Pak. J. Bot., 58(6): 1385-1391, June 2026.

ARTICLE DOI: http://dx.doi.org/10.30848/PJB2026-6(17)

IN SILICO AND *IN VITRO* STUDIES ON THE SYNERGISTIC EFFECT OF *PUNICA GRANATUM* L. AND *NIGELLA SATIVA* L. EXTRACTS IN INHIBITING THE GROWTH OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Abstract

Antimicrobial resistance (AMR) is a major public health concern due to antibiotic misuse. The prevalence of drug resistance has drawn attention to the antibacterial qualities of plants and their metabolites. *Punica granatum* and *Nigella sativa* are well-known plant species for their bioactive antimicrobial phytochemicals. However, the current research does not provide enough insight into the specific interactions between these phytochemicals and bacterial target proteins. The present study employed molecular docking to investigate the likely interactions of bioactive phytochemicals from *P. granatum* and *N. sativa* with the known antibacterial target proteins to fill this critical missing link. The drug-likeliness of the phytochemicals was predicted by SwissADME. Among the tested phytochemicals, punicalin, a compound unique to *P. granatum*, exhibited the highest binding affinity (-19.4 kcal/mol) followed by dithymoquinone (-12.9 kcal/mol), derived from *N. sativa* for iron-regulated surface determinant protein A of *Staphylococcus aureus*. Nonetheless, SwissADME predicted more favorable drug-likeness properties for dithymoquinone than punicalin. To validate the predicted results, powdered samples of *N. sativa* and *P. granatum* were extracted separately in methanol, ethyl acetate, and distilled water. Analysis of Variance (ANOVA) followed by Tukey HSD test was calculated at $p \leq 0.05$ to measure statistically significant differences among the means. In a well diffusion assay, the combination of methanolic extracts of *P. granatum* and *N. sativa* showed significantly the highest value (2.71 cm) of bacterial growth inhibition zone compared to the rest of the extracts, whereas the lowest value of bacterial growth inhibition zone was shown by aqueous extract of *N. sativa* (0.39 cm). Similar results were recorded in the disc diffusion assay. These results indicate that combining *P. granatum* and *N. sativa* extracts leads to synergistic antimicrobial activity against *S. aureus* which may prove a promising strategy for combating AMR.

Key words: Dithymoquinone; Punicalin; Antimicrobial resistance; Bioactive phytochemicals

Introduction

Antimicrobial resistance (AMR) is a serious global health threat that undermines the effectiveness of antimicrobials. Various factors, including the overuse and misuse of antimicrobials, globalization, increased human mobility, pollution, ecosystem degradation, and climate change, are responsible for the increase in AMR. By 2050, it is predicted that AMR could cost the world economy $100 trillion resulting in over 10 million deaths annually if action is not taken to slow down the current rates of emergence and spread. (Anthony *et al*., 2021). The microbes in general and bacteria in specific acquire AMR by modifying drug receptor sites, enzymatic degradation and efflux pump activation of drugs, and mutation in RNA polymerase, and DNA gyrase to make the drug inactive (Ayaz *et al*., 2019). This escalating problem has intensified the demand for innovative therapeutic strategies capable of overcoming the limitations of existing antimicrobial agents. Within this context, plant-derived bioactive molecules have gained considerable attention as promising candidates for antimicrobial drug discovery (Li *et al*., 2024).

In the last decade, there has been a notable shift in the focus of antimicrobial discovery towards natural sources, including plants, fungi, lichens, endophytes, and various marine organisms such as seaweeds and corals. Several reports show medicinal plant-derived compounds exhibit better interaction with receptor sites of microorganisms and inhibit or evade drug efflux pumps (Seukep *et al*., 2020). Among the medicinal plants, pomegranates (*Punica granatum* L.) and black seeds (*Nigella sativa* L.) have long been favored as functional foods and sources of nutraceuticals due to their antioxidant, anti-carcinogenic, and anti-inflammatory qualities. Pomegranate peel, juice, seeds, and fruits are all rich in bioactive compounds that have been shown to have therapeutic properties and promote health. These compounds include ellagic acid, ellagitannins, punicalagins, punicic acid, flavonoids, anthocyanidins, and anthocyanins, as well as other fatty acids and flavones that can be used for medicinal purposes (Maleš *et al*., 2023). Seeds of *N. sativa* are used in ancient medicine for several ailments including back pain, asthma, fever, bronchitis, cough, chest congestion, dizziness, paralysis, chronic headache, inflammation, infertility, and

Received: 09-04-2025 Revised: 03-12-2025 Accepted: 13-12-2025 Online: 25-02-2026

other gastrointestinal disorders like dyspepsia, flatulence, diarrhea, and dysentery (Ahmad *et al*., 2021). Important bioactive metabolites of *N. sativa* include α-pinene, p-cymene, thymoquinone, thymohydroquinone, nigellicine, dithymoquinone, nigellicimine and nigellicimine (Abbas *et al*., 2024). *Staphylococcus aureus* is a Gram-positive bacterium responsible for a wide spectrum of diseases, ranging from mild skin and soft tissue infections to severe respiratory tract infections (Onyeaka & Nwabor, 2022). Despite being named for its methicillin resistance, MRSA has evolved resistance to a broad range of antibiotics. The virulence of the pathogen is attributed to a diverse range of toxins, adhesins, and immune-evasion factors that facilitate efficient colonization of hosts and the establishment of persistent infections (Loggenberg *et al*., 2022). Consequently, *S. aureus* represents a significant target for the advancement of innovative antimicrobial strategies.

Phytochemicals' characterization as antimicrobial agents have been proved very effective for combating AMR. Phytoalexins, phytoanticipins, and phytoavengins are three major classes of plant compounds are reported to have antimicrobial activities. Phytoalexins are low molecular weight plant secondary metabolites produced in response to pathogen attack. Examples of phytoalexins include glyceollin and stilbene (Lin *et al*., 2024). Contrary to phytoalexins, phytoanticipins are unique in their ability to be synthesized even before the onslaught of a pathogen or infection. In their physiologically active compound (constitutive), they exist in a healthy state. Examples include maysin, glucosinolates, and dhurrin (Yactayo-Chang *et al*., 2020). Phytoavengins previously categorized as phytalexins are produced in response to tissue damage and include danielone and sakuranetin (Kliebenstein & Kvitko, 2023**)**. Combinatorial application of phytochemicals is an effective strategy to synergize the bioactive effects of individual compounds. A synergistic interaction between two compounds is preferable since it permits the use of lower dosages of the combination constituents, thus shortening the course of the therapy and lowering the risk of dose-dependent toxicity (Bonincontro *et al*., 2023). Moreover, synergism also helps overcome pathogens' resistance by facilitating the compounds in building a viable interaction with their target receptor sites (Chen *et al*., 2024). A synergistic interaction of the phytochemicals with their target antimicrobial proteins may make the microbes more susceptible to the compounds of medicinal plants.

Based on this, we selected the molecular docking technique to predict the interaction between phytochemicals of selected medicinal plants and their target antimicrobial proteins. The predicted interaction was validated by *In vitro* antimicrobial assays. Furthermore, it was hypothesized that bioactive compounds of two medicinally well-known plants, *P. granatum* and *N. sativa*, may act synergistically to inhibit the growth of a methicillin-resistant strain of *S. aureus*.

Materials and Methods

In-silico Analyses

Retrieval of phytochemicals and target proteins: Table 1 shows a list of *N. sativa* and *P. granatum* phytochemicals obtained through an extensive literature review. The structures of selected phytochemicals were downloaded from PubChem in sdf format. OpenBabel was used to convert sdf to pdbqt (O'Boyle *et al*., 2011). Antibacterial target proteins of *S. aureus* listed in Table 2 were downloaded from the protein data bank.

Table 1. List of bioactive phytochemicals of *N. sativa* and *P. granatum* as ligands for molecular docking.

Plant	Phytochemical	PubChem ID
N. sativa	Flaccidoside 2	24799010
	Nigellicine	11402337
	Linolenic acid	5280934
	Rutin	5280805
	Linoleic acid	5280450
	Apigenin	5280443
	Dithymoquinone	398941
	Sapindoside	161686
	Thymohyroquinone	95779
	Isosalsolidine	20725
	Tetradecanoic acid	11005
	Thymoquinone	10281
	P-CYMENE	7463
	Lauric acid	3893
	Palmitic acid	985
	Pyrocatechol	289
	Nigelloside A	275794242
P. granatum	Punicalin	92131301
	Ttriterpenoids	71597391
	Granatin B	50903199
	Punicalagin	44584733
	Ellagic acid	5281855
	Punicic acid	5281126
	Pedunculagin	442688
	Tellimagrandin 2	151590
	Anthraquinone	6780
	Gallic acid	370
	Salicylic acid	338

Table 2. List of target antibacterial proteins of *S. aureus* against the selected ligands for molecular docking.

Protein ID	Name
2ITE	Iron-regulated surface determinant A (IsdA) NEAr iron Transporter (NEAT) domain
2Z8L	Staphylococcal superantigen-like protein SSL5
3T41	Epidermin Leader Peptide Processing Serine Protease (EpiP)
6LXH	Surface protein serine-aspartate repeat-containing protein C (SdrC)
6LXS	Cell Wall Anchored (CWA) serine-aspartate repeat-containing protein C protein (SdrC)

Molecular docking: All the proteins listed in Table 2 were docked against every phytochemical in Table 1. A blind docking was performed, in which the grid covered the whole protein. The selected proteins were docked against the phytochemicals using PyRx software (Dallakyan & Olson, 2014). The binding affinity values were recorded. Discovery Studio Visualizer visualized protein-ligand interaction.

In-silico ADME and drug-likeliness: Swiss ADME was used to evaluate the in-silico ADME and drug-likeliness of the ligands that showed the best binding affinity with targeted antibacterial proteins. Parameters such as Lipinski's rule of five violations, GI absorption, solubility, and bioavailability were recorded (Daina *et al*., 2017).

Collection and Extraction of Plant Sample: Seeds of *N. sativa* and *P. granatum* were collected from the herbal store. The seeds were washed with tap water and shade-dried at room temperature for approximately two weeks. The dried samples were ground separately into fine powder using an electric blender. 10 g of each plant powder was soaked in 100 mL of methanol, ethanol, distilled water, and ethyl acetate separately for 1 week. The samples were then sonicated for 20 min and the resultant solution was filtered with Whatman filter paper and dried. The dried extract was accurately weighed, and 0.5 mg of each extract was dissolved in 1 mL of DMSO. The pure extracts (P) were serially diluted in DMSO in a concentration of 1:10 (D1), 1:00 (D2), and 1:1000 (D3). Similar dilutions were prepared for the combinatorial effect of extracts of *P. granatum* and *N. sativa*.

Antibacterial: Assay *Staphylococcus aureus* strain resistant to methicillin (ATCC 43300) obtained from COMSATS University, Abbottabad Campus was used to validate the antibacterial potential of plant extracts. 28 g of the nutrient agar and 14 g of the nutrient broth were mixed separately in 1 L distilled water. The mixtures were sterilized in an autoclave at 121℃ for 20 min at 15 psi. The sterilized broth was inoculated with a bacterial strain to get the desired optical density. A spectrophotometer was used to determine the optical density of the bacterial strain. In a biosafety cabinet, the sterilized nutrient agar media were poured into sterile petri plates for solidification under aseptic conditions. Using a sterile loop, a known concentration of bacteria was streaked onto petri dishes.

With some modifications, the agar well diffusion method was employed to investigate the antibacterial activity of *N. sativa* and *P. granatum* seed extracts. The wells were made using a sterile cork borer on nutrient agar media after solidifying and streaking with a bacterial strain. A total of five wells were made on each 9 mm agar plate. A positive control of amoxicillin solution of 30 µg/mL concentration whereas a negative control of DMSO was used. Three concentrations of *N. sativa* and *P. granatum* seed extracts alone (P, Dil1, and Dil2) and combined were added to the wells. After incubation at 37℃ for 24 hours, all plates were examined; the diameter of zones of growth inhibition was measured (Silvero *et al*., 2018).

Statistical analysis

The *In vitro* antibacterial activity was carried out in triplicate. Two-way ANOVA was carried out using Statistics 8.1. The independent variables were plant type and solvent type. Tukey HSD test was used to compare mean values. Graphs were made by GraphPad Prism 8.0. All the values were given as mean ± SD.

Results

In-silico Analyses

Molecular docking of *P. granatum* phytochemicals: Interestingly, punicalin, unique to *P. granatum*, showed the highest binding affinity with all the selected proteins. Of the proteins, the highest binding affinity (-19.4 kcal.mol) of punicalin was observed for IsdA (2ITE) of *S. aureus*. The binding affinity of ellagic acid followed punicalin for all the selected proteins. Ellagic acid showed its highest binding affinity (-11.9 kcal/mol) with EpiP (3T41) (Fig. 1).

Molecular docking of *N. sativa* phytochemicals: Among the phytochemicals of *N. sativa*, dithymoquinone showed the highest binding affinity with the selected proteins. The highest binding affinity of dithymoquinone (-12.9 kcal/mol) was observed for IsdA (2ITE). Dithymoquinone was followed by rutin which showed its binding affinity of -11.3 kcal/mol with EpiP (3T41) and -11.2 kcal/mol with SrdC (6LXH) (Fig. 2).

ADME and drug-likeliness: Among the 4 ligands (punicalin, ellagic acid, dithymoquinone, and rutin) with the highest binding affinities with target proteins, two molecules, namely ellagic acid and dithymoquinone, showed drug-likeliness and lead-likeness, 0 violations of Lipinski's rule of five, had high GI absorption, and were soluble in water. The other two molecules, punicalin and rutin, owing to their high molecular weight, had no drug-likeliness and lead-likeliness, each showed 3 violations of Lipinski's rule of five, had low GI absorption, and moderate to less solubility in water (Supplementary Tables 1).

In vitro Analyses

***S. aureus* growth in well diffusion assay:** It was observed that D3 (1:1000) did not significantly inhibit the growth of *S. aureus*. Figure 3 shows the inhibition zones observed for pure, D1 (1:10), and D2 (1:100). Two-way ANOVA for the three concentrations was significant at $p \leq 0.05$. The inhibition potential of the methanolic extract was highest, followed by the ethyl acetate extract. The inhibition of *S. aureus* growth was relatively the least for aqueous extracts. The combination of the extracts produced better inhibition of bacterial growth than the application of *N. sativa* and *P. granatum* extracts alone. The combination of pure methanolic extracts of *N. sativa* and *P. granatum* was most effective in producing the highest zone of inhibition (2.71 cm) against *S. aureus*. The combined methanolic extracts of *N. sativa* and *P. granatum* were equally effective in marking the highest zone of inhibition in response to D1 (2.46 cm) and D2 (2.09 cm). The least zone of inhibition (0.39 cm) was observed for D2 of aqueous extracts.

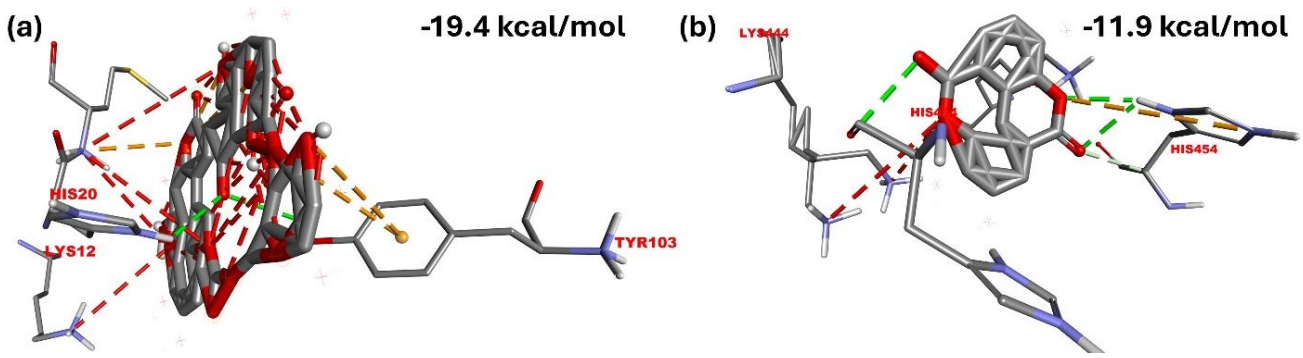

Fig. 1. Molecular docking of (a) IsdA protein with punicalin (-19.4 kcal/mol) and (b) EpiP with ellagic acid (-11.9 kcal/mol). The dotted line shows the binding of ligands with amino acids of the target proteins through hydrogen bonds (green), attractive charges (orange), and unfavorable positive-positive interaction (red).

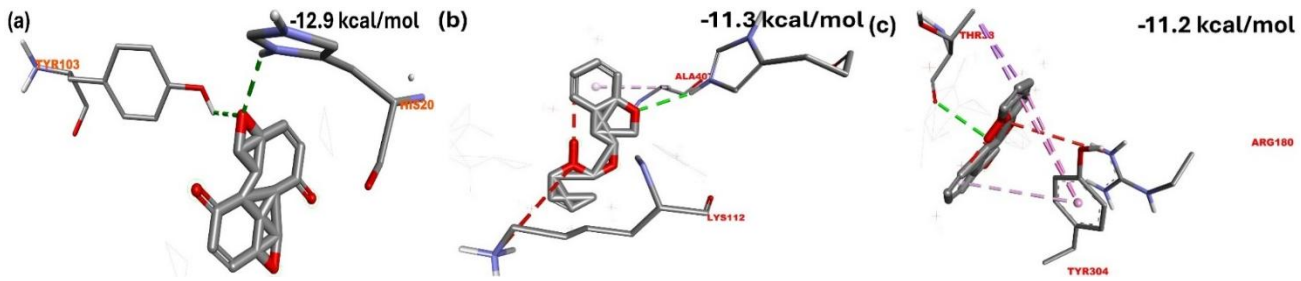

Fig. 2. Molecular docking of (a) IsdA protein with dithymoquinone (-12.9 kcal/mol), (b) EpiP with rutin (-11.3 kcal/mol) and (c) and SrdC with rutin (-11.2 kcal/mol). The dotted line shows the binding of ligands with amino acids of the target proteins through hydrogen bonds (green), pi-sigma (indigo), pi-alkyl (light pink), pi-pi-T shaped (dark pink), and unfavorable positive-positive interaction (red).

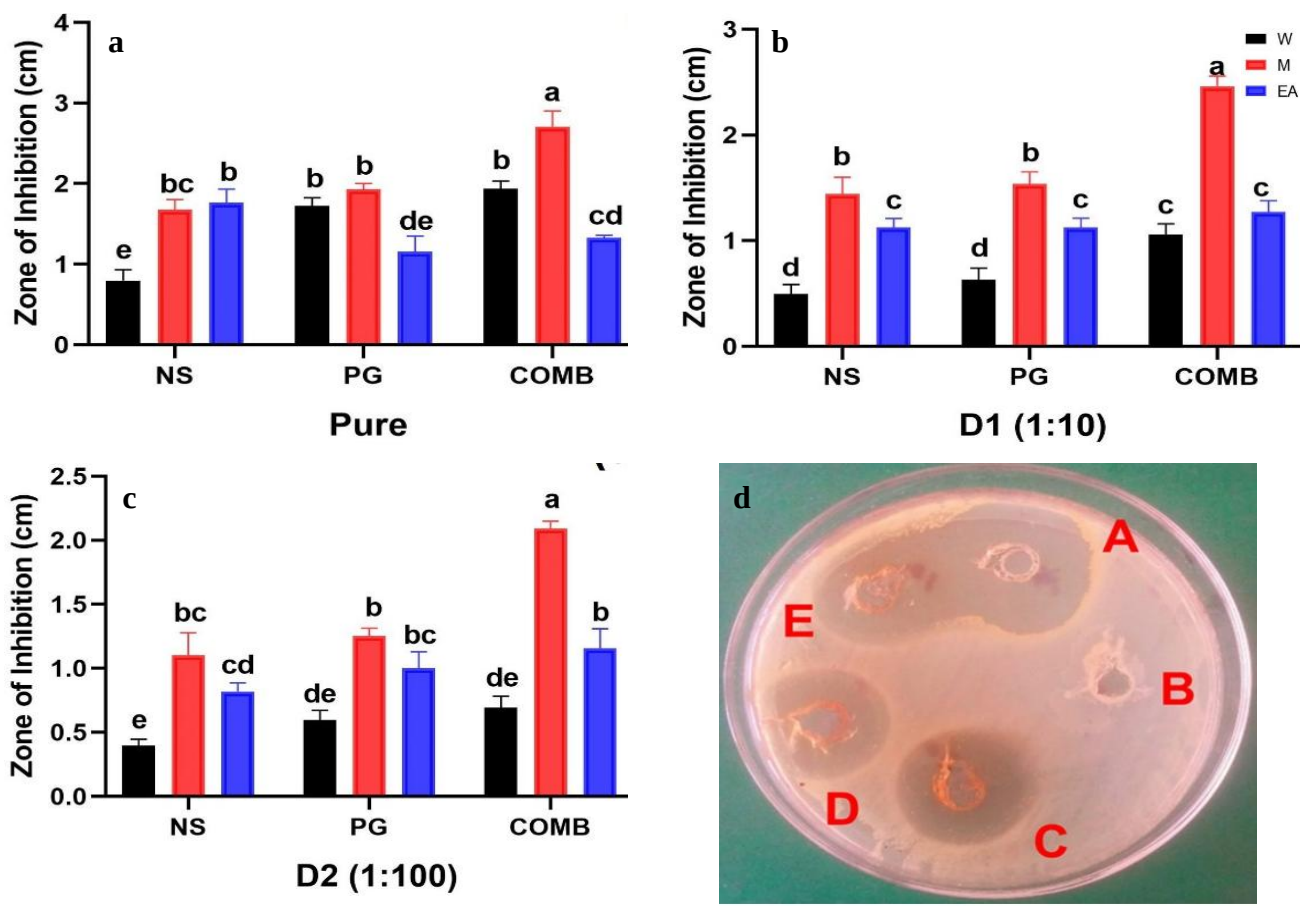

Fig. 3. Zone of inhibition of *S. aureus* growth in response to the application of aqueous (W), methanolic (M) and ethyl acetate (EA) extracts of *N. sativa* (NS) and *P. granatum* (PG) and the combination of NS and PG (COMB) as (a) pure, (b) dilution 1 (1:10) and (c) dilution 2 (1:100). (d) shows the zone of inhibition of A (COMB), B (negative control), C (*N. sativa* extract), D (*P. granatum* extract), and E (positive control of 30 µg/mL). Different alphabets (a-e) show statistically significant differences among the mean values as per Tukey HSD test.

Supplementary Table 1. ADME and drug-likeliness of punicaline, ellagic acid, dithymoquinone and rutin.

Molecule	**Punicaline**	**Ellagic acid**	**Dithymoquinone**	**Rutin**
Formula	$C_{34}H_{22}O_{22}$	$C_{14}H_6O_8$	$C_{20}H_{24}O_4$	$C_{27}H_{30}O_{16}$
MW	782.53	302.19	328.4	610.52
Heavy atoms	56	22	24	43
Aromatic heavy atoms	28	16	0	16
Fraction Csp3	0.18	0	0.6	0.44
Rotatable bonds	0	0	2	6
H-bond acceptors	22	8	4	16
H-bond donors	13	4	0	10
MR	180.45	75.31	91.24	141.38
TPSA	385.24	141.34	68.28	269.43
iLOGP	0.18	0.79	2.46	0.46
XLOGP3	-0.29	1.1	2.07	-0.33
WLOGP	0.02	1.31	2.71	-1.69
MLOGP	-2.83	0.14	1.74	-3.89
Silicos-IT Log P	-1.23	1.67	4.11	-2.11
Consensus Log P	-0.83	1	2.62	-1.51
ESOL Log S	-4.88	-2.94	-3.05	-3.3
ESOL Solubility (mg/ml)	1.03E-02	3.43E-01	2.94E-01	3.08E-01
ESOL Solubility (mol/l)	1.32E-05	1.14E-03	8.95E-04	5.05E-04
ESOL Class	Moderately soluble	Soluble	Soluble	Soluble
Ali Log S	-7.34	-3.66	-3.13	-4.87
Ali Solubility (mg/ml)	3.57E-05	6.60E-02	2.42E-01	8.30E-03
Ali Solubility (mol/l)	4.57E-08	2.18E-04	7.36E-04	1.36E-05
Ali Class	Poorly soluble	Soluble	Soluble	Moderately soluble
Silicos-IT LogSw	-2.71	-3.35	-4.18	-0.29
Silicos-IT Solubility (mg/ml)	1.51E+00	1.36E-01	2.19E-02	3.15E+02
Silicos-IT Solubility (mol/l)	1.93E-03	4.49E-04	6.67E-05	5.15E-01
Silicos-IT class	Soluble	Soluble	Moderately soluble	Soluble
GI absorption	Low	High	High	Low
BBB permeant	No	No	Yes	No
Pgp substrate	Yes	No	No	Yes
CYP1A2 inhibitor	No	Yes	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	No	No	No	No
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
log Kp (cm/s)	-11.28	-7.36	-6.83	-10.26
Lipinski #violations	3	0	0	3
Ghose #violations	3	0	0	4
Veber #violations	1	1	0	1
Egan #violations	1	1	0	1
Muegge #violations	5	0	0	4
Bioavailability Score	0.17	0.55	0.55	0.17
PAINS #alerts	1	1	0	1
Brenk #alerts	4	3	0	1
Leadlikeness violations	1	0	0	1
Synthetic Accessibility	6.74	3.17	4.65	6.52

Discussion

S. aureus is responsible for diverse skin and soft tissue infections, endocarditis, and osteomyelitis. Its pathogenicity is attributed mainly to various virulence factors, including adhesion proteins and toxins, which facilitate its survival and proliferation in host environments (Adhikari, 2021). Additionally, the emergence of antibiotic resistance, particularly in MRSA, poses a critical challenge in clinical settings and highlights the urgent need for novel treatment strategies and effective management protocols (Thomas *et al*., 2024).

P. granatum and *N. sativa* have a rich history of traditional medicinal uses in eastern medicines including Ayurveda and Chinese medicine, for various ailments (Li *et al*., 2022). Based on this, seeds of both the plants were selected for the current study. The type of solvent utilized in the extraction procedure significantly impacts the isolation of physiologically active chemicals from plant sources. Organic solvents like ethyl acetate, acetone, chloroform, and hexane are frequently used to extract bioactive phytochemicals; nonetheless, aqueous and alcoholic extracts are preferred for their safety and lower cytotoxicity in herbal medicines (Waszkowiak *et al*., 2015). The current study revealed the highest efficacy for *S. aureus* growth inhibition by methanolic extracts, followed by ethyl acetate whereas the least was recorded for aqueous extracts. *P. granatum* is rich in phenolic compounds such as gallic acid, punicic acid, and chlorogenic acid, which exhibit strong antibacterial properties by disrupting bacterial cell membranes and inhibiting resistance mechanisms (Mendes *et al*., 2023). Similarly, *N. sativa* contains thymoquinone, which has been shown to possess significant antibacterial effects against various pathogens, including *S. aureus* (Rahman *et al*., 2024). Interestingly, the *In silico* evaluation of the binding affinity of methanol soluble punicalin and dithymoquinone was highest among all tested phytochemicals against the selected antibacterial target proteins, which explained the reason behind the higher antibacterial activity of methanolic extracts of both the selected plants. Likewise, aqueous extracts tend to have the least antibacterial effect due to lower concentrations of soluble bioactive compounds, often more effectively extracted using organic solvents. Similar results were reported by Jung *et al*., (2022), where a variable degree of zone of inhibition was observed with different plant extracts. While methanolic extracts show superior antibacterial activity, aqueous extracts may be preferred in certain contexts for their safety and lower toxicity, despite their reduced efficacy. This finding also highlights the need for a balanced approach in selecting extraction methods based on the intended application.

In the current study, the antibacterial activity of *P. granatum* extracts was higher than that of *N. sativa*, particularly when both extracts were combined. Previous studies showed that a combination of pomegranate extracts with Areca nut enhanced antibacterial effects, particularly against *S. aureus* and *Escherichia coli* (Gharbani *et al*., 2023). Similarly, studies also indicated that the combined extracts yield greater inhibition zones compared to individual extracts, suggesting a synergistic interaction (Alghamdi *et al*., 2024). Similar synergistic interaction was recorded in the current study, where a significantly higher zone of inhibition was observed when extracts of both plants were applied in combination. *Pomegranate* extracts, though they exhibited superior antibacterial properties, nonetheless, the potential of *N. sativa* should not be overlooked, as it also possesses significant antimicrobial effects that could complement pomegranate extracts in therapeutic applications.

Punicalin exhibited the highest binding affinity of -19.4 kcal/mol with the antibacterial target protein IsdA, indicating a strong interaction that may enhance its antibacterial efficacy. Following this, ellagic acid showed a binding affinity of -11.9 kcal/mol with EpiP, suggesting a moderate interaction. Dithymoquinone, with a binding affinity of -12.9 kcal/mol to IsdA, also demonstrates significant potential, while rutin showed binding to EpiP with a slightly lower affinity of -11.3 kcal/mol. These findings highlight the varying degrees of interaction between these compounds and their respective targets, which can influence their therapeutic applications. IsdA facilitates the uptake of heme iron, critical for *S. aureus* growth and pathogenicity and is an ideal candidate for vaccine development (Conroy *et al*., 2019). Compounds like kaempferol, apigenin, and quercetin have shown potential in molecular docking studies, indicating strong binding affinities to IsdA, suggesting their role as natural anti-*S. aureus* agents (Kumar *et al*., 2023). Similarly, glucomoringin isothiocyanate from Moringa oleifera demonstrated strong binding to various *S. aureus* proteins, indicating its potential as an antibacterial agent against MDR strains (Bhattacharya *et al*., 2024). Targeting IsdA, EpiP, and other selected proteins presents a novel approach to combat *S. aureus* infections; the complexity of bacterial resistance mechanisms necessitates a multifaceted strategy, including the exploration of combinatorial therapies with existing antibiotics and phytochemicals. Furthermore, the current study is limited to *In silico* evaluation of the interaction between the phytochemicals of *P. granatum* and *N. sativa*; an *In vitro* confirmation of the binding of these phytochemicals with the target proteins may provide supporting evidence as potential therapeutic agents.

Conclusion

Based on the results, it was concluded that a combination of extracts of two or more plants acts synergistically to inhibit the growth of MRSA. Furthermore, the molecular docking pointed out the potential of *P. granatum* specific punicalin and *N. sativa specific* dithymoquinone as potential antibacterial phytochemicals targeting IsdA, EpiP, and SrdC proteins of *S. aureus*, thus broadening our insights on molecular aspects of bacterial growth inhibition. ADME properties of dithymoquinone also support its potential as a functionalized antimicrobial phytochemical.

Declaration: The authors declare no conflicts of interest.

Data Availability: Data will be made available when required.

Author Contributions: SNA, MH: Experimentation, draft preparation, AH, AM: Manuscript review, editing, lab facilitation, AHS, JA: Supervision, conceptualization, and study design.

Acknowledgements

The authors are grateful to Taha, Department of Biotechnology, COMSATS University Islamabad Abbottabad Campus, for his help in *In vitro* assays.

References

Abbas, M., M.A. Gururani, A. Ali, S. Bajwa, R. Hassan, S.W. Batool, M. Imam and D. Wei. 2024. Antimicrobial properties and therapeutic potential of bioactive compounds in *Nigella grava*: A review. *Molecules*, 29(20): 4914.

Adhikari, R.P. 2021. Staphylococcal infections: host and pathogenic factors. *Microorganisms*, 9(5): 1080.

Ahmad, M.F., F.A. Ahmad, S.A. Ashraf, H.H. Saad, S. Wahab, M.I. Khan, M. Ali, S. Mohan, K.R. Hakeem and M.T. Athar. 2021. An updated knowledge of black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. *J. Herb. Med.*, 25: 100404.

Alghamdi, M.A., F. Al-Sarraj, W.H. Alamshani, I. Alotibi, M. Al-Zahrani, R. Albiheyri, N.M. Nass, B.H. Sajer, N.M. Bataweel, M.A. Al-Matary and T.M. Alqahtani. 2024. Antibacterial power of pomegranate extracts against beta-lactamase producing *Escherichia coli*. *Caryologia*, 77(1): 83-99.

Anthony, W.E., C.D. Burnham, G. Dantas and J.H. Kwon. 2021. The gut microbiome as a reservoir for antimicrobial resistance. *J. Infect. Dis.*, 223(Suppl 3): S209-S213.

Ayaz, M., F. Ullah, A. Sadiq, F. Ullah, M. Ovais, J. Ahmed and H.P. Devkota. 2019. Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance. *Chem. Biol. Interact.*, 308: 294-303.

Bhattacharya, S., A. Dutta, P.K. Khanra, N. Gupta, R. Dutta, N.T. Tzvetkov, L. Milella and M. Ponticelli. 2024. *In silico* exploration of 4(α-L-rhamnosyloxy)-benzyl isothiocyanate for combating multidrug-resistant *Staphylococcus aureus*. *Comput. Biol. Med.*, 179: 108907.

Bonincontro, G., S.A. Scuderi, A. Marino and G. Simonetti. 2023. Synergistic effect of plant compounds with antimicrobials against biofilms of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida* spp. *Pharmaceuticals*, 16(11): 1531.

Chen, C., J. Shi, D. Wang, P. Kong, Z. Wang and Y. Liu. 2024. Antimicrobial peptides as promising antibiotic adjuvants to combat drug-resistant pathogens. *Crit. Rev. Microbiol.*, 50(3): 267-284.

Conroy, B.S., J.C. Grigg, M. Kolesnikov, L.D. Morales and M.E. Murphy. 2019. *Staphylococcus aureus* heme and siderophore-iron acquisition pathways. *Biometals*, 32: 409-424.

Daina, A., O. Michielin and V. Zoete. 2017. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.*, 7(1): 42717.

Dallakyan, S. and A.J. Olson. 2014. Small-molecule library screening by docking with PyRx. In: *Chemical Biology: Methods and Protocols*, pp. 243-250. Springer, New York.

Gharbani, P., N. Jam, H. Doshmanfekan and A. Mehrizad. 2023. Optimization of synergic antibacterial activity of *Punica granatum* L. and *Areca* nut extracts. *Sci. Rep.*, 13(1): 6098.

Jung, I.G., J.Y. Jeong, S.H. Yum and Y.J. Hwang. 2022. Inhibitory effects of selected medicinal plants on bacterial growth of methicillin-resistant *Staphylococcus aureus*. *Molecules*, 27(22): 7780.

Kliebenstein, D.J. and B.H. Kvitko. 2023. Better living through phytochemistry: "phytoavengins" and reappraising defensive phytochemicals. *Physiol. Mol. Plant Pathol.*, 125: 101978.

Kumar, P., P. Bansal, V.K. Garg, S. Sangwan, K. Dhama, D. Chandran, G.K. Bhatia, B. Gupta and H.S. Tuli. 2023. *In silico* targeting of enterotoxin from *Staphylococcus aureus* with flavonoids: potential natural anti-mastitis agents. *J. Exp. Biol. Agric. Sci.*, 11(1): 132-139.

Li, S., S. Jiang, W. Jia, T. Guo, F. Wang, J. Li and Z. Yao. 2024. Natural antimicrobials from plants: Recent advances and future prospects. *Food Chem.*, 432: 137231.

Li, X., L. Wu, R. Wu, M. Sun, K. Fu, T. Kuang and Z. Wang. 2022. Comparison of medicinal preparations of Ayurveda in India and five traditional medicines in China. *J. Ethnopharmacol.*, 284: 114775.

Lin, J., I. Monsalvo, H. Kwon, S. Pullano and N. Kovinich. 2024. The WRKY transcription factor GmWRKY72 represses glyceollin biosynthesis in soybean. *Plants*, 13(21): 3036.

Loggenberg, S.R., D. Twilley, M.N. De Canha, D. Meyer, E.C. Mabena and N. Lall. 2022. Evaluation of wound healing and antibacterial potential of *Greyia radlkoferi* Szyszyl. ethanolic leaf extract. *Front. Pharmacol.*, 13: 806285.

Maleš, I., A. Dobrinčić, Z. Zorić, S. Vladimir-Knežević, I. Elez Garofulić, M. Repajić, D. Skroza, I. Jerković and V. Dragović-Uzelac. 2023. Phenolic and sensory profile and antioxidant capacity of fruit juice enriched with *Salvia officinalis* L. and *Thymus serpyllum* L. extracts. *Molecules*, 28(9): 3656.

Mendes, P.M., G.M. Gomes Fontoura, L.D.S. Rodrigues, A.S. Souza, J.P.M. Viana, A.L. Fernandes Pereira, R.P. Dutra, A.G. Nogueira Ferreira, M.S. Neto, A.S. Reis and M.C.G. Maciel. 2023. Therapeutic potential of *Punica granatum* and isolated compounds against bacterial infections. *Int. J. Microbiol.*, 2023: 4026440.

O'Boyle, N.M., M. Banck, C.A. James, C. Morley, T. Vandermeersch and G.R. Hutchison. 2011. Open Babel: an open chemical toolbox. *J. Cheminform.*, 3: 1-14.

Onyeaka, H.N. and O.F. Nwabor. 2022. Microbial food contamination and foodborne diseases. In: *Food Preservation and Safety of Natural Products*, pp. 19-37.

Rahman, A.U., A. Abdullah, S. Faisal, B. Mansour and G. Yahya. 2024. Unlocking the therapeutic potential of *Nigella sativa*: phytochemical analysis and antimicrobial and antioxidant activities. *BMC Complement. Med. Ther.*, 24(1): 266.

Seukep, A.J., V. Kuete, L. Nahar, S.D. Sarker and M. Guo. 2020. Plant-derived secondary metabolites as efflux pump inhibitors and methods for identification. *J. Pharm. Anal.*, 10(4): 277-290.

Silvero C, M.J., D.M. Rocca, E.A. de la Villarmois, K. Fournier, A.E. Lanterna, M.F. Perez, M.C. Becerra and J.C. Scaiano. 2018. Selective photoinduced antibacterial activity of amoxicillin-coated gold nanoparticles. *ACS Omega*, 3(1): 1220-1230.

Thomas, T., A. Moore, S. Nath and G. Kearns. 2024. *Staphylococcus aureus* infections in wrestlers. *Arch. Dermatol. Res.*, 317(1): 24.

Waszkowiak, K., A. Gliszczyńska-Świgło, V. Barthet and J. Skręty. 2015. Effect of extraction method on phenolic and cyanogenic glucosides of flaxseed extracts. *J. Am. Oil Chem. Soc.*, 92(11-12): 1609-1619.

Yactayo-Chang, J.P., H.V. Tang, J. Mendoza, S.A. Christensen and A.K. Block. 2020. Plant defence chemicals against insect pests. *Agronomy*, 10(8): 1156.logg