amino acids are fundamental components or building elements of proteins and significant biomolecules. Their structures contain a side

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chain, one carboxyl group, and at least one amino group. Protein polymers are created when a number of amino acids are united by amide bonding, sometimes referred to as peptide bonding, in such a way that the carboxyl group of one amino acid joins to the amino group of another amino acid while losing a water molecule (per each peptide bond) (Idrees *et al.*, 2020). Amino acids are categorized as hydrophilic (serine, threonine, and tyrosine), hydrophobic (alanine, glycine, leucine, phenylalanine, proline, and valine), basic (arginine, histidine, and lysine), and acidic (glutamic and aspartic acids) (Assunção Bicca *et al.*, 2022). Their structure is composed of a hydrophilic moiety that includes peptides or amino acids for cations or anions; di-, mono-, or polysaccharides; and a hydrophobic moiety that includes saturated and unsaturated fatty acids (Rasheed and Haydar, 2023).

Antibacterial activity of mannoprotein

The inhibitory activity of the purified mannoprotein was tested against five isolates of *P. aeruginosa* which is characterized by being multi-resistant to antibiotics and by its ability to form a biofilm to a high degree. The findings showed that mannoprotein had antibacterial properties. The highest growth inhibition rate was 98.40%, the lowest growth inhibition rate was 27.27%, while the growth inhibition rates of the remaining isolates ranged between 72.88%, 92.92% and 97.23% as shown in figure (5).

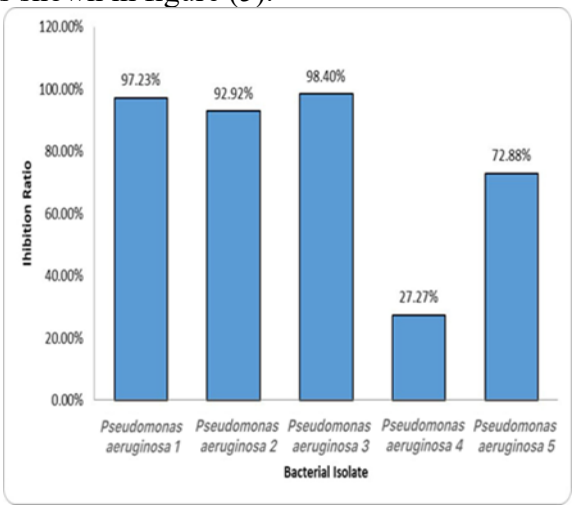

Figure 5. Antibacterial activity of purified mannoprotein against MDR *Pseudomonas aeruginosa* isolates

Kadhem *et al.* (2019) studied the inhibitory activity of the bio-emulsion mannoprotein against bacteria and concluded that it has an inhibitory effect against many bacterial isolates, including *P. aeruginosa*, as the strongest inhibitory activity was against these bacteria. Biosurfactants disturb protein conformation, which ultimately alters vital membrane activities, and interact with cytoplasmic membranes, causing metabolite leakage and cell lysis (Rasheed and Haydar, 2023). It has been suggested that yeast mannoproteins are paraprobiotics with prebiotic and antibacterial qualities. They can be utilized in food as biopreservatives and in the treatment of disease. The antibacterial activity varied depending on the type of preparation and its dose, as well as the pathogenic bacteria exposed to it. Supplementing with probiotics and prebiotics, such as yeast mannoptoteins, mannan-oligosaccharides, and β-(1,3/1,6)-glucans, can improve gut microbiota composition and improve human and animal health, including prevention of pathogenic infections. Probiotic yeasts have been shown to trap pathogenic bacteria that express type I fimbriae on their surface using yeast cell-wall mannose-containing glycoprotein residues, preventing pathogens from infecting the host (Bzducha Wróbel *et al.*, 2022).

Antibiofilm Effect of Mannoprotein

When applied to biofilm-forming bacterial isolates, mannoprotein showed a clear effect in inhibiting biofilm formation, with the highest inhibition rate being 60.09%, as shown in table (2). The concentration and the microorganism under test determine the antiadhesive action (Giovani *et al.*, 2010). Bioemulsifiers have the ability to prevent the adhesion of pathogens to solid surfaces or infection sites, which makes them useful in the treatment of many diseases due to their role as therapeutic agents or bio-enhancers (Barth, 2000). Probiotics, natural medications, and other natural products (such as those derived from yeast cell walls) can be utilized as alternatives to antibiotics in the treatment of infectious diseases. It is well known that biofilms cause various pathogenic bacteria, including *P. aeruginosa*, to become more harmful and resistant to antibiotics

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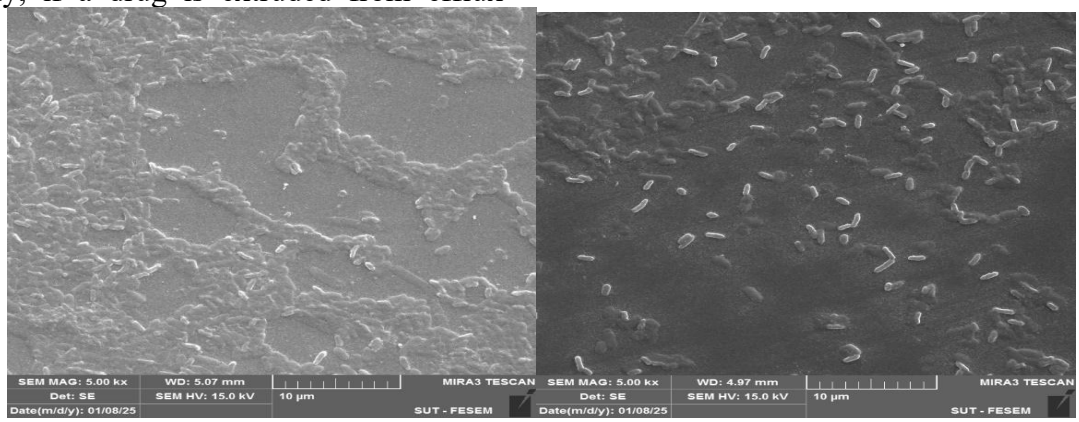
(Saleh *et al.*, 2020). Mannoproteins are readily available biomaterials that are separated from yeast biomass. They can be made by using a variety of inexpensive agricultural byproducts, such as lignocellulotic biomass (Bzducha Wróbel *et al.*, 2022).

Table 2. Antibiofilm effect of Mannoprotein against MDR *Pseudomonas aeruginosa* isolates

Bacterial isolate	OD control 630 nm	OD treatment 630 nm	Percentage of inhibition of biofilm formation %
Pseudomonas aeruginosa 1	**0.772**	**0.394**	**48.89%**
Pseudomonas aeruginosa 2	**0.583**	**0.423**	**27.35%**
Pseudomonas aeruginosa 3	**0.433**	**0.384**	**11.41%**
Pseudomonas aeruginosa 4	**0.600**	**0.457**	**23.83%**
Pseudomonas aeruginosa 5	**1.101**	**0.439**	**60.09%**

Reduced planktonic cell viability is probably one factor contributing to the decrease in biofilm and the rate of cell attachment following mannoprotein exposure that contain several amino acids. It is possible that the underlying mechanism involves increased drug absorption or reduced efflux. Adjuvants are often intended to inhibit the mechanism of drug resistance. For example, if an enzyme inactivates a medicine, the adjuvant can boost the drug's activity by blocking the enzyme. Similarly, if a drug is extruded from efflux pumps, the adjuvant inhibits the efflux. So the two basic ways used to increase medication accumulation in resistant bacteria are these (Khaleel and Haydar. 2024).

Antibiofilm of purified mannoprotein by Field Emission Scanning Electron Microscopy (FE-SEM): Figure (6) shows that the mannoprotein had good antibiofilm effect, where the biofilm formed by *P. aeruginosa* which treated with purified mannoprotein reduced and cells dispersed.



A B

Figure 6. The antibiofilm effect of purified mannoprotein from *Saccharomyces cerevisaie*. A- Control, B- Treatment with mannoprotein

This ability of mannoprotein to destroy the biofilm formed by the multidrug-resistant bacteria *Pseudomonas aeruginosa* may be due to its containing a large group of amino acids. Amino acids are gaining popularity as biofilm disruptors, with potential applications in treating cystic fibrosis and medical device infections. Also they can be used to combat biofilms, which cause spoiling and raise infection risk in the food sector and environment. Amino acids can also be used as antibiofilm agents, to increase the solubility of already-approved medications, and to combine with antimicrobial medications to increase their effectiveness through synergism or additive effects. Where amino acids destroy biofilms, allowing antibiotics to penetrate deeper levels and reduce the concentration needed to treat biofilm related infections (Idrees *et al.*, 2020).

Biofilm Degradation activity of mannoproteins: This study showed that mannoprotein biosurfactant was penetrate the biofilm and kill microorganisms with effective-ness reached to 21.12% as showed in the table (3). The concentration of mannoprotein used is 37.61 mg/ml and with two repetition.

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Table 3. Degradation results of biofilm by mannoprotein from *Saccharomyces cerevisiae* against MDR *Pseudomonas aeruginosa* isolates from different source

Bacterial isolate	OD control 630 nm	OD treatment 630 nm	Percentage of biofilm degradation %
Pseudomonas aeruginosa 1	0.815	0.708	13.13%
Pseudomonas aeruginosa 2	0.604	0.476	21.12%
Pseudomonas aeruginosa 3	0.481	0.507	-
Pseudomonas aeruginosa 4	0.512	0.421	17.77%
Pseudomonas aeruginosa 5	0.286	1.118	-

***- = No effect.**

Communities of sessile bacteria known as biofilms live in a self-produced matrix or matrix of extracellular polymeric substances, that helps the microorganisms survive (Warraich *et al.*, 2020). Yeast mannoproteins have surfactant action and can prevent and reduce bacterial surface adherence and biofilm formation (Bzducha Wróbel *et al.*, 2022). Mnif and Ghribi (2015) explained that the bioemulsion is well known for its membrane permeability property, forming pores in cell membranes which in turn causes the flow of ions across the membrane, including sodium +Na and potassium +K, leading to membrane disruption and cell death. In addition, it is able to interfere with the formation of bacterial biofilms and inhibit their formation (Mujumdar *et al.*, 2014). As a study mentioned that, aspartic acid and glutamic acid are known to enhance the solubility of Cip (Warraich *et al.*, 2020). So, the ability of mannoprotein to break down the biofilm may be attributed to its high content of amino acids that enhance its solubility and thus its penetration and breakdown of the biofilm. Furthermore, as previously mentioned, amino acids have been found to improve medication penetration into cells. Another suggested mechanism is the ability of amino acids to function as chelating agents (Khaleel and Haydar. 2024). A study conducted by Warraich *et al.* (2021) demonstrated that acidic amino acids function as adjuvants that can improve antimicrobial activity. Walencka *et al.* (2007) found no direct antibiotic activity of *S. cerevisiae* mannoprotein preparations against *Staphylococcus aureus* and *Staphylococcus epidermidis* cells. However, mannoproteins were effective in reducing staphylococci initial deposition, reducing biofilm formation, and accelerating mature staphylococcal biofilm detachment. The quoted authors concluded that mannoproteins' anti-biofilm action was related to their effect on cell surface hydrophobicity (Walencka *et al.*, 2007). On the other hand, beneficial bacteria use α-Mannan to multiply in the gut, which is then fermented into short-chain fatty acids. This provides energy for intestinal cells and maintains intestinal building and function. Additionally, yeast mannoproteins improved the adhesion of lactic acid bacteria to Caco-2 cells and positively affected their survival in simulated gastrointestinal juice. Mannoproteins' sensitivity to digestion by bacterial cell-secreted enzymes and their interactions with cell receptors may be influenced by the particular structure and chemical makeup of the preparations themselves. It was claimed that aggregation of lactic acid bacteria (LAB) with yeasts in stomach or intestinal fluids could increase LAB tolerance in the intensive track and improve adhesion specificity to Caco-2 cells (Bzducha Wróbel *et al.*, 2022). To summarize, the findings reveal that mannoproteins found naturally in the cell wall of *S. cerevisiae* have prebiotic qualities that may help the production and enrichment of fermented foods, as well as potentially modulating intestinal microbiota. At the same time, they demonstrate antibacterial action, which limits the proliferation of unwanted microorganisms. On the other hand, when Khaleel and Haydar, (2024) study the synergistic effect of lipopeptide with bacteriocin, according to the results, the pathogens became more sensitive and the range of inhibitory zones expanded when lipopeptide and bacteriocin were combined. Bacteriocin's different modes of action on microorganisms may be attributed to its effect on cell membrane permeability or integrity, which was disrupted and caused leakage of intracellular components including Na^+K^+-ATP, AKP, nucleic acids, and proteins,

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or to its bactericidal and cytoxicity effect, which led to cell death depending on the type of microorganism (Jebur and Auda, 2020). Due to the amphiphilic nature of the surfactants, which destabilize the lipid packing of biological membranes, altering their integrity, and penetrate these coats through hydrophobic interactions, biosurfactants collectively disrupt bacterial membranes and create pores that allow antibiotics to enter the cells, increasing the antimicrobial activity (Khaleel and Haydar. 2024). Ultimately, the literature contains relatively little information on the relationship between the structure and content of mannoproteins and yeast species, as well as the impact of mannoprotein characteristics on their biological activity. In conclusion, baker's yeast *S. cerevisiae* had the ability to produce glycoprotein emulsifier (mannoprotein) that had Antibacterial and Antibiofilm activity.

CONCLUSION

In conclusion, baker`s yeast S. cerevisiae had the ability to produce glycoprotein emulsifier (mannoprotein) that had Antibacterial and Antibiofilm activity.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

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AUTHOR'S CONTRIBUTION STATEMENT

Zainab Jawad Salman conducted the experiments and wrote the research paper. Dr. Jehan Abdul Sattar Salman designed and supervised the experiments, reviewed the results, and verified the research. Dr. Raghad Akram Aziz reviewed the purification results and verified the research.

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الفعالية الحيوية للمانوبروتين المنقى من خميرة الخبز تجاه Pseudomonas aeruginosa

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المستخلص

هدفت هذه الدراسة إلى تقييم النشاط البيولوجي للمانوبروتين المنقى من خميرة الخبز Saccharomyces cerevisiae المتوفرة في الأسواق المحلية. تم استخلاص المانوبروتين وتنقيته وكشف الفعالية الاستحلابية في كل خطوة. كانت الفعالية الاستحلابية للمانوبروتين الخام 67.85٪. تم تنقية المانوبروتين الخام جزئيًا بواسطة الترسيب بالإيثانول البارد وبلغت الفعالية الاستحلابية 75٪. ثم تم تنقيته بشكل كامل باستخدام كروماتوغرافيا الترشيح الهلامي باستخدام هلام السيفادكس G–100، وكان المانوبروتين المنقى يحتوي على 37.61 ملغم/مل بروتين و32.93 ملغم/مل كاربوهيدرات مع فعالية استحلابية 87.55%. وتم توصيف المانوبروتين باستخدام تحليل FTIR وتحليل الأحماض الأمينية والتي أظهرت أن المانوبروتين عبارة عن جلايكوبروتين. وتم اختبار النشاط المضاد للبكتيريا والمضاد للأغشية الحيوية للمانوبروتين ضد عزلات Pseudomonas aeruginosa المعزولة من مصادر سريرية مختلفة، وأظهر تأثيرًا عاليًا جدًا في تقليل نمو البكتيريا مع معدل بلغ 98.4%. كما قام المانوبروتين بتثبيط تكوين الأغشية الحيوية للعزلات البكتيرية بمعدلات مختلفة، كان أعلى معدل 60.09%، بينما كانت معدلات تحلل الأغشية الحيوية أقل ووصلت الى 21.12%. وأوضح فحص FE–SEM اختزال تكوين الغشاء الحيوي.

الكلمات المفتاحية: الأحماض الأمينية، النشاط المضاد للأغشية الحيوية، FE–SEM، FTIR، المانوبروتين.