Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Biochemical Capabilities of Saccharomyces cerevisiae*

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Received: 25/04/2026; Revised: 02/05/2026; Accepted: 07/05/2026.

Abstract

along with biochemical tests to determine its biological characteristics. A yeast culture was prepared on a medium, and four different concentrations (1, 0.5, 0.25, and 0.12 mg/ml) were prepared for testing. An antioxidant activity of yeast was evaluated. The four concentrations mentioned above were also used in this test with the reagent (DPPH). The results indicated the ability of yeast to remove DPPH free radicals, and the removal activity was as follows: 0.1522, 0.1684, 0.3035, 0.3559 absorbency. Then, a hemolysis test was performed using a sample of healthy human blood, and the yeast demonstrated the ability to induce beta-hemolysis (β-hemolysis), with the following values: 0.4652, 0.3966, 0.3772, and 0. 3558µL.In the Biological efficacy test (BET), five types of bacteria were used, namely (Staphylococcus, Escherichia coli, Acinetobacter baumannii, Klebsiella, Pseudomonas). The yeast showed a clear antibacterial and anti-biofilm effect that depended on concentration, with strong inhibition of all tested bacteria at high concentrations (1mg/ml) and minimal activity at low concentrations, which confirms its effectiveness mainly at high doses. The results showed that yeast has a variable capacity to remove heavy metals, with the removal process depending on several factors. These factors include the components of the cell wall, which plays a crucial role in determining biosorption efficiency, as the cell structure dictates the

interaction between the microorganism and the heavy metal. Other factors include nutrition, biomass growth, age, pH, the microorganism's growth medium, temperature, and the microorganism's metabolic activity, such as metabolite release and respiration, which in turn alters the microenvironment.

Keywords: Microbiology, Yeasts, Heavy Metals, Biochemical Tests, Environmental, Pollution,water

1. Introduction

Recent research highlights the importance of Saccharomyces cerevisiae as a microorganism possessing diverse biochemical properties relevant to environmental bioremediation technologies, including heavy metal treatment and biosorption mechanisms. Yeast biomass exhibits efficient biosorption of toxic heavy metal ions such as zinc, lead, cadmium, and mercury from aqueous solutions. The cell wall's structural properties, such as hydroxyl and carboxyl groups, facilitate electrostatic adsorption processes, which are quantitatively considered isothermal and kinetic models. This underscores its suitability as a low-cost biosorbent for metal removal under optimal conditions (pH, contact time, and biomass dosage) in aqueous systems (Savastru, Bulgariu, Zamfir, & Bulgariu, 2022). In addition to the biosorption of heavy metals, various biological activities associated with the yeast Saccharomyces cerevisiae have been recorded, such as oxidative stress. Studies of yeast responses to oxidative stress confirm the role of glutathione and superoxide dismutase systems in mitigating reactive oxygen species produced by heavy metals, linking biosorption processes to cellular adaptation pathways to stress (Gerenot and Yi, 2001).

2. Literature Review

Biochemical tests are performed on the yeast Saccharomyces cerevisiae for several scientific and practical reasons related to its ability to remove heavy metals and other pollutants. The main reasons for using these tests are as follows:
Biological Effects: Several tests, such as oxidative stress and biological reactions, contribute to understanding the environmental effects of yeast on other organisms. Yeast can be considered as a source of environmental toxicity or for its antibacterial or antioxidant effects in the medical or environmental industries (Fakruddin, Hossain, & Ahmed, 2017). Other studies have also shown that yeast and its

compounds, such as beta-glucan, have significant antioxidant activity in vitro, capable of protecting biomolecules from oxidative damage, confirming that they contribute to modulating reactive oxygen species and oxidative stress responses in process systems (Pereira, 2021).

2.1. Diagnostic Description of Saccharomyces cerevisiae

Saccharomyces cerevisiae is a type of yeast belonging to the genus Saccharomyces within the family Saccharomycetaceae. One of its characteristics is that it is a single-celled, eukaryotic organism widely used in biotechnology, fermentation, and baking due to its ability to ferment sugars. First described by Carl Linnaeus in 1753, this yeast is widely known for its ability to ferment glucose through alcoholic fermentation (Parapouli et al., 2020). The diameter of Saccharomyces cerevisiae yeast cells ranges between 5 and 10 micrometers. They are spherical to oval in shape. They are characterized by a thick cell wall, the composition of which is mainly glucans, which provide structural protection and constitute about 30% of the dry cell weight. This yeast reproduces by budding, through the formation of new cells that separate after maturation is completed (Banwarth-Kuhn, 2020; Parapouli et al., 2020). Another of its characteristics is It is a facultative anaerobic, meaning that it is has the ability to grow in different conditions, rich and poor in oxygen. It switches to aerobic respiration, using glucose and other sugars, when available of oxygen, Ethanol is produced from the fermentation of glucose. and carbon dioxide in the absence of oxygen (Duncan et al., 2024). This yeast grows in a temperature range of 30-35°C, while it tolerates high temperatures under dedicated pressure, and grows at a pH range of 4.0-6.0 (Ma et al., 2025). The yeast Saccharomyces cerevisiae possesses a complete genome sequence consisting of 16 chromosomes, thus classifying it as a eukaryotic organism. This yeast has been the subject of numerous applications, including genetic engineering, pharmaceutical production, protein synthesis, and biofuel production (Parapouli et al., 2020; Tullio, 2022). It is considered to have essential metabolic pathways in the production of vitamins and amino acids, especially in industrial systems (Perli, 2020; Wang et al., 2024). Furthermore, recent studies have demonstrated the probiotic potential of the yeast Saccharomyces cerevisiae, through its ability to balance gut microbiota and strengthen immune responses (Cui et al., 2024; Onyema et al., 2023). Its importance in both modern and traditional industries has been confirmed by its use in fermented products such as bread, food processing,

and beverage production. In addition, S. cerevisiae yeast has specialized mechanisms for a strong stress response, giving it a chance to survive and resist harsh conditions, including oxidative stress and high concentrations of ethanol (Postaru et al., 2023; Yuan et al., 2024).

2.2. Bioremediation

The American Academy of Microbiology defines bioremediation as the utilization of living organisms or components of their metabolic processes to eliminate or neutralize hazardous pollutants in the environment (Qattan, 2025). To remediate polluted sites by It entails utilizing natural or genetically modified microorganisms via biological degradation or transformation processes. increases the industrialization in developing countries has led to significant environmental pollution, discharging substantial volumes of hazardous chemical wastes, including Aromatic hydrocarbons, polycyclic (PAHs) as well as heavy metals (Frontiers in Environmental Science, 2024). Among the problems and damages caused by the accumulation of these harmful substances are widespread problems in public health and the environment, especially in water and soil ecosystems. Activated-carbon adsorption, chemical oxidation, evaporation, and chemical precipitation are all common physicochemical methods for disposal pollutants. However, they are expensive, Production of toxic byproducts, and are not quite good on a large scale (Frontiers in Agronomy, 2023). Therefore, biological methods, which are more important to the environment, are replacing these traditional methods. Bioremediation is the way to clean up toxic substances because it's cheap and ecofriendly by turning them into less harmful or non-toxic forms through the natural activity of microbes in soil and water (Frontiers in Agronomy, 2023). This transformation is done by microorganisms that effectuate during enzymatic and metabolic pathways that facilitate degradation of intricate oxidation and reduction contaminants (Frontiers in Plant Science, 2024). That microbes can remove metals in two main ways, Biosorption is considered a passive process physicochemical interaction between metal ions and cell-wall functional groups. While bioaccumulation is a metabolic process in which living cells actively transport ions across membranes, bioaccumulation is a metabolic process in which living cells actively transport ions across membranes (Nnaji *et al*., 2024). These methods and mechanisms enable microorganisms to stabilize or transform metals efficiently across various environmental conditions. Can both improve bioremediation or

bioaugmentation, which involves adding certain strains of microbes (native, exogenous, or genetically modified) to contaminated areas, or bio stimulation and bioventing, which boost the activity of native microbes (Frontiers in Bioengineering; Karnwal, 2024, 2024 and Biotechnology, 2023). People often use these strategies to clean up soils and industrial waste contaminated with hydrocarbons and metals. This method has several benefits: it cuts down on secondary waste, does away with the need for chemical additives, and uses cheap, plentiful biological materials while still being adaptable to changing environmental conditions (Frontiers in Agronomy, 2023; Applied Sciences, 2023). Bioremediation has thus become a key strategy in contemporary environmental management. Heavy metals are dangerous to the environment because they are toxic, they can build up in living things, and they can move around in food chains. This puts human health at risk (Mustapha et al., 2025; Das et al., 2025). While the bacterial mechanisms for heavy metal removal are extensively researched, yeast-based processes are still inadequately comprehended, despite their significant potential. Several types of yeast have been developed, such as Schizosaccharomyces, Candida sp., and Saccharomyces cerevisiae have developed various methods for detoxifying pollutants, including biosorption, ion exchange, bioaccumulation, and biotransformation. S. cerevisiae is a cost-effective and A genetically modified organism that can be used to eliminate many metals (Cd, Co, Cr, Ni, Pb, Cu, Zn, and Ag) The ease of recovering precious metals and recycling their use in industry (Microbial Bioremediation Review, 2023).

3. Materials and Methods

3.1. Materials

3.1.1. apparatus and equipment

The instruments and equipment enumerated below were employed in this study to conduct the necessary laboratory analyses and experimental procedures:

Table (3-1): Laboratory Equipment Used in Biochemical Tests and Their Countries of Origin

NO.	Equipment	Manufacturer	Country of Origin
1	Thermocycler – PCR	Biorad	USA
2	pH Meter	-	Germany
3	Elisa	Paramedical	Italy

4	Atomic Absorption Spectrophotometer AA-7000	Shimadzu	Japan
5	Nano Drop Microvolume Spectrophotometer	Philips	Holland
6	Vortex Mixer	Amato	Japan
7	Spectrophotometer UV-Vis	Hitachi	Japan
8	Electrophoresis	Shimadzu	Germany
9	Eppendorf Tubes	Bio basic	Canada

3.1.2. Chemicals and Biological Materials

The following materials were employed in this study:

Table (3-2): Biochemical Materials and Their Countries of Origin

NO.	Material	Company	Country of Origin
1	2,2—1-Diphenyl picrylhydrazyl (DPPH)	-	China
2	Sabouraud agar	Himedia	India
3	NaOH	BDH	England
4	HNO3	BDH	England
5	Glycerol	BDH	England
6	Ethidium bromide	LKB	Sweden
7	Agarose gel	Promega	USA
8	Phosphate buffer saline	RPI-Grop	-
9	Crystal violet	Solarbio	China
10	Ethanol	BDH	England
11	Glucose	Fluka	Switzerland
12	Protease	Promega	USA
13	Nuclei lysis	Promega	USA
14	RNase Solution	Promega	USA

3.1.3. Kits

The following Kits were employed in this study:

Table (3-3): Biochemical Kits Used in the Study and Their Countries of Origin

NO.	Kit	Company	Country of Origin
2	Go Taq® G2 Green Master Mix	Promega	Korea
1	Hipura® fungal DNA purification Kit	Himedia	India

3.1.4. Media for Cultures

The culture media that used in study:

The table (3-4) The culture media that used in study

NO.	Culture media	Company	Country of Origin
1	Sabouraud Agar	Himedia	India

3.2. Methods

3.2.1. DPPH reagent

Added 400 microliters of cashew were to various concentrations of the yeast sample and used to evaluate the yeast's ability to remove free radicals in oxidation assay.

3.2.2. tritonx100 reagent

Added 15 microliters of cash were to flexible blood tubes and used as a positive test sample. and used in Hemolysis test.

3.2.3. Phosphate buffer saline

200 microliters of solution were added for the purpose of washing the test samples anti-biofilm.

3.2.4. Sodium acitate

200 microliters of the solution were added to the anti-biofilm test samples and left on the samples for half an hour.

3.2.5. MeOH methanol solution

Dissolve 200 mg of DPPH in 100 ml of solution, and the mixture was used as a control sample in the antioxidant test.

3.3. Collected Samples

Yeast samples were collected. In this study, suitable culture media were used, such as (Saburro Dextrose Broth), which is an ideal medium for yeast growth. The yeast was obtained from laboratories.

3.4. Biochemical Identification Tests

3.4.1. Antioxidant Test

Four concentrations were taken from a yeast sample grown on Sabouraud Dextrose medium. The concentrations were (0.12, 0.25, 0.5, 1 mg/ml). After preparing a solution, 200 µg of DPPH was dissolved in 100 ml of MeOH in an ELISA plate. In another well, 100 µL of DPPH reagent was added with 100 µL of the first concentration. The mixture was incubated for 30 minutes in the dark at room temperature. The absorbance was then measured using a spectrophotometer (ELISA device). The experiment was repeated with the remaining three concentrations, and the free radical scavenging activity was calculated according to the following formula: Radical scavenging = (control – sample) / control × 100 (Alam et al., 2013).

3.4.2. Hemolysis Test

This test was performed using blood from a healthy donor, and 285 µL was added to 6 tubes. Then, 15 µL of a lysis reagent (Triton X-100) was added to the first tube, which was considered the positive control. Tube number 2 is the negative control tube, as it contains only blood (285 microliters). Specific concentrations were added to tubes numbered 3, 4, 5 and 6, where 15 microliters of the first concentration (1 mg) were added to tube 3, 0.5 mg to tube 4, 0.25 mg to tube 5 and 0.12 mg to tube 6. The samples were then incubated for 4 hours at room temperature. After incubation, the samples were centrifuged at 10,000 rpm for 5 minutes. The supernatant (plasma) was collected, and the degree of degradation was measured using ELISA. A positive result was indicated when the sample turned yellow (signifying degradation) (Bhattacharya et al., 2021).

3.4.3. Biological efficacy test (BET)

One gram of glucose was added to 100 ml of the prepared heart and brain extract broth. Then 100 microliters of the medium were mixed with 100 microliters of Saccharomyces cerevisiae yeast culture (5 microliters for each type of bacteria). A

series of five dilutions of each bacterial strain were performed on an ELISA plate. Five bacterial strains were used: Staphylococcus, Escherichia coli, Klebsiella, Salmonella, and Bacillus. The cultures were then placed with distilled water in a turbidity meter, with a reading of 0.5. Ten microliters of each dilution, except for the last one which served as a control sample, were added to the plate. The samples were incubated at 37°C for 24 hours. After incubation, the samples were washed twice with 200 microliters of BBS (phosphate-buffered saline) solution. Then, 200 µL of sodium acetate solution was added, and the samples were washed with crystal violet (200 µL) for 30 minutes. Afterward, ELISA was used to measure absorbance. For lysis, the samples were washed and dried, and then 200 µL of ethanol was added. ELISA was then used to measure absorbance and assess lysis efficiency (Azeredo et al., 2017).

Figure (3-1) shows an image of the ELISA device used in biochemical taste

4.Results and Discussion

4.Biochemical Tests

Several biochemical tests were conducted to identify the capabilities of yeast as follows:

4.1. Antioxidant Test

The antioxidant activity of the yeast was evaluated using the DPPH assay. S. cerevisiae demonstrated a significant ability to scavenge free radicals, as evidenced by the reduction of the DPPH purple color to yellow, indicating the formation of a stable compound, DPPH-H, which does not cause further oxidation. The ability of S. cerevisiae to scavenge free radicals was measured by the disappearance of the purple color, which is converted to a stable non-radical compound. This transformation is indicative of the yeast's antioxidant potential. The results are shown in Figure (4-1), (4-2) and Table (4-1). (Zhou et al., 2021).

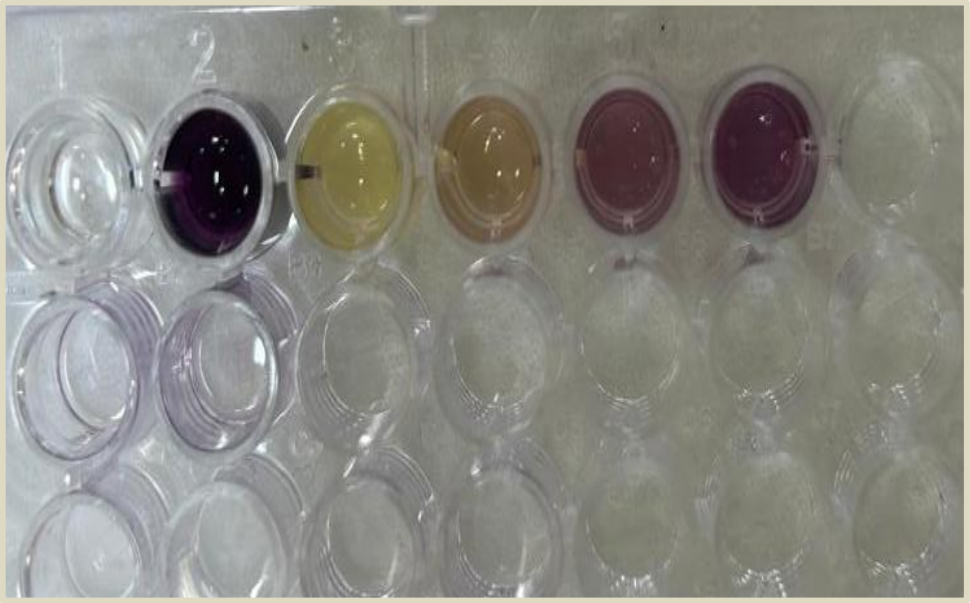

Figure (4-1) shows the antioxidant test

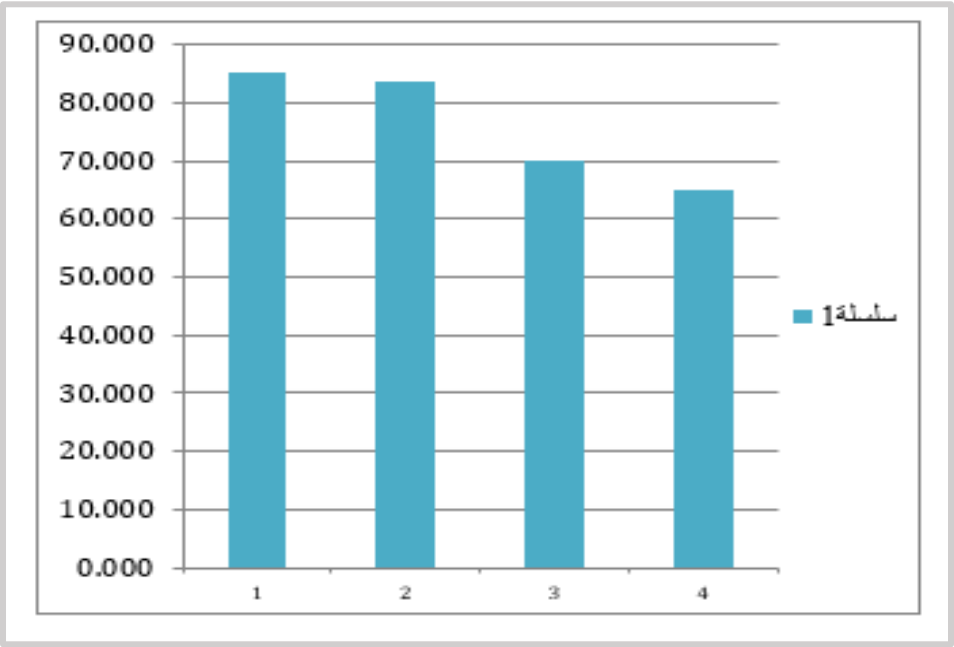

Figure (4-2) shows a graph of the antioxidant test

Table (4-1) shows the antioxidant test

sample name	Concentration	absorbency	scavenging %
1	1 mg/ml	0.1522	85.049
2	0.5 mg/ml	0.1684	83.458
3	0.25 mg/ml	0.3035	70.187
4	0.125 mg/ml	0.3559	65.039
#	Control	1.018	

The results of the DPPH assay showed that Saccharomyces cerevisiae yeast possesses significant antioxidant activity, demonstrated by its ability to reduce stable DPPH radicals. This is evidenced by the gradual decrease in absorption values from the control sample (1.018) to the treated samples, which is directly related to the conversion of DPPH from its purple radical form to the yellow non-radical

compound (DPPH-H). The highest free radical scavenging activity was observed at a concentration of 1 mg/ml (85.049%), followed by 0.5 mg/ml (83.458%), indicating strong antioxidant capacity at higher concentrations. Conversely, a gradual decrease in the free radical scavenging rate was observed with decreasing concentrations, reaching 65.039% at 0.125 mg/ml. This concentration-dependent behavior indicates that the antioxidant compounds produced by yeast are most effective at high doses, due to the increased availability of reducing factors such as phenolic compounds, proteins, and polysaccharides in the cell wall. Finally, these results confirm that Saccharomyces cerevisiae possesses a high capacity for scavenging free radicals, supporting its potential as an important source of antioxidants in biological and environmental applications. These results are consistent with recent studies that have confirmed the strong antioxidant activity of yeast-derived bioactive compounds and their effectiveness in DPPH free radical removal assays (Zhou et al., 2021; Liu et al., 2022).

4.2. Hemolysis Test

It was observed that yeast is capable of causing hemolysis with an increase in its concentration in blood samples, as shown in Table (4-2). This test is a classification trait for the yeast, and there are three types of hemolysis:

α-type hemolysis: Also known as partial or incomplete hemolysis, where the sample turns green.

β-type hemolysis: Or complete hemolysis, where the sample turns yellow. This is the type caused by the yeast in our current study, as shown in Figure (4-3).

γ-type hemolysis: Or non-hemolytic type, where no hemolysis occurs and the color of the sample does not change.

The results were matched with those of (Perini et al., 2019).

Figure (4-3) shows the hemolysis Test

Table (4-2) shows the hemolysis test

concentration (mg/ml)	test result	hemolysis %
1	0.4652	0
0.5	0.3966	0
0.25	0.3772	0
0.12	0.3558	0
POSITIVE	1.8647	100
NEGATIVE	0.3341	0

The hemolysis test results show that Saccharomyces cerevisiae does not exhibit hemolytic activity under laboratory conditions. This is evidenced by the hemolysis percentage recorded in the test, which remained at 0% at all tested concentrations (1, 0.5, 0.25, and 0.12 mg/ml), despite slight variations in absorbance values. In contrast, the positive control sample showed complete hemolysis (100%) with

significantly higher absorbance (1.8647), while the negative control sample maintained a value consistent with non-hemolytic behavior (0.3341).Although β-type hemolysis is characterized by complete lysis of red blood cells and a clear or yellowish appearance, the laboratory data in this experiment do not support the occurrence of this type of hemolysis by Saccharomyces cerevisiae, as no measurable hemolytic effect was recorded. The absorbance values recorded in the yeast-treated samples closely matched those of the negative control sample, confirming the integrity of the red blood cell membrane and the absence of any toxic cellular damage. These results indicate that Saccharomyces cerevisiae yeast is bio harmless and safe with respect to red blood cells, lacking the damaging factors that cause hemolysis under the studied conditions. This non-hemolytic behavior enhances its suitability for biotechnological and environmental applications, including fields where biosafety is a fundamental requirement, consistent with recent studies that reported the non-hemolytic and safe profile of S. cerevisiae in biological applications (Parapouli et al., 2020; Hatoum et al., 2012).

4.3. Biological efficacy test (BET)

The yeast Saccharomyces cerevisiae demonstrated the ability to inhibit the growth of the bacteria used in this study, thereby reducing their ability to form a biofilm, as shown in Figure (4-4) and Table (4-3). Inhibition of the growth of all targeted bacterial species was observed at high concentrations of the yeast, consistent with the results shown in previous studies (Kim et al., 2020).

Figure (4-4) shows the biological efficacy test

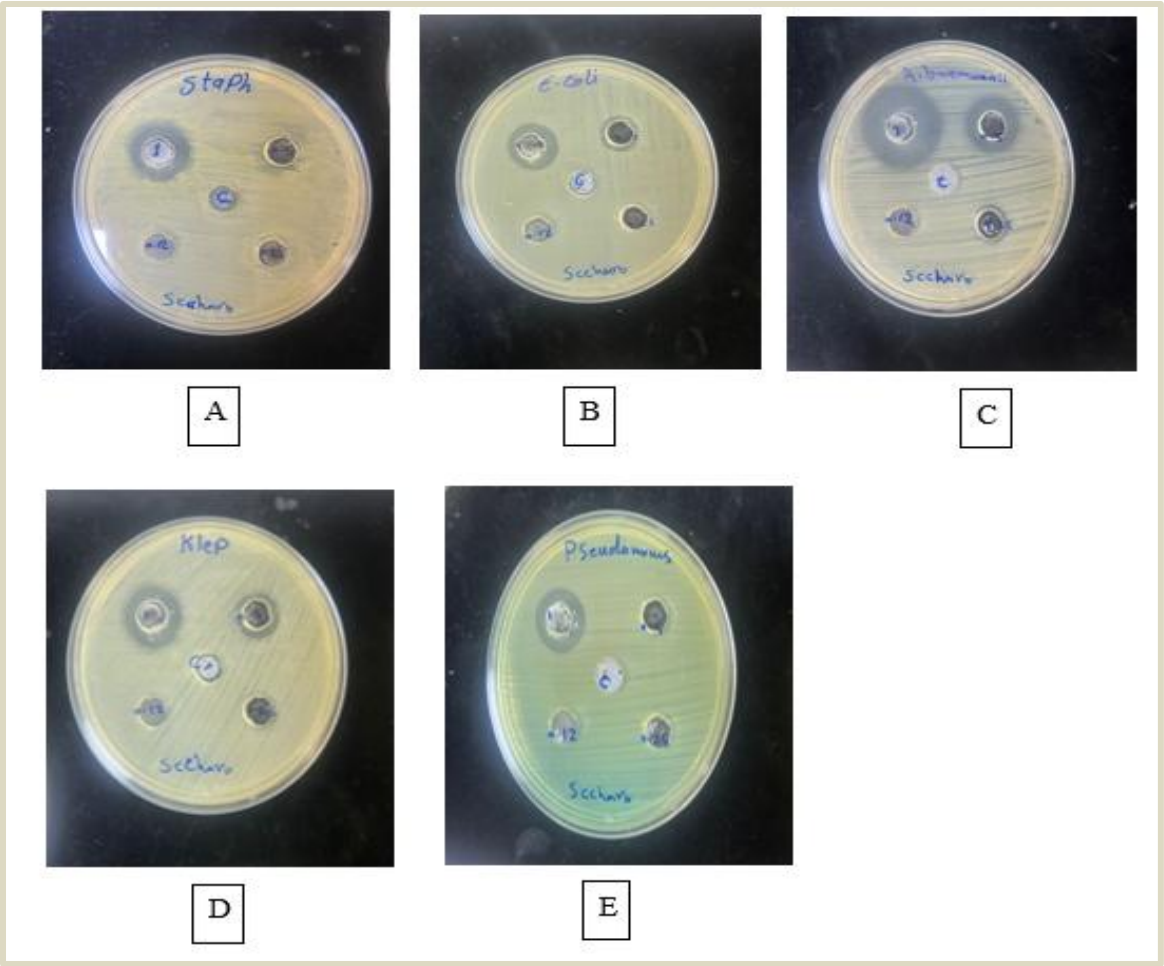

Table (4-3) shows the biological efficacy test

no.	Sample name	Con.	Staph.	E. Coli	A. B	Kleb.	Pseudo.
1	saccharomyces	1	18	17	26	18	17
2		0.5	11	12	17	12	0
3		0.25	0	0	11	0	0
4		0.12	0	0	0	0	0
5		control	0	0	0	0	0

The results of the Biological Efficacy Test (BET) confirm that Saccharomyces cerevisiae exhibits an inhibitory effect against the bacterial species used in the test, with this effect being strongly dependent on concentration. At the highest concentration (1 mg/mL), the yeast showed notable antibacterial activity, with inhibition zones of 18 mm against Staphylococcus, 17 mm against Escherichia coli, 26 mm against Acinetobacter baumannii, 18 mm against Klebsiella, and 17 mm against Pseudomonas, indicating broad-spectrum efficacy. This concentration-dependent pattern indicates that the biologically active compounds produced by Saccharomyces cerevisiae—such as organic acids, peptides, and secondary metabolites—require a certain threshold level to exert their antimicrobial effect. The marked sensitivity of Acinetobacter baumannii suggests greater susceptibility compared to the other tested species, whereas Pseudomonas shows intrinsic resistance, likely due to its well-known adaptive defense mechanisms. Overall, these results demonstrate that S. cerevisiae possesses significant antibacterial and anti-biofilm potential at high concentrations, supporting its use as a biological agent for controlling pathogenic microorganisms. A noticeable decrease in inhibition was recorded at a concentration of 0.5 mg/mL, where activity persisted but at lower levels, with complete loss of activity against Pseudomonas, reflecting higher resistance of this bacterium. At a concentration of 0.25 mg/mL, the inhibitory effect became highly selective and very weak, being limited to Acinetobacter baumannii (11 mm), while all other bacterial species showed no response. At the lowest concentration (0.12 mg/mL), no antibacterial activity was observed, which is consistent with the control results (0 mm), confirming the absence of spontaneous inhibition. This finding is consistent with previous studies that reported the antibacterial and antibiofilm activity of Saccharomyces cerevisiae and its metabolites against pathogenic bacteria (Zhang et al., 2025; Khadam & Salman, 2024).

Conclusions

1. The study showed that yeast has integrated biological activity, including concentration-dependent antioxidant effects, enhanced antibacterial activity at high concentrations, and non-hemolytic safety.
2. The ability of yeast to remove heavy metals varied due to the different tolerance mechanisms of yeast, as well as the different types of heavy metals,

their ion properties, and their interactions with the functional groups present on the yeast cell wall.

Recommendations

1. This research aims to improve antioxidant activity testing using different concentrations of Saccharomyces cerevisiae yeast, compared to other pollutants and chemicals. The goal is to achieve optimal free radical scavenging effects of the yeast, thereby enhancing its use in bioremediation applications.
2. Contributing to the development of new safety tests for yeast: To expand the scope of safety testing, this includes studying the effects of yeast at high concentrations on human cells. This is essential for the safe use of yeast in environmental and medical applications.
3. Developing bioabsorption tests to determine the ability of yeast to remove heavy metals. To evaluate the ability of Saccharomyces cerevisiae yeast to remove heavy metals from contaminated water and environments using bioabsorption tests, with a focus on the reactions of yeast with metals, such as lead, cadmium, mercury and zinc.

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