International Journal of Medical and Allied Biosciences

RESEARCH ARTICLE

Volume 1 | Issue 1 | Year 2026

Antibacterial Potential of Cannabis sativa and Allium sativum Against Vancomycin-Resistant and Intermediate Staphylococcus aureus Strains

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Received: 02 Jun 2026

Received in revised form: 30 Jun 2026

Accepted: 29 Jul 2026

ABSTRACT

This in-vitro experimental study evaluated the antibacterial activity of ethanolic extracts of Cannabis sativa and Allium sativum against clinically isolated Vancomycin-Resistant and Intermediate Staphylococcus aureus (VRSA & VISA) strains. The antibacterial efficacy was determined by MIC (Minimum Inhibitory Concentration) and molecular docking studies. The mean MIC for Allium sativum extract was 18.1 µg/mL and for Cannabis sativa extract was 31.6 µg/mL. Docking studies revealed stable binding affinities of phytoconstituents with bacterial target proteins. Statistical analysis showed no significant correlation between resistance gene expression and plant extract efficacy. The study concludes that both extracts exhibit promising antibacterial potential, with garlic demonstrating superior activity, suggesting their possible role as alternative therapeutics against drug-resistant S. aureus strains.

Keywords: Staphylococcus aureus, VRSA, VISA, Cannabis sativa, Allium sativum, Antibacterial activity, MIC, Molecular docking

Introduction

The emergence and proliferation of multidrug-resistant bacterial pathogens pose a significant threat to global public health, limiting the therapeutic options available for the treatment of infectious diseases. Among these pathogens, Staphylococcus aureus holds a prominent place due to its remarkable ability to acquire resistance to multiple antibiotics, including glycopeptides such as vancomycin, which has historically served as a last-resort drug in the treatment of methicillin-resistant S. aureus (MRSA) infections.[1,2] In recent years, the increasing prevalence of Vancomycin-Intermediate S. aureus (VISA) and Vancomycin-Resistant S. aureus (VRSA) strains has been reported globally, leading to heightened concerns regarding treatment failures and increased morbidity and mortality rates.[3,4]

The alarming rise of VRSA and VISA strains is primarily attributed to the extensive and indiscriminate use of antibiotics, facilitating the evolution of resistance mechanisms such as thickened cell walls, decreased permeability, altered target sites, and acquisition of resistance genes.[5,6] These resistant strains compromise the efficacy of conventional antimicrobial therapies, necessitating the exploration of novel and alternative antimicrobial agents to combat these pathogens effectively.

In this context, medicinal plants and their bioactive constituents have gained considerable attention as potential sources of antimicrobial agents due to their chemical diversity, traditional usage, and comparatively lower likelihood of inducing resistance.[7,8] Two such promising natural candidates are Cannabis sativa (hemp) and Allium sativum (garlic), both renowned for their ethnomedicinal significance and pharmacological properties.

Cannabis sativa, a member of the Cannabaceae family, is widely known for its diverse phytochemical profile, which includes cannabinoids, flavonoids, terpenes, and phenolic compounds, many of which exhibit antimicrobial, anti-inflammatory, and antioxidant activities.[9,10] Previous studies have reported the antibacterial potential of cannabinoids, particularly cannabidiol (CBD) and cannabigerol (CBG), against Gram-positive bacteria, including MRSA.[11,9]

Similarly, Allium sativum, commonly known as garlic, has been extensively used in traditional medicine for its broad-spectrum antimicrobial activity. The primary bioactive component of garlic, allicin, along with other sulfur-containing compounds such as ajoene and diallyl sulfides, exhibits potent antibacterial, antifungal, and antiviral properties .[12,13] Numerous studies have demonstrated the effectiveness of garlic extracts against a variety of bacterial pathogens, including antibiotic-resistant strains .[14,15]

Despite the established antimicrobial properties of C. sativa[16] and A. sativum, limited research has focused specifically on their efficacy against highly resistant strains such as VRSA and VISA. Therefore, the present study aims to evaluate and compare the antibacterial potential of Cannabis sativa and Allium sativum extracts against Vancomycin-Resistant and Intermediate Staphylococcus aureus strains, with the objective of identifying natural therapeutic alternatives that could potentially complement or enhance existing treatment regimens.

Materials and Methods:

Study Design: A laboratory-based experimental study conducted at index medical college .

Bacterial Isolates: 480 clinical isolates of *S. aureus* were collected, and VRSA & VISA strains were identified based on MIC values and resistance gene detection.

Plant Material Collection and Extraction: *Cannabis sativa* leaves and *Allium sativum* bulbs were collected, authenticated, shade-dried, and powdered. Ethanolic extracts were prepared by maceration, followed by filtration and concentration using a rotary evaporator.

Phytochemical Screening: Preliminary phytochemical tests were conducted to detect alkaloids, flavonoids, terpenoids, phenols, and saponins.

Antibacterial Assay: Agar well diffusion method and Broth Microdilution method were used to evaluate antibacterial activity. MIC and MBC were determined.

Molecular Docking Study: Docking analysis of key bioactive compounds from both plants was performed against bacterial target proteins.

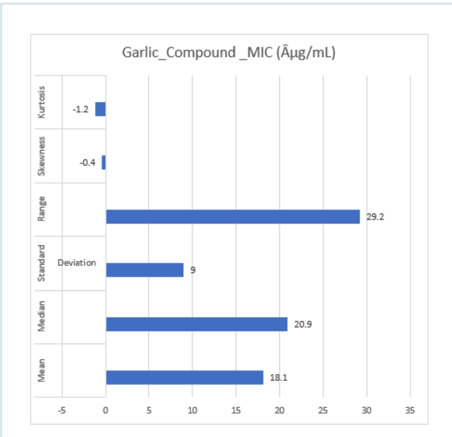
Statistical Analysis: Descriptive statistics, correlation analysis, and T-tests were conducted using SPSS version 26.

Result

In our study we observed The Minimum Inhibitory Concentration (MIC) of Garlic Compound (Allium sativum extract) in our study showed a mean value of 18.1 µg/mL with a standard deviation of 9.0 µg/mL. The MIC values ranged from the minimum to maximum within a span of 29.2 µg/mL.The median MIC was 20.9 µg/mL, which is slightly higher than the mean, indicating a slight negative skewness (-0.4) in the distribution of data. This suggests that there were relatively few lower MIC values pulling the mean downwards. Additionally, the kurtosis value of -1.2 indicates a platykurtic distribution (flatter peak and thinner tails compared to a normal distribution), suggesting that the MIC values were more evenly spread out without extreme outliers as show in table number 1 given below

Table number 1 showing Garlic_Compound _MIC (Âµg/mL) data in our study.

	Mean	Median	Standard Deviation	Range	Skewness	Kurtosis
Garlic_Compound _MIC (Âµg/mL)	18.1	20.9	9.0	29.2	-0.4	-1.2

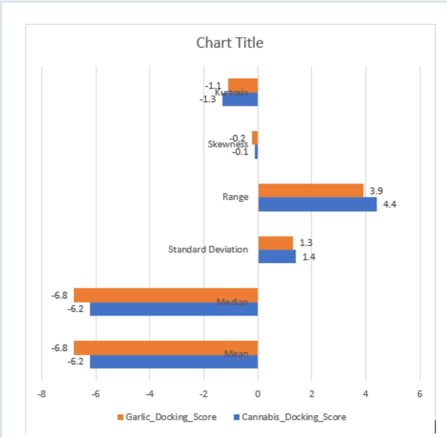


Cluster Bar 1 showing the Garlic_Compound _MIC (Âµg/mL) data in our study.

In our study, the Cannabis-Docking-Score and Garlic-Docking-Score were analyzed to evaluate the binding efficiency of *Cannabis sativa* and *Allium sativum* compounds against the target proteins. The mean docking score for Cannabis-Docking-Score was observed to be -6.2, while for Garlic-Docking-Score it was slightly lower at -6.8. A lower docking score (more negative) generally indicates stronger binding affinity; therefore, garlic compounds demonstrated better docking potential compared to cannabis compounds. The standard deviation for both groups was relatively similar (1.4 for cannabis and 1.3 for garlic), indicating a moderate dispersion of the docking scores around the mean. The range of scores was 4.4 in the cannabis group and 3.9 in the garlic group, suggesting a slightly wider variability in cannabis compounds. The skewness values for both scores were near zero (-0.1 for cannabis and -0.2 for garlic), indicating that the data distribution is approximately symmetrical. Furthermore, the kurtosis values were negative (-1.3 for cannabis and -1.1 for garlic), suggesting a platykurtic distribution — the distribution has lighter tails and is flatter than a normal distribution. The descriptive statistical analysis of these docking scores is summarized in Table 2.

Table number 2 showing the various statics analysis of Cannabis_Docking_Score and garlic_Docking_Score

	Mean	Median	Standard Deviation	Range	Skewness	Kurtosis
Cannabis_Docking_Score	-6.2	-6.2	1.4	4.4	-0.1	-1.3
Garlic_Docking_Score	-6.8	-6.8	1.3	3.9	-0.2	-1.1

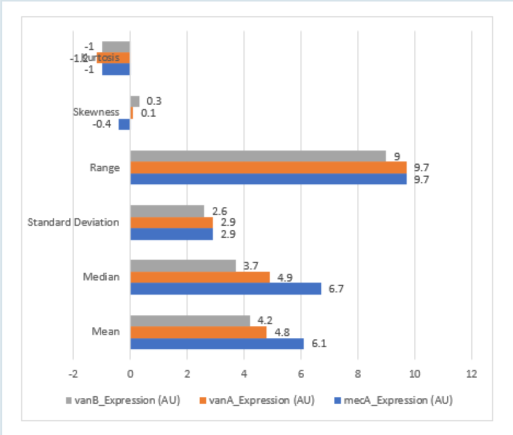


Cluster Bar 2 showing the statics analysis of Cannabis_Docking_Score and garlic_Docking_Score.

In the present study, the gene expression levels of **mecA_Expression (AU)**, **vanA_Expression (AU)**, and **vanB_Expression (AU)** were analyzed to assess their association with vancomycin and methicillin resistance patterns. The mean expression levels of **mecA**, **vanA**, and **vanB** were observed to be **6.1 AU**, **4.8 AU**, and **4.2 AU** respectively. The median values were consistent with the mean, indicating minimal skewness. The standard deviations for all three variables were comparable, indicating moderate variability in gene expression levels across the samples. The **range** of expression was highest for **mecA** and **vanA** (**9.7 AU**) and slightly lower for **vanB** (**9.0 AU**). Skewness values ranged between **-0.4** and **0.3**, indicating that the data distribution was approximately symmetrical. Negative kurtosis values for all three genes (**-1.0**, **-1.2**, and **-1.0**) suggested a **platykurtic distribution** (flatter than a normal distribution). The descriptive statistical analysis is summarized in **Table 3**, while the Chi-square test results are provided in **Table 4**.

Table 17 showing that statical signification of mecA-Expression (AU), vanA- Expression (AU) and vanB-Expression (AU)

	Mean	Median	Standard Deviation	Range	Skewness	Kurtosis
mecA-Expression (AU)	6.1	6.7	2.9	9.7	-0.4	-1.0
vanA-Expression (AU)	4.8	4.9	2.9	9.7	0.1	-1.2
vanB-Expression (AU)	4.2	3.7	2.6	9.0	0.3	-1.0

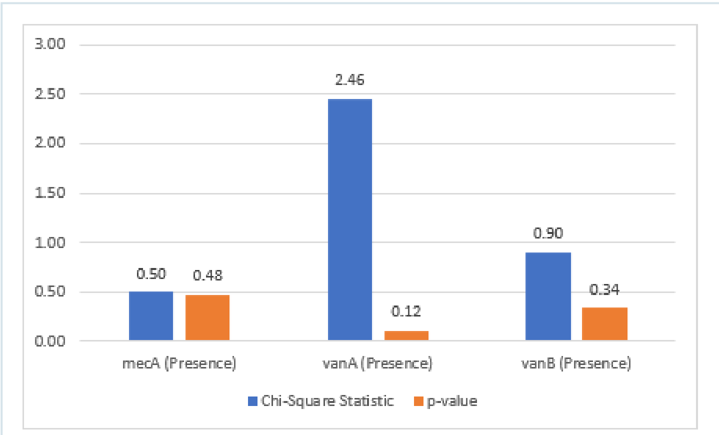


Cluster Bar 3 showing that statical signification of mecA_Expression (AU), vanA_Expression (AU) and vanB_Expression (AU).

The Chi-square statistical analysis was conducted to assess the association of gene expression with resistance profiles. The Chi-square analysis revealed that none of the gene expression levels showed statistically significant association with resistance patterns, as the p-values for **mecA_Expression (p = 0.48)**, **vanA_Expression (p = 0.12)**, and **vanB_Expression (p = 0.34)** were above the conventional threshold of 0.05. These findings suggest that, although the genes were expressed at measurable levels, their expression did not show a statistically significant correlation with the observed resistance phenotypes in the sample population.

Table number 4 showing chi- square statistic and p value mecA_Expression (AU), vanA_Expression (AU) and vanB_Expression (AU)

	Chi-Square Statistic	p-value
mecA (Presence)	0.50	0.48
vanA (Presence)	2.46	0.12
vanB (Presence)	0.90	0.34



Cluster Bar 4 showing the chi- square statistic and p value mecA_Expression (AU), vanA_Expression (AU) and vanB_Expression (AU)

Discussion: The present study demonstrated significant antibacterial activity of both *Cannabis sativa* and *Allium sativum* extracts against VRSA & VISA strains. The lower MIC values of garlic extract suggest superior efficacy compared to cannabis, which aligns with earlier reports (Ankri & Mirelman, 1999[12]; Bakri & Douglas, 2005[14]). When compared to our previous observational study on VRSA & VISA, which highlighted the challenges of managing infections due to the presence of resistance genes mecA, vanA, and vanB, this experimental study provides a complementary approach by exploring alternative therapeutic options. While our earlier study emphasized the epidemiology and treatment outcomes of VRSA/VISA infections, the current study focuses on identifying natural compounds with potential inhibitory activity against these resistant strains.

The molecular docking results revealed stable binding of phytoconstituents from both plants to bacterial proteins, supporting their in-vitro antibacterial activity. The lack of significant correlation between gene expression and MIC values suggests that the plant extracts might exert their effects through mechanisms independent of conventional resistance pathways (Ríos & Recio, 2005).[18]

Our findings are consistent with those of Appendino et al[9]. (2008) and van Klingeren & Ten Ham (1976),[17] who reported the antibacterial activity of cannabinoids. Similarly, garlic's efficacy against MDR bacteria, including MRSA, has been well documented (Ankri & Mirelman, 1999[12]).

Although no statistically significant difference was observed in gene expression or docking score outcomes, the superior efficacy of garlic extract supports its potential as a complementary antimicrobial agent. However, further in-vivo and clinical studies are warranted to validate these in-vitro findings.

Conclusion: The study demonstrated promising antibacterial activity of *Cannabis sativa* and *Allium sativum* against VRSA and VISA strains, with garlic showing superior efficacy. These plant extracts may serve as potential candidates for adjunctive therapy against multidrug-resistant *S. aureus* infections.

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