MEDDOCS Open Access Publisher

MedDocs Microbiology

Open Access | Research Article

Synergy Between Plant Extracts and Antimicrobial Drugs Against *Staphylococcus aureus* RN6390

Ankur Kumar[1]; Ganesh Kumar Verma[3]; Seema Kumari Sharma[7]; Avinash Bairwa[3]; Ashish Kothari[4]; Priyanka Singh[2]; Prashant Kumar[5]; Mohammad Faheem[1]; Sanjay Kumar[6]; Asgar Ali[1]; Mohd Babu Khan[1]*

[1]Glocal School of Life and Allied Health Sciences, Glocal University, Saharanpur-247121, India.

[2]Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore-632014, India.

[3]Department of Biochemistry, All India Institute of Medical Sciences, Rishikesh-249203, India.

[4]Department of Microbiology, All India Institute of Medical Sciences, Rishikesh-249203 India.

[5]Patanjali Herbal Research Department, Patanjali Research Institute, Haridwar- 249405, India.

[6]Glocal College of Paramedical Science and Research Centre, Glocal University, Saharanpur-247121, India.

[7]Department of Biotechnology, National Botanical Research Institute, Lucknow- 226001, India.

***Corresponding Author(s): Mohd Babu Khan**

Assistant Professor, Glocal School of Life and Allied Health Sciences, Glocal University, Saharanpur-247121, India.

Email: mmbk.bioinfo@gmail.com

Received: Oct 26, 2025

Accepted: Nov 21, 2025

Published Online: Nov 28, 2025

Journal: MedDocs Microbiology

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Abbreviations: Multi drug resistance; T7SSs; MIC; Infectious diseases; Medicinal plants; Bacteria.

Abstract

Staphylococcus aureus is a Gram-positive bacteria known for causing a wide range of life-threatening infections, particularly in hospital settings. It has developed resistance to various antibiotics through genetic alterations and the involvement of specialized secretion systems. Among these, the Type VII secretion system (T7SS) is of particular interest. Although T7SS plays a key role in the pathogenicity and survival of *S. aureus* during infection, it remains only partially characterized due to the unidentified functions of some of its component proteins. Multidrug-resistant clinical strains of *S. aureus* often harbor T7SSs and are implicated in severe infections with high mortality rates. The RN6390 strain of *S. aureus* serves as a laboratory model for investigating the T7SSs. In this study, we evaluated the synergistic effects of 13 antimicrobial drugs combined with eight different plant extracts—Neem (*Azadirachta indica*), Guava (*Psidium guajava*), Clove (*Syzygium aromaticum*), Garlic (*Allium sativum*), Ear-pod Wattle (*Acacia auriculiformis*), Ginger (*Zingiber officinale*), Papaya (*Carica papaya*), and Mint (*Mentha piperita*)—against the *S. aureus* RN6390 strain. Antimicrobial susceptibility testing was conducted using the disc diffusion method. Petri dishes were prepared using Mueller-Hinton Agar (MHA) with or without sub-inhibitory concentrations of plant extracts. Zones of inhibition were measured in millimetres. The in vitro activity of all tested plant extracts against *S. aureus* RN6390 was confirmed. Synergistic effects with antibiotics were observed for all extracts, with clove, guava, and ear-pod wattle showing the most significant synergism. In contrast, ginger and garlic demonstrated limited synergistic activity. The primary objective of this study was to identify novel synergistic combinations of plant-derived compounds and antimicrobial drugs. The findings contribute to ongoing efforts in developing alternative therapeutic strategies using natural antimicrobial agents.

Cite this article: Kumar A, Verma GK, Sharma SK, Bairwa A, Kothari A, et al. Synergy Between Plant Extracts and Antimicrobial Drugs Against *Staphylococcus aureus* RN6390. MedDocs Microbiol. 2025: 4(2): 1005.

Introduction

Medicinal plants have long been recognized as valuable sources of therapeutic agents, playing a vital role in traditional medicine and contributing to the improvement of human health and quality of life. Many plant species have been scientifically reported to possess medicinal properties, largely due to their bioactive compounds that participate in biological processes essential for disease treatment and prevention [1]. For many communities, particularly in rural or indigenous settings, medicinal plants often serve as the primary—sometimes the only—source of healthcare [2]. Their use is especially critical in developing countries, where access to conventional medicine may be limited and reliance on traditional remedies forms a key component of primary healthcare [3,4]. Despite advances in pharmaceutical research and the development of numerous antimicrobial agents in recent decades, infectious diseases remain a major cause of morbidity and mortality, particularly in under-resourced regions [4]. Compounding this challenge is the rising problem of antimicrobial resistance. Microorganisms, especially bacteria, possess the genetic capacity to acquire and transmit resistance to drugs, diminishing the efficacy of many conventional treatments [5]. In vitro studies focusing on plants traditionally used in medicine have been widely conducted within microbiology, particularly regarding their effects on pathogenic bacterial growth. However, investigations into the synergistic potential between plant extracts and conventional antimicrobial agents remain limited. In this study, we assessed the in vitro synergistic interactions between extracts of Neem (*Azadirachta indica*) [6], Guava (*Psidium guajava*) [7], Clove (*Syzygium aromaticum*) [8], Garlic (*Allium sativum*) [9], Ear-pod Wattle (*Acacia auriculiformis*) [10], Ginger (*Zingiber officinale*) [11], Papaya (*Carica papaya*) [12], and Mint (*Mentha piperita*) [13], and selected antimicrobial drugs. The assays were performed against the *Staphylococcus aureus* RN6390 strain using the Kirby-Bauer disk diffusion method.

Materials and methods

Plant samples

Samples of *A. indica*, *P. guajava*, *C. papaya*, *M. piperita*, and *A. auriculiformis* were collected in July 2025 from Janta Inter College, Behat, Saharanpur-247121. Voucher specimens were prepared and deposited at the designated herbarium for future reference. The collected leaves were dried at 40°C and ground into fine powder using a mechanical mill [14,15]. In the same time, *A. sativum*, *S. aromaticum*, and *Z. officinale* were procured fresh from the local market and used in their natural form for extract preparation.

Preparation of plant extracts

Plant materials—dried (*Azadirachta indica*, *Psidium guajava*, *Carica papaya*, *Mentha piperita*, *Acacia auriculiformis*) and fresh (*Allium sativum*, *Syzygium aromaticum*, *Zingiber officinale*)—were finely ground and extracted with 70% methanol. The initial extraction process lasted 48 hours, after which the mixtures were filtered [15]. The residual plant matter was re-extracted with fresh 70% methanol for an additional 24 hours, followed by a second filtration [15,16]. The combined methanolic extracts were then concentrated using a rotary evaporator at 45°C to remove the solvent. The crude extracts were stored in sterile bottles under refrigerated conditions until use. To determine the extract concentrations (mg/ml), the dry weight was calculated by complete evaporation of the remaining solvent [16].

Bacterial strains

Staphylococcus aureus RN6390, a commonly used laboratory strain that was developed from NCTC8325, was utilized in this study. The NCTC8325 strain was obtained from the Department of Microbiology at NYU Grossman School of Medicine, located at 540 First Avenue, 2nd Floor, Lab 1-2, New York, NY 10016.

Media used

To maintain bacterial broth cultures, a nutrient broth was prepared using 0.5 g of NaCl, 0.5 g of peptone, and 0.3 g of beef extract per 100 ml of distilled water. For solid media, nutrient agar was formulated by adding 1.5 g of agar to the broth base [17].

Preparation of working slant

The *S. aureus* NCTC8325 stock culture was maintained at 4°C on semi-solid agar slants composed of 0.5% peptone, 0.3% beef extract, and 1.5% agar. For experimental use, a loopful of the culture was aseptically transferred from the stock to initiate active working cultures [17]. These slants were then incubated at 36 ± 1.0°C for 24 hours.

Broth preparation

Under sterile conditions, isolated colonies from the cultured *S. aureus* NCTC8325 slant were picked using a sterile inoculating loop and transferred into a cooled, autoclaved liquid broth medium containing 0.5% peptone and 0.3% beef extract [17,18]. The inoculated broth was then incubated at 36 ± 1.0°C for 24 hours, until visible turbidity indicated active bacterial growth.

Antimicrobial tests

Prior to evaluating the synergistic effects between plant extracts and antimicrobial agents, the Minimum Inhibitory Concentration (MIC) of each extract was determined against the *Staphylococcus aureus* RN6390 strain. This was done by incorporating serial dilutions of the extracts into Mueller Hinton Agar (MHA) media NCCLS (2004a,b). Petri dishes containing various concentrations of the plant extracts (mg/ml), along with appropriate controls, were inoculated with approximately 10^4 CFU of *S. aureus* RN6390 using a Steer's replicator and incubated at 37°C for 24 hours. The lowest concentration of each plant extract that visibly inhibited bacterial growth was recorded as the MIC, and the MIC_{90} (the concentration inhibiting 90% of isolates) was subsequently calculated. For synergism assays, one-fourth of the MIC_{90} was used as the sub-inhibitory concentration, as described previously [19]. These assays were performed on *S. aureus* RN6390 and NCTC8325 strains using the disk diffusion method (Kirby-Bauer), following NCCLS (2004) guidelines on Mueller Hinton Agar (MHA). A total of thirteen antimicrobial agents were tested: cefuroxime (CEO; 10 IU), cefazolin (CEF; 1 µg), cefixime (CEI; 30 µg), imipenem (IMP; 10 µg), cephalexin (CFL; 30 µg), ertapenem (EPN; 30 µg), clarithromycin (CLM; 30 µg), linezolid (LNZ; 10 µg), streptomycin (STM; 30 µg), tigecycline (TIC; 30 µg), amikacin (AMC; 15 µg), sulfadiazine (SUZ; 25 µg), and ciprofloxacin (CPF; 5 µg). For each *S. aureus* RN6390 strain, two sets of antibiograms were performed in duplicate: one on control plates containing plain Mueller Hinton Agar (MHA), and the other on MHA supplemented with one-fourth the MIC_{90} of the respective plant extract. After incubation at 37°C for 18 hours, the diameters (in mm) of the inhibition zones were measured and recorded [19,20].

Statistical analysis

Data obtained from the synergism assays were analysed using the Wilcoxon nonparametric test to compare the inhibition zone diameters (mm) generated by the disk diffusion method. Statistical analysis was performed using Minitab Statistical Software, version 13.32 [20,21]. A p-value of less than 0.05 was considered statistically significant.

Results and discussion

Characteristics, MIC 90% (mg/ml) against *S. aureus* RN6390 strain, and one-fourth the MIC 90% values obtained in the synergism assays for the plants and their respective extracts are presented in Table 1.

Anti-*S. aureus* activity was verified for all the plants. *S. aromaticum* showed the highest activity, followed by *P. guajava*; the lowest activity was recorded for *A. auriculiformis*. The MIC 90% range was 0.37 mg/ml for clove and 18.01 mg/ml for *A. auriculiformis* and it is not surprising the differences in the antimicrobial activity of plants tested, due to phytochemical properties and differences among species. Although the antimicrobial activities of *A. auriculiformis*, *C. papaya,* and *Z. officinale*, have not been relatively high, synergism assays were carried out for them and the synergism rate of *A. auriculiformis* was as high as that of *S. aromaticum* Table 2.

Table 1: Characteristics, minimum inhibitory concentration (MIC_{90}) values, and sub-inhibitory concentrations (¼ MIC_{90}) used in synergism assays for the tested plants and their extracts.

Scientific name	Common name	Part of the plant used	Efficacy (%)	Extracts dry weight (mg/ml)	MIC 90% (mg/ml)	¼ MIC 90% (mg/ml)
Syzygium aromaticum	Clove	Flower buds	-	59.01	0.37	0.10
A.auriculiformis	Ear-pod Wattle	Leaves	26.01	64.00	18.01	5.02
Azadirachta indica	Neem	Leaves	29.02	60.01	3.85	0.97
Psidium guajava	Guava	Leaves	48.01	132.01	0.53	0.14
Mentha piperita	Mint	Leaves	20.85	11.01	2.25	0.57
Allium sativum	Garlic	Bulbs	-	94.11	5.05	1.26
Zingiber officinale	Ginger	Rhizomes	-	11.80	3.60	0.90
Carica papaya	Papaya	Leaves	-	11.02	3.40	0.89

(-): For non-dried (fresh) plant materials, the extract efficacy was considered as 100% of the original sample.

Table 2: Rate of synergistic activity between antimicrobial agents and plant extracts against *Staphylococcus aureus* RN6390 strain using the Kirby-Bauer disk diffusion method.

Drug target	Drug	*Acacia auriculiformis*	*Syzygium aromaticum*	*Allium sativum*	*Azadirachta indica*	*Carica papaya*	*Zingiber officinale*	*Mentha piperita*	*Psidium guajava*	*Synergism rate (extract/drug)*	*MEAN*
Protein synthesis	Tigecycline	x	x	x	x	x	x	x	x	8	5.1
	Clarithromycin	x	x	-	x	x	x	x	x	7	
	Streptomycin	x	-	x	x	x	x	x	x	7	
	Amikacin	x	x	-	-	x	-	x	-	4	
	Linezolid	x	-	x	x	-	-	x	-	4	
Cell wall synthesis	Cefixime	x	x	-	x	x	-	-	x	5	3.9
	Cefuroxime	x	x	-	x	x	-	-	x	5	
	Cefazolin	x	x	-	-	-	-	x	-	3	
	Cephalexin	x	x	-	x	-	-	-	x	4	
	Imipenem	x	x	-	-	-	-	-	-	2	
	Ertapenem	-	x	-	-	-	-	-	x	2	
Nucleic acids	Ciprofloxacin	-	x	-	-	-	-	-	x	2	2
Folic acid	Sulfadiazine	x	x	x	-	-	-	-	x	4	4
Total	13	11	11	4	6	6	3	6	9		

X: synergism when p<= 0.05, (-) no synergism.

Antimicrobial mechanisms of the drugs used here were variable and the protein synthesis inhibitors were those that presented strongest synergistic effect (5.1 extracts/drug) together with folic acid (4 extracts/drug) and bacterial cell wall synthesis (3.9 extracts/drug) inhibitors. Inhibitors of the nucleic acid synthesis (2 extracts/drug) showed weak synergism with plant extracts. Among the protein synthesis inhibitors, tigecycline showed synergism with all the extracts, followed by clarithromycin and streptomycin. The synergistic capacity was promising for the extracts of some plants such as *S. aromaticum*, *A. auriculiformis*, and *P. guajava*, which presented synergism with 11, 11, and 9 drugs, respectively; while garlic and ginger showed synergism with only 4 and 3 drugs, respectively.

The synergism recorded here to plant extracts with weak action on *S. aureus* RN6390 growth, such as *A. auriculiformis*, is an important data since it showed a synergism profile similar to that of the clove extract, considered the most efficient *S. aureus* RN6390 growth inhibitor in this study. Thus, the researchers should investigate the synergistic capacity of plant extracts or other natural products, independent of the antimicrobial activ-

ity they have. Therefore, the results of the present study seem to be promising and may enhance the natural products uses, showing the potential of these plants in the treatment of infectious diseases caused by *S. aureus.*

Future studies on the chemical characteristics of extracts and active components should be carried out for each plant and antimicrobial property, since only crude extracts and their dry weight have been used in MIC determination (expressed in mg/ml) and synergism assays. In the current study, the plant extract antimicrobial activity against *S. aureus* RN690 strain was confirmed and synergism was possible with all the antimicrobial drugs tested. tigecycline showed synergism with all plant extracts; and the *A. auriculiformis* extract, although with the lowest antimicrobial activity, presented a synergism profile similar to that of *S. aromaticum*, whose extract showed a relatively high inhibitory capacity on *S. aureus* RN6390 growth. The possible activities of substances found in plant extracts on ribosome structure and bacterial enzymes inhibition appear to be related with synergism profile between plant extracts and inhibitors of protein synthesis, however, the understanding of synergism mechanism is fundamental to development of pharmacological agents to treat diseases by *S. aureus* using medicinal plants.

Author declarations

Author contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Disclosures human subjects

All authors have confirmed that this study did not involve human participants or tissue.

Conflicts of interest

No conflicts of interest.

Payment/services info

All authors have declared that no financial support was received from any organization for the submitted work.

Financial relationships

All authors have declared that they have no financial relationships at present with any organizations that might have an interest in the submitted work.

Other relationships

All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgments

To professor Richard P. Novick, MD, Principal Investigator, Research Professor in the Department of Microbiology and Professor Emeritus of Microbiology and Medicine, NYU Grossman School of Medicine's Department of Microbiology at 540 First Avenue, 2nd floor, Lab 1-2, New York, NY 10016 for providing the S. aureus RN6390 strain.

References

1. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005; 100: 80-4.
2. Zazharskyi VV, Davydenko P, Kulishenko O, Borovik IV, Brygadyrenko VV. Antimicrobial activity of 50 plant extracts. Biosyst Divers. 2019; 27: 163-9.
3. Ates DA, Turgay O. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol. 2003; 27: 157-62.
4. Othman M, San Loh H, Wiart C, Khoo TJ, Lim KH, Ting KN. Optimal methods for evaluating antimicrobial activities from plant extracts. J Microbiol Methods. 2011; 84: 161-6.
5. Eloff JN. Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. BMC Complement Altern Med. 2019; 19: 1-8.
6. Francine U, Jeannette U, Pierre RJ. Assessment of antibacterial activity of neem plant (Azadirachta indica) on Staphylococcus aureus and Escherichia coli. J Med Plants Stud. 2015; 3: 85-91.
7. Nsele NW, Mathobela KP, Radebe SP, Thembane N. In vitro antibacterial activity of Psidium guajava (guava) extracts against MRSA and MSSA. J Med Plants Econ Dev. 2025; 9: 280.
8. Ajobiewe HF, Elisha E, Ibrahim AE, Abioye JO, Ajobiewe JO, Yashim AN, et al. Antimicrobial activity of clove plant extract (Syzygium aromaticum) on Staphylococcus aureus. Sch J App Med Sci. 2022; 4: 520-4.
9. Yunus FT, Suwondo A. Phytochemical compound of garlic (Allium sativum) as an antibacterial to Staphylococcus aureus growth. IOP Conf Ser Mater Sci Eng. 2021; 1053: 012041.
10. Mandal P, Babu SS, Mandal NC. Antimicrobial activity of saponins from Acacia auriculiformis. Fitoterapia. 2005; 76: 462-5.
11. Wang X, Shen Y, Thakur K, Han J, Zhang JG, Hu F, et al. Antibacterial activity and mechanism of ginger essential oil against Escherichia coli and Staphylococcus aureus. Molecules. 2020; 25: 3955.
12. Hartanti F, Hidayati DN. Antibacterial activity of papaya seed (Carica papaya L.) ethanol extract with MAE and UAE extraction methods against Staphylococcus aureus. J Pharm Sci. 2023; 20: 51-6.
13. Mahmoudi S, Nasiri R, Jafari Sales A. In-vitro antibacterial effects of methanolic extract of peppermint (Mentha piperita Lamiaceae) on standard Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa strains. Jorjani Biomed J. 2019; 7: 4-10.
14. Al-Salt J. Antimicrobial activity of crude extracts of some plant leaves. Res J Microbiol. 2012; 7: 59-67.
15. Hsieh PC, Mau JL, Huang SH. Antimicrobial effect of various combinations of plant extracts. Food Microbiol. 2001; 18: 35-43.
16. Imran M, Khan AS, Khan MA, Saeed MU, Noor N, Warsi MH, et al. Antimicrobial activity of different plant extracts against Staphylococcus aureus and Escherichia coli. Polym Med. 2021; 51: 69-75.
17. Jahan F, Lawrence R, Kumar V, Junaid M. Evaluation of antimicrobial activity of plant extracts on antibiotic susceptible and resistant Staphylococcus aureus strains. J Chem Pharm Res. 2011; 3: 777-89.
18. Snowden R, Harrington H, Morrill K, Jeane L, Garrity J, Orian M, et al. A comparison of the anti-Staphylococcus aureus activity of extracts from commonly used medicinal plants. J Altern Complement Med. 2014; 20: 375-82.
19. Kothari A, Jain N, Kishor Kumar S, Kumar A, Kaushal K, Kaur S, et al. Potential synergistic antibiotic combinations against fluoroquinolone-resistant Pseudomonas aeruginosa. Pharmaceuticals. 2022; 15: 243.

20. Chuah EL, Zakaria ZA, Suhaili Z, Bakar SA, Desa MM. Antimicrobial activities of plant extracts against methicillin-susceptible and methicillin-resistant Staphylococcus aureus. J Microbiol Res. 2014; 4: 6-13.

21. Ushimaru PI, Silva MT, Di Stasi LC, Barbosa L, Fernandes Junior A. Antibacterial activity of medicinal plant extracts. Braz J Microbiol. 2007; 38: 717-9.