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Evaluation of Anti- biofilm activity of Purified Coagulase from a Local Pathogenic *Staphylococcus aureus* Isolate

[1]Marwa Ali Athab AL_ shimmery, [2]Sawsan Hassan Authman, [3]Mohammed Fadhil Abo Ksour

1- Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.,
Email: marwaali1997@uomustansiriyah.edu.iq
2- College of science/Biology department/Mustansiriyah University.
Email: dr.sawsanh@uomustansiriyah.edu.iq
3- Microbiolgy department, College of Science, Mustansiriyah university,
Email: m.aboksour@uomustansiriyah.edu.iq
Corresponding author: Marwa Ali Athab AL_ shimmery**,** Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.,
Email: marwaali1997@uomustansiriyah.edu.iq

Abstract

Coagulase is one of the important enzyme produced by pathogenic strains of Staphylococcus that has been used as a marker to classify them into coagulase-positive - (CoP) or coagulase-negative staphylococci (CoNS), that affects the clotting mechanism of blood. This enzyme reacts with prothrombin, which then clots the plasma or serum. The aim from this study is the determine the anti- biofilm activity of Coagulase purified from *Staphylococcus.aureus* on five different local clinical bacterial isolates from wound and vagina swab from Al-Shahed Alsadder Teaching Hospital and Ibn Al-Baladi Hospital from 1 December 2023 unitl 15 April 2024 that was biofilm former : *P.aeruginosa* ,*E. coli* , *S,aureus* , *S. pneumonie*and *Candida. albican*s.; Coagulase was used at different concentration (4,2,1,0.5,0.25,0.12, 0.06 mg/ml).twenty hundred samples from various clinical sources were collected from hospitals throughout Baghdad, and under aseptic lab conditions,about One hundred thirty single isolates were identified . The optimum condition of coagulase enzyme is pH-7.5, temperature 37C is the ideal environment for *Staphylococcus.aureus* to produce Coagulase . The crude Coagulase extracted had an activity of 1.7 (U/ml) and a specific activity of (U/mg).

The enzyme was purified by precipitating it with ammonium sulphate at a saturation level of 50-80% and utilizing SDS-PAGE. Further purification was achieved using ion exchange chromatography using DEAE cellulose and gel filtering using Sephadex G150. The molecular weight of the Coagulase enzyme was found to be 36 kilodaltons (KD).

Keywords: Staphylococcus, Purification , Coagulase ,DEAD-cellulose,Sepharose6B,SDS –PAGE, Anti-biofilm.

Introduction:

Staphylococcus aureus is a Gram-positive bacterium living as a commensal on the skin, mouth and upper respiratory system, making it a risk factor for opportunistic and nosocomial infections [1]. *Staphylococcus aureus (S. aureus*) has a spherical shape , It is salt tolerant and facultatively anaerobic. It may grow in mannitol-salt agar medium with 7.5% of sodium chloride and give positive hemolysis, coagulase, and catalase reactions but negative oxidase reactions. It is non-spore-forming, non-motile, and rarely capsulated[2].During the 1880s, there was a widespread outbreak of "puerperal fever" (later known as "childbed fever") in Great Britain. The emergence of "puerperal fever" was initially linked to *Staphylococcus aureus*. Bacterial coagulase is a virulence determinant of *S. aureus*. This enzyme converts the soluble plasma protein fibrinogen to insoluble fibrin clots, which have been suggested to protect *S. aureus* from opsonization and phagocytosis by host immune cells, thereby preventing clearance by the immune system [3].

S. aureus coagulase, the best known virulence factor among clumping factor family proteins also secreted with the same proprotein form, is the only coagulase of bacteria that has been characterized biochemically and genetically. *S. aureus* produces at least 50 different virulence factors and can cause diseases ranging from localized skin infections to life-threatening systemic infections. Pathogenicity is mediated by a number of virulence factors, including toxins, enzymes, and factors that interfere with host defence mechanisms such as the immune system or blood clotting [4].

Biofilms are communities of microorganisms attached to surfacesthrough a matrix of extracellular polymeric substance (EPS) [5].

These structures are adhered to surfaces and are largely composed of exopolymeric substances. Biofilm organisms are typically more resistant to stresses, such as antimicrobial therapy or host immune response, when compared to their planktonic counterparts. In addition to the increased resistance to stress factors, microbial cells that reside within biofilms are also afforded additional environmental protection and enhanced growth environments in comparison to their free-living planktonic counterparts[6].

Biofilms form through a complex multistep process where pathogenic cells adhere to surfaces, proliferate into a microbial community, and later disengage to establish new resident cells. They can take various structural

shapes, and their development is typically categorized into three progressive phases. The steps include:

The initial attachment of microbial cells is vital for biofilm formation. Cell adhesion to surfaces occurs through three mechanisms: physical interactions from mechanical forces, charge interactions, or specific chemical recognition. This adhesion is mediated by weak intermolecular forces like van der Waals, electrostatic, and hydrophobic forces. Surface roughness and texture significantly influence initial cell adhesion.. Surface hydrophobicity also plays an important role in the initial attachment of cells to solid surfaces, The period of attachment is followed by later stages of biofilm development, with growth and maturation as subsequent phases. Growth refers to cell proliferation; i.e., an organism increases in size and mass. The growth results in an increase in the thickness of the biofilm as well as a higher cell density. During maturation, member organisms interact in a cooperative manner, producing a community that has greater complexity than its individual parts and higher resilience to environmental stress. Endemic species will release chemical signals that modulate the structure of the biofilm matrix and recruit secondary species to the biofilm,

the three step of biofilm formation is Detachment It involves the release of biofilm cells into the environment, occurring naturally in mature biofilms. This process allows detached cells to colonize new areas or even form new biofilms. Environmental conditions can also trigger detachment events, influencing the dynamics of biofilms[7].

Coagulas has the properties of an adhesion factor and induction factor. Coagulas is used by bacteria in the process of colonization, biofilm formation, and immune evasion and has potential as a drug target for the treatment of some infectious diseases. The Coagulas polypeptide chain has 288 amino acids, including an N-terminal interface of 21 amino acids and a C-terminal interface of 29 amino acids. Coagulas and coagulase are competitive in their reactions with fibrinogen but differ in their products. At pH 5.5, the main products of CoaC to hydrolyze fibrinogen are proposed as the peptide A279, Bβ1–14, and Bβ15–20. The reported data indicated that the fibrinogen was digested by CoaC or coagulase from S. aureus with different amino acid sequences and cutting sites[8].

Methodology:

2.1. Collection of Sample

A total of two hundred samples from blood, biopsies, wounds, vaginal swabs, and urine were collected from the hospitals in Baghdad included: Al-Shahed Alsadder Teaching Hospital and Ibn Al-Baladi Hospital. From 1 December 2023 unitl 15 April 2024 , All samples were cultivated on selective media for isolation of *S.aureus* The media (from Himedia and others) were autoclave sterilized at 121°C for 15 minutes. All solutions and other materials have been prepared in advance [9].

***2*.2. Isolation of Bacteria**

In the laboratory and under aseptic conditions, the collected samples were cultured directly on a suitable selective media; the selective media used mannitol salt agar for 24 hours at 37ºC. Then sub-culture on the nutrient agar plate by streaking and incubation at 37°C for 24 hours to obtain pure well-isolated colonies, for identification [10].

2.3.Identification of Bacteria

In identifying *S. aureus*, as cultural , morphological& biochemical testBergey's textbook on systematic bacteriology proved to be crucial [11].

2.4 Phenotypic detection of Coagulase enzyme

The various methods used to detect coagulase rely on the principle of the coagulase enzyme reaction. Coagulase production (coagulase-free coagulase) binds to prothrombin. Several bacterial species frequently produce coagulase and blood clotting properties by converting prothrombin (Factor II) to staphylocoagulase, thrombin (Factor IIa). This induces the formation of a fibrin clot [12].It was generated utilizing [13].

The slide test was conducted to isolate bound coagulase. This involved suspending a single, uncontaminated bacterial colony in a mixture of normal saline and human plasma, while gently stirring. The test is classified as coagulase-positive, and the formation of clumps within a few seconds is observed. The control group consisted of regular saline and bacteria without the addition of plasma. This was done to verify that the bacteria did not form clusters spontaneously in normal saline.

Tube test; for this tube experiment according to [13] , a solitary colony of the bacteria was mixed with milliliters of human plasma (diluted in normal saline at a ratio of 1:6) and incubated for 24 hours at a temperature of 37 °C. The process of testing the tubes required a duration of one to four hours. The result was affirmative when a clot formed, and negative when the tube was incubated for a further 24 hours and examined again.

2.5 Extraction of Coagulase from *S.aureus*

The extraction of coagulase was performed following the methods outlined in reference [14] and the specified agencies. At first, a 250 ml volume of BHIB medium was produced and then inoculated with 7 $\times 10^3$ cell/ml Coagulase-producing *S. aureus*. The medium was inoculated with four specifically chosen isolates of *S. aureus* bacteria, with the isolate that demonstrated the highest level of anti-growth activity being selected, and incubated at a temperature of 37 °C. The combination was left to incubate for a period of 24 to 48 hours. Next, the bacterial culture underwent centrifugation at a speed of 10,000 revolutions per minute for a duration of 30 minutes, and the solid particles were removed. Subsequently, the filtrate was subjected to heating at a temperature of 70 °C for a duration of 3 minutes. The liquid portion, which included undiluted *S,aureus*, was gathered after cooling.

2.6 Determination of Coagulase Protein Concentration

The Bradford [15] method was used to estimate the protein concentration of each Coagulase extract produced from the isolate of *S.aureus* bacteria. This was done by using the standard curve of bovine serum albumin (BSA) at concentrations of (10, 20, 30, 40, 50…100) µg/ml. The following test was carried out; 0.5 ml of the sample and 0.5 ml of each bovine serum albumin concentrate were separately added to the test tubes. Then, 4.5 millimeters of Commasie Brilliant Blue dye solution was added to each one. After shaking, the tubes were left for two minutes, and the absorbance was read at a wavelength of 590 nm. A phosphate buffer was used instead of the model in a blank efficiency solution to miniaturize the spectrophotometer. The protein concentration was calculated in the model, by referring to the standard curve of bovine serum albumin. This was drawn based on the relationship between albumin concentration and absorbance values.

2.7 CoagulasePurification byAmmonium sulphate precipitation

The previously acquired supernatant, obtained by subjecting the mixture to heating and subsequent cooling to a temperature of 4 ℃, was gradually combined with the crude enzyme, along with a precise amount of ammonium sulfate. The mixture was agitated incessantly for 60 minutes to attain a saturation level ranging from 50% to 80%. The precipitate was diluted in a liter of Tris-HCl buffer (0.1 M, pH 7.5) and then tested for coagulase activity using a thermally stable assay to determine the ideal saturation percentage [16,17].

Dialysis

Ammonium sulfate precipitate was filtered via an active dialysis membrane which the molecular cut-off weight of the dialysis membrane (8-14 KDa) and then added to a buffer solution in a test tube (0.01Mphosphatebuffer)[18].

Ion-exchange chromatography

In order to create the DEAE-cellulose column, Whitaker and Bernhard [19] combined 20 grammes of resin with 1 litre of distilled water. The liquid underwent repeated rinses with distilled water until it achieved clarity after being allowed to settle. After being discharged, the suspension was filtered using a Buchner funnel with a Whattman No. 1 filter. The resin was dissolved in a 0.25 M solution of sodium hydroxide and sodium chloride. The suspension underwent many filtrations and rinses using a 0.25 M hydrochloric acid solution, as previously stated. Subsequently, it was balanced with a 0.05 M phosphate buffer solution at a pH of 7. After washing the column with an equal volume of the same buffer, the bound proteins were eluted progressively by adding increasing quantities of sodium chloride (0.1–1 M). The absorbance of each fraction was determined at 280 nm using a UV-VIS spectrophotometer, with the column operating at a flow rate of 30 ml/h. We quantified the coagulase activity of each component

Gel filtration

According to [19] Sephadex G-150 was formulated based on the guidelines provided by Pharmacia Fine Chemicals Company. A glass column measuring 2×40 cm was filled with Sephadex G-150. The Sephadex G-150 was then treated to remove any trapped gases and placed in a suspension in a Tris-HCl buffer with a pH of 7.5, at a concentration of 0.1 M. Subsequently, the column was permitted to reach equilibrium. Following pre-equilibration with a 0.1 M Tris-HCl buffer at pH 7.5, a concentrated amount of Serratiopeptidase was added to the matrix. The elution process was carried out using the identical equilibration buffer at a flow rate of 3 ml each fraction. The measurement of the absorbance of each fraction was conducted at a wavelength of 280 nm.

2.8 Characterization of Coagulase

2.8.1. Molecular weight determination

The molecular weight was determined using gel-filtration chromatography. The column used was a Sephadex G-150 (2x40 cm) that was prepared and washed with a 0.05M phosphate buffer solution at pH 7. Alcohol dehydrogenase, with a molecular weight of 150,000, albumin with a molecular weight of 66,000, carbonic anhydrase with a molecular weight of

29,000, and lysozyme with a molecular weight of 14,300, were used as molecular weight markers. The void volume at 600 nm was measured using Blue Dextran. At a wavelength of 280 nm, the UV-Vis Bio-Rad spectrophotometer was used to find out how much of each reference protein was eluted. The molecular weight of Coagulase was measured by comparing it to the molecular weight of established reference proteins and the elution volume [20].

2.9 Anti- biofilm activity of Coagulase produced from *S.aureus*

In this experiment, the bacteria used were obtained as biofilm components. Anti-biofilm of Coagulase was achieved using micro titer plates with 96 flat bottom wells accordind to [21], as seen below:

***** Bacterial suspension prepared from overnight culture on B.H.I. agar for five different isolates using B.H.I. with (2%) sucrose.

* All microtiter plates contained the same arrangement of different volumes of B.H.I. with (2%) sucrose inoculated with constant volume (20µl) of bacterial suspension. •

* First one which subjected to the treatment at zero time before had been incubated in 37C° as the following: Stock solution was prepared from Coagulase extract from *staphylococcus aureus* by add 10mg of Coagulase to 5 ml of PBS (pH 7.4) to obtain 2000 µg/ml then continuous dilution.

*Wells contained 200 µl of B.H.I only considered as negative control, whereas wells contained (180 µl of B.H. I+20 µl of bacterial suspension) was indicated as positive control.

* All these enclosed microtiter plates have been parafilm-sealed then incubated for 72 hrs.

* As shown in the biofilm formation assay, these microtiter plates were washed after incubation.

* Optical density at 630 values determined by the ELISA reader according to the ODc of the Positive control. The collagenase inhibitory effect was calculated using the equation:

inhibitory effect of Coagulase = OD control − OD treatment / OD control X100%

Results & Discussion

3.1. Collection of Samples

A total of two hundred samples from blood, biopsies, wounds, vaginal swabs, and urine were collected from the hospitals in Baghdad included: Al-Shahed Alsadder Teaching Hospital and Ibn Al-Baladi Hospital. From 1 December 2023 unitl 15 April 2024 , All samples were cultivated on selective media for

isolation of S. *aureus*. The media (from Himedia and others) were autoclave sterilized at 121°C for 15 minutes.

3.2. Isolation & Identification of *Staphylococcus aureus*

In the laboratory and under aseptic conditions, the collected samples were cultured directly on a suitable selective media; the selective media used mannitol salt agar for 24 hours at 37ºC. Then sub-culture on the nutrient agar plate by streaking and incubation at 37°C for 24 hours to obtain pure well-isolated colonies, for identification also by using the microscopic examination. As well as biochemical tests were done by VITEK 2 compact system.[22] *Staphylococcus aureus* colonies appear in creamy yellow colonies on mannitol salt agar.

Figure1. Colonies of *Staphylococcus aureus* on Mannitol Salt Agar (Selective media)

The fermentation of mannitol by pathogenic *Staphylococcus aureus* on MSA causes the medium's color to change from red to yellow.A total of 130 isolates were obtained from a sample size of 200, using the technique outlined below: 75 isolates were obtained from urine samples, 11 from biopsy samples, 39 from wound and ear swabs, 22 from fluid samples, and 33 from sputum samples. A total of 45 isolates were collected from urine samples, 5 isolates were obtained from biopsy samples, 28 isolates were obtained from lesion samples, and 20 isolates were obtained from ear samples. In addition, 15 isolates were collected from fluids, and 27 isolates were collected from sputum. Table 1 displays the distribution of isolates within the total samples categorized by their type and origin.

Table (1): *S.aureus* isolates isolated from different clinical sources

Samples	No. of Samples	No. of isolates	Percentage of isolates according total samples%	Percentage of isolates according samples source%
Urine	75	45	22.5	60
Biopsy	11	5	2.5	45.4
Ear Swabs	20	10	5	50
Wound Swabs	39	28	14	71.7
Fluids	22	15	7.5	68.1
Sputum	33	27	13.5	81.8

3.3 Phenotypic detection of Coagulase enzyme

The coagulase test can be performed using two different procedures:

Slide Coagulase Mehtod

This technique entails creating a mixture of bacterial cells suspended in a solution, which is then blended with a small amount of rabbit plasma treated with EDTA on a slide for microscopic observation. If the bacterial cells have bound coagulase, the presence of plasma will cause them to come together and form aggregates. The cells will adhere to fibrinogen in the plasma due to the presence of the adhesin, a type of adhesion molecule belonging to the MSCRAMM family, which is responsible for the formation of clusters. This phenomenon will result in the observable clustering of bacterial cells on the microscope slide.

The tube coagulase test

The technique involves pooling bacterial cells with higher plasma concentration in a tiny test tube. The staphylocoagulase secreted by the bacteria into the plasma stimulates their proliferation. The activation of prothrombin by staphylocoagulase initiates the cycle of blood coagulation. staphylocoagulase catalyzes the rapid breakdown of fibrinogen into fibrin by creating a complex with it, therefore bypassing the blood clotting process and facilitating the immediate formation of a fibrin clot. Upon the completion of twenty-four hours, the formation of a clot indicates a successful reaction.

Despite the potential for false negative outcomes, the slide test is user-friendly and provides data within a reasonable 10-second timeframe. Although the tube test is the exclusively reliable method, it may require a duration of up to 24 hours to provide results. The presence of any degree of

aggregation or clotting in either test indicates a positive outcome [23, 24]. Furthermore, the level of bacterial infection is unaffected by the coagulation rate of the blood.

3.4 Detection Enzyme Activity

According to [25] Using a dilution ratio of 1:10, the plasma was prepared by combining 0.2 ml of plasma with 1.8 ml of physiological saline. Three small test tubes labelled T (Test), P (Positive Control), and N (Negative Control) were obtained. Test duration ranges from 18 to 24 hours. An 18 to 24 hour incubated broth culture of *S. aureus* constitutes the positive control. An aseptic broth constitutes the negative control. Each tube was pipetted with 0.5 ml of diluted plasma. Five 0.1 ml drops of the Test organisms were introduced to tube "T", five droplets of *S. aureus* culture were placed in tube "P", and five droplets of sterile broth were added to tube "N". The three tubes were combined and then positioned in an incubator operated within the temperature range of 35 to 37 degrees Celsius. Analysis of coagulation after one hour of physical exertion. In order to investigate the agglutination events induced by tube coagulase, we conducted an experiment that mirrored the methodology outlined by Katz in 2010, but with certain modifications.

Calculate Enzyme Activity The enzyme activity was calculated using the following equation:

Enzyme activity =rate × reaction volume.

The specific activity is obtained by dividing the enzyme units (U) by the protein content.

The following equation was used to calculate the overall activity: The overall activity is determined by multiplying the enzyme activity by the whole volume produced during each stage. The purity (fold) was determined using the following equation: Purification (fold) is calculated by dividing the specific activity (for phases) by the specific activity (crude enzyme).

3.5 Extraction of Cured enzyme

Compound coagulase was synthesised from cardiac infusion broth.The staphylococci were cultivated in cardiac infusion broth over a period of three days on a rotating shaker set at 37°C. The orgamism was cleared by centrifugation in a cold centrifuge at 20,000 revolutions per minute. Table 2 displays a protein yield concentration of 10.5mg/ml, along with Coagulase activity and specific activity measurement of 1.7 units per millilititer and 0.16 units per millilitre, respectively. As show in table 2

Table 2. Coagulase enzyme extracted by four different methods (Crude extract , Dialyzes,DEAD-cellulose,Sepharose6B).

Purification Step	Vol ume (ml)	Enzyme Activity U/ml)	Protein Concentration (mg/ml)	Specific Activity (U/mg)	Total Activity (U)
Crude enzyme	450	1.7	10.5	0.16	765
Ammonium Sulfate	30	2.6	10.8	0.24	78
Enzyme dial yzes	27	2.8	9.6	0.29	75.6
DEAD-cel l ul ose	25	2.3	1.6	1.4	57.5
Sepharose 6B	15	1.3	2	0.65	19.5

3.6 Purification of Coagulase

3.6.1 Ion exchange chromatography

After ammonium sulfate precipitation, negatively charged Coagulase was purified using ion exchange chromatography. As shown in Fig. (2). Due to the gradient concentrations of sodium chloride, the washing stage produced one protein peak, while the subsequent elution step produced one protein peak. These protein peaks were examined to see if they had Coagulase activity. According to the findings, the proteins eluted (Fractions 25 to 30) accounted for the bulk of Coagulase activity. Analysis of pooled fractions was conducted to determine Coagulase activity at 2.3 U/ml, specific activity at 1.4 U/mg, and protein production at 1.6 mg/ml. The charge difference principle is the foundation of ion exchange chromatography. Therefore, the detection of a negatively charged Coagulase during the elution stage confirmed that it was produced by *S. aureus*.

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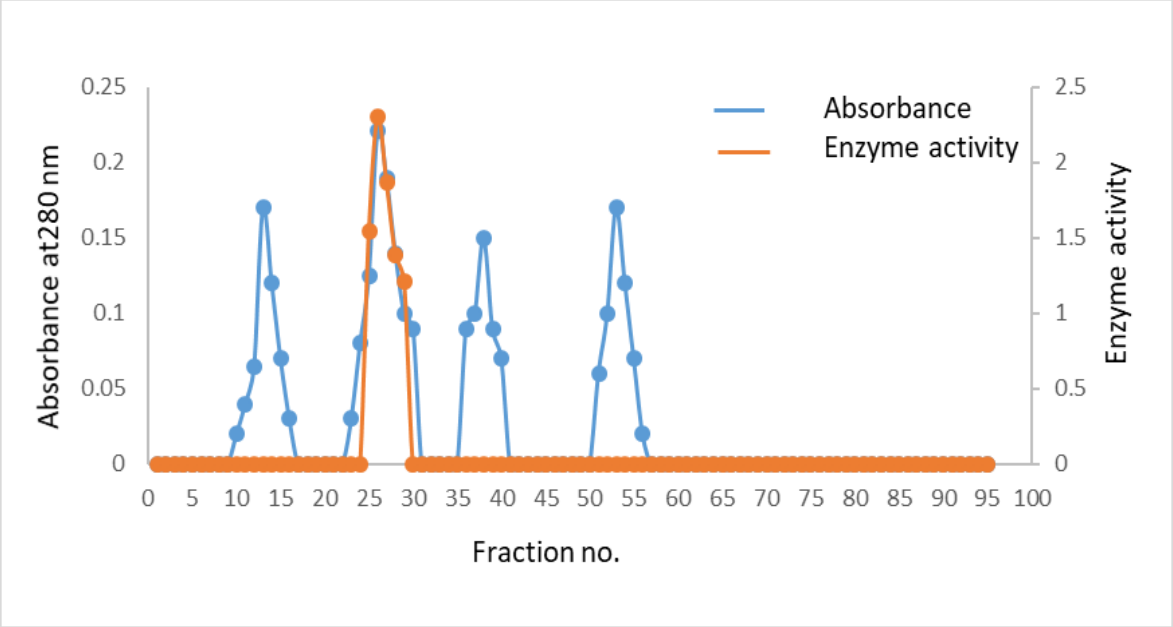

Figure2. Ion exchange chromatography for Purification of Coagulase produced by *S.aureus* Using G.150colum(2*40) cm with aflow rate of 30ml/hr.

3.6.2. Gel filtration chromatography

Coagulase-reflecting fractions were pooled and put into a Sephadex G-150 column following ion exchange purification. This column's separation limits range from (5,000) to (600,000) Dalton to produce superior separation efficiency and purification level. It may be utilized repeatedly for protein separations due to its minimal maintenance, ease of preparation, rapidity, and excellent recovery [26]. The results in Fig. (3) show thatCoagulase activity showed up as one peak after the Tris-HCl buffer was used to wash the sample. These separate parts were put together to make one protein. Coagulase was also measured for its concentration, activity, and specific activity. The fractionation yielded one protein peak with an absorbance reading of 280 nm. This peak (fractionation tubes 6-8) contained Coagulase activity (1.3U/ml), and specific activity (0.65 U/mg) with a yield of protein(2mg/ml) .

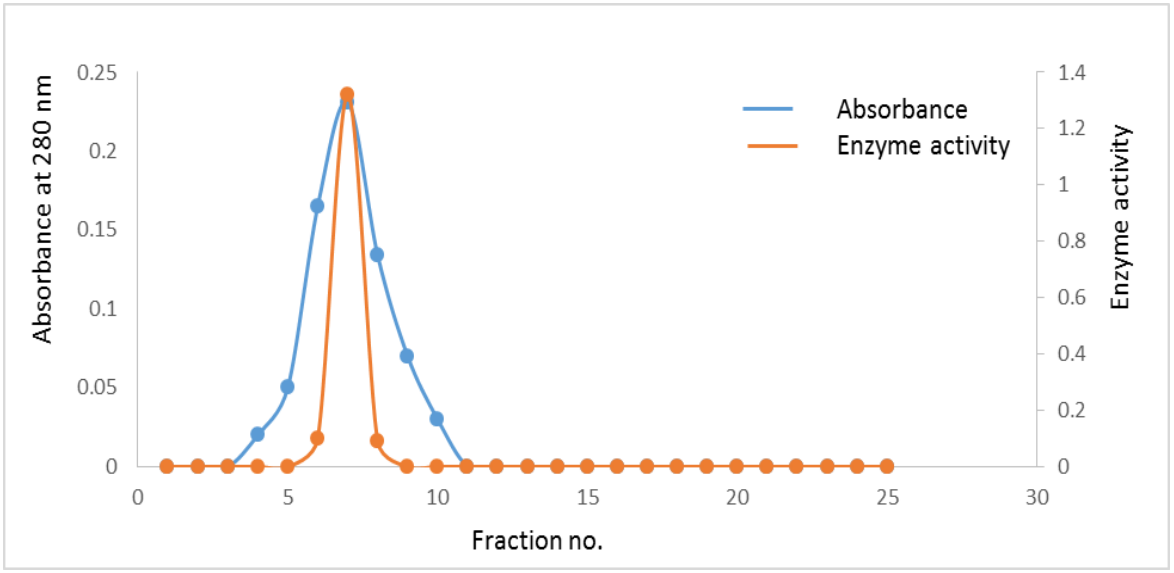

Figure3. Gel filtration chromatography for Purification Coagulase produced by *S.aureus* Using G.150colum(2*40) cm with aflow rate of 30ml/hr.

3.6.3 Determination of Protein Concentration

Fig. 4 demonstrates the application of the Bradford approach with Coomassie brilliant blue-250 and BSA in purification procedures to determine the crude protein level obtained by supernatant filtering.

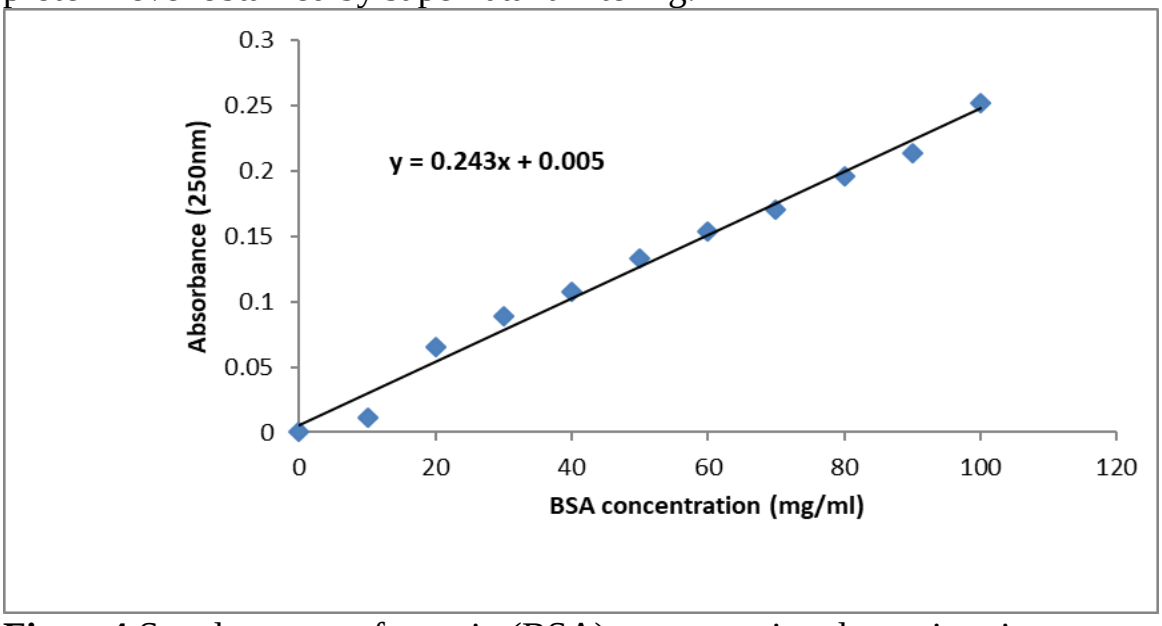

Figure4:Stander curve of protein (BSA) concentration determinatrion

The initial concentration of Coagulase was 9.6 Mg/ml, which decreased to 1.6 Mg/ml after ion exchange purification and further stabilised to 2 Mg/ml after gel filtration purification.

3.6.4 Molecular weight determination by SDS-PAGE

By using SDS-PAGE, it was discovered that Coagulase had an apparent molecular weight of 36 kDa.as shown in **figure 5.**

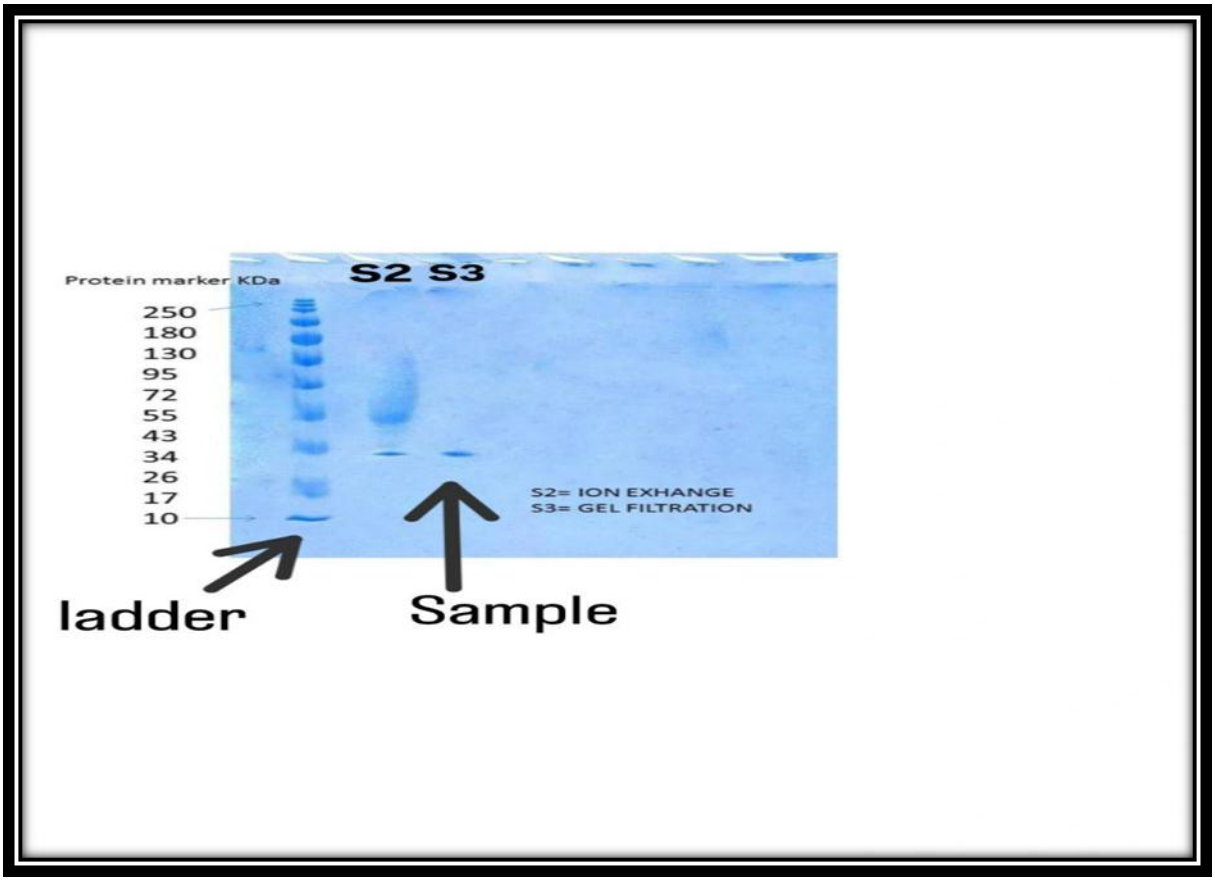

Figure5. SDS-PAGE after purification

3.6.5 Anti-biofilm activity of Coagulase Enzyme produced from *S.aureus*

Antibiofilm activity of Coagulase was examined against five different local clinical bacterial isolates that was biofilm former: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Candida albicans*. Coagulase was used at different concentration (4, 2,1,0.5, 0.25, 0.12, 0.06 mg/ml).

For *Pseudomonas aeruginosa*, inhibition peaked at 64.71% with 4 mg/ml of the compound, dropping to 3.9% at 0.06 mg/ml, indicating moderate effectiveness against this strong biofilm-forming pathogen Figure 6.

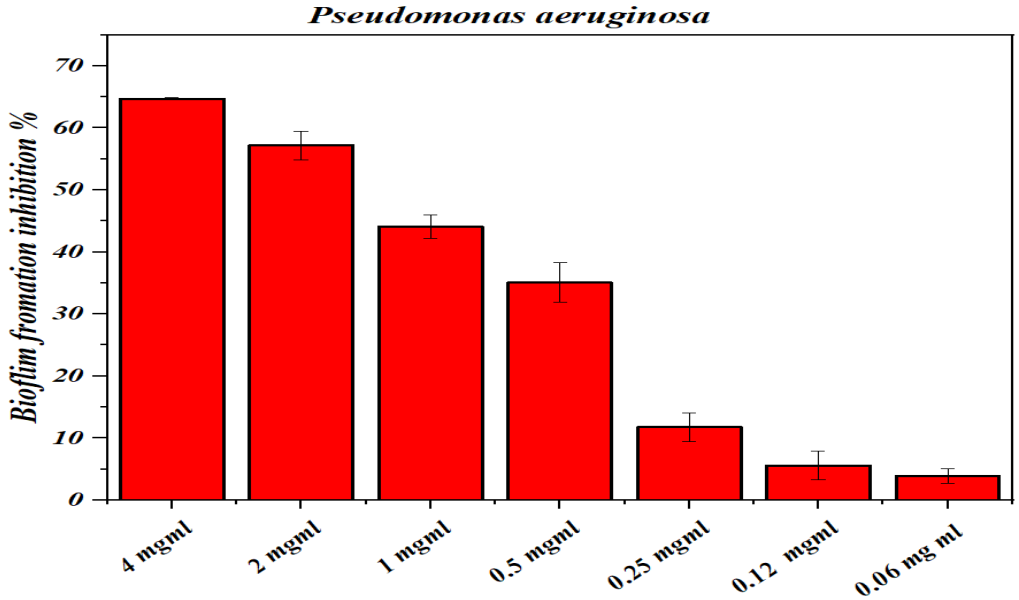

Figure6. Anti-biofilm activity of purified Coagulase on *P. aeruginosa*

Escherichia coli demonstrated the highest biofilm inhibition, reaching 93.45% at 4 mg/ml and 73.5% at 2 mg/ml, suggesting significant disruption of biofilm structure at higher concentrations Figure 7.

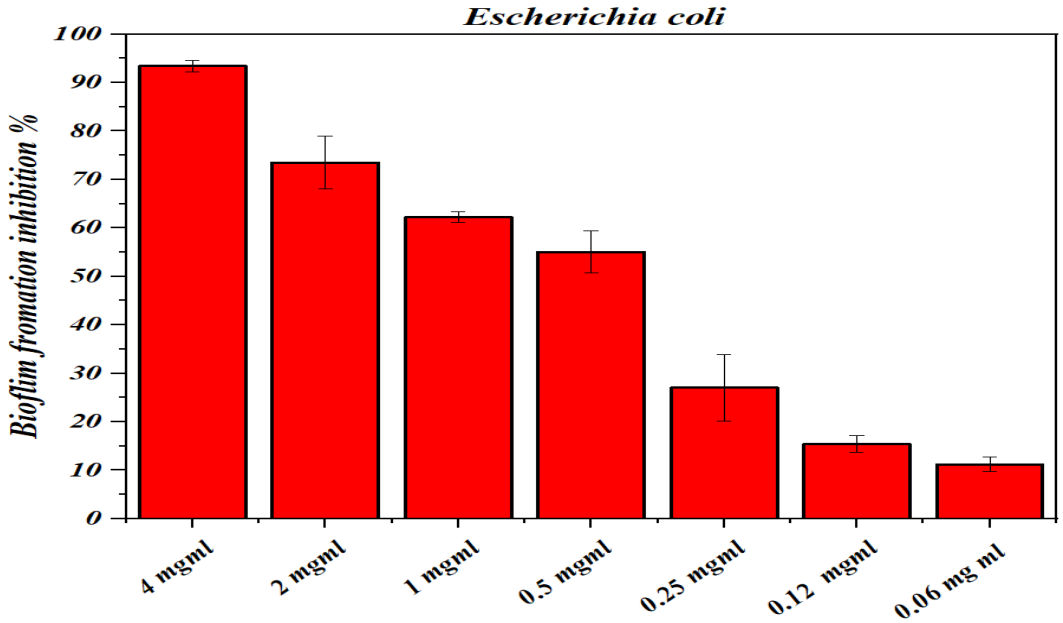

Figure7. Anti-biofilm activity of purified Coagulase on*E.coli*.

Staphylococcus aureus showed a similar trend, with 87.22% inhibition at 4 mg/ml, and a sharp decline to 13.11% at 0.06 mg/ml Figure 8.

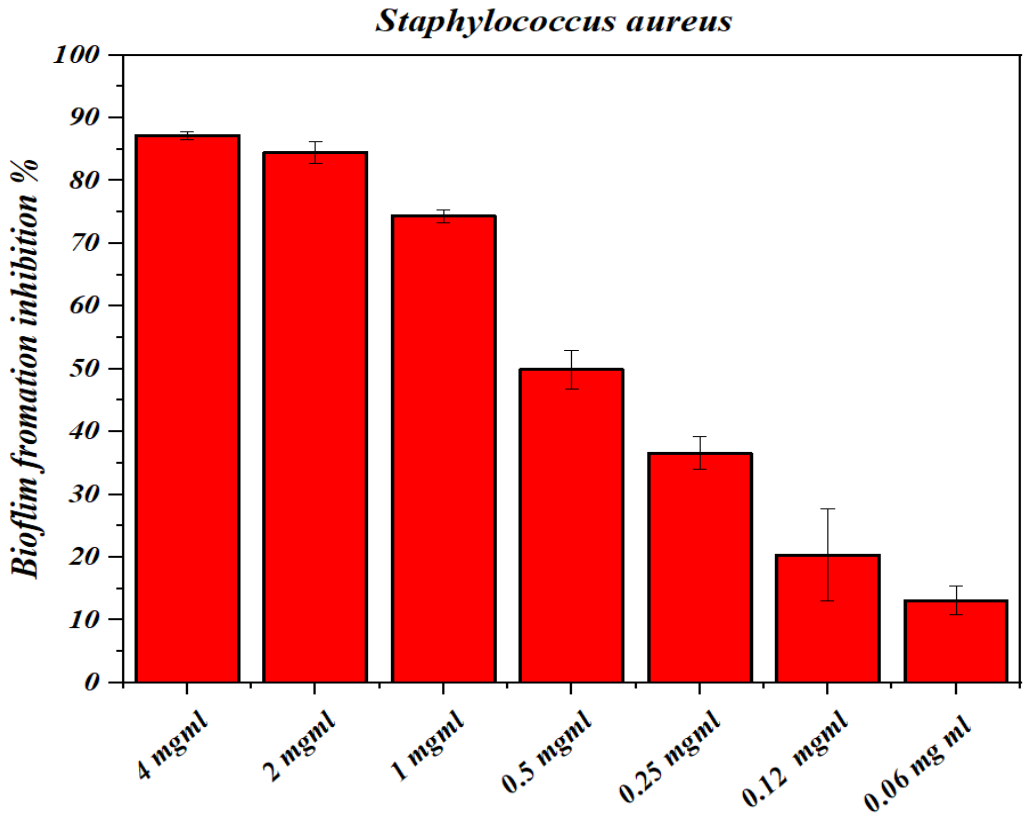

Figure8 Anti-biofilm activity of purified Coagulase on *S.aureus.*

Interestingly, *Streptococcus pneumoniae* exhibited strong inhibition even at lower concentrations, with 91.48% inhibition at 4 mg/ml and 55.75% at 0.06 mg/ml, indicating high susceptibility to the test compound.Figure 9.

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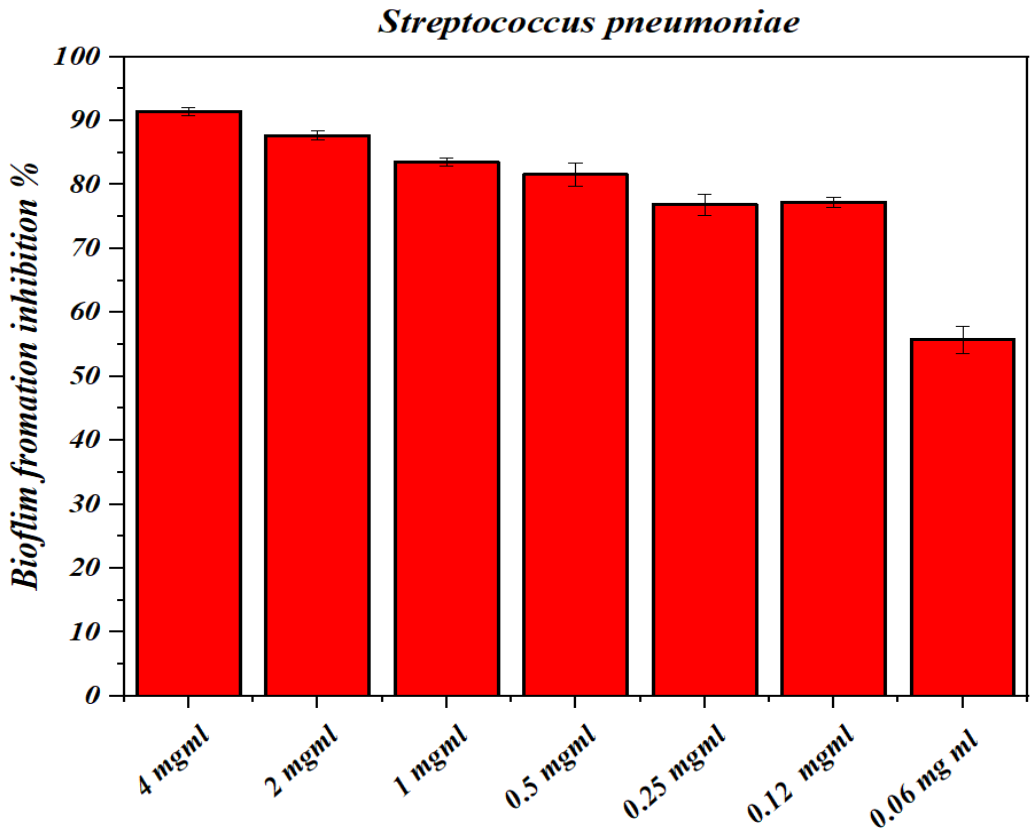

Figure9.Anti-biofilm activity of purified Coagulase on *S.pneumoniae*

In contrast, *Candida albicans*, a fungal pathogen, was less susceptible, with biofilm inhibition ranging from 75% at 4 mg/ml to 15.07% at 0.06 mg/ml. This suggests that while the compound is effective against bacterial biofilms, its efficacy against fungal biofilms may be somewhat limited, although notable inhibition was still observed at higher concentration Figure 10.

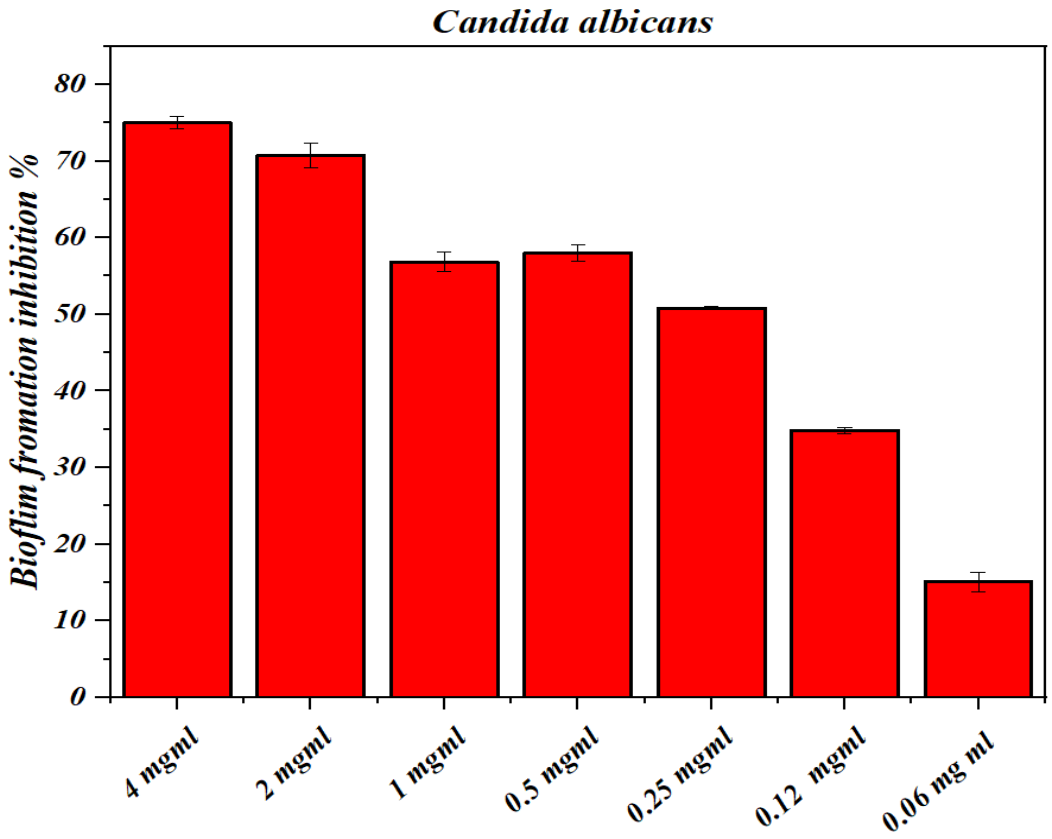

Figure10. Anti-biofilm activity of purified Coagulase on*Candida albicans*

Biofilms pose a significant challenge in clinical settings due to their enhanced resistance to antibiotics and immune responses. The ability of the tested compound to inhibit biofilm formation in a dose-dependent manner is promising, especially against Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*, which are common in hospital-acquired infections. The high inhibition levels against *E. coli* suggest that the compound disrupts early biofilm formation or interferes with biofilm maturation. The effectiveness against *Staphylococcus aureus* and *Streptococcus pneumoniae*, two common Gram-positive pathogens, further underscores its broad-spectrum antibiofilm potential.[27].

The relatively lower inhibition of *Candida albicans* biofilm could be attributed to the structural and functional differences between bacterial and fungal biofilms. Fungal biofilms are more complex, with a thicker extracellular matrix that may shield cells from the compound. Nevertheless, the observed inhibition at higher concentrations indicates that the compound could still be effective, especially when combined with other antifungal agents.[28].

This study highlights the potential of the compound as a broad-spectrum antibiofilm agent. However, additional research is needed to explore its mechanism of action, synergistic effects with conventional antibiotics, and effectiveness in in vivo models. The ability to target both Gram-positive and Gram-negative bacteria, as well as fungal pathogens, could make it a valuable candidate for combating biofilm-related infections, particularly in medical devices and chronic infections.[29]

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Conclusion:

A total of two hundred samples from blood, biopsies, wounds, vaginal swabs, and urine were collected from the hospitals in Baghdad included: Al-Shahed Alsadder Teaching Hospital and Ibn Al-Baladi Hospital. From 1 December 2023 unitl 15 April 2024 , From two hundred sample 130 of *Staphylococcus aureus* was producing Coagulase enzyme were produced under optimal condition , a molecular weight of coagulase enzyme was 36 kDa. In this study the purified Coagulase was used as anti-biofilm on various bacterial and fungal strains, with varying levels of biofilm inhibition observed at different concentrations. For *Pseudomonas aeruginosa*, inhibition peaked at 64.71% with 4 mg/ml of the compound while the effects the enzyme on . *Escherichia coli* demonstrated the highest biofilm inhibition, reaching 93.45% at 4 mg/ml, *Staphylococcus aureus* showed a similar trend, with 87.22% inhibition at 4 mg/ml, and a sharp decline to 13.11% at 0.06 mg/ml. Interestingly, *Streptococcus pneumoniae* exhibited strong inhibition even at lower concentrations, with 91.48% inhibition at 4 mg/ml, indicating high susceptibility to the test compound. In contrast, *Candida albicans*, was less susceptible whene using the purified enzyme with biofilm inhibition ranging from 75% at 4 mg/ml to 15.07% at 0.06 mg/ml. This suggests that while the compound is effective against bacterial biofilms, its efficacy against fungal biofilms may be somewhat limited, although notable inhibition was still observed at higher concentrations. The relatively lower inhibition of *Candida albicans* biofilm could be attributed to the structural and functional differences between bacterial and fungal biofilms. Fungal biofilms are more complex, with a thicker extracellular matrix that may shield cells from the compound. Nevertheless, the observed inhibition at higher concentrations

indicates that the compound could still be effective, especially when combined with other antifungal agents.

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تقييم النشاط المضاد للغشاء الحيوي لإنزيم الكواجيوليز المنقى من عزلة محلية لبكتيريا المكورات العنقودية الذهبية الممرضة

مروة علي عذاب الشمري 1
سوسن حسن عثمان 2
محمد فاضل أبوكسور3
قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق1
marwaali1997@uomustansiriyah.edu.iq

مستخلص البحث:

تم جمع ما مجموعه مئتي عينة من الدم والخزعات والجروح ومسحات المهبل والبول من المستشفيات في بغداد، شملت: مستشفى الشهيد الصدر التعليمي ومستشفى ابن البلدي. من 1 ديسمبر 2023 حتى 15 أبريل 2024. من بين مئتي عينة، تم إنتاج إنزيم التجلط (Coagulase) في 130 عينة من بكتيريا المكورات العنقودية الذهبية (*Staphylococcus aureus*) تحت ظروف مثلى، حيث كان الوزن الجزيئي لإنزيم التجلط 36 كيلو دالتون. في هذه الدراسة، تم استخدام إنزيم التجلط المنقى كمضاد لتكوين الأغشية الحيوية على سلالات بكتيرية وفطرية مختلفة، حيث لوحظت مستويات متفاوتة من تثبيط الأغشية الحيوية بتركيزات مختلفة. بالنسبة لبكتيريا الزائفة الزنجارية (*Pseudomonas aeruginosa*)، بلغ التثبيط ذروته عند 64.71% بتركيز 4 ملغ/مل من المركب، بينما أظهرت تأثيرات الإنزيم على بكتيريا الإشريكية القولونية (*Escherichia coli*) أعلى نسبة تثبيط للأغشية الحيوية، حيث وصلت إلى 93.45% بتركيز 4 ملغ/مل. وأظهرت بكتيريا المكورات العنقودية الذهبية (*Staphylococcus aureus*) اتجاهًا مشابهًا، مع تثبيط بنسبة 87.22% بتركيز 4 ملغ/مل، وانخفاض حاد إلى 13.11% بتركيز 0.06 ملغ/مل. من المثير للاهتمام أن بكتيريا المكورات الرئوية (*Streptococcus pneumoniae*) أظهرت تثبيطًا قويًا حتى عند تركيزات منخفضة، مع نسبة تثبيط بلغت 91.48% بتركيز 4 ملغ/مل، مما يشير إلى حساسية عالية للمركب المختبر. في المقابل، كانت فطريات المبيضات البيضاء (*Candida albicans*) أقل حساسية عند استخدام الإنزيم المنقى، حيث تراوح تثبيط الأغشية الحيوية بين 75% بتركيز 4 ملغ/مل و 15.07% بتركيز 0.06 ملغ/مل. يشير ذلك إلى أنه بينما يكون المركب فعالًا ضد الأغشية الحيوية البكتيرية، فإن فعاليته ضد الأغشية الحيوية الفطرية قد تكون محدودة إلى حد ما، على الرغم من ملاحظة تثبيط ملحوظ عند التركيزات العالية. قد يُعزى التثبيط المنخفض نسبيًا لأغشية المبيضات البيضاء الحيوية إلى الاختلافات الهيكلية والوظيفية بين الأغشية الحيوية البكتيرية والفطرية. تُعد الأغشية الحيوية الفطرية أكثر تعقيدًا، مع مصفوفة خارج خلوية أكثر سمكًا قد تحمي الخلايا من تأثير المركب. ومع ذلك، يشير التثبيط الملاحظ عند التركيزات العالية إلى أن المركب قد يكون فعالًا، خاصة عند استخدامه مع عوامل مضادة للفطريات الأخرى.

Keyword: Staphylococcus, Purification , Coagulase , DEAD-cellulose, Sepharose6B ,SDS –PAGE, Anti-biofilm

ملاحظة :البحث مستل من رسالة الماجستير.