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Inhibition of Hyaluronidase Enzyme Produced by *Staphylococcus* Bacteria Using Curcumin Phenolics of *Curcuma* Plant

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Abstract: Bacterial infections that caused by *Staphylococcus* bacteria remain a significant global health concern, necessitating the development of alternative treatment strategies. Hyaluronidase an enzyme produced by *Staphylococcus* enzyme that breaks down primarily hyaluronic acid thereby disrupting the structural integrity of the extracellular matrix (ECM) found in connective tissues which considered as virulence factor. The aim of this research was Study the effect of curcumin that separated from *Curcuma zanthorrhiza* as inhibitor agent for Hyaluronidase enzyme that isolated from *Staphylococcus aureus* which considered as virulence factor. The 120 clinical isolates were collected, Hyaluronidase enzyme was extracted and purification from *Staphylococcus aureus* bacteria that identified by performing standard laboratory techniques, and genotypic detection by *16S rRNA* gene, and the enzyme was inhibited using effective plant compounds that were isolated from plant extracts. *Results*: showed that 100 out of 120 clinical samples growth of bacteria hen cultured on blood agar medium. Also, it was found that from the 100 isolates, the 56 (56%) isolates belonged to *S. aureus*. In order to test the ability of *S. aureus* isolates to produce hyaluronidase enzyme, the inhibition zones were measured by using brain heart serum albumin medium. The results revealed some that *S. aureus* isolates were able to produce hyaluronidase with zones of hydrolysis ranged between 5-20 mm. Among them, isolate Staph 21 was the most efficient in on by giving highest diameter of hydrolysis (20 mm) on brain heart serum albumin medium. It was concluded that the burn samples had more *S. aureus* than other bacterial isolates. A synthesis medium containing 0.5% yeast extract and 0.25 mg hyaluronic acid, pH7, and 37°C, and ammonium saturation to generated the enzyme. Curcumin inhibits hyaluronidase in certain quantities.

Keywords: Curcumin, Hyaluronidase Inhibitor, and *S. aureus*.

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Introduction

Curcumin is a natural polyphenolic compound found in the spice turmeric (*Curcuma zanthorrhiza*). It has been extensively studied for its various biological activities, including anti-inflammatory, antioxidant, and anti-microbial properties. Some research suggests that curcumin may also have inhibitory effects on hyaluronidase (1).

Hyaluronidase is an enzyme involved in the breakdown of hyaluronic acid, a major component of the extracellular matrix. Inhibiting hyaluronidase activity could potentially help in preventing the spread of infections and reducing tissue damage caused by certain pathogens (2).

Studies have investigated the potential of curcumin as an inhibitor of hyaluronidase activity. Curcumin is believed to inhibit hyaluronidase through its anti-inflammatory and antioxidant properties. By reducing inflammation

and oxidative stress, curcumin may indirectly inhibit the activity of hyaluronidase (3).

In the context of bacterial infections, hyaluronidase can be produced by certain bacteria, including Staphylococcus species, such as *Staphylococcus aureus*. *Staphylococcus aureus* is a pathogenic bacterium that can cause a range of infections in humans, from skin and soft tissue infections to more severe systemic infections (4). The best method for identification of bacteria is based on molecular techniques by amplification of the *16S rRNA* gene (5). The role of hyaluronidase in *Staphylococcus* infections is thought to involve breaking down the host's connective tissues, including hyaluronic acid, which can facilitate the spread and invasion of the bacteria within the host. By breaking down the extracellular matrix, hyaluronidase can contribute to tissue damage and inflammation, allowing the bacteria to access deeper tissues and potentially evade the host's immune responses (4).

Inhibition of hyaluronidase activity could potentially be a therapeutic strategy to limit the invasive potential of *Staphylococcus aureus* and other bacteria that produce this enzyme. However, it's important to note that the development of such inhibitors is a complex process and requires thorough research to ensure their safety and effectiveness (6).

It's worth mentioning that while hyaluronidase is associated with bacterial infections, the development of treatments targeting hyaluronidase activity is just one aspect of the broader efforts to combat bacterial infections. Antibiotics, vaccines, and other strategies remain crucial in managing and preventing bacterial infections caused by *Staphylococcus* and other pathogens (7).

Materials and methods

Sampling

Medical samples were collected from 120 patients at the Al-Yarmouk Educational Hospital in Baghdad, Iraq. The specimens were burn swabs.

Isolation of bacteria

Bacteria were first isolated as pure colonies on Blood agar and Mannitol salt agar, and then they were examined microscopically using the Gram's stain technique. Symmetry tests were then carried out on all the bacterial isolates to determine their cultural, physiological, and morphological properties (8).

Primary screening of hyaluronidase

The primary screening of hyaluronidase activity typically involves the detection of its ability to break down hyaluronic acid. This can be done using a variety of methods, such as the following:

1. Hydrolysis zone test: This is a common screening test used in microbiology laboratories to identify bacteria that produce hyaluronidase.
2. Gel electrophoresis: Hyaluronidase can be detected by running a sample of the enzyme through a gel electrophoresis system.
3. Spectrophotometric assay: This method measures the ability of hyaluronidase to break down hyaluronic acid by monitoring the absorbance of light at a specific wavelength.

Overall, the primary screening of hyaluronidase activity is important for identifying potential sources of the enzyme and for characterizing enzymatic properties (9).

Extraction and isolation of hyaluronidase

Plate method: Brain heart serum albumin (BHSA) agar inoculation with *S. aureus* $10^8 \times 1.5$. The plate was then incubated at 37°C for 24hrs. After incubation, the plat was submerged for

(2N) acetic acid for 10minutes. Diameters of the hydrolyzed or clear zone (production of hyaluronidase) by the isolates were measured in mm (10).

Enzyme purification

S. aureus in thin tryptic soy broth was grown overnight at 37°C, and it was centrifuged on (8,000Xg) for 30minutes using a cold centrifuge. Take the extracellular hyaluronidase-containing supernatant. Hyaluronidase is concentrated by ethanol precipitation. The supernatant was centrifuged on (8,000Xg), using cooling centrifuge for 30minutes, and then chilled with dry ice and mixed with ethanol (95%) to get the final concentration of 33% ethanol. The precipitate, which had the majority of the enzyme activity in it, was then suspended in 10 ml of sodium phosphate buffer (pH6), containing 0.05 M NaCl, after being taken. The dialysate was run against a phosphate buffer (11).

Optimum conditions for hyaluronidase production

The optimum conditions for hyaluronidase production may vary depending on the source of the enzyme, but here are some general factors that can affect hyaluronidase production:

1. Media: BHIB, nutrient broth and BHIBSA are used, the best are BHIB
2. pH: The pH range for optimum hyaluronidase production can vary, but it is typically around pH 7.
3. Temperature: Hyaluronidase production is typically highest at temperatures between 37°C.
4. Incubation periods: Effect of different Incubation periods of the selected isolate of *Staphylococcus aureus* on hyaluronidase production was studied.

Optimization of these factors is typically required to achieve the highest possible levels of enzyme production.

Hyaluronidase activity assay

By utilizing HA sodium salt as a substrate in a turbidity reduction test, hyaluronidase activity is evaluated spectrophotometrically (12). When 1mL of HA at 70mg/mL was incubated with 1mL of enzyme sample in the presence of 0.05M sodium phosphate buffer with 0.05M NaCl at pH 7, the turbidity was reduced by enzymes. A decrease in turbidity was detected by measuring the absorbance at 600nm after the mixture had been treated for 30min with 2.5mL of acidified protein solution (1%w/v) bovine serum albumin fraction (BSA) in 0.5M sodium acetate buffer of pH 3.1. The blank was culture broth that hadn't been infected. The quantity of enzyme that results in a decrease in turbidity at 600nm (A600) under specific conditions was used to define one unit of hyaluronidase activity.

Isolation of *Curcuma zanthorrhiza* crude phenol (13)

Collection and preparation of plant material: Harvest fresh or dried turmeric plant roots. Clean and chop the plant material into smaller pieces to increase the surface area for extraction.

extraction:

a. Solvent extraction: Use a suitable solvent, (ethanol) to extract the alkali from the plant material. This can be done through techniques such as maceration.

Filtration and concentration:

a. After extraction, filter the solvent extract to remove solid plant residues.
b. Concentration of the solvent extract using techniques rotary evaporation under reduced pressure to obtain a crude extract.

Fragmentation and separation:

a. Perform liquid-liquid fractionation using an appropriate solvent system water and organic solvent to separate different classes of compounds.

b. Consider column chromatography, such as silica gel columns, to further separate the crude extract into fractions based on polarity.

Extraction acid-base extraction to selectively isolate phenols from the fraction) and purification of alkaloid by high-performance liquid chromatography (HPLC)

High performance liquid chromatography

The effective compounds (curcumin) were examined by using high performance liquid chromatography (HPLC) depending on condition HPLC (Shimadzu LC-2010 ATH) chromatogram of curcumin, the column of examine that is 250*4.6 mm 5-micron C18, flow rate1.5 ml/min, wave length λ 280 nm, mobile phase (80%-15% -5%-H_2O-methanol –Acetomatral), oven 40ºC, volume injection 20 µl, and weight 10g /100 ml. The results agree with this condition

Minimum inhibitory concentration (MIC) of curcumin

The minimum inhibitory concentration (MIC) of curcumin refers to the lowest concentration of an extract or compound derived from curcumin that can inhibit the growth of a particular microorganism, typically a bacterium or fungus. The MIC values for the curcumin against *S. aureus* ranged from 156.2- 20 µg/mL.

Minimum bactericidal concentration (MBC) of curcumin

The MBC (minimum bactericidal concentration) of curcumin refers to the lowest concentration of an extract or compound derived from curcumin that can completely kill a particular microorganism, typically a bacterium. Studies have investigated the antibacterial properties of curcumin phenols against various bacterial strains using the broth dilution method to determine the MBC. The MBC values for the different curcumin ranged from 156.2 µg/mL to 20.000 µg/mL Overall, these studies suggest that curcumin phenols have potential as a natural antibacterial agent, and further research is needed to determine the efficacy and safety of using curcumin phenol for this purpose

Inhibitory assay

The method adopted was described by Adu *et al*. (14) with slight difference. briefly 0.2ml of the crude enzyme extract and different concentration of curcumin severally (0.2,0.4,0.6,0.8,1ml] of 10 % w/v of plant crude in 1.8 of hyaluronic acid solution was added this reaction mixture was mixed and incubated at 37ºC for 10minutes this reaction was stopped by adding 2 ml of 5% tris-HCl acid assays was carried out and procedure was repeated without inhibitor (15).

Molecular assay

From a 1ml culture, genomic DNA was isolated with the use of a PrestoTM Mini gDNA Bacteria Kit (Geneaid, Thailand). Chromosomal DNA after purification was kept at -4°C. Nanodrop device was used to measure DNA concentration and purity. One µl of each DNA sample was used to measure optical density (O.D) at a wavelength 260 nm and 280 nm

Polymerase Chain Reaction (PCR) was performed to amplify specific DNA fragments using specific primer (The forward primer was 5'-ACG GTC TTG CTG TCA CTT ATA-3' and reverse primer was 5'-TAC ACA TAT GTT CTT CCC TAA TAA-3') that was performed for the detection of the gene associated with the identification of *S. aureus* (*16S rRNA*) (16). The reaction mixture contained template DNA (for chromosomal DNA or plasmid) (3 µl),

primers [1 µl from each of forward and reverse primers (10 poml)], and GoTaq®Green Master Mix (10 µl) and Complete the PCR mixture at 20 µl by adding deionized nuclease-free water. PCR was performed under the conditions for the uniplex PCR, 1 cycle for initial denaturation at 94˚C for 5mins, then 35 cycles from denaturation at 94˚C for 1mins, annealing at 52˚C for 30secs, and extension at 72˚C for 1mins, and 1 cycle for final extension at 72˚C for 8mins. The temperature and time of the PCR program were optimized using gradient PCR. The PCR products were visualized by gel electrophoresis using a safe stain.

Statistical analysis

The data obtained from this study were subjected to analysis using a one-way ANOVA test to determine the potential impact of the active compound on the inhibition of the Hyaluronidase enzyme.

Results and discussion

Collection of samples

Results showed that (100) out of 120 clinical samples growth of bacteria when cultured on blood agar medium. Also, it was found that from the 100 isolates the 56 (56%) isolates belonged to *S. aureus*. Our study additionally included genotypic identification of all isolates by PCR to identify the *16S rRNA* gene. Using an amplified size of 257bp from the *16S rRNA*, the results showed that all of the isolates were *S. aureus*. (Figure 1) shows the positive result bands bright in compared with a DNA ladder (100pb). This result like in (16) study is 53%, and more than in (17) study is 36%. Molecular techniques based on PCR have emerged as an alternate way for reliable identification and classification of bacterial species where the targeted gene for amplification is the *16S rRNA*. One of the most important factors in the categorization is the stability and conservation of the *16S rRNA* gene across time. This gene may play a significant role in diagnosis and typing after current diagnostic approaches are exhausted since it contains regions of high covariance among bacterial kinds, providing a unique sequence for each type (5).

Primary screening of the isolates

Table 1 showed larger inhibition zone (mm) of staph 6, staph21, staph33, staph50, and staph90, respectively upon treated with hyaluronic acid as a substrate solution around colony, then selected for secondary screening by shake flask fermentation method and further for hyaluronidase activity (Table 2). Results showed that 5 (8.9%) out of 56 isolates of *S. aureus* there were able to production of enzyme.

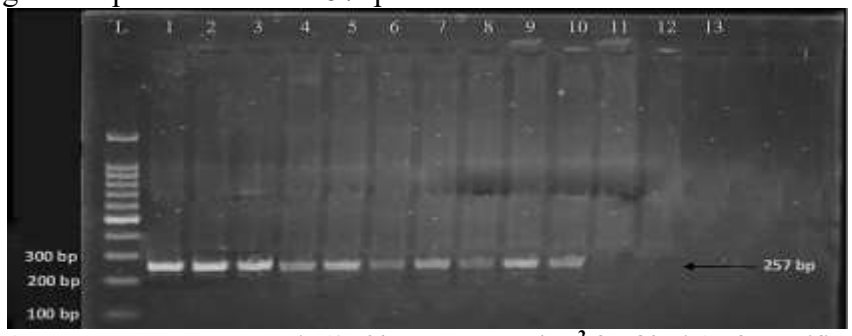

Figure (1): Agarose gel electrophoresis (1.5% agarose, 7 V/cm^2 for 80min) of amplified *16S rRNA* gene (257bp) for *S. aureus*. Lane L: 100bp DNA ladder. Lane 1-10: Amplicons *16S rRNA* gene for *S. aureus*. Lane 11-12: Control, DNA extracted from deferent bacteria isolates (*S. epidermidisand* and *Streptococcus pneumoniae*). Lane 13: Negative control (replacement of DNA template with water in the PCR mixture).

Table (1): Diameters of clear zones around colonies of *S. aureus* grown on brain heart serum albumin for 24 hours at 37°C.

Isolate	Diameter of clear zone (mm)
staph 6	16
staph 21	20
staph 33	15
staph 50	17
staph 90	18

Hyaluronidase production was detected in the 100 isolates the 56 (56%) isolates belonged to *S. aureus* bacteria while 44 isolates showed no hyaluronidase activity.

Table (2): Activities of hyaluronidase produced by *S. aureus* after 24hrs. with incubation at 37 C°, pH 7 on brain heart broth.

Number of isolates	Enzymatic activity (U/ml)
staph 6	6.321
staph 21	9.222
staph 33	6.7
staph 50	6.465
staph 90	6.512

The hyaluronidase plate assay method was employed to quantitatively estimate the production of hyaluronidase. The highest inhibition zone, measuring 20 mm, was observed with the isolation staph 21. A standard curve was used to calculate the hyaluronidase concentration, with the diameter of the clearance zone being directly proportional to the logarithmic concentration of the hysA enzyme. Among the *S. aureus* isolates, staph 21 exhibited the highest level of hyaluronidase production, measuring 6.7 U/ml (Table 3).

Table (3): Purification steps of hyaluronidase produced from *S. aureus* (staph 21).

Purification Step	Volume (ml)	Activity (U/ml)	Protein Conc. (mg/ml)	Specific Activity (U/mg)	Total Activity (U)	Purification Fold	Yield (%)
Crude Extract	50	6	0.070	97.1	300	1	100
Concentration by sucrose	18	7	0.044	159	126	1.8	42
Gel Filtration Chromatography (by Seph acryl S-300)	24	9	0.03	300	48	1.8	38

Result showed that this ratio gave specific activity of Concentration by sucrose 159 U/mg protein (Table 3), indicating observable increase in the specific activity compared to that of the crude extract (97.1 U/mg protein). Upon dialysis of crude hyaluronidase, results indicated in (Table 3) showed that an increasing in the activity of hyaluronidase when the specific activity reached to 300 U/mg protein. While the total activity was decrease at 58% from crude extract [we opinion because of laboratory environment and/ or because of the compound used in sedimentation (can using another such as Ammonium sulfate)]. Isolate staph 21 was characterized by being the most prolific in producing the enzyme by giving it the highest enzyme specific activity

estimated at 96 U.mg^{-1} proteins. The difference in the enzymatic activity of the different isolates may be due to the difference in the sources of isolating samples and to the difference in the expression rate of the genes encoding the enzyme.

Optimum conditions for hyaluronidase production

Due to its highest production of hyaluronidase, Staph 21 isolate of *S. aureus* was used to detection the optimum production conditions. Result show in (Figure 2).

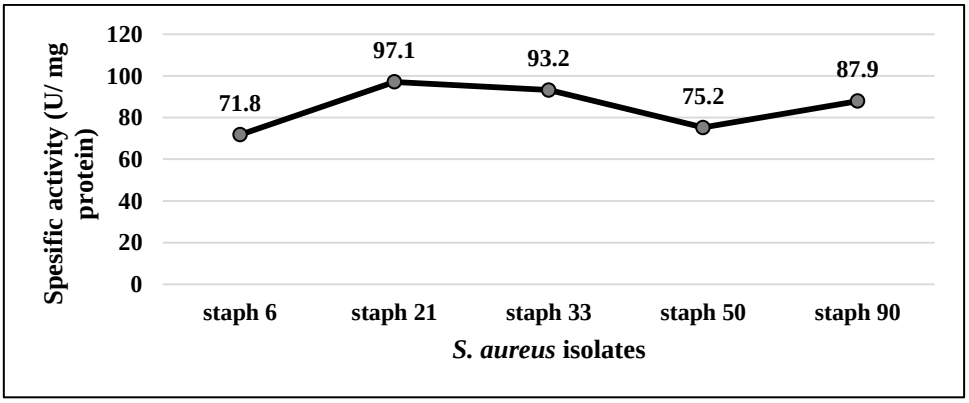

Figure (2): Specific activity of hyaluronidase produced by five isolates of *S. aureus.*

The hyaluronidase producer from *S. aureus*

I) Effect of media compositions

The Effect of media compositions, the results showed (Table 4).

Table (4): The significant differences among ability of *S. aureus* to Hyaluronidase production on different media.

Media	Enzyme activity (U/ml)	Standard Deviation	F-Value	P-Value	Significance
BHIB	**6.8**	**0.6**	0.126	0.886^{NS}	Non- Significant
Nutrient broth	6.6	0.2			
BHBSA	6.3	1.8			

Data presented as One-way ANOVA. ^{NS} At a probability ($p< 0.05$).

Effect of different culture media on the production of hyaluronidase from the isolate of *S. aureus* spp. Many researchers used BHI medium alone or supplemented with 0.5% of yeast extract to produce hyaluronidase. BHI medium was used to produce and purify the enzyme in large quantities from *S. aureus* (18). Also, the Ponomareva *et al*(19) indicated the use of BHI medium supplemented with 0.5% of yeast extract to produce hyaluronidase to produce and purify the enzyme in large quantities from *Poptostreptococcus* bacteria, while yeast extract was added at a rate of 0.2% to Todd-Hewitt broth to produce The enzyme from Group A bacteria *Streptococci* that the difference in the components of the food medium such as carbohydrates, proteins and inorganic materials present in the medium leads to a difference in the production of enzymes from microorganisms, and that enrichment (20).

II) Effect of pH media

During different incubation pH that ranged between 6.5-9.2, and the results showed (Table 5).

Table (5): The significant differences among ability of *S. aureus* to Hyaluronidase production at different pH.

pH	Enzyme activity (U/ml)	Standard Deviation	F-Value	P-Value	Significance
4	6.5	0.1	261.4	<0.0001	Significant
5	7.5	0			
6	8.1	0.02			
7	**12.3**	**0.5**			
8	6.4	0.1			
9	9.2	0.03			

Data presented as One-way ANOVA. **Statically significant at p <0.01.

Tallet *et al*. (21) declared that best production of the hyaluronidase enzyme from *S. aureus* bacterium was achieved at pH 7.2.

III) Effect of incubation temperature

During different incubation temperature that ranged between 30-55°C, and the results showed (Table 6).

Table (6): The significant differences among ability of *S. aureus* to Hyaluronidase production at different temperature.

Temperature (°C)	Enzyme activity [U/ml)	Standard Deviation	F-Value	P-Value	Significance
30	6.2	0.3	95.036	<0.0001	Significant
37	**11.4**	**0.6**			
40	8.1	0.02			
45	11.3	0.5			
50	6.2	0.3			
55	9.2	0.1			

Data presented as One-way ANOVA. **Statically significant at p <0.01.

Other studies revealed that the incubation temperature of 37°C was optimum for the production of hyluronidase enzyme by: *Staphylococcus* spp, *Streptococcus* spp, *Citrobacter freundii* (11). Kadhum (22) discussed depressed enzyme activity in low and high temperatures due to the inadequacy of these degrees for the growth of bacterial cells, leading to slow growth. The temperature may also have a negative effect on the dynamic energy of the molecules, speed of reactions and metabolic processes in the cell.

IV) Determination of optimum incubation time

During different incubation periods that ranged between 24-96hours, and the results showed (Table 7).

Table (7): The significant differences among ability of *S. aureus* to Hyaluronidase production for different period.

Incubation period (h)	Enzyme activity (U/ml)	Standard Deviation	F-Value	P-Value	Significance
24	**11.8**	**0.01**	372.820	<0.0001	Significant
48	9.6	.03			
72	7	0.01			
96	6.6	0.4			

Data presented as One-way ANOVA. **Statically significant at p <0.01.

Characterization of curcumin compound

I- By reagent

Formation of blue-green colour is indicating the presence of phenols.

II- by HPLC analysis of curcumin

The analysis of active plant extract (curcumin) using HPLC revealed several peaks that had important bioactive-natural chemical compounds shown in (Figure 3) for curcumin.

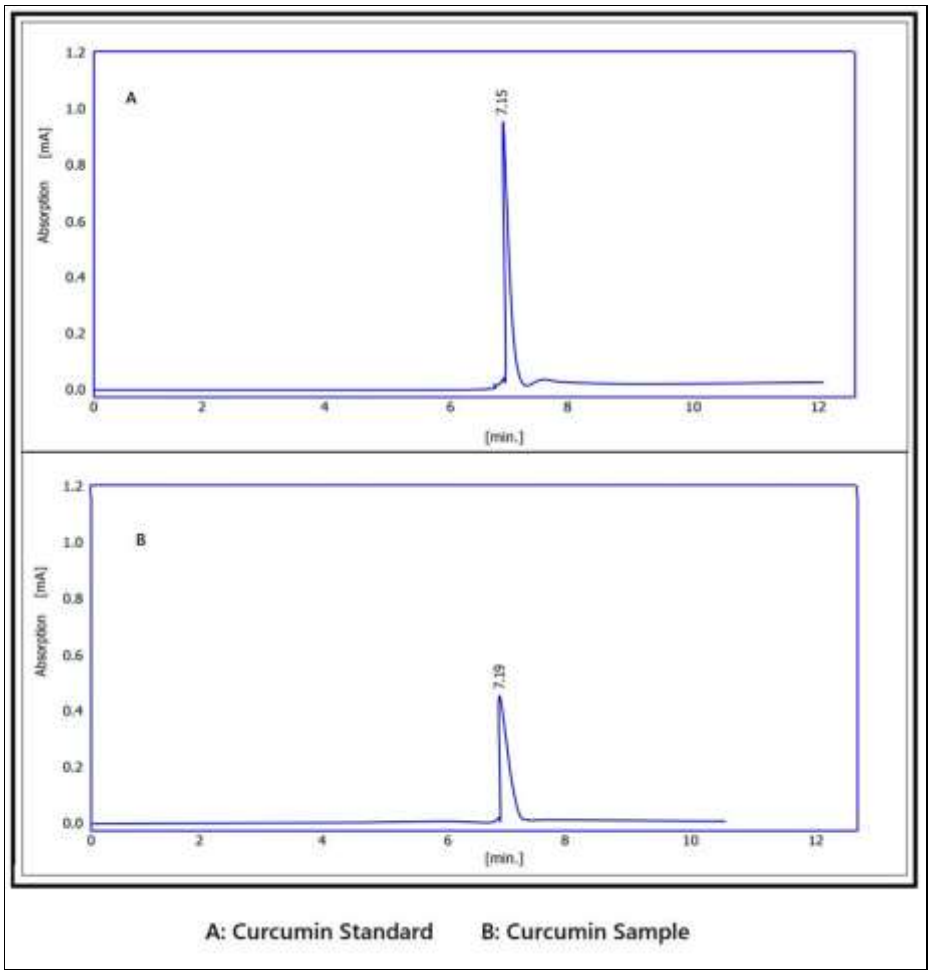

Figure (3): HPLC chromatogram for curcumin.

The quantity of documents pertaining to the HPLC of Phenols is substantial, indicating the significance of this methodology for the examination of these chemical compounds (23). This article demonstrated that Phenols from various primary groups have been effectively scrutinized through the utilization of HPLC. Hence, it is imperative to contemplate the implementation of this technique for the analysis of plant materials that encompass this essential category of naturally occurring substances.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of Curcumin phenols (Table 8) has been studied against a variety of microorganisms.

Table (8): The MIC and MBC of plant extract.

Plant Extract	MIC (µg/ml)	MBC (µg/ml)
Curcumin	312.5	1.250

Determining the MIC of Curcumin or any specific compound derived from it requires conducting laboratory tests and studies on various microorganisms. MIC values can vary depending on the specific microorganism tested, the form of Curcumin or compound used, the solvent or medium, and other factors. The MIC of Curcumin against *S. aureus* was found to be 312.5 µg/mL and MBC was 1.250 µg/Ml.

Inhibitory assay

The result of a hyaluronidase inhibitory assay (Table 9) would be the concentration of the tested compound required to inhibit the activity of hyaluronidase, which is an enzyme that degrades hyaluronic acid. The inhibition of hyaluronidase is important in the context of various medical conditions, as it can help preserve the structural integrity of hyaluronic acid-containing tissues such as the skin, joints, and eyes.

Table (9): Determination of inhibition activity of active compounds depending on its concentration.

Active compounds	Concentration of active compounds (µg/ml)	Inhibition activity %
Curcumin	7	67.47
	15.125	75.11
	31.25	76.86
	62.5	77.29
	125	79.04
	250	79.69
	500	83.84

The inhibition of hyaluronidase by Curcumin phenols may have significant implications in various areas. For instance, hyaluronic acid degradation plays a role in inflammatory processes, tissue damage, and wound healing. By inhibiting hyaluronidase, Curcumin phenols could potentially help maintain the integrity of the extracellular matrix, promote tissue repair, and reduce inflammation

Conclusion

In conclusion, infection and wound samples were characterized by a high proportion of staphylococcus compared with other samples. The enzyme was produced through a production medium fortified with 0.5% yeast extract and 0.25 mg hyaluronic acid, with a pH of 7, and incubated at 37 °C. the Curcumin exhibits the capacity to inhibit the hyaluronidase enzyme at specific concentrations. Further research and experimentation are required to determine the precise mechanisms of inhibition and explore the extract's potential in practical settings, such as pharmaceuticals, cosmetics, or other industrial applications. Additionally, it is essential to evaluate the extract's safety, stability, and effectiveness under different conditions to ascertain its viability for future development and commercialization.

References

1. Manoharan, Y.; Haridas, V.; Vasanthakumar, K. C.; Muthu, S.; Thavoorullah, F. F. and Shetty, P. (2020). Curcumin: A wonder drug as a preventive measure for COVID19 management. Indian Journal of Clinical Biochemistry, 35(3): 373-375.
2. Bala, E.; Hazarika, R.; Singh, P.; Yasir, M. and Shrivastava, R. (2018). A biological overview of Hyaluronidase: A venom enzyme and its inhibition with plants materials. Materials Today: Proceedings, 5(2): 6406-6412.
3. Amalraj, A.; Pius, A.; Gopi, S. and Gopi, S. (2017). Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives–A review. Journal of Traditional and Complementary Medicine, 7(2): 205-233.
4. Tigabu, A. and Getaneh, A. L. E. M. (2021). Staphylococcus aureus, ESKAPE Bacteria Challenging Current Health Care and Community Settings: a Literature

Review. Clinical Laboratory, 67(7): 1539-1549.
5. Matsuo, Y.; Komiya, S.; Yasumizu, Y.; Yasuoka, Y.; Mizushima, K.; Takagi, T., *et al*. (2021). Full-length 16S rRNA gene amplicon analysis of human gut microbiota using MinION™ nanopore sequencing confers species-level resolution. BMC Microbiology, 21(2021): 1-13.
6. Bonar, R.; Favaloro, E. J.; Mohammed, S.; Ahuja, M.; Pasalic, L.; Sioufi, J., *et al*. (2016). The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology, 48(1): 60-71.
7. Ahmad-Mansour, N.; Loubet, P.; Pouget, C.; Dunyach-Remy, C.; Sotto, A.; Lavigne, J. P., *et al*. (2021). *Staphylococcus aureus* toxins: An update on their pathogenic properties and potential treatments. Toxins, 13(10): 677-699.
8. Monaam, Z. A. and Khadhum, K. J. (2022). Effect of Chitosan on Biofilm Formation of Multi-Drug Resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Iraqi Journal of Biotechnology, 21(2): 651-662.
9. Melis, C.; Billing, A. M.; Wold, P. A. and Ludington, W. B. (2023). Gut microbiome dysbiosis is associated with host genetics in the Norwegian Lundehund. Frontiers in Microbiology, 14(2023): 1-12.
10. He, Z.; Sun, Y.; Chu, M.; Zhu, J.; Zhang, Y.; Tang, Q., *et al*. (2023). Screening of a Novel Fibrinolytic Enzyme-Producing Streptomyces from a Hyper-Arid Area and Optimization of Its Fibrinolytic Enzyme Production. Fermentation, 9(5): 410-426.
11. Grootveld, M.; Page, G.; Bhogadia, M. and Edgar, M. (2022). Updates and original case studies focused on the NMR-linked metabolomics analysis of human oral fluids Part I: Emerging platforms and perspectives. Applied Sciences, 12(3): 1235-1308.
12. Treffon, J.; Fotiadis, S. A.; van Alen, S.; Becker, K. and Kahl, B. C. (2020). The virulence potential of livestock-associated methicillin-resistant Staphylococcus aureus cultured from the airways of cystic fibrosis patients. Toxins, 12(6): 360-378.
13. Tripathy, S.; Verma, D. K.; Thakur, M.; Patel, A. R.; Srivastav, P. P.; Singh, S., *et al*. (2021). Curcumin extraction, isolation, quantification and its application in functional foods: A review with a focus on immune enhancement activities and COVID-19. Frontiers in Nutrition, 8(2021): 747956-747984.
14. Adu, O. B.; Folorunso, O. S.; Adeola, S. A. and Godfrey, C. (2013). Antioxidant and Antimicrobial properties of volatile oil of Terminalia catappa and its inhibition on the extracellular protease of *Pseudomonas aeruginosa*. Research Journal of Life Sciences, 1(1): 1-8.
15. Horosanskaia, E.; Yuan, L.; Seidel-Morgenstern, A. and Lorenz, H. (2020). Purification of curcumin from ternary extract-similar mixtures of curcuminoids in a single crystallization step. Crystals, 10(3): 206-221.
16. Omar, N. N. and Mohammed, R. K. (2021). A Molecular Study of Toxic Shock Syndrome Toxin gene (tsst-1) in β-lactam Resistant *Staphylococcus aureus* Clinical Isolates. Iraqi Journal of Science, 62(3): 825-837.
17. Al-Hayali, O. Z.; Marjani, M. F. A. and Maleki, A. (2023). Evaluation of Rosmarinus officinalis Leaves Essential Oils Activity Against Vancomycin Intermediate Staphylococcus aureus (VISA) Isolated from Baghdad Hospital Patients. Iraqi Journal of Science, 64(5): 2153-2167.
18. Kadhum, H. H. and Abood, Z. H. (2022). Staphylococcus aureus Incidence in Some Patients with a Topic Dermatitis in Baghdad City. Iraqi Journal of Biotechnology, 21(2): 13-20.
19. Ponomareva, T.; Sliadovskii, D.; Timchenko, M.; Molchanov, M.; Timchenko, A. and Sogorin, E. (2020). The effect of hepatopancreas homogenate of the Red king crab on HA-based filler. Peer Journal, 8(2020): 8579-8594.
20. Kuryłek, A.; Stasiak, M. and Kern-Zdanowicz, I. (2022). Virulence factors of Streptococcus anginosus–a molecular perspective. Frontiers in Microbiology, 13(2022): 1-16.
21. Tallet, L.; Gribova, V.; Ploux, L.; Vrana, N. E. and Lavalle, P. (2021). New smart antimicrobial hydrogels, nanomaterials, and coatings: Earlier action, more specific, better dosing?. Advanced Healthcare Materials, 10(1): 2001199.
22. Kadhum, F. M. (2023). Optimization of hyaluronidase enzyme conditions produced from pseudomonas aerigenosa isolated from diabetic foot ulcer patients. Journal of Medical and Life Science, 5(1): 23-30.
23. Kamona, Z. K. and Alzobaay, A. H. (2021). Effect of essential oil extract from lemongrass (Cymbopogon citratus) leaves on vaiability of some pathogenic bacteria and sensory properties of fish balls. Iraqi Journal of Agricultural Sciences, 52(2): 268-275.