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INTEGRATED COMPUTATIONAL AND EXPERIMENTAL EVALUATION OF ALLICIN AS A NATURAL INHIBITOR OF PENICILLIN-BINDING PROTEINS IN METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT

Context

Antimicrobial resistance is a major global health challenge that contributes to treatment failure and increased mortality. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important multidrug-resistant pathogen associated with severe infections and bacteremia, with mortality rates ranging from 30% to 40%. MRSA produces several virulence factors, including staphylococcal enterotoxins, toxic shock syndrome toxin-1 (TSST-1), leukocidins, hemolysins, and exfoliative toxins, which contribute to its pathogenicity. Garlic (*Allium sativum*) contains bioactive compounds such as allicin, flavonoids, saponins, and tannins that exhibit antibacterial activity. Allicin (diallyl thiosulfinate), generated from alliin by the enzyme alliinase in the presence of water, possesses broad-spectrum antimicrobial activity against several drug-resistant bacteria. One mechanism of β-lactam resistance in MRSA involves alterations in penicillin-binding proteins (PBPs), resulting in reduced antibiotic affinity. In the present study, allicin was evaluated as a potential inhibitor of penicillin-binding protein 3 (PBP3) of *Staphylococcus aureus* using molecular docking analysis. The results demonstrated favorable binding interactions between allicin and PBP3, suggesting its potential as an alternative therapeutic candidate against MRSA infections.

Methods: The three-dimensional structure of PBP3 of *Staphylococcus aureus* was obtained from the Protein Data Bank, while the structure of allicin was retrieved from the PubChem database. Molecular docking was performed to predict the binding affinity and interaction pattern of allicin with PBP3. Protein–ligand interactions and docking scores were analyzed to evaluate the inhibitory potential of allicin against MRSA.

KEYWORDS: Allicin, *Methicillin Resistant Staphylococcal aureus* (MRSA), penicillin binding protein, molecular docking, death rate, infected.

INTRODUCTION

Garlic (Allium sativum L.), a bulbous plant belonging to the Liliaceae family, is originally indigenous to Central Asia. However, it has established itself in numerous countries worldwide (**1).** Garlic is a well-known spice widely used in traditional medicine for its wide variety of therapeutic properties, especially its potent antimicrobial effects (**2**). It has been claimed to be more effective against many diseases. Traditionally, it has been used to treat colds, hay fever, coughs, asthma, abdominal discomforts, etc (**3**). Garlic is an aromatic herbaceous plant that is consumed worldwide as food and traditional remedy for various diseases. It has been reported to possess several biological properties including anti-carcinogenic, antioxidant, anti-diabetic, anti-atherosclerotic, antibacterial, antifungal, and antihypertensive activities in traditional medicines (**4**). Allicin is the precursor of sulfur containing compounds, which are responsible for the flavor, odor, and pharmacological properties (**5).** More studies have been provided compelling evidence that garlic has antimicrobial properties. In 1858, Pasteur noted garlic's antibacterial activity and it was used as an antiseptic agent. The biochemical characteristics of the fresh garlic are highly variable, and numerous studies have been demonstrated that the concentration of organosulfur compounds (**6**).Allicin is a significant organo-sulfuric substance found commonly in garlic. Allicin is one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit antibacterial activity against a wide range of Gram Negative and Gram Positive bacteria, antifungal activity, anti-parasitic and antiviral activity. *Staphylococcus aureus* is a gram-positive cocci that contains wall teichoic acid, which plays a crucial role in pathogenicity. The fundamental structural component of the bacterial cell wall is peptidoglycan, which is important

for life and is the target of critical antibiotics. (**7**).The natural habitats of Staphylococcus are human skin and the nasopharynx. It can carry numerous infections that damage the skin, soft tissue, endovascular sites, and internal organs. *Staphylococcal aureus* is an essential infection in hospitals, leading to high rates of mortalility and morbidity (**8**). This pathogen can cause a wide variety of disease ranging from moderately from severe skin infections to fatal pneumonia and sepsis. *Staphylococcal aureus* is presently the second cause of death by antibiotic-resistance infection worldwide (**9**). Antibiotic resistance makes it difficult to treat *Staphylococcus aureus* infections, and there is no viable vaccination available (**10**). Patients with severe staphylococcal infections usually had very poor consequences until the introduction of penicillin in early 1940s. A few years later, the first penicillin-resistant strain of *Staphylococcus aureus* was identified (**11**). Methicillin Resistant *Staphylococcus aureus* is a clinically significant strain of this thoroughly researched antibiotic-resistant bacteria. The first MRSA strains appeared in 1960–1961 and were characterized by resistance to all the beta-lactam antibiotics then used in the treatment (**12**). Upto 60% of clinically isolated S. aureus strains have been reported to be methicillin-resistant. The mortality rate for patients with MRSA is about 30% - 40%, which is about twice that reported for methicillin susceptible *Staphylococcus aureus* bacteraemia (**13**). MRSA cannot be efficiently treated with antibiotics such as methicillin, nafcillin, cephalosporin or penicillin but it can be typically treated with vancomycin. Vancomycin is a glycopeptide antibiotic used for the treatment of Gram-positive infections. Traditionally it was used as a drug of last resort; however, clinical isolates of methicillin-resistant Staphylococcal aureus strains with decreased susceptibility to vancomycin and more recently with high-level vancomycin resistance have been described in the clinical literature (**14**). Vancomycin has many shortcomings including poor tissue penetration and slow killing time (**15**). Over the decades, *Staphylococcus aureus* has refined resistance mechanisms against penicillin, which are then countered by new generations of beta lactam structures (**16**). Penicillin was the first line treatment of infection caused by *Staphylococcus aureus*, which inhibits *Staphylococcus aureus* cell wall formation. However, in a few years of penicillin use, 50% of S. aureus infections were resistant to the drug due to the catalytic function of β-lactamase (**17**). The first resistance mechanism of staphylococcus aurues to beta lactams was due to the production of a beta lactamase (penicillinase), an inducible extracellular enzyme released in response to betalactams exposure hydrolyzes the beta lactam ring, resulting in inactive derivatives. *Staphylococcus aureus* has four penicillin binding proteins (PBPs): PBP1, PBP2, PBP3, and PBP4. PBP2a confers the highest protection against two beta-lactams that are known to have a high specific affinity toward the transpeptidase PBP3 of *Staphylococcus aureus* (**18**). *Staphylococcus aureus* is resistant to most beta-lactamases because it expresses an additional PBP3 with low beta-lactam affinity. Molecular docking simulations are essential in discovering novel medications. AutoDock is a software application widely used for predicting molecular interactions at close distances (**19**). The aim of this study is to identify the efficacy of the garlic extract (allicin) against the penicillin binding protein in *Methicillin Resistant Staphylococcal aureus* by in vitro method.

MATERIALS AND METHODS:

The study was conducted at tertiary care hospital over the period of six months, from Aug 2023 to Jan 2024. Samples received to clinical Microbiology Laboratory were processed using conventional method.
MRSA were identified by standard microbiological techniques by studying their characteristics of colony, morphology and biochemical reactions.

Microscopic examination:

Gram Stain:

Crystal violet stain is used as the primary stain. After 1 minute, the stain is drained off; any surplus is rinsed away with water. The goal is to remove the discoloration without damaging the fixed culture. Iodine solution is applied on the smear for 1 minute. This technique is referred to as "fixing the dye." The iodine solution is drained off, and the slide is cleaned under running water. Excess water on the surface is shook off. A few drops of acetone are applied to the slide for decolorizing. Decolourizers are frequently combined solvents of ethanol and acetone. This step is known as "solvent treatment." The slide is rinsed with water for 5 seconds. To avoid excessive decolourization in gram-positive cells, discontinue use of the decolourizer once the solvent no longer appears colored as it travels over the plate. The smear is counterstained with a carbol fuchsin solution for 30-45 seconds. The fuchsin solution is washed away with water, and any surplus water is wiped with bibulous paper. After removing the extra water, the slide can be air-dried **(20).**

Characteristics of colony:

Catalase test – Slide Method:

Place a microscope slide inside a petri dish. Keep the petri dish cover available. The use of a petri dish is optional the slide catalase test can be performed properly without it. However, in order to avoid catalase particles, which have been reported to contain live bacterial organisms, applying a petri dish is highly recommended. Gather a tiny quantity of organism from a well-isolated 18–24 hour colony using a wooden applicator stick or sterile inoculating loop, then

transfer it to the microscope slide. Take care to avoid picking up any agar. This is especially crucial if the colony isolate was cultivated on red blood cell-containing agar. Red blood cell carryover into the test could provide a false-positive result. Apply one drop of 3% H2O2 to the organism on the microscope slide using a dropper or Pasteur pipette. Avoid mixing. To reduce aerosols, quickly put a lid onto the petri dish and examine the formation of bubbles (O2 + water = bubbles). Readability is improved by looking for the development of bubbles on a dark background. Instant effervescence (bubble formation) indicates a positive reaction. To view faint positive reactions, place a microscope slide over a dark backdrop and use a magnifying glass or microscope **(21).**

Tube coagulase test:
The tube coagulase test involves mixing bacterial cells into a larger volume of plasma in the smallest test tube. As bacteria proliferate in the plasma, they produce staphylocoagulase. Staphylocoagulase causes blood coagulation by activating prothrombin. Staphylocoagulase binds to fibrinogen, generating a complex that cleaves fibrinogen into fibrin by bypassing the blood clotting cascade and causes a clump of fibrin to develop. A clot formation will be noted within 24 hours after getting a positive response. **(22)**

Detection of MRSA:
Disc diffusion test using cefoxitin disc (30µg):
All the 81 isolates were done with cefoxitin (30µg) disc using Muller Hinton agar plate. The plates were incubated at 37°C for 24 hours.
After incubation period of 24hours, zone of inhibition was observed. The strains with an inhibition zone diameter with less than or equal to 19mm will be determined as MRSA **(23).**

16sRNA detection for the confirmation of MRSA:
Confirmation of MRSA was done by using gene sequencing method. A single band of large molecular weight DNA was seen after the DNA was separated from the culture and its quality assessed on a 1.0% agarose gel. 16S rRNA-F and 16S rRNA-R primers were used to amplify the 16S rRNA gene fragment. On an agarose gel, a single distinct 1500bp PCR amplicon band was seen. PCR amplification is purified to be free of impurities. By using the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic analyser, the forward and reverse DNA sequencing reactions of the PCR amplicon were performed using 16SrRNA-F and 16SrRNA-R primers. BLAST was performed using the 16S rRNA gene sequence and the NCBI GenBank database's "nr" database. The highest ten sequences selected based on the maximum identity score and aligned using Clustal W, a multiple alignment software application. A distance matrix and phylogenetic tree were created using MEGA 10. **(24).**

High Performance Liquid Chromatography
The HPLC equipment utilized for the analysis included a pump, UV-visible detector, a C-18 column measuring 254mm × 4.60mm, and data software unit. The garlic extract was optimally resolved with a retention period of 1.990 ± 0.5 minutes utilizing a column and analytical solvent system consisting of HPLC water and methanol in a 50:50 (v/v) ratio. The degassed mobile phase was passed through a 5 µm filter. The solvent flow rate was maintained at a constant 1ml/min, with absorption detected at 254nm. The standard sample was injected and the average detector response was recorded. Similarly, the extracts from leaves were analyzed, and the peak areas corresponding to garlic extract were compared with the calibration curve.

Authentication:
The plant was planted on October 2024 and the plant was authenticated on March 2025.

Extraction of allicin:
5 grams of garlic cloves were peeled and grinded in a sterile mortar and pestle, mixed with 10 ml of demineralised water. The solid components of the garlic were removed using filtration.

Lyophilisation:
The extracted garlic sample was poured in a petri-dish plate and stored in -20°C for 24 hours. Then the sample was placed in lyopholiser machine. The water content in the sample was removed.

Gas Chromatography Mass Spectrometry (GCMS):
GC-MS QP2010 Ultra (Shimadzu) system was utilized. The system included an auto injector (AOC-20i), a head space sampler (AOC-20s), a mass selective detector with an ion source (240°C), and a 250°C interface. The SH-Rxi-5 Sil MS (crossbond, comparable to 5%diphenyl/95%dimethyl pol) capillary column with dimensions of 30 mm X 0.25 mm X diameter and film thickness of 0.25 µm was utilized for MS analysis. The injector was configured in the split

injection mode with 250°C of temperature. The ratio applied for split mode was 30.0. The starting temperature was adjusted to 100°C, which afterwards increased to 170°C with a ramp rate of 3°C/min, which afterwards increased to 270°C with a ramp rate of 5°C/min and hold for 2 min. Helium (>99.99%) with 37.2 cm/s of linear velocity was employed as a carrier gas.
The system was programmed with 34.0 ml/min of total flow rate and 1.0 ml/min of column flow. Total program or run time for the sample was 45min.

Antibiotic susceptible testing:
The Kirby-Bauer disc diffusion technique is used to screen for antibiotic susceptibility. The Muller Hinton agar (MHA) plate is used for testing of antimicrobial. The sterile swab was dipped in the inoculated Nutrient broth, rubbed against the edge of the test tube to remove any surplus inoculums, and then streaked across the whole agar surface. The operation was performed three times, with the plate turned at a 60°C angle between each streak. Antibiotic discs are placed in a MHA plate as per CLSI guidelines by using sterile forceps.
The antibiotic used against *Staphylococcus aureus* is gentamycin, cotrimaxazole, ciprofloxacin, vancomycin, penicillin, lenezolid, cefoxitin, erythromycin, clindamycin and tetracycline.

Antibacterial Susceptible Test using allicin:
Antibacterial susceptible test was done on the agar cup diffusion method. Inoculum was prepared in a nutrient broth and incubated for overnight. After incubation the inoculum was lawn on Muller Hinton agar plate and the cylindrical bore was made. Then the bore was filled with water extraction of allicin with the concentration of 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml, and 50mg/ml. Then the plates are incubated overnight at 37 degree celcius. After incubation the plates are seen for any zone of inhibition was considered effective.

Minimum Inhibitory Concentration (MIC):
The minimum inhibitory concentration was done by broth dilution method with various concentration of allicin. Following that, a millilitre of allicin was placed into a tube containing a similar amount of nourishing broth incubated with the test organism (MRSA), resulting in final concentrations of 70%, 60%, 50%, 40%, 30%, 20%, and 10%. The tube containing nutrient broth and allicin without organism and a tube containing nutrient broth and test organism without allicin were used as positive and negative control. Then the tubes were incubated for 24 hours and visible growth were assessed. When compared to control tubes, the concentration of allicin that displays no observable development was considered the minimal inhibitory concentration **(25).**

Scanning Electron Microscopy (SEM):
Get a test tube and measure the organisms with the allicin water extract using the Mc Farland Standard. Then inoculate the 0.5µl of organisms in 50µl of allicin water extract incubate at it 30 mins at 37°C in an incubator. After the incubation period of 30 mins, take loopful of organisms which were incubated and do smear in grease free slides. Then air dry the slides. Then the slides are examined in Scanning Electron Microscopy under the 2000x magnification.

Molecular Docking:
Molecular docking was done against PBP3 using a software called Autodock. The ligand and allicin complex (Pubcmem ID: 65036) were straight away downloaded as mol 2 files. The downloaded ligand was then converted into PDBQT (Protein (P), Data bunk (DB), Partial charge (Q) and Atom type (T) format using the open label. Autodock employed gradient computations and Monte Carlo techniques to locate and rank docking postures based on a scoring mechanism. The wizard-like interface was used to choose converted ligands along with to PBP3 protein. The crystallographic structure was used to build a grid around active site residues (N450, Q524, T621, S448, T603, P660, and E623) to acquire coordinates (**26).** The allicin ligand was allowed to dock with the desired protein targets, and the results were obtained. The lowest intermolecular energy configurations were chosen for study. The DIMPLOT server was used to study non-bonded interactions involving hydrogen bonds and Van de Waals forces. The interactive image was created using chimera software.

RESULT
The study was conducted at Saveetha Medical College and Hospital over the period of six months, from Aug 2023 to Jan 2024. Samples received to clinical Microbiology Laboratory were processed using conventional method.
In this study, total 81 isolates were collected from Department of Clinical Microbiology Laboratory of Saveetha Medical College and Hospital. Out of 81 isolates, all the isolates showed uniformly stained Gram Positive cocci arranged in pairs and clusters. All the isolates showed coagulase positive and clumping factor positive. The antibiogram pattern of *Staphylococcus aureus* isolates are showed in **Table: 1**. From 81 isolates, 81 isolates (100%)

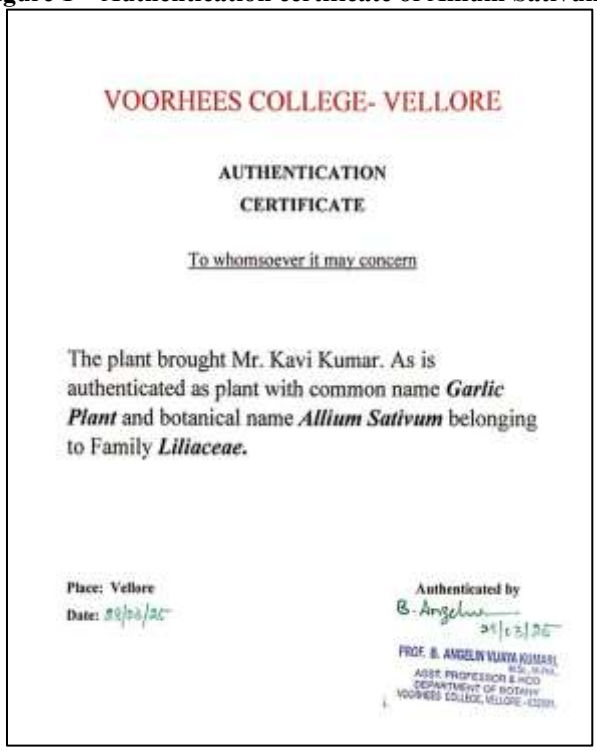
shows resistant to cefoxitin. We can classify it as MRSA in accordance with Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines, because it is resistant to cefoxitin.

Table 1 shows the antibiogram pattern of *Staphylococcus aureus* isolate.

Drugs	Sensitive	Resistance
Gentamycin	36 (44%)	45 (56%)
Cotrimoxazole	37 (46%)	44 (54%)
Ciprofloxacin	42 (52%)	39 (48%)
Vancomycin	81 (100%)	0 (0%)
Penicillin	79 (98%)	2 (2%)
Linezolid	81(100%)	0 (0%)
Cefoxitin	0 (0%)	81 (100%)
Erythromycin	34 (42%)	47 (58%)
Clindamycin	44 (55%)	37 (45%)
Tetracycline	43 (53%)	38 (47%)

In figure 1 shows the plant was authenticated as common name ***Garlic plant*** with botanical name ***Allium sativum*** belonging to the family ***Liliaceae.***
The plant was authenticated by **B. Angeline Vijayakumari** M.sc, M.Phil. Professor and Head of the Department, Department of Botany, Voorhees college of Arts and Science, Vellore.

Figure 1 – Authentication certificate of Allium Sativum:



VOORHEES COLLEGE- VELLORE

AUTHENTICATION
CERTIFICATE

To whomsoever it may concern

The plant brought Mr. Kavi Kumar. As is authenticated as plant with common name ***Garlic Plant*** and botanical name ***Allium Sativum*** belonging to Family ***Liliaceae.***

Place: Vellore
Date: 29/12/25

Authenticated by
B. Angeline
29/12/25
PROF. B. ANGELIN VIJAYA KUMARI
ASST. PROFESSOR & HOD
DEPARTMENT OF BOTANY
VOORHEES COLLEGE, VELLORE - 632001.

A 2012 study (Rahman et al) found that, other from HPLC, no other technologies is reliable to research allicin. The study found that the retention duration of allicin varied from 3 to 3.7 minutes/ml in many garlic varieties.
Nishu sekar (2015) showed that the retention time of garlic extract was noted to be 2.2 minutes/ml. In their study garlic oil was used as a standard and the retention time for garlic oil is 1.990 minutes/ml.
In our study, HPLC analysis of garlic extract was performed using a mobile phase consisting of HPLC-grade water and methanol, with a flow rate of 1 mL/min and a total run time of 20 minutes. Absorbance was monitored at a wavelength of 254 nm. The retention time of the garlic extract was observed to be 1.992 minutes, which was comparable to that of the standard garlic oil sample **(Fig. 2).**

Figure 2: retention time of garlic oil used as a standard

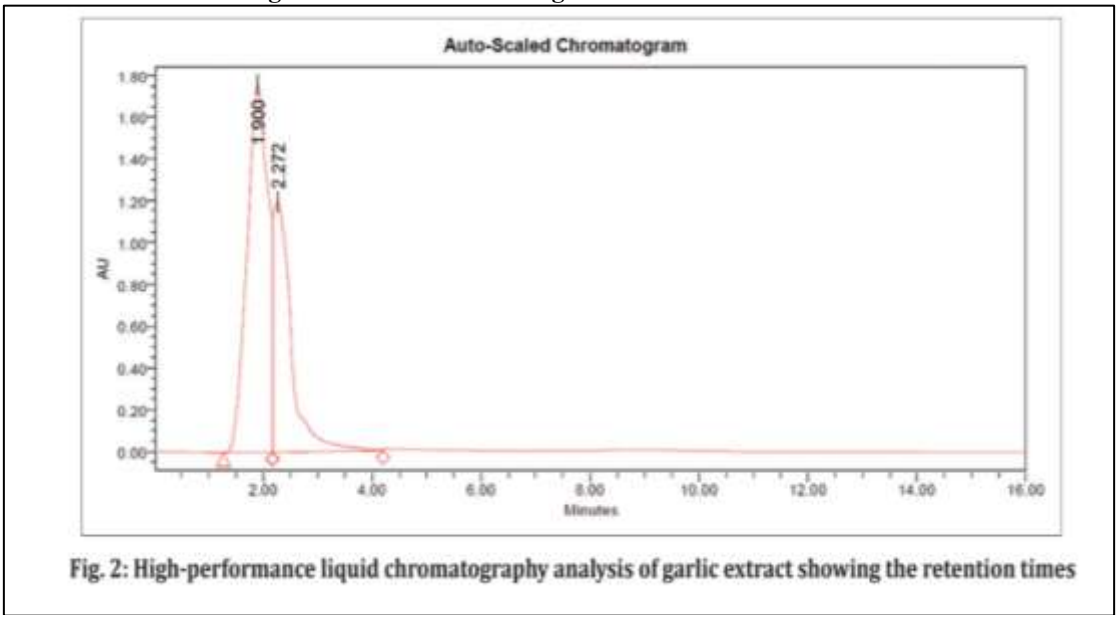
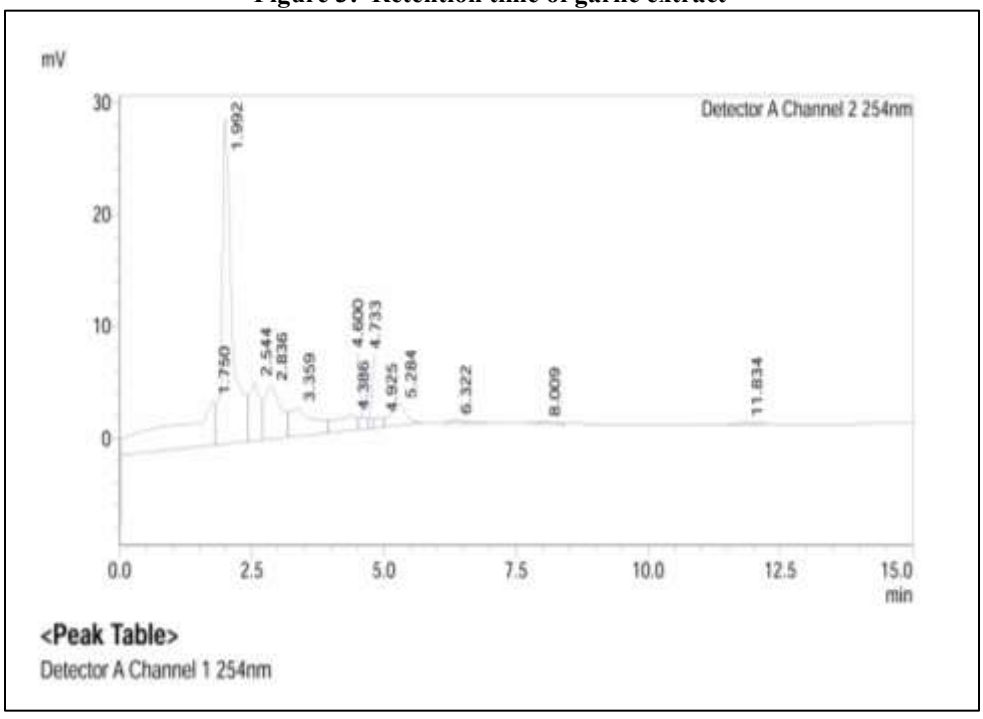


Fig. 2: High-performance liquid chromatography analysis of garlic extract showing the retention times

The HPLC chromatogram of the garlic extract showed a retention time of 1.992 minutes under the optimized chromatographic conditions, indicating the presence of allicin in the extract. The chromatographic profile and retention peak of the garlic extract are shown in **Fig. 3**.

Figure 3: Retention time of garlic extract



The lyophilized garlic extract was analyzed using GC-MS to identify its bioactive constituents. Several compounds with known antimicrobial, antioxidant, and anti-inflammatory properties were detected in the sample. The GC-MS chromatogram showing the peak profile and corresponding retention times of the identified compounds is presented in **Fig. 4**, while the identified compounds and their biological roles are summarized in **Table 2.**

Figure 4: displays the GC-MS peak profile and the associated retention times of the detected compounds.

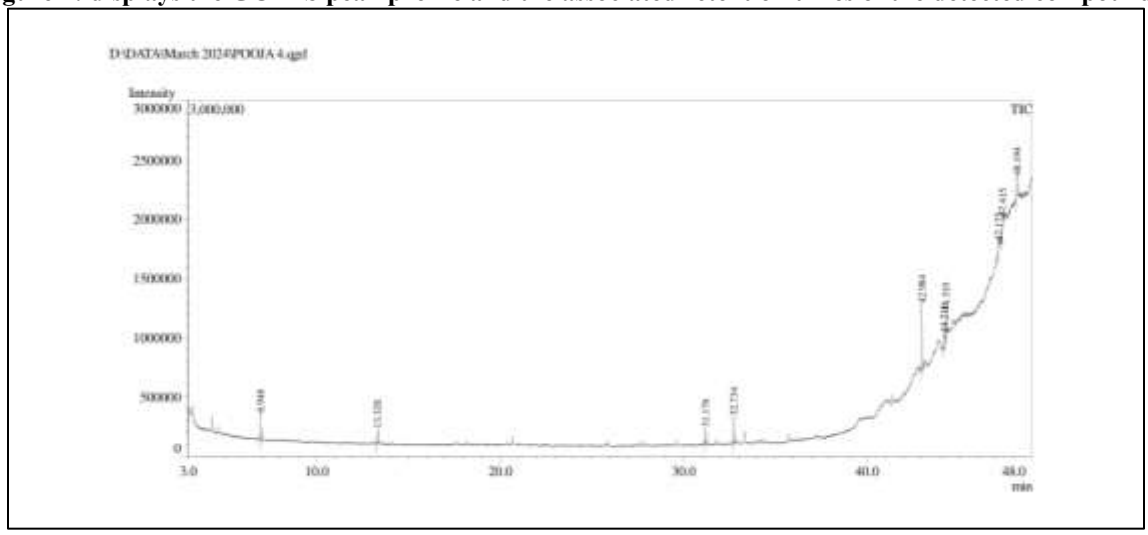


Table 2: shows the compounds detected and their roles:

Peak #	Name of the compound detected	RT(min)	Area %	Molecular roles
1	**Dodecane**	6.948	9.03	Microbial infection prevention properties against microorganisms such *as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cere.*
2	**Tetradecane**	13.328	5.20	Antibacterial and antifungal
3	**Dibutyl phthalate**	31.176	6.62	Strong activity against Gram-positive and Gram-negative bacteria, as well as unicellular and filamentous fungi.
4	**Dibutyl phthalate**	23.734	12.39	Strong activity against Gram-positive and Gram-negative bacteria, as well as unicellular and filamentous fungi.
5	**Hexanedioic acid bis(2-ethylhexyl) ester**	42.984	21.70	Primarily in the areas of antioxidant and antibacterial properties
6	**ETHYL(9Z,12Z)-9,12 – OTADECADIENO**	44.210	4.00	It's commonly found in plant-based oils and is known for its moisturizing, antibacterial, and anti-inflammatory properties
7	**9-Octadecenoic acid, 1,2,3-propanteriyl ester**	44.310	9.07	it exhibits antioxidant properties and can act as an immune modulator
8	**CELIDONIO, DEOXY-**	47.175	8.85	biological activities, including antimicrobial, anticancer, antioxidant, and anti-inflammatory properties
9	**Stigmasterol**	47.415	5.73	Bioactive compound to prevent skin cancer.
10	**Stigmast-5-en-3-ol, oleate**	48.194	17.40	Antibacterial activity as well as in water vapor treatments for skin tumors.

The distribution of major virulence factors among *Staphylococcus aureus* isolates and their association with antibiotic susceptibility patterns were analyzed. The virulence factors evaluated included protease, lecithinase, hemolysin, staphylokinase, and lipase production. The relationship between these virulence factors and antibiotic resistance profiles is summarized in **Table 3**.

Table 3: shows the virulence factor of *Staphylococcus aureus*

DRUGS		**PROTEASE**		**LECITHINASE**		**HEAMOLYSIN**		**STAPHYLO KI-NASE**		**LIPASE**	
		+	-	+	-	+	-	+	-	+	-
Gentamycin	Sensitive	22	14	19	17	20	16	18	18	21	15
	Resistance	23	22	18	27	24	21	19	26	25	20
	Chi- square P value	0.810 0.3681		1.316 0.2513		0.040 0.8419		0.488 0.4850		0.063 0.8020	
Cotrimoxazole	Sensitive	22	15	20	17	16	21	25	12	18	19
	Resistance	24	20	19	25	27	17	25	19	23	21
	Chi- square P value	0.707 0.4003		0.120 0.7288		9.449 0.0021		1.286 0.2567		11.216 0.008	
Ciprofloxacin	Sensitive	22	20	25	17	15	27	27	15	23	19
	Resistance	26	13	23	16	17	22	18	21	21	18
	Chi- square P value	0.098 0.7543		17.001 0.0001		1.130 0.2878		0.844 0.3583		0.425 0.5145	
Vancomycin	Sensitive	50	31	58	23	54	27	55	26	57	24
	Resistance	0	0	0	0	0	0	0	0	0	0
	Chi- square P value										
Penicillin	Sensitive	58	21	61	18	59	20	44	35	53	26
	Resistance	2	0	1	1	2	0	2	0	0	2
	Chi- square P value	1.563 0.2113		0.709 0.3998		0.772 0.3795		0.283 0.5946		9.763 0.0018	
Linezolid	Sensitive	68	13	59	22	60	21	69	12	52	29
	Resistance	0	0	0	0	0	0	0	0	0	0
	Chi- square P value										
Cefoxitin	Sensitive	0	0	0	0	0	0	0	0	0	0
	Resistance	63	18	57	24	59	22	46	35	58	23
	Chi- square P value										
Erythromycin	Sensitive	22	12	24	10	19	15	20	14	21	13
	Resistance	25	22	29	18	28	19	30	17	29	18
	Chi- square P value	5.700 0.0170		0.022 0.8821		2.998 0.0834		0.000 0.9859		1.211 0.2711	
Clindamycin	Sensitive	25	19	26	18	23	21	24	20	26	18
	Resistance	25	12	22	15	23	14	21	16	23	14
	Chi- square P value	6.143 0.0132		1.233 0.2668		1.211 0.2712		0.275 0.5997		0.275 0.6001	

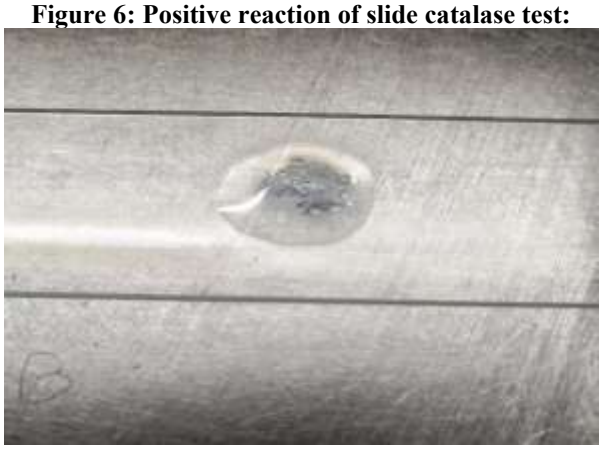
Tetracycline	Sensitive	32	11	22	21	28	15	30	13	29	14
	Resistance	26	12	28	10	24	14	23	15	29	9
	Chi- square P value	0.158 0.6906		7.575 0.0059		0.608 0.4356		3.885 0.0487		1.554 0.2126	

The coagulase test was performed by mixing the bacterial cells with plasma in a small test tube. After 24 hours formation of clot was noted. The clot formed indicates the positive reaction. In **figure 5** shows the positive reaction of tube coagulase test. The positive reaction of coagulase clearly indicates the organism of *Methicillin Resistant Staphylococcus aureus*.

Figure 5: shows the positive reaction of tube coagulase test:

Catalase test was done by slide catalase method. The small amount of organism was picked from well isolated colony and treated with 1 drop of 3% hydrogen peroxide in a glass slide, the slide was noted for bubble formation (effervescence). **In figure 6**, it shows the positive reaction of catalase test by forming the effervescence. The formation of effervescence reveals the organism of *Methicillin Resistant Staphylococcus aureus*

Figure 6: Positive reaction of slide catalase test:



In the study conducted by Mukarramah Zainal, Nurhayati Mohamad Zain, reveals the bactericidal activity of allicin against *Staphylococcus aureus* with the concentration of 8µg/ml.

Our study demonstrates that the aqueous garlic extract, containing allicin, exhibits potent bactericidal activity against MRSA isolates. Even at the lowest dilution, no growth was observed, indicating excellent efficacy in eliminating the bacteria. This suggests that allicin has strong antibacterial properties, making it a promising candidate for treating MRSA infections

Angnina Listya Anggraini (2020) reported that allicin exhibits significant antibacterial activity against *Staphylococcus aureus*, with the minimum inhibitory concentration (MIC) ranging from 40 mg/mL to 50 mg/mL. In the present study, the antibacterial susceptibility test was performed using the agar well diffusion method on Mueller–Hinton agar plates. Following incubation at 37°C for 24 hours, a clear zone of inhibition with a diameter of 24 mm was observed around the wells containing allicin extract, indicating its antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA). The susceptibility pattern of allicin against MRSA as determined by the agar well diffusion method is shown in **Fig. 7**.

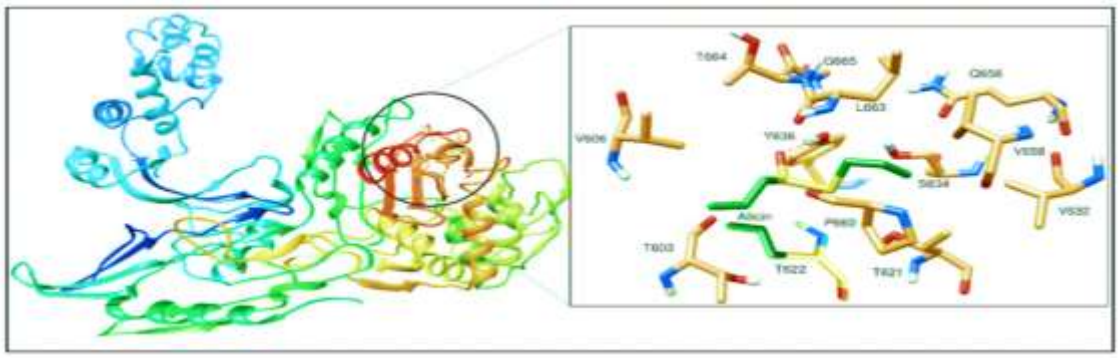
Figure 7: shows the susceptibility test of allicin against *Methicillin Resistant Staphylococcus aureus.*

Mohammed A Eltaweel (2004) reported that the aqueous garlic extract has more antimicrobial activity than the methanol extract against *Staphylococcus aureus*.

The molecular docking study revealed that allicin exhibits stronger interactions with PBP3 compared to PBP2a, a key penicillin-binding protein involved in MRSA resistance. The docking results showed a favorable score of -3.09 and a binding energy of -3.384 Kcal/mol, indicating a stable and energetically favorable complex formation between allicin and PBP3. This suggests that allicin may effectively inhibit PBP3, disrupting cell wall synthesis and contributing to its antibacterial activity against MRSA.

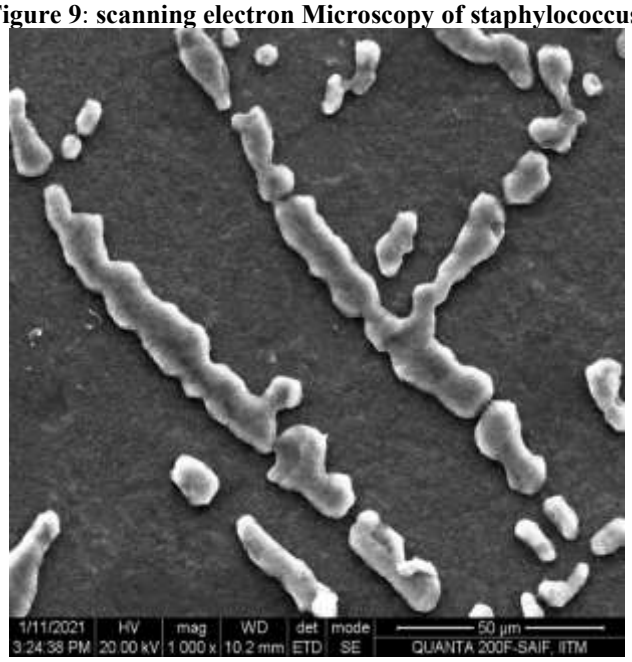
At a contact distance of 2.58A, a hydrogen bond forms between the oxygen atom of allicin and Y636 of the PBP3 protein. Allicin and T603, V606, T664, G665, L663, S634, Q656, V658, V632, P660 and T621 of the PBP3 protein have several kinds of hydrophobic interactions. The molecular docking analysis demonstrated a stable interaction between allicin and PBP3 of *Staphylococcus aureus*. A hydrogen bond was observed between the oxygen atom of allicin and the Y636 residue of PBP3, while multiple hydrophobic interactions were identified with residues T603, V606, T664, G665, L663, S634, Q656, V658, V632, P660, and T621. The magnified view of the PBP3–allicin biomolecular complex illustrating the hydrogen bond and hydrophobic interactions is shown in **Fig. 8**.

Figure 8: The magnified picture of the PBP3-Allicin bio-molecular complex demonstrates the formation of a hydrogen and hydrophobic connection between them.



The interaction between allicin and Staphylococcus aureus was confirmed using Scanning Electron Microscopy (SEM). The results revealed significant damage to the bacterial cell membrane, indicating that allicin disrupts the membrane's structure and function, ultimately leading to cell lysis and death. This mechanism supports allicin's potential as an antibacterial agent against S. aureus, including MRSA strains, and highlights its potential therapeutic application. In **figure 9** shows the Scanning Electron Microscopy of cell membrane damage of *Methicillin Resistant Staphylococcus aureus* when treated with allicin.

Figure 9: **scanning electron Microscopy of staphylococcus.**



The Scanning Electron Microscopy (SEM) images reveal significant cell membrane damage in Methicillin-Resistant Staphylococcus aureus (MRSA) treated with the water extract of allicin. The images show clear morphological changes, indicating that allicin disrupts the cell membrane, leading to cell lysis and death. This visual evidence supports allicin's potential as an effective antibacterial agent against MRSA.

DISCUSSION AND CONCLUSION

Garlic's been used for centuries for its medicinal properties. People use it for everything from boosting immunity to potentially helping with heart health and antimicrobial effects. Many studies revealed that allicin has no antibacterial growth at lower concentrations. This study demonstrates that allicin possesses potent bactericidal activity against *Methicillin-resistant Staphylococcus aureus*, as no bacterial growth was observed even at the lowest tested concentration of 3.12 µl.

In 2019 a study conducted by K. S. Vikraman, V. Vishnpriya, R. Ponnulakshmi **(27)** found that allicin has strong antimicrobial activity against some oral pathogens. This is consistent with other studies that have shown allicin's effectiveness against various microorganisms, including bacteria, viruses, and fungi. Allicin's antimicrobial properties make it a potential natural remedy for preventing and treating oral infections. Benshiga et al. found that allicin has good antimicrobial activity when combined with ZnO nanoparticles **(28).**

The molecular docking analysis revealed that allicin exhibits strong interactions with PBP3, a crucial penicillin-binding protein in Staphylococcus aureus. This suggests that allicin could be an effective inhibitor of PBP3, making it a potential alternative treatment option for Methicillin-Resistant Staphylococcus aureus (MRSA) infections. - Allicin's ability to interact with PBP3, a key protein involved in cell wall synthesis, highlights its potential as a novel antibacterial agent against MRSA.

Fujisawa et al (2009) reported that antibiotic-resistance mechanisms of MRSA have no impact on susceptibility to garlic and they suggest allicin might be effective against drug-resistant strains like MRSA, making it a potential therapeutic option.

Anna Marchese, Ramona Barbieri and Ana Sanches-Silva (**29**) revealed that allicin has shown promise as an antimicrobial agent against a range of microorganisms. With the increasing prevalence of multi-drug resistant microbes, there's growing interest in alternative therapeutic agents like allicin.

Combining allicin with conventional antibiotics may offer a synergistic approach to tackling antimicrobial resistance. However, rigorous preclinical and clinical studies are necessary to establish allicin's safety, efficacy, and potential adverse effects, paving the way for its potential use as a feasible antibacterial medication in humans. The synergistic effects of combining allicin with antibiotics could lead to reduced antibiotic resistance and improved treatment outcomes. Allicin's broad-spectrum antimicrobial activity may also contribute to its potential as a treatment option for co-infections.

The favorable binding energy and score indicate a stable and energetically favorable complex formation between allicin and PBP3, supporting its therapeutic potential. Allicin's natural origin and potential low toxicity make it an attractive candidate for further development as an anti-MRSA agent. Further in vivo studies and clinical trials are necessary to validate the efficacy and safety of allicin as a treatment option for MRSA infections.

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Consent to Participate: Not relevant. The study involved retrospective analysis of anonymised laboratory data only. No patients, volunteers, or human subjects were recruited or directly involved, and therefore no informed consent to participate was required.

Ethics Approval: Ethical review was not required for this study as it involved no direct intervention, experimentation, or interaction with human subjects. The work was based solely on retrospective examination of de-identified laboratory records. Institutional human ethical clearance was granted for use of the data.

Clinical Trial Registration: Not relevant. This study does not report on a clinical trial. No prospective assignment of participants to health-related interventions was performed, therefore trial registration is not necessary.