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# EVALUATION OF THE INHIBITION ACTIVITY OF SOME LOCAL PLANTS EXTRACT TOWARD THE *STAPHYLLOCOCCUS AUREUS*

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## ABSTRACT

**The study attempted blocking a pure gelatinase generated by *S. aureus* using local plant inhibitors. One hundred local isolates of *S. aureus*, identified through biochemical testing, underwent initial as well as secondary testing for identifying actively gelatinase-producing *S. aureus* isolates. From these isolates, forty-four exhibiting the highest hydrolysis capacity in the first screening (with a Z/G ratio over 10 mm) were chosen for secondary screening. *S. aureus* R54 demonstrated the highest specific enzyme activity, recorded at 12.3 U/mg protein. Identified through the Vitek test, this isolate exhibited the highest gelatinase activity. Optimal conditions for gelatinase production via submerged fermentation were determined as follows: medium 1 as the most effective production medium, 2% fructose as the best carbon supplier, as well as A mixture containing 2% extracted yeast with 0.5% nitrogen from peptone. The optimum pH as well as temperatures had been 9 as well as 37°C. Following 24 hour incubating, this specific activity attained 54.3 U/mg. Gel filtration chromatography using Sephadex G-150 purified enzyme, resulting in a 1.2-fold enhancement in purity and An enzyme yielding 98.2%. An isolated enzymatic demonstrated its optimum efficiency at 37°C as well as maintained stable at this temperature.The isolated enzyme had peak activities at pH 9.0 also maintained stable at pH 7.0. The maximum rate of pure enzyme specificity seen with gelatin. Gelatinase was suppressed by local plant extracts, with avocado extract completely inhibiting 100% of gelatinase activity. It also exhibits antibacterial action against *S. aureus* R54.**

**Keywords: bacteria, avocado, plant inhibitors, characterization, Sephadex G-150.**



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## INTRODUCTION

*Staphylococcus aureus (S. aureus)* is an essential part of the microbiome present on human and animal skin, within the nasal cavity, and on mucous membranes. However, Staphylococci are linked to various significant medical conditions. These gram-positive bacteria appear in clusters, with each spherical cell with a diameter ranging from 0.5 for 1.5 µm. Their unique division in multiple planes leads to their cluster formation. They are non-motile, do not form spores, and require specific nutrients for growth. Known for their resistance to heat and ability to withstand high salt concentrations, they prefer aerobic environments but can also grow in the absence of oxygen as facultative anaerobes. (Lima *et al*, 2020; Joshi *et al*, 2007). All living organisms manufacture enzymes; however, the vast catalytic range of microbial enzymes makes them better than plants or animals enzymes, reduced cost, and enhanced stability. Bacterial species such as *Staphylococcus aureus* produce gelatinases, which are proteases analogous to human matrix metalloproteinases (MMP 2 as well as 9). These enzymes destroy the extracellular matrix, facilitating developing embryos,

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morphogenesis, procreation, also tissue restructuring, while also being associated with illnesses such as arthritis, cardiovascular and neurological disorders, and cancer metastasis. Therefore, they represent crucial objectives for pharmacological advancement. Bacterial metalloproteases are linked to virulence, while eukaryotic matrix metalloproteases are essential for processing precursors that affect tumor growth (Lima *et al*, 2020). Microorganisms produce gelatin enzymes that breakdown gelatin producing polypeptides, peptides, as well as amino acid which enter within the cell membranes and are digested. Currently, gelatinases have garnered significant interest as targets for pharmacological development due to their probable involvement with the deterioration of connective tissues associated with metastatic tumors (Cheng et al, 2019; Mazotto et al, 2011). The choice of optimal medium components and their concentrations is crucial in product development. Moreover, environmental elements such as pH and temperature have a significant effect in this context (Buchholz et al, 2005). During the enzymatic stage of fermentation, Raw extract comprise diverse combinations of protein as well as undesired byproducts, like organic acids as well as metabolite. The intended product must be purified using several ways. (Mazotto et al, 2011). Influences numerous chemical components in enzyme activity, as certain chemicals function as enzyme activators, while others serve as inhibitors, such as MnCl2, CaCl2, ZnCl2, EDTA, and Cysteine (Fernebro, 2011). The challenge that of resistance to antibiotics among human as well as animal is expected to endure for a considerable period. As a result, There are exists an immediate require to accelerate the develops for newly classes of medications to combat these infections (Chiavaroli et al, 2011). Plant produces various secondary metabolite includes alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones, as well as coumarin. These compounds are foundational for plant-based antibacterial agents (PDAms) (Chiavaroli et al, 2011). Certain naturally occurring compounds exhibit significant efficacy in the treatment of bacterial illnesses (Fernebro, 2011). The purpose for this investigation aimed to produce, purification, as well as identify gelatinase form a local isolate of *S. aureus*. The study evaluated the inhibition efficacy of the plant extracts towards *S. aureus* gelatinase.

## MATERIALS.AND.METHODS

**Chemicals:** Nutrient broth and agar, cerebral cardiac broth and agar, peptone, yeast extract, Coomassie Blue 250, KCl, $Na_2HPO_4$, $NaH_2PO_4$, HCl, gelatin, ferric compounds, $CaCl_2$, lactose, sucrose, glucose, fructose, Tris-HCl, $K_2HPO_4$, $KH_2PO_4$, Sephadex G-150, agar-agar, Hi-Media (India) as well as Sigma Aldrich (Switzerland) supplied reagent-grade chemical.

**Sample collection and bacterial isolation**

We collected 170 samples from Medical City Hospital, Al-Kadhimiya Hospital, Al-Yarmouk Hospital/Burn Center, and Al-Hariri Hospital between September 15 and December 15, 2022. Such samples had been obtained by various sources such as skin, burns, wounds, urine, blood, tonsils, and cases of otitis media. The initial cultivation was performed using brain heart broth, followed by plating on brain heart agar, and later on mannitol salt agar, and selective substrate for isolating *Staphylococcus* species. Identification of *Staphylococcus aureus* was achieved through biochemical tests, including the Vitek 2 system. The isolated *S. aureus* strains were prepared for subsequent screening experiments, with a focus on evaluating gelatinase producing.

**Assessment of *S. aureus* Isolation to obtain Gelatinase Synthesis:**

**Initial Assessment (Qualitative Assessment):** We assessed 100 S. aureus isolated by employing a plate-based test that utilized a gelatinous solid medium for identifying which were most proficient gelatinase producers. This medium comprised 10g of gelatin, 13g of nutritional broth, and 25g of agar, dissolving within a liter of distilled water, with a pH adjusted to 7.0. A sterile cork borer was aseptically inserted into the agar surface plate to create a well. Fifty microliters of the active bacterial isolate were deposited inside the holes using gelatin agar sheet with incubation at 37°C for 24 hours. A

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distinct zone surrounding the colony wells was seen as a sign of gelatinase secretion (Cappuccino and Welsh, 2011).

**Secondary Screening (Quantitative screening):** The isolates demonstrating the highest yield during the primary screening were cultured on a solid gelatin medium. For submerged cultivation, 50 ml of media was prepared with 250 mL flask, consisting of the following components for each liter in purified water: 0.25 g $K_2HPO_4$, 0.125 g $KH_2PO_4$, 0.125 g $MgSO_4$, 0.00025 g $FeSO_4$, 0.05 g NaCl, 0.05 g $CaCl_2$, 2.5 g gelatin, and 2.5 g glucose, adjusted to a pH of 7.0. The media was sterilized via autoclaving and A 1.0 mL overnight culture comprising $3x10^9$ cells/mL was injected. For 24 hours, the flask underwent incubation using a rotary shaker at 37°C and 120 rpm. After incubation, the culture had been spun at 10,000 rpm for 10 minutes for obtaining the basic enzyme extraction. This extract has been evaluated to gelatinase action as well as protein content, with both measurements performed in duplicate (Bajaj et al, 2014).

**Gelatinase activity**

The method described by (Hamzah et al, 2006) was used to assess gelatinase activity, employing gelatin as the substrate. Gelatin hydrolysis was determined utilizing a spectrophotometer for detecting 280 nm absorption. The amount of protein had been determined Bradford-style. (Bradford, 1976).

**Optimal factors for gelatinase synthesis**

**Impact of the medium of fermentation:** Six distinct media formulations were evaluated to determine the most effective medium for gelatinase production by the *S. aureus* R54 isolate.The media compositions were as follows:

(1) 5 g/L of extracted yeast, 20 g/L peptone, 20 g/L sucrose, and 20 g/L KCl (28). (2)5 g/L yeast extract, 20 g/L peptone, 20 g/L sucrose, 20 g/L KCl, and 20 g/L gelatin. (3)0.25 g $K_2HPO_4$, 0.125 g $KH_2PO_4$, 0.125 g $MgSO_4$, 0.00025 g $FeSO_4$, 0.05 g NaCl, 0.05 g $CaCl_2$, and 2.5 g glucose (20). (4)0.25 g $K_2HPO_4$, 0.125 g $KH_2PO_4$, 0.125 g $MgSO_4$, 0.00025 g $FeSO_4$, 0.05 g NaCl, 0.05 g $CaCl_2$, 2.5 g glucose, and 2.5 g gelatin. (5)13 g/L Nutrient Broth. (6)13 g/L Nutrient Broth with 10 g/L gelatin (Ohta et al, 2004). Two 250 mL Erlenmeyer flask flask s, all containing 50 mL of the specified media, have been created in duplicated, sterilized using autoclaving, and injected via 1.0 mL from an overnight suspension with an amount of $3\times10^9$ cells/mL from a chosen isolate. The flasks were then incubated at 37°C for 24 hours in a shaker incubator set to 120 rpm. Following incubation, the cultures underwent centrifuged, as well as their resulte filtrates have been assessed for enzyme activity, protein content, and specific activity (Hamzah et al, 2006, Bradford, 1976).

**Optimal Carbon Sources**

50 mL of purified fermentation media was produced in 250 mL flake Erlenmeyer, with the pH calibrated at 7.0. The mixture underwent an autoclave at 121°C for a duration of 15 minutes. 2% for every solution's sugar after sterilizing (lactose, glucose, sucrose, fructose, and starch) was individually introduced into the flasks in duplicate, employing a 0.22 µm filter for sterilization. Each flask was infected with 2% of an overnight culture. A culture of *S. aureus* an amount of $3\times10^9$ cells/mL was left to incubate at 37°C for 24 hours in a shaking incubator rotating at 120 rpm (Pathak and Rathod, 2017). Subsequent to incubation, the cultures underwent centrifugation, and the supernatants were collected for the assessment of enzyme activity, protein concentration, and specific activity (Hamzah et al, 2006, Bradford, 1976).

**Impact of nitrogen source**

The gelatinase production medium was enriched with various combinations of peptone and yeast extract in different ratios, including 0.5 g yeast extract with 2 g peptone, 2 g yeast extract with 0.5 g peptone, 0.5 g yeast extract alone, 2 g peptone alone, and 1.25 g yeast extract with 1.25 g peptone. The pH of the medium was adjusted to 7.0. Following sterilization, 2% of an overnight culture containing $3\times10^9$ cells/mL of the selected isolate was introduced into the medium. The flasks were incubated at 37°C for 24 hours in a shaking incubator at 120 rpm. Following incubation, the cultures were subjected to centrifugation, and the supernatants were harvested to assess enzyme activity, protein

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content, and specific activity (Hamzah et al, 2006, Bradford, 1976).

**The impact of pH**

After determining which nitrogen source had the maximum gelatinase production, the medium's starting pH was changed to evaluate enzyme synthesis. 50 mL of the optimized medium was placed in each of 250 mL Erlenmeyer flasks, pH was adjusted to 5, 6, 7, 8, and 9. Following autoclave sterilization, a S. *aureus* culture was added to the flasks at a concentration of $3\times10^9$ cells/mL. The cultures were kept in a shaking incubator running at 120 rpm for 24 hours at 37°C. Enzyme activity, protein content, and specific activity were assessed after incubation.

**Extraction of crude gelatinase**

Following cultivation in the appropriate producing medium, cells have been separated using centrifuged in 10,000 rpm for 10 minutes. The resulting supernatant has been employed to assess enzyme action, the concentrated of protein, and additional purifying procedures.

**Gelatinase production**

The gelatinase in the S. aureus R54 isolated has been isolated through gel filter chromatography (on a Sephadex G-150 column. The column (1.7 × 29 cm) was packed and prepared according to the manufacturer's instructions (Pharmacia, Sweden). The crude enzyme was loaded onto the column, and elution was carried out using 0.2 M sodium phosphate buffer at pH 7.0, with a flow rate of 20 mL/h, collecting fractions of 3 mL each. Protein concentration in the fractions was measured at 280 nm, and enzymatic activity was determined. The active fractions were combined, and their volume, enzymatic activity, and protein concentration were documented. The purified enzyme was then concentrated, aliquoted, and stored at low temperatures for future use (Mäkinen and Mäkinen, 1994).

**Characterization of partial purified gelatinase**

**Impact of temperature of gelatinase action:** The activity of the partially purified gelatinase was assessed at several temperatures (30°C, 37°C, 45°C, and 50°C) to identify the best temperature for enzymatic performance. The correlation between temperature and enzyme activity was examined to determine the optimal temperature for enhancing gelatinase activity (Mazotto et al, 2011).

**Impact of temperature on gelatinase stability:** After 15 minutes of exposure to different temperatures (30, 37, 45, and 50°C), partially purified gelatinase was promptly cooled in an ice bath. The percentage of residual activity was then calculated when the enzyme activity was assessed at its optimal operating temperature (Shen, 2015).

**Impact of pH on gelatinase action**

The activity of partially purified gelatinase was assessed at different pH levels using gelatin as a substrate. Various buffer solutions were employed, including 0.02 M sodium acetate buffer (pH 5 and 6), 0.02 M sodium phosphate buffer (pH 7.0), and 0.02 M Tris-base buffer (pH 8.0 and 9.0). Enzyme activity was measured, and a graph was plotted to illustrate the relationship between activity and pH, allowing determination of an appropriate pH to gelatinase action (Mazotto et al, 2011).

**Impact of different pH levels on stability of partially purified gelatinase**

Equal volumes of the partially purified enzyme and buffers with pH levels ranging from 5 to 9 were mixed in a 1:1 ratio. The mixtures were incubated at 37°C in a water bath for 15 minutes, then immediately cooled in an ice bath to stop the reaction. The residual enzymatic activity (%) was measured, and a graph was plotted to analyze the relationship between residual activity and pH, determining the optimal pH for gelatinase stability (Bhagwan *et al.* 2015).

**Gelatinase specificity**

Gelatin, BSA, and casein were used to investigate how various substrates affected the activity of gelatinase. One gram of each substrate was dissolved in 100 ml of 0.02 M sodium phosphate buffer at pH 7.0. The relationship between enzyme activity and the various substrates was assessed to identify the most effective substrate for optimal gelatinase activity (Ekpenyong et al, 2016).

**Impact of certain chemical substances on gelatinase action**

The influence of chemical substances such as CaCl2, NaCl, HgCl2, ZnSO4,

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mercaptoethanol, EDTA, and cysteine on the activity of pure gelatinase was examined. Each solution was prepared at doses of 5 mM and 10 mM by dissolving in 0.02 M phosphate buffer at pH 7.0. The enzyme solution was incubated with a metal ion solution at a 1:1 (v/v) ratio for 15 minutes at 37 °C. Thereafter, the enzyme activity was evaluated and contrasted with a control representing the untreated enzyme, followed by the calculation of the percentage of residual activity (Femi-Ola et al, 2014).

**Impact of certain plant extracts upon gelatinase action:** The plant utilized during this research was procured from the local market; they include: Avocado (*Persea americana*), Tomato (*Solanum lycopersicum*), Potato (*Solanum tubersum*) Soy bean **(***Glycine max*), Aloe vera (*Aloe vera*), and Turmeric (*Curcuma longa*). Tomato extract was prepared using gauze, the inhibitors extract from Avocado, One gm of each plant was homogenized with 10 milliliters of 0.02M phosphate buffer pH 7.0 to extract the aloe vera and potato. And the inhibitors extracts from Turmeric and Soy bean were grinding by electronic grinder then their extracts prepared with 0.02 M of phosphate buffer pH 7.0. All plant extracts were centrifuged at 10,000 rpm for 15 minutes, and the resulting clear supernatant was considered the crude extract. This extract was used to evaluate gelatinase inhibitor activity by incubating each plant extract individually with an equal volume of the enzyme (1:1 ratio) for 30 minutes at room temperature. Subsequently, gelatinase activity was measured. A control sample, representing 100% enzyme activity, was prepared under the same conditions but without any inhibitory extract (Lima et al, 2020).

The following formula was used to estimate the inhibitory activity:

$$\textit{Gelatinase inhibition activity} = \frac{E\ without\ I - Ewith\ I}{E\ without\ I} \times 100$$

Where:

E= Enzyme, I= Inhibitor (Priya et al, 2020).

**Antibacterial assay**

The agar well diffusion method was used to evaluate antibacterial activity. The bacterial strain employed in this study, *S. aureus* R54, was cultured in nutrient broth at 37°C for 24 hours to achieve a concentration of $1\times10^8$ CFU/mL. The bacteria were then spread evenly on the surface of agar plates. Wells were aseptically created using a sterile cork borer and filled with 100 µL of crude plant extracts, including Avocado, Tomato, Potato, Soybean, Aloe Vera, and Turmeric. The plates were incubated at 37°C for 24 hours. Antimicrobial activity was assessed in triplicate Through determining inhibitory area diameter (in millimeters). Water played the role of a negative controls (Bisht et al, 2016).

## RESULTS.AND.DISCUSSION

**Initial Assessment of Gelatinase (Qualitative Evaluation Utilizing Gelatin Plate Agar Media):** A total of 100 isolated bacteria had been qualitative assessed employing gelatin plates agar media as well as a clear hydrolysis zone assay. The performance of the bacterial isolates has been determined through calculating the width of colorless zones surrounding the wells. Among all *S. aureus* isolates, forty-four demonstrated gelatinase production, indicated by a distinct zone of hydrolysis observed around the wells containing the isolates on the gelatin plate agar (Fig. 1). The isolate's area of clarity proportion ranged from 1.3 to 4.5 cm. These isolates were selected for additional quantitative evaluation. The size associated with clear area varied across various isolates. This result aligns with (Balan et al, 2012), who investigated the capacity of bacteria to create gelatinase on solid plate medium by creating wells. He observed variability in the diameter of the clear zones surrounding the wells, which depends on the bacterial species and the kind of medium used.

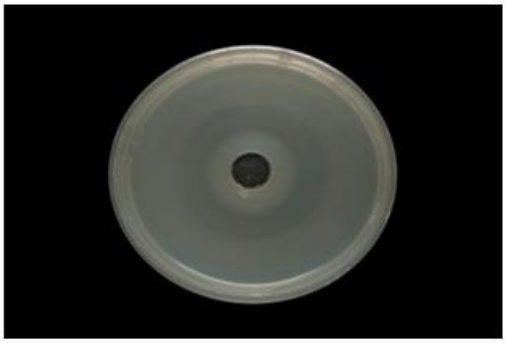

**Figure 1. Gelatinase activity of *S. aureus* on solid medium at 37◦C for 24 h.r**

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**Secondary Screening (quantitative screening) of gelatinase production**: To further the analysis and selection process, *S. aureus* isolates exhibiting the most extensive hydrolysis zones from the initial screening were subsequently assessed for their enzymatic activity by the submerged fermentation method. *S. aureus* R54 demonstrated the greatest enzymatic activity, displaying 12.3 U/mg protein gelatinase action within crude supernatant. Other isolated had 0.339–9.93 U/mg particular action. Due to its superior specific activity, the *S. aureus* R54 isolate was chosen for subsequent studies. The production of gelatinase, which is an enzyme that hydrolyzes gelatin, can indeed vary among members of the same species due to several factors. Genetic variation plays a significant role, as not all bacteria have a gene for a gelatinase enzyme. The type and source of isolates can also influence enzyme production, as well as cultivation conditions like media components, temperature, pH, aeration, and stirring (Ekpenyong et al, 2016). These factors influence enzyme expression and activity, affecting the bacteria's capacity to degrade gelatin and potentially enhancing their pathogenicity. They also enhance the capability of the R54 Separate for creating enzymes in a fluid environment.

**Optimal Conditions to get Gelatinase Synthesis**

**Impact for Fermentation Medium:** We grew this S. aureus R54 isolate in six different media, was used to evaluate the effects of different Impact of fermenting medium for gelatinase synthesis. Medium 7 had been considered to be greatest effective, attaining the highest particular action being 14.8 U/mg proteins. In comparison, exact action by gelatinase in media 2, 3, 4, 5, and 6 was lower, recorded as 7.4, 3.2, 12, 12.5, and 6.3 U/mg protein, respectively (Fig. 2). Consequently, medium 7 was selected to optimize the remaining fermentation parameters. Since each microorganism has unique requirements for maximizing gelatinase production, Therefore crucial for optimizing the fluid's composition along with the surrounding circumstances. optimum circumstances to creating enzymes differ markedly based upon the microorganisms, necessitating careful adjustment about the growth as well as culture parameters (Walpola,2013).

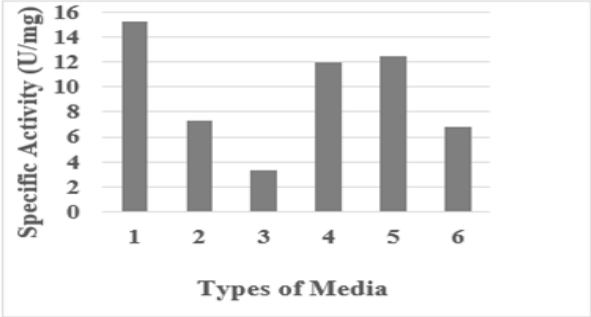


**Figure 2. Production of gelatinase in different liquid media by *S. aureus* R54 using submerged culture using shaker incubator 120 rpm at 37°C for 24 hrs.**

**Optimum carbon source:** Suppliers of carbon were introduced into the synthesis mixture around 2% (w/v) to determine their effect upon gelatinase production. Some of these five tested suppliers of carbon, fructose demonstrated the highest efficacy, enhancing the specific activity to 16.3 U/mg, compared to 4.41 U/mg in the glucose-containing medium (Fig. 3). Carbon is fundamental to microbial growth, serving as a primary source for many microorganisms. Thus the substance is extensively utilized as a fundamental element for enhancing microbe proliferation along with metabolic synthesis. (Pathak and Rathod, 2017).

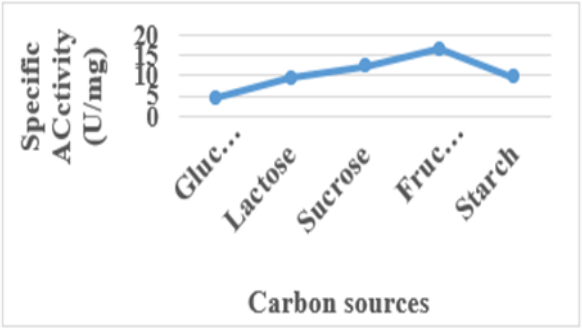


**Figure 3. Effect of carbon sources on gelatinase produced by *S. aureus* R54 utilizing submerged culture pH 7.0 at 37°C for 24hrs**

**Optimal sources of nitrogen:** Different nitrogen sources influence on gelatinase production was assessed utilizing the optimal carbon sources. The combination of extracting yeast along with peptones in a 2:0.5 ratio was

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determined to be the most efficacious, facilitating the proliferation of *S. aureus* R54 and augmenting enzyme production, with a specific activity of 33.5 U/mg (Fig. 4). Medium for cultivation nitrogen types as well as concentration are critical parameters for gelatinase synthesis. The highest enzyme action occurred within a medium supplemented with extracted yeast and peptones. Extracted yeast, being an easily digestible protein source, is especially conducive to microbial proliferation. Consequently, the amalgamation about extracted yeast and peptones had been recognized to be an optimal nitrogen supply in protease creation. (Joseph et al, 2019).

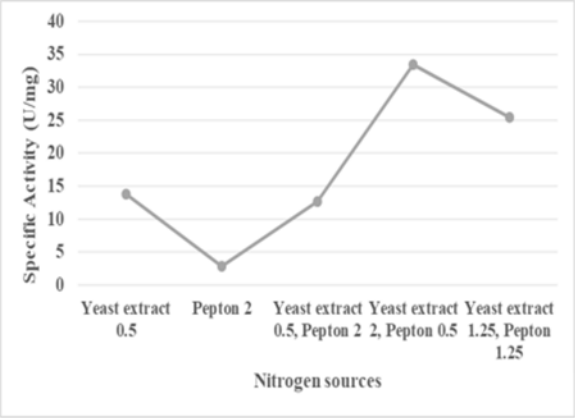


**Figure 4. Effect of nitrogen sources on gelatinase production from *S. aureus* R54 at 37°C in shaker incubator 120 rpm pH 7.0 for 24 hrs.**

## Ideal pH creation

*S. aureus* R54 had been grown within an extraction medium having initial pH valus of 5, 6, 7, 8, as well as 9 to assess the impact for pH levels on gelatinase synthesis. This peak specific activity about gelatinase, 50.3 U/mg had been attained with pH 9.0, shown illustrated in Fig. 5. Any variation from this pH, whether elevated or diminished, led to a reduction in enzyme activity. The pH is crucial for enzyme production as it impacts nutrient solubility in the media, modifies substrate ionization and availability for microorganisms, and affects enzyme stability (Mazotto et al, 2011).

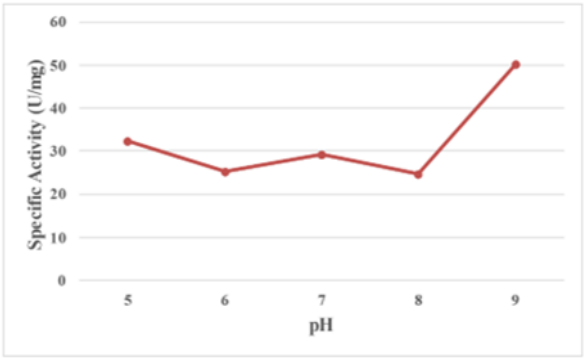


**Figure 5. Influence of various values of Ph (5.0-9.0) on partial purified gelatinase activity from *S. aureus* R54**

**Impact of Temperature upon Gelatinase** data in Fig. 6 demonstrate thier ability about *S. aureus* R54 to growing and producing gelatinase across a broad temperature range (30, 37, 45, and 50°C). The highest gelatinase production, exhibiting a particular action at 53.737 U/mg , was achieved at 37°C. Lower specific activities were observed at 30°C and 50°C, yielding 43.3 and 43.2 U/mg protein, respectively. Temperature is a critical factor influencing microbial growth and metabolic processes, significantly impacting enzyme creation (Mazotto et al, 2011).

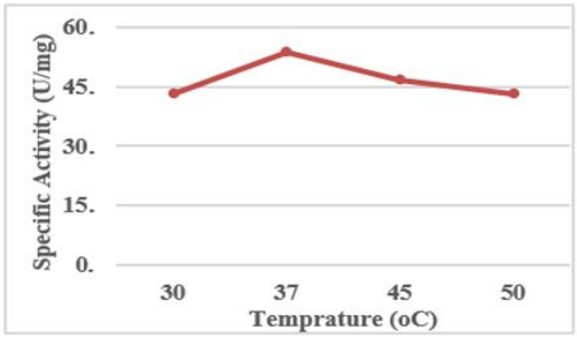


**Figure 6. Impact of temperature of gelatinase production from *S. aureus* R54 using shaker incubator 120 rmp for 24hr**

**Impact of Incubation Periods on Gelatinase Synthesis:** optimal incubating duration for bacterial growth and gelatinase production was investigated. As shown in Fig. 7, The maximum specificity of 54.3 U/mg protein had been achieved following 24 hours of incubated by S. aureus R54. Beyond this period, specific activity declined, likely as a result of alterations of cultural conditions, including a lack of oxygen as well as nutritional resources or the accumulation of toxic metabolites that hinder bacterial growth. Incubation time serves as a critical determinant affecting microbial

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growth and enzyme secretion. Researchers have noted that enzyme production typically begins within the beginning phases of grown as well as continues during following few hours of microbial development (Nour El Dein et al, 2022).

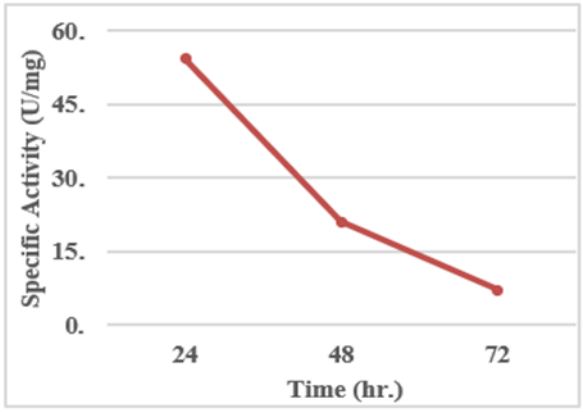


**Figure 7. Effect of incubation period on gelatinase production from *S. aureus* R54 in shaker incubator 120 rpm at 37°C**

## Purification of gelatinase

*S. aureus* R54 was cultured under optimal conditions to maximize gelatinase production. The enzyme has been extracted via centrifuged at 10,000 rpm for 10 minutes. The crude gelatinase had been further refined to eliminate extraneous proteins as well as additional constituents via gel filtration chromatography with a Sephadex G-150 column, in accordance with the designated protocol:

## Gel filtration chromatography

A 1.7 x 29 cm Sephadex G150 gel filtration column that had been previously equilibrated utilizing a 0.2 M sodium phosphate buffer at pH 7.0 was exposed to a crude enzyme solution. In addition to a peak of gelatinase activity in fractions (19-25), the data showed a distinct proteins pattern within the drained fraction across this column (Figure 8). As shown in Table 1, the yield was 98.2% and the activity reached 90 U/mg having a purifying coefficient of 1.2.

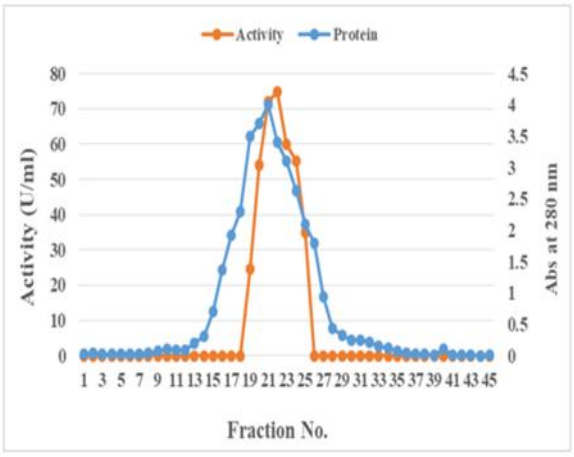


**Figure 8. Gel Filtration chromatography for gelatinase purification from *S. aureus* R54 by using Sephadex G150 column (1.7 x 29) cm equilibrated and eluted with phosphate buffer (0.2 M, pH 7), in flow rate 20 ml/hr. 3 ml for each fraction**

**Table 1. The purifying procedures for gelatinase derived from *S. aureus* R54**

| Sample | Volume (ml) | Activity (U/ml) | Protein (mg/ml) | Specific Activity (U/mg) | Total Activity (U) | Purification Fold | Yield (%) |
|---|---|---|---|---|---|---|---|
| Crude Gel | 10 | 154 | 2.07 | 74.4 | 1540 | 1 | 100 |
| Filtration with Sephadex G-150 | 21 | 72 | 0.8 | 90 | 1512 | 1.2 | 98.2 |

## Characterization of gelatinase

**Impact of temperature on gelatinase activity:** The action of partly refined gelatinase has been assessed with temperature ranging from 30°C to 50°C. Figure 9 illustrates that the activity reached its zenith at 37°C, recording a value of 102.6 U/mL. Exceeding this temperature, the activity diminished, attaining a nadir of 6 U/mL at 45°C and thereafter declining to 3 U/mL at 50°C. At 30°C, the specific action was measured at 49.8 U/mL. Temperature, conversely, exerts numerous impacts on enzyme reactions, including pH (Mäkinen and Mäkinen, 1994, Gaurav et al, 2014).

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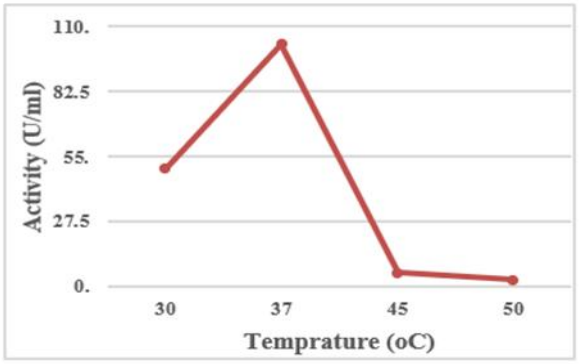


**Figure 9. Effect of different temperature on partial purified gelatinase activity from *S. aureus* R54**

**Impact of temperature on stability of gelatinase :** The durability for gelatinase with various temperatures has been tested with incubation the enzyme that had been isolated at temperatures ranging from 30°C to 50°C for 1 minute. Followed by measuring the percentage of residual activity. The current study demonstrated that gelatinase action had been maintained at temperatures ranging from 30 to 50°C (Fig. 10); however, a decline in activity was noted when temperatures rose from 45°C to 50°C. The reduced activity of gelatinase at temperatures exceeding 45°C is due to its sensitivity to elevated temperatures; The enhanced thermal endurance for gelatinase via immobilized has become advantageous for the disinfection of effluents at elevated temperatures. S. aureus R54 gelatinase had similar thermal endurance compared with different enzymes, as shown in reference (Flood et al,2000), which demonstrated that the enzyme from Bacillus subtilis maintained stability when incubated for 30 minutes at 30-40°C.

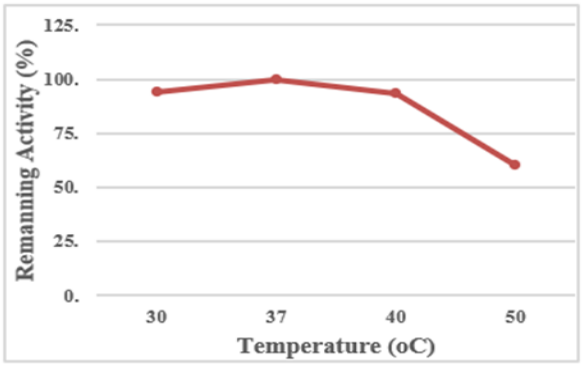


**Figure 10. Effect of different ranges of temperature on stability of partial purified gelatinase from *S. aureus* R54**

**Impact of pH on gelatinase action**

The impact of pH upon the partially isolated gelatinase form *S. aureus* R54 was assessed across a pH spectrum of 5.0 to 9.0, as illustrated in Fig. 11. The optimal activity occurred at pH 9.0, achieving 228.6 U/ml, while the activities at pH 5, 6, 7, and 8 were 60 U/ml, 85.8 U/ml, 115.8 U/ml, and 193.8 U/ml, respectively. The alteration in the ideal pH level has been credited by electrical interactions affected through the carrier's environment (Mazotto et al, 2011).

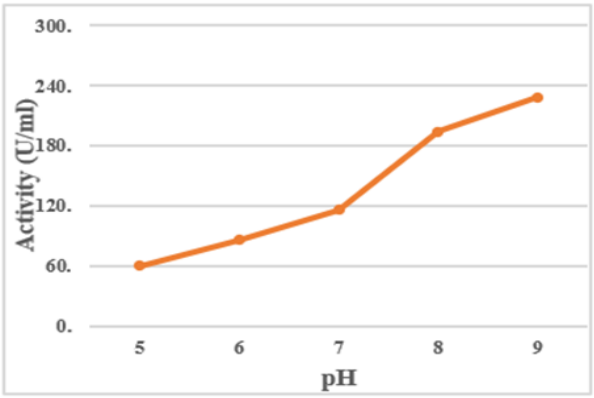


**Figure 11. Effect of different pH values (5.0- 9.0) on partial purified gelatinase activity from *S. aureus* R54**

**Impact of pH values on stability of gelatinase:** Figure 12 illustrates that the optimal pH range for gelatinase stability is between 6.0 and 8.0, with the enzyme maintaining approximately 100% activity at pH 7.0; enzyme activity diminishes on either side of this optimal pH range. The residual activities were 20% at pH 6.0, whereas enzymatic activity was minimal at nearly acidic pH, reaching 6.7% at pH 5.0. Enzyme activity diminished with alkaline pH levels, exhibiting a reduction of 61.3% at pH 8.0 and 57.3% at pH 9.0. (Bhagwan *et al,* 2015).

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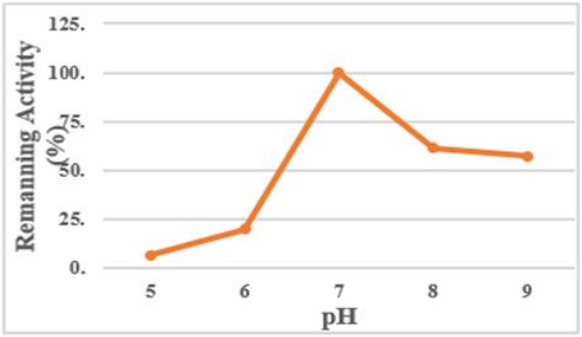


**Figure 12. Effect of different pH values (5.0-9.0) on partial purified gelatinase stability from *S. aureus* R54**

**Substrates specificity of gelatinase**

The ideal substrate for gelatinase activity was determined by incubating the purified enzyme with several substrates, such as BSA, gelatin, and casein, at pH 9.0 and 37°C. Figure 13 demonstrates an increase in enzyme activity with gelatin as a substrate, reaching 232 U/ml, followed by BSA at 186 U/ml, and casein at 157.8 U/ml. The results aligned with prior studies that identified gelatin as a specific substrate for the gelatinase enzyme produced by microorganisms, in accordance with the reference (Jones et al, 1998).

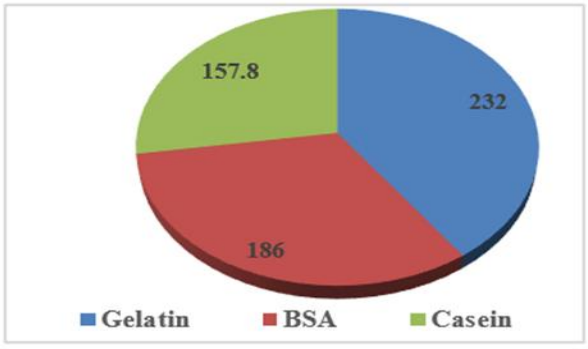


**Figure 13. Effect of different substrates on purified gelatinase activity produced *by S. aureus* R54 at pH 7**

**Impact some chemical compounds on gelatinase activity:** Chemical agents were employed to process pure gelatinase derived from S. aureus R54. The results in Table 2 indicate that certain compounds activated the gelatinase enzyme, while others inhibited it. Metal ions affect gelatinase activity differently based on the enzyme's source; nonetheless, at concentrations of 5 and 10 mM, ZnSO4, CaCl2, and NaCl were demonstrated to enhance gelatinase activity beyond the control value. At concentrations of 5 mM and 10 mM, ZnSO4 activated the enzyme, resulting in residual activities of 123.7% and 110.09%, respectively. CaCl2 and NaCl enhanced enzyme activity, exhibiting residual activities of 111.11% and 131.0% at concentrations of 5 mM, and 125% and 112.15% at 10 mM, respectively. This indicates that enzyme activity fluctuates with increasing concentrations, as shown in Table 2.Recent findings reveal that ions affect enzyme activity differently depending on their type and concentration, with these effects often becoming more pronounced at higher concentrations (Li et al, 2022). The findings demonstrated that HgCl2 inhibited the enzyme at doses of 5mM and 10mM, resulting in residual activities of 30% and 18% respectively, implying the presence of SH groups in the enzyme's active site, which were oxidized by HgCl2. Furthermore, the inclusion of $HgCl_2$ in the substrate processing solution may result in the formation of a complex with the enzyme, hindering its binding to the substrate and blocking product synthesis. EDTA was utilized to assess the effect of inhibitors on gelatinase activity. The residual enzyme activities exhibited a decline in enzyme activity with an elevation in inhibitor concentration. Incubation of pure gelatinase with EDTA at doses of 5 mM and 10 mM resulted in residual activities of 25% and 16%, respectively (table 2). The findings demonstrated that the enzyme was a metalloenzyme and that divalent ions were essential for the reported activity. The enzyme exhibited a reduction of 90% and 85% in activity when treated with cysteine and β-Mercaptoethanol at a concentration of 5mM, respectively. And lost 96% and 91.1% of its activity, respectively, at 10 Mm. Incubation of an enzyme with reducing agents like cysteine and β-Mercaptoethanol diminishes the disulfide bonds within the protein's structure, leading to fragmentation into its constituent parts, hence adversely affecting its activity (Priya et al, 2020). The findings indicated that the enzyme being studied included disulfide linkages. The reduction in activity results from the formation of complexes with the enzyme, inhibiting its binding to the substrate and subsequent conversion to product (Mohamed et al, 2017).

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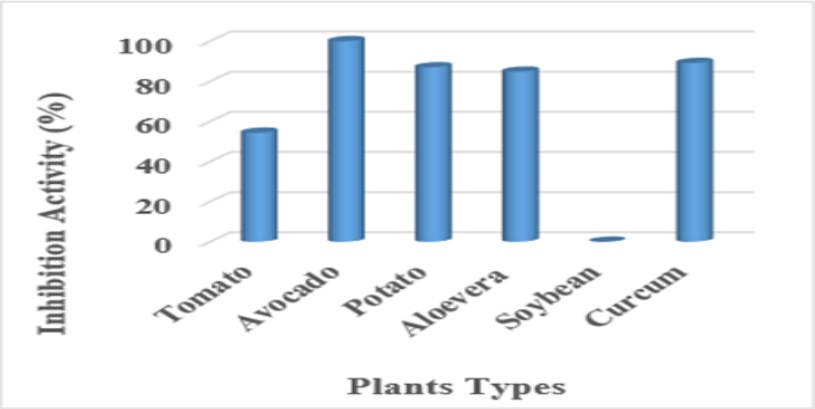


**Figure 14. Effect of some plants extracts on purified gelatinase activity from *S. aureus* R54**

**Table 2. The effect chemical compounds on purified gelatinase activity for 15 min at 37 °C**

| Chemical Compounds | Concentrations | Remaining Activity |
|---|---|---|
| Control | - | 100% |
| ZnSO4 | 5 | 123.7 |
| | 10 | 110.09 |
| CaCl2 | 5 | 111.11 |
| | 10 | 125 |
| NaCl | 5 | 131.0 |
| | 10 | 112.15 |
| HgCl2 | 5 | 30 |
| | 10 | 18 |
| EDTA | 5 | 25 |
| | 10 | 16 |
| Cysteine | 5 | 10 |
| | 10 | 4 |
| β- Mercaptoethanol | 5 | 15 |
| | 10 | 8.9 |

**Effect some plants sources on gelatinase activity:** This work utilized indigenous plants as a source of inhibitory material for pure gelatinase derived from *S. aureus* R54. The results in Fig. (14) indicated that Avocado inhibited gelatinase by 100%, followed by Aloe vera at 97.82%. The inhibition ratio of gelatinase decreased with the application of other plant materials, with Tomato exhibiting the lowest inhibition activity at 54.34%. Although soybean exhibits no inhibitory activity, refer to Fig. (14). Plants are a crucial group for gelatinase inhibition and are regarded as essential for human health. Natural products are secondary metabolites synthesized by living organisms, including plants and microbes (Dias et al, 2012). The plant world serves as a plentiful and readily available source of natural compounds, with a significant fraction yet to be investigated for potential bioactive metabolites (Dias et al, 2012). These compounds exhibit significant chemical and structural diversity unmatched by synthetic compound libraries (Cragg et al,2014) and have developed inherent bioactive properties owing to their evolutionary biological functions in living organisms (Beutler, 2009). Consequently, natural products represent a compelling source of medicinal compounds, and extensive research is dedicated to the identification of pharmaceuticals derived from natural product extracts, including those from plants (Rodríguez-Carpena et al, 2011).

**Antibacterial activity:** The result showed that the highest inhibition zone was found in the extract of Avocado plant. inhibition zones was filled the whole plate, Figure 16 an inhibitory effect was found in turmeric extracts (13.1) mm. Variable degrees of antibacterial activity

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were found from the other plants extracts of Tomato, Potato, Soy bean and Aloe vera, with inhibition zone diameters as (18) mm, (37.5) mm, (20.7) mm, (22) mm respectively. The antimicrobial activity of avocado extracts may be as cribable to its chemical composition. Phytochemical screening highlighted the presence of phenolic compounds in avocado tissues, whose antimicrobial activity is well documented (Phuong et al, 2021).


**Figure 16. Antibacterial activity of Avocado extract against *Staphylococcus aureus***

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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# تقييم الفعالية التثبيطية لبعض المستخلصات النباتية تجاه الجيلاتينيز المنقى من المكورات العنقودية

رائد مضاء صبيح، سحر رحيم حسين

جامعه بغداد / كليه العلوم – قسم التقنيات الاحيائية

**المستخلص**

**هدفت الواسه الحالية الى تثبيط انزيم الجيلاتينيز المنقى من الغزلة المحلية لبكتريا *S. aureus* بوساطه مستخلصات بعض النباتات المحلية، تم عزل 100 عزلة من بكتريا *S. aureus*، شخصت هذه العزلات بوساطه الاختبارات الكيموحيويه وخضعت لعملية الغربلة الاولية باستخدام الوسط الصلب لتحديد العزلة الاكفأ في انتاج الانزيم، شخصت 44 عزلة كفوءة اعتماده على نسبه (النمو/ الهاله الشفافه) على الوسط الصلب. ادخلت هذا العزلات الى الغربلة الثانوية باستخدام الوسط السائل، انتخبت العزلة *S. aureus* R54 بكونها الاكفا في انتاج الانزيم اذا بلغت الفعالية النوعية 12.3 وحدة / ملغم بروتين. حددت الظروف المثلى لانتاج الانزيم اذا بلغت 54.3 وحدة / ملغم بروتين باستخدام الوسط (1) المجهز بـ 2% فركتوز كمصدر كربوني امثل و 2% مستخلص خميرة/ 0.5 ببتون كمصدر نتروجيني امثل بدرجه حرارة 37م وبرقم هيدروجيني 9 بعد 24 ساعه من الحضانة. نقي انزيم الجيلاتينيز باستخدام كروماتوغرافيا الترشيح الهلامي بوساطه هلام السيفادكس ج-150 بعدد مرات تنقيه 1.2 وبحصيلة انزيمية 98.2 %. اظهر الانزيم المنقى اعلى فعاليه وثباتيه عند درجه حرارة 37 م. كما اظهر الانزيم المنقى اعلى فعاليه عند رقم هيدروجيني 9.0 واعلى ثباتية عند رقم هيدروجيني 7.0، سجلت اعلى فعالية نوعيه للانزيم المنقى باستخدام الجيلاتين كافضل مادة اساس. ثبط الانزيم المنقى باستخدام مستخلصات بعض النباتات المحلية، اذا بلغت اقصى نسبه مئوية لتثبيط الانزيم المنقى عند معاملته مع مستخلص نبات الافوكادو اذا بلغت 100% كما امتلك مستخلص نبات الافكادو فعالية ضد البكتريا قيد الواسه (*S. aureus* R54).**

**كلمات مفتاحية: بكتريا، افوكادو، مثبطات نباتية، توصيف، سيفادكس ج-150**