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Research Article
Synergistic Interaction of Salicylic Acid and Ciprofloxacin against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Serratia marcescens*

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Abstract

Background and Objective: Salicylic acid (SA), a notable member of phytoconstituents is known to possess remarkable antibacterial potential. The present investigation deals with the synergistic interaction of salicylic acid and ciprofloxacin (CIP), evaluating their antibacterial potential against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Serratia marcescens*. **Materials and Methods:** The antibacterial potential of CIP, SA and a combination of CIP and SA was validated by MIC, MBC, broth checkerboard method, biofilm inhibition by crystal violet assay, membrane stability and time-kill kinetics. Expression levels of genetic markers such as lasB, MrkA and bsmB in *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*, respectively were analyzed using PCR. **Results:** The MIC and MBC of *E. coli* and *S. aureus* of CIP was 5 µg/mL and for SA it was found to be 5 mg/mL for all the two microbes. *Escherichia coli* and *Staphylococcus aureus* tend to be resistant to SA. The combination of CIP and SA showed exceptional antibacterial capability toward *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*. In the checkerboard assay, *K. pneumoniae* exhibited a partial synergistic effect with a FICI value of 0.502. *Serratia marcescens* and *P. aeruginosa* with FICI values of 0.49 and 0.48, respectively demonstrated synergistic action. The combination of CIP and SA effectively inhibits biofilm formation in *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* in crystal violet assay. From membrane stability, it can be found that the combination of CIP and SA causes destabilization of the outer membrane of tested microbes. The results of the time-kill assay demonstrated that the combination of CIP and SA remarkably inhibited the proliferation of the tested bacterial population. The combination of CIP and SA exhibited significant downregulation of lasB, MrkA and bsmB. **Conclusion:** The combination of CIP and SA exerts enhanced antibacterial capability against tested microbes compared to CIP and SA alone. The antibacterial efficacy was greater against *P. aeruginosa* than against *K. pneumoniae* and *S. marcescens*.

Key words: Synergy, ciprofloxacin, salicylic acid, antibacterial activity, phytoconstituents

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The antimicrobial drug resistance has created greater demand for the need of antimicrobial against infection causing pathogens[1]. Because of their exceptional capacity for environmental adaptation, bacteria can survive at clinically relevant quantities of currently available antibiotics, which leads to the selection of resistant strains. The utilization of phytochemicals as a substitute for current antimicrobials appears to have surged recently[2]. The natural compounds typically exhibit lower antibacterial activity than conventional antibiotics; hence, it is challenging for them to successfully substitute existing antibiotic in clinical applications. But it has been demonstrated that certain antimicrobial substances originating from plants might increase antibiotic action in a synergistic effect. The combination of naturally occurring compounds with commercially available antibiotics can work synergistically to reduce the minimum inhibitory concentration (MIC) of both the antibiotics and the natural product, potentially making it as effective as the antibiotic alone[3,4].

Salicylic acid (SA), is a phenolic acid constituent that has functional derivatives with an aromatic ring connected to a hydroxyl group and is also a well-known representative in the class of phytoconstituents that have positive impacts on human health[5]. Several studies showed the anti-inflammatory activity of SA and it also possesses antibacterial activity with minimal toxicity[6]. Salicylic acid and other phenolic compounds may interact with the proteins in bacterial cell membranes resulting in a lack of chemiosmotic control and eventually cell death[7]. It is also linked to synthesis of components that contribute to bacterial virulence and to decrease in resistance to a range of antimicrobial[8,9].

Ciprofloxacin (CIP), a fluorinated quinolone, exhibits antibacterial action against a broad range of bacterial species among all the more recent quinolones that have been commercialized to date. The mode of action of ciprofloxacin involves the targeting of alpha subunits of DNA gyrase thereby preventing the supercoiling of bacterial DNA which ultimately results in the termination of replication[10].

A great deal of recent investigations on antimicrobials have been concentrated on the development of novel antimicrobial agents or their individual compounds. Nonetheless, there is limited knowledge regarding the underlying mechanism of synergistic antibacterial effects. It has been proven that employing natural compounds and phytoconstituents in combination with antimicrobial agents significantly improves antibacterial efficacy compared to using solely natural compounds or antimicrobial agents. Within this framework of synergistic activity, the present investigation is one of the novel approaches involved in testing the antimicrobial capability of CIP and SA alone and in combination with CIP and SA against different gram-negative and gram-positive bacteria.

MATERIALS AND METHODS

Study area: The study was carried out at University of Jeddah, College of Medicine, Department of Medical Microbiology and Parasitology, Jeddah, Saudi Arabia from June to December 2023.

MIC and MBC: The MIC of CIP, SA alone and the combination of CIP and SA were determined by CLSI MO7-A9[11]. The *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* were cultured in the MHB separately and absorbance was adjusted to 0.1-0.4 at 600 nm. The SA was added in serially two-fold dilution. The tested microbes alone in the MHB without SA were used as control and then incubated for 24 hrs at 37°C. The apparent turbidity in each well was analyzed. Following this, the plates were observed in a UV-Vis spectrophotometer at 600 nm. The same protocol was performed for CIP. The MIC denotes the minimal concentration of SA and CIP that visibly inhibits bacterial growth. The minimal concentration of SA and CIP that completely inhibits bacterial growth is referred to as MBC.

Broth checkerboard method: Using the broth microdilution method, two-dimensional checkerboard titrations were carried out, where the concentration of SA decreased vertically and the concentration of antibiotic (CIP) decreased horizontally. The antibiotic stock solution preparation, SA suspension preparation and further steps were followed as performed in Fadwa *et al.*[12]. The Fractional Inhibitory Concentration Index (FICI) was computed from the formula to ascertain the association between the two drugs. The interpretation ranges were followed for interpreting the FICI value as mentioned in Fadwa *et al.*[12].

Crystal violet assay: The crystal violet assay was used for evaluating biofilm growth in accordance with the earlier steps were carried out with slight modifications. After carefully emptying each well, the plate was washed using a sterile saline solution to get rid of cells which do not adhere. For 15 min, each well was incubated after 200 µL of pure methanol was introduced. After removing the methanol and completely drying the plates at RT, 200 µL of 0.5% crystal violet was applied and kept for 15 min. Following this, the

stain was removed and the wells were dried and rinsed with water. After this, each well received 200 µL of 95% ethanol. The absorbance was measured utilizing a Biotek microplate reader at OD 450. Eradication of biofilm was verified using the formula given by de Oliveira Negreiros *et al.*[13].

Membrane stability: Membrane stability was performed in accordance to Reddy *et al.*[14]. The antibacterial potential of CIP, SA alone and combination of CIP and SA on the outer membrane of the tested microbes *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* was evaluated by SDS treatment of pre-treated cells (with CIP, SA alone and combination of CIP and SA). Live cultures of tested microbes were mixed in PBS buffer containing different concentrations of CIP, SA and a combination of CIP and SA, then incubated for 30 mins. After centrifuging the cells, the pellet was combined with the equivalent amount of PBS. Following this, SDS (0.15%) was mixed in CIP, SA alone and a combination of CIP and SA treated cells. For every 2 min, absorbance was measured at 565 nm.

Time-kill kinetics assay: Time-kill kinetics studies have been used to analyse several antimicrobial drugs and are also the basis for *in vitro* investigations on pharmacodynamic medication interactions. The MHB was inoculated with tested organisms (5×10^6-1×10^7 CFU) with various concentrations of CIP, SA alone and a combination of CIP and SA for all the tested organisms and analysed for survival at 0, 4, 8, 12 and 24th hrs. The UV-Vis spectrophotometer evaluated bacterial growth inhibition at 600 nm. The absorbance was measured and assessed statistically[15].

Gene expression studies on lasB, MrkA and bsmB: The DNA from *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* isolates was extracted by the phenol-chloroform method. The lasB, MrkA and bsmB were amplified by PCR (Takara). The lasB (Forward: GGAATGAACGAGGCGTTCTC & Reverse: GGTCCAGTAGTAAGCGGTTGG), MrkA (Forward: CACCAAACAG GATGATGTGAG & Reverse: CGCATAGCCGACGTAGTAAG) and bsmB (Forward: CCGCCTGCAAGAAAGAACTT & Reverse: AGAGATCGACGGTCAGTTCC). The initial denaturation condition was set at 95°C for 2 min, followed by 95°C for 1 min. The annealing temperatures of lasB, MrkA and bsmB are 55.6°C, 56.8°C and 56.8°C, respectively for 30 sec. The extension cycle was set at 72°C for 1 min, followed by the final extension at 72°C for 5 min. The final products of PCR were loaded onto 1.0% agarose gel with EtBr and electrophoresis was performed. The results were visualized under UV transilluminator (UV-3,600 Shimadzu, Japan).

Statistical analysis: The statistical evaluation was conducted utilizing version 8.1 of GraphPad Prism. Each experiment was carried out twice and one-way ANOVA was applied to assess the outcomes. To compare the treatment and control groups, Tukey's multiple comparison test was employed. Data are represented as Mean±SD. The $p<0.0001$ was denoted as significant.

RESULTS

MIC and MBC: The MIC and MBC of *E. coli* and *S. aureus* of CIP was 5 µg/mL and for SA it was 5 mg/mL for *E. coli* and *S. aureus*. Both microbes were found to be resistant and exhibited uncontrolled growth to SA, hence for further parameters both microbes were eliminated. The CIP and SA had inhibitory effects on *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*. The MIC and MBC of CIP for *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* were depicted in Fig. 1(a-c) and Fig. 2(a-c) and Table 1. In the same manner, MIC and MBC of SA for *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* were demonstrated in Fig. 3(a-c) and Fig. 4(a-c) and Table 2.

Broth checkerboard assay: In broth checkerboard assay, for *K. pneumoniae*, the synergistic effect was partially observed, with a FICI value of 0.502. With FICI values of 0.48 and 0.49, respectively, the combination of CIP and SA demonstrated synergistic interaction for *P. aeruginosa* and *S. marcescens*. The findings of the checkerboard assay were represented in Table 3.

Crystal violet assay: After treating *P. aeruginosa* with a combination of CIP and SA at the dosage of 0.019 µg/mL and

Table 1: MIC and MBC of *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* for CIP

Microbe	MIC (µg/mL)	MBC (µg/mL)
P. aeruginosa	0.078	0.15
K. pneumoniae	1.25	2.5
S. marcescens	2.5	5

Table 2: MIC and MBC of *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* for SA

Microbe	MIC (µg/mL)	MBC (µg/mL)
P. aeruginosa	400	800
K. pneumoniae	500	1000
S. marcescens	500	1000

Table 3: Type of interaction for the combination of CIP and SA towards *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* in broth checkerboard assay

Microbe	CIP+SA (µg/mL)	Type of interaction
P. aeruginosa (FICI)	0.48	Synergy
K. pneumoniae (FICI)	0.502	Partial synergy
S. marcescens (FICI)	0.49	Synergy

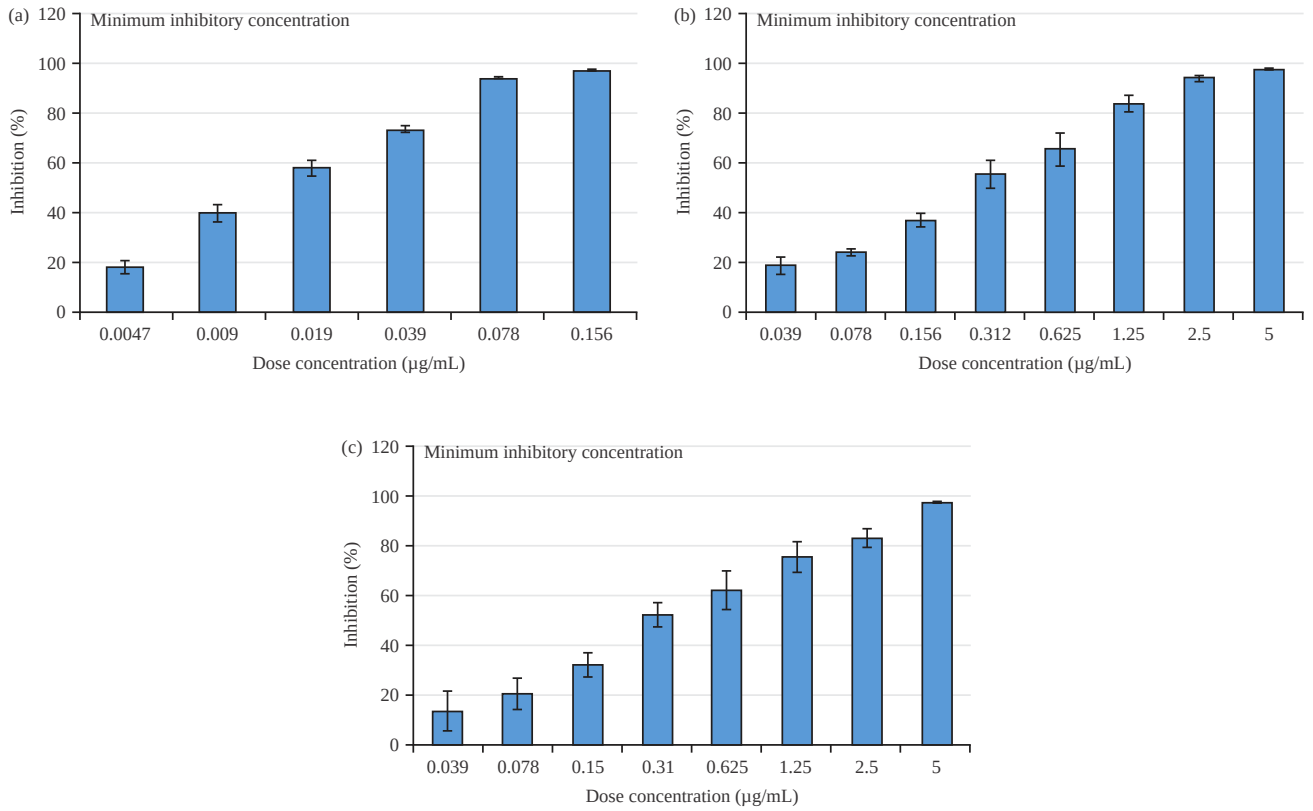


Fig. 1(a-c): MIC of CIP towards (a) *Pseudomonas aeruginosa*, (b) *Klebsiella pneumoniae* and (c) *Serratia marcescens* in a dose-wise manner

100 µg/mL, respectively, the biofilm development was eliminated. For *K. pneumoniae*, the combination of CIP and SA at the dosage of 0.312 and 125 µg/mL, respectively showed excellent biofilm inhibition. For *S. marcescens*, CIP and SA (0.62 and 125 µg/mL) showed biofilm inhibition. The biofilm inhibition of *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* was depicted in Fig. 5-7.

Membrane stability: The results of membrane stability were represented in Fig. 8-10. The treatment with the combination of CIP and SA destabilizes the outer membrane of tested microbes. The CIP and SA at the dosage of 0.019 and 100 µg/mL, respectively disrupted the cell membrane of *P. aeruginosa* which gradually decreased on increasing time. For *K. pneumoniae*, the combination of CIP and SA in the range of 0.312 and 125 µg/mL, respectively showed rupturing of the cell wall. For *S. marcescens*, CIP and SA (0.62 and 125 µg/mL) cause leakage of cellular contents.

Time kill kinetics assay: The findings of the time-kill kinetics assay revealed that all three tested microbes were found to be highly sensitive to the combination of CIP and SA than CIP and SA alone. At 24th hrs, the growth was completely inhibited for all three microorganisms after treatment with the combination of CIP and SA. Figure 11-13 depicted the time-kill kinetics of *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*.

Gene expression studies on lasB, MrkA and bsmB: The PCR has been employed for assessing the gene expression profile of lasB (Elastase) in *P. aeruginosa*, MrkA in *K. pneumoniae* and bsmB in *S. marcescens* was depicted in Fig. 14. The combination of CIP and SA demonstrated a noticeable downregulation of lasB in *P. aeruginosa* in comparison to CIP and SA alone. For *K. pneumoniae*, the expression of MrkA had been negatively regulated after being subjected to the CIP, SA and a combination of CIP and SA. The expression of bsmB was also downregulated in *S. marcescens* after treatment with CIP, SA and a combination of CIP and SA. The same pattern was observed for the other two genes in the case of *K. pneumoniae* and *S. marcescens*, the combination of CIP and SA demonstrated remarkable downregulation in comparison with CIP and SA alone.

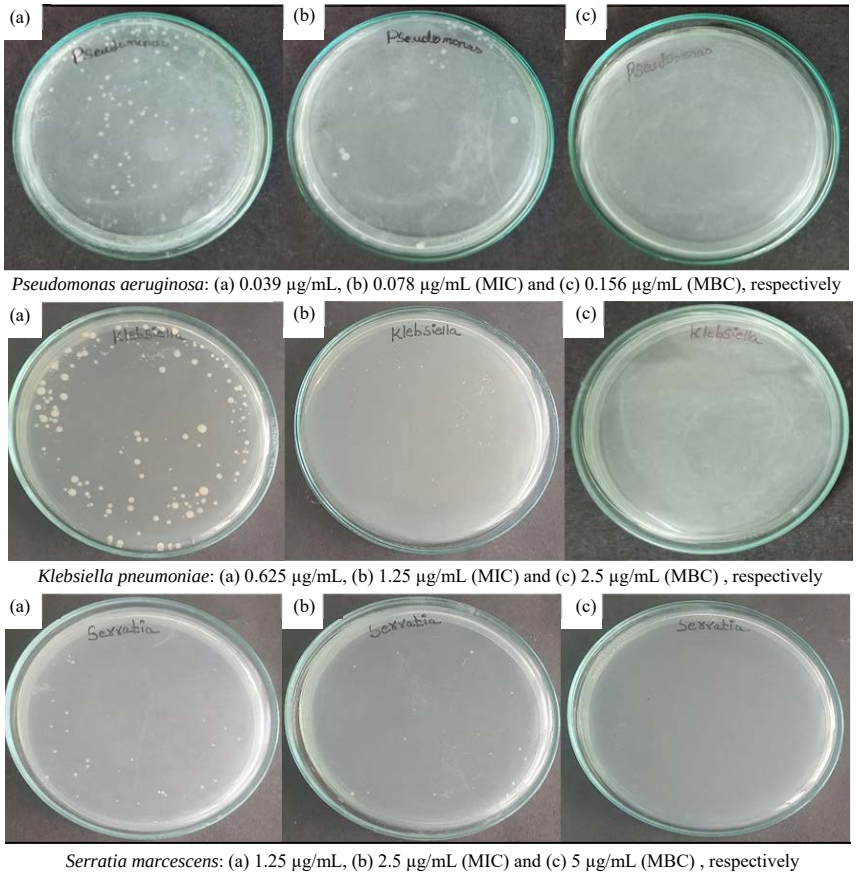


Fig. 2(a-c): MBC of CIP towards (a) *P. aeruginosa*, (b) *K. pneumoniae* and (c) *S. marcescens* in a dose-wise manner

DISCUSSION

The application of phytochemicals as a substitute for conventional antimicrobials has seen a sharp rise in recent times. The SA is one of the well-known phenolic acid compounds with antibacterial action. The current study deals with the antibacterial potential of SA along with their synergistic capability with CIP against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*. The *E. coli* and *S. aureus* were resistant with MIC of 5 µg/mL for CIP and 5 mg/mL for SA. In previous work, salicylic acid microcapsules exhibited MIC and MBC of 4 mg/mL toward *E. coli* and *S. aureus*[16]. Hence further studies were performed on *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*. The MIC of *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* was 0.078, 1.25 and 2.5 µg/mL, respectively for CIP. The MBC was 0.15, 2.5 and 5 µg/mL for the same microbes against CIP. For SA, *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* had MIC values of 400, 500 and 500 µg/mL, respectively. The MBC for SA, *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* had 800, 1000 and 1000 µg/mL, respectively. The MIC and MBC values of CIP and SA for the tested microbes in the present study are in correlation with previously available literature[1,17]. In current investigation, the impact of the combination of CIP and SA was evaluated using the checkerboard assay and FICI was computed by assessing the degree of interaction between CIP and SA against *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*. The synergistic impact was partial for *K. pneumoniae* with a FICI value of 0.502. The combination of CIP and SA demonstrated synergistic interaction against *P. aeruginosa* and *S. marcescens* with FICI values of 0.48 and 0.49, respectively. The results strongly suggested that both CIP and SA synergistically had excellent antibacterial activity.

Furthermore, in the crystal violet assay, there was a notable variation in the biofilm development by bacteria subjected to CIP, SA and combination of CIP and SA, in comparison with the control. For *P. aeruginosa*, compared to CIP and SA alone, the combination of the CIP and SA (0.019 µg/mL and 100 µg/mL, respectively) demonstrated nearly complete biofilm eradication. The same pattern of biofilm inhibition was observed for the other two microbes. For *K. pneumoniae*, CIP and SA at the dosage of 0.312 µg/mL

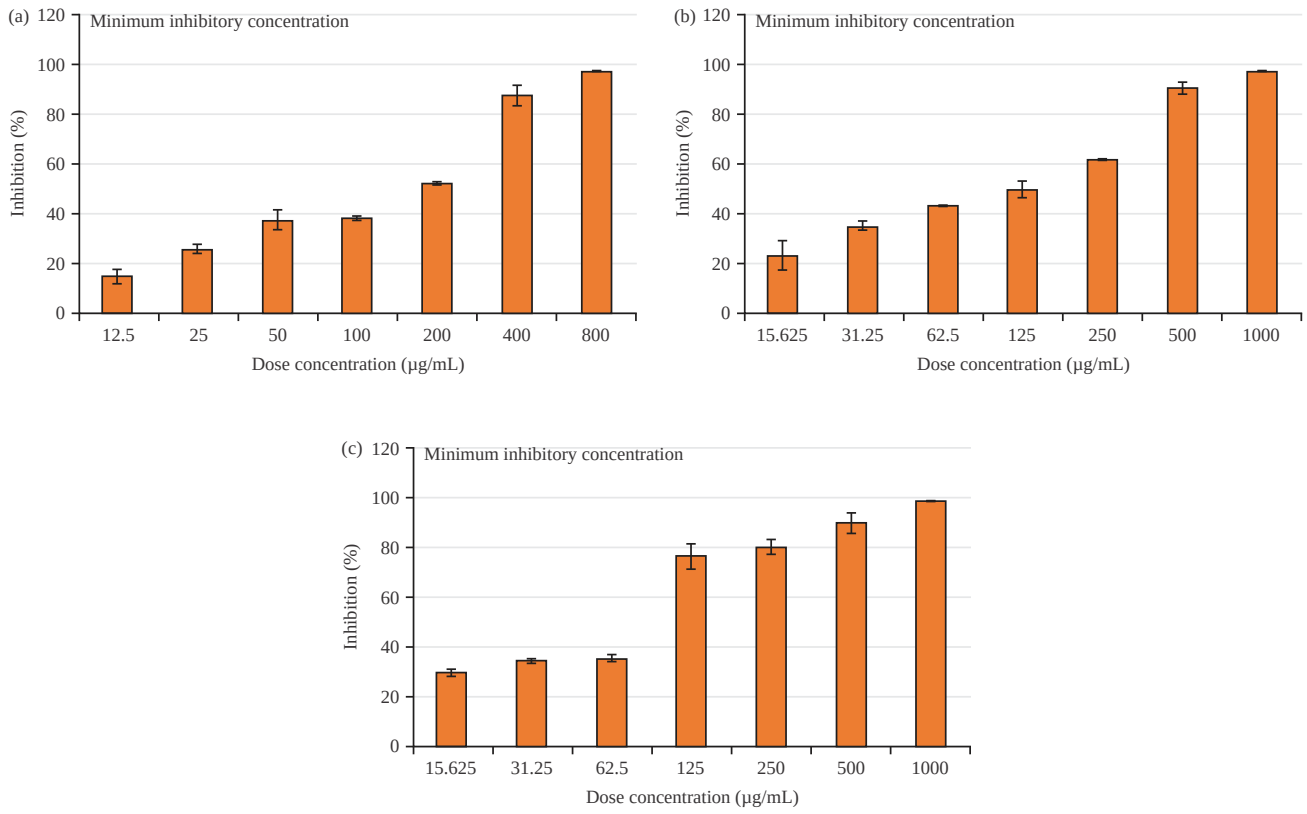
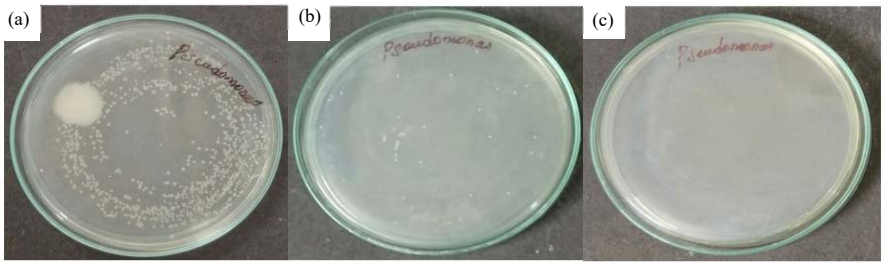


Fig. 3(a-c): MIC of SA towards (a) *Pseudomonas aeruginosa*, (b) *Klebsiella pneumoniae* and (c) *Serratia marcescens* in a dose-wise manner

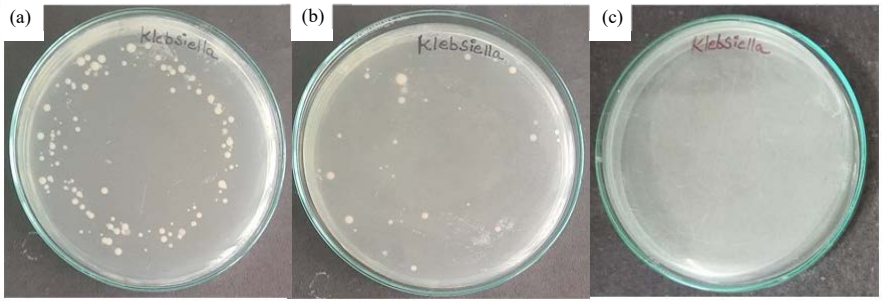
and 125 µg/mL, respectively showed exceptional biofilm inhibition. For *S. marcescens*, CIP and SA at the dosage of 0.62 and 125 µg/mL, respectively showed outstanding biofilm inhibition. The earlier studies showed that the phenolic compounds, especially at higher doses, can influence biofilm formation by affecting quorum sensing, motility and adhesion structures, which are vital for the development of biofilms[4].

A healthy state of the cell membrane is crucial for the proliferation and metabolism of bacteria. The outcomes of the membrane stability revealed that the treatment with the combination of CIP and SA for all three microbes exhibited destabilization of the outer membrane. Following exposure to CIP, SA and the combination of CIP and SA at different intervals of time, there was a gradual release of proteins from the tested microorganisms as it was monitored by the detection of absorbance in the range of 565 nm. Based on these data, it was evident that the combination of CIP and SA ruined the integrity of the bacterial cell membrane, possibly resulting in cellular death. Studies revealed that phenolic acid compounds can interact with the bacterial cell membrane, causing the cell wall to rupture and the intracellular macromolecules like proteins and nucleic acids to leak out. On cascading effect, damage to the cell wall reduces the cell's ability to withstand adverse circumstances and other environmental factors leading to cell death[4]. Moreover, time kill assay was conducted further to examine the antibacterial potential of CIP, SA and combination of CIP and SA. The results demonstrated that *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* were gradually killed during treatment. From the findings, it can be noted that the combination of CIP and SA exhibited prompt bactericidal effect which ultimately resulted in the reduction of bacterial population at 24th hrs. Phenolic acid compounds can deteriorate the inner cell membrane of bacteria by associating with the proteins of the cell membrane, which impairs chemiosmotic regulation and eventually culminates in cell death[4].

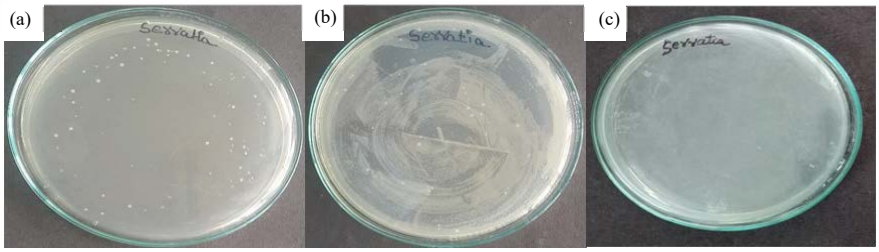
Lastly, an in-depth analysis of the genetic alterations in the tested microbes after treatment with CIP, SA and a combination of CIP and SA was carried out. The gene expression profile of lasB in *P. aeruginosa*, MrkA in *K. pneumoniae* and bsmB in *S. marcescens* were investigated using PCR. The combination of CIP and SA demonstrated



Pseudomonas aeruginosa: (a) 200 µg/mL, (b) 400 µg/mL (MIC) and (c) 800 µg/mL (MBC), respectively



Klebsiella pneumoniae: (a) 250 µg/mL, (b) 500 µg/mL (MIC) and (c) 1000 µg/mL (MBC), respectively



Serratia marcescens: (a) 250 µg/mL, (b) 500 µg/mL (MIC) and (c) 1000 µg/mL (MBC), respectively

Fig. 4(a-c): MBC of SA towards (a) *P. aeruginosa*, (b) *K. pneumoniae* and (c) *S. marcescens* in a dose-wise manner

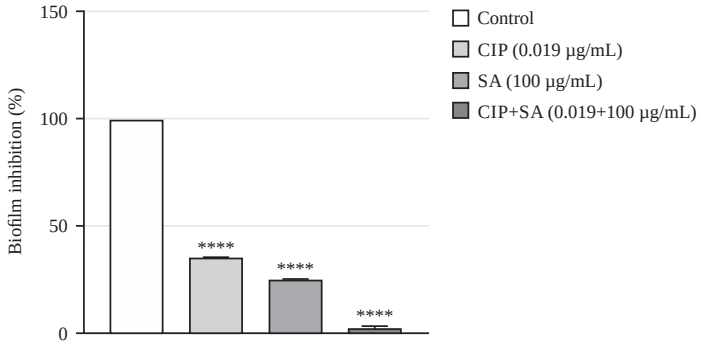


Fig. 5: Biofilm inhibition of *P. aeruginosa*

Results are denoted as mean and SD and subjected to one way ANOVA (Tukey's Multiple Comparisons Test) which represented noticeable differences between control and treated groups ($p<0.0001$)

notable downregulation of lasB, MrkA and bsmB in *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*, respectively. The *P. aeruginosa* has three main, intricately connected quorum sensing mechanisms: las, rhl and pqs[18]. The generation of virulence elements such as elastase, pyocyanin and biofilm development in *P. aeruginosa* has been reported to be effectively repressed by phenolic acid compounds, particularly SA[19,20]. At the same time, CIP could target the quorum sensing mechanism of *P. aeruginosa* hindering biofilm development[21].

Fig. 6: Biofilm inhibition of *K. pneumoniae*

Results are denoted as mean and SD and subjected to one way ANOVA (Tukey's Multiple Comparisons Test) which expressed noticeable differences between control and treated groups ($p<0.0001$)

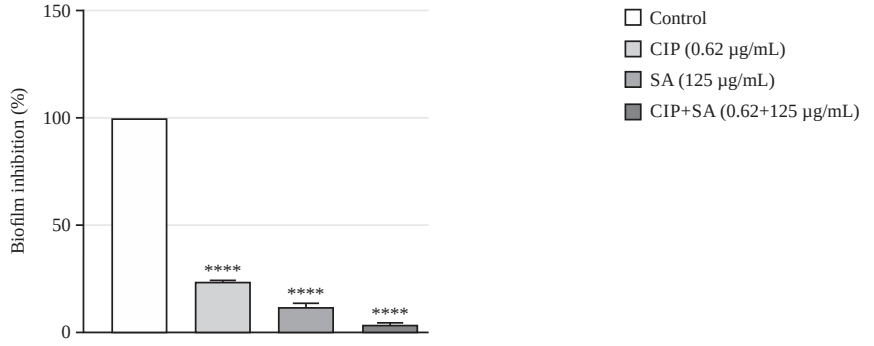


Fig. 7: Biofilm inhibition of *S. marcescens*

Outcomes were depicted as mean and SD and subjected to one way ANOVA (Tukey's Multiple Comparisons Test) which demonstrated noticeable differences between control and treated groups ($p<0.0001$)

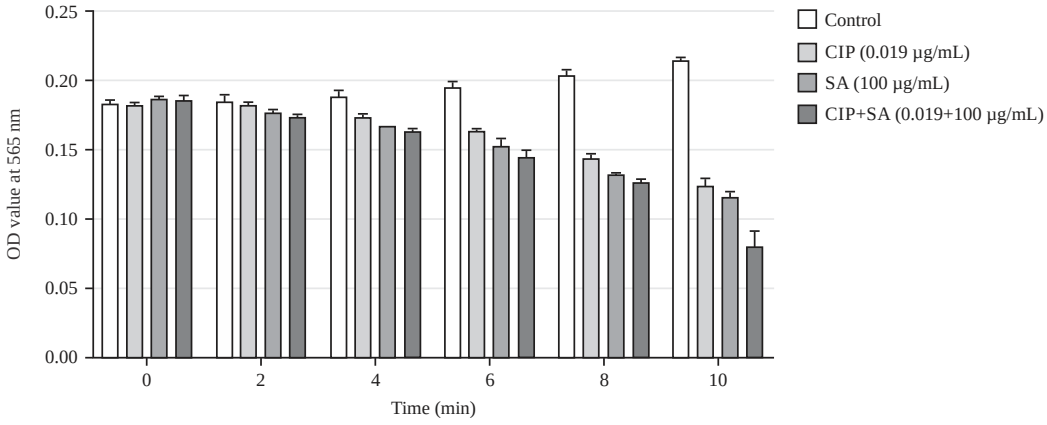


Fig. 8: Membrane stability of *P. aeruginosa* at different intervals

Outcomes are denoted as mean and standard deviation and subjected to one way ANOVA (Tukey's multiple comparisons test) which expressed noticeable differences between control and treated groups ($p<0.0001$)

In the case of *K. pneumoniae*, MrkA protein one of the vital structural components of type 3 fimbriae which acts as appendages in the formation of biofilm had been negatively regulated after being subjected to CIP, SA and a combination of CIP and SA. The combination of CIP and SA downregulated the expression levels of MrkA, up to the mark that eventually impacted the organism's pathogenicity[19,20]. Quorum sensing regulatory genes namely bsmA and bsmB in *S. marcescens* were required for biofilm formation and also involved in adhesion to the abiotic

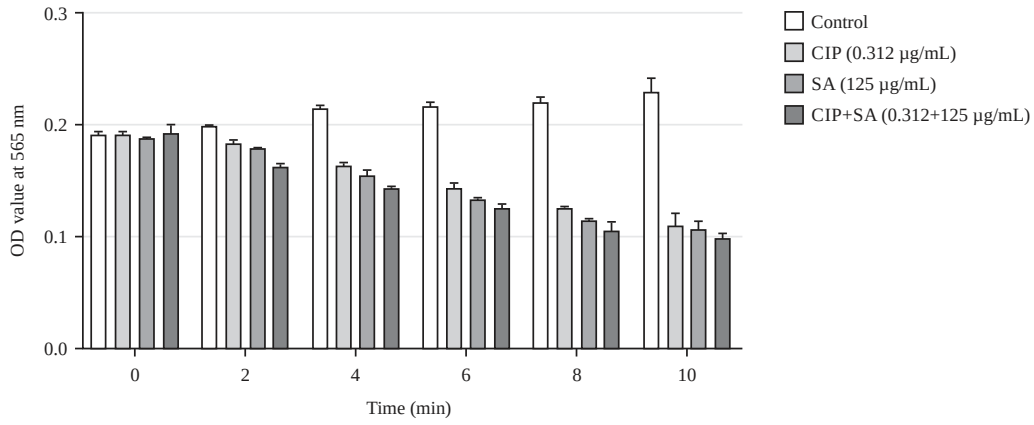


Fig. 9: Membrane stability of *K. pneumoniae* at different intervals

Findings are denoted as mean and standard deviation and subjected to one way ANOVA (Tukey's multiple comparisons test) which expressed noticeable differences between control and treated groups ($p<0.0001$)

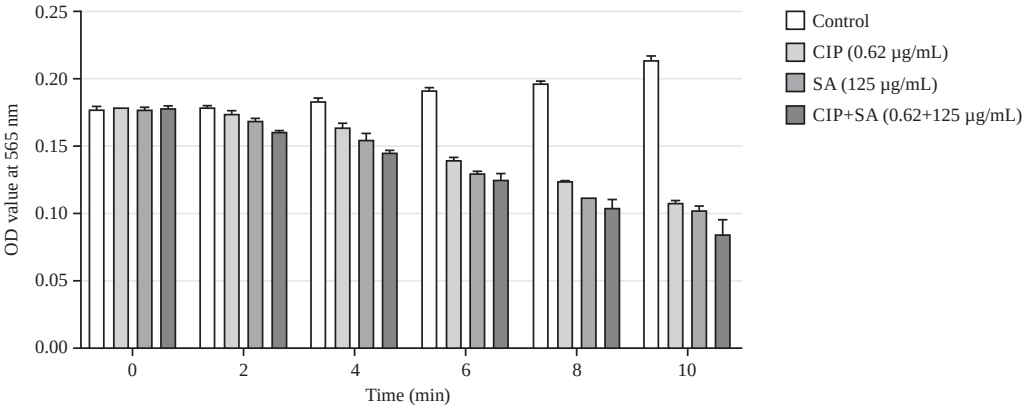


Fig. 10: Membrane stability of *S. marcescens* at different intervals

Outcomes are denoted as mean and standard deviation and subjected to one way ANOVA (Tukey's multiple comparisons test) which expressed noticeable differences between control and treated groups ($p<0.0001$)

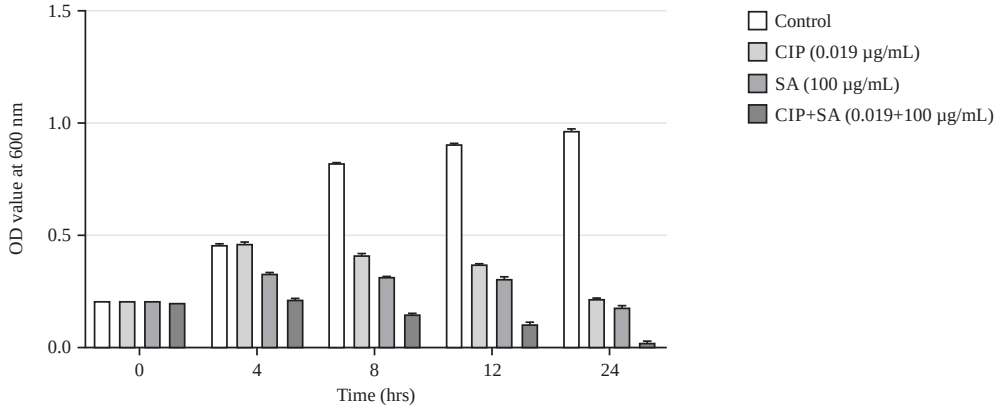


Fig. 11: Time-kill kinetics of *P. aeruginosa*

Findings are denoted as mean and standard deviation and subjected to one way ANOVA (Tukey's multiple comparisons test) which expressed noticeable differences between control and treated groups ($p<0.0001$)

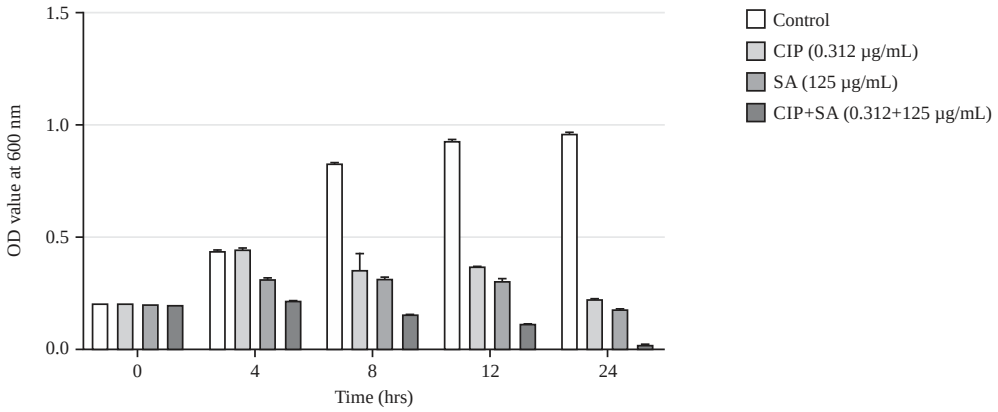


Fig. 12: Time-kill kinetics of *K. pneumoniae*

Outcomes are represented as mean and standard deviation and subjected to one way ANOVA (Tukey's multiple comparisons test) which expressed noticeable differences between control and treated groups (p<0.0001)

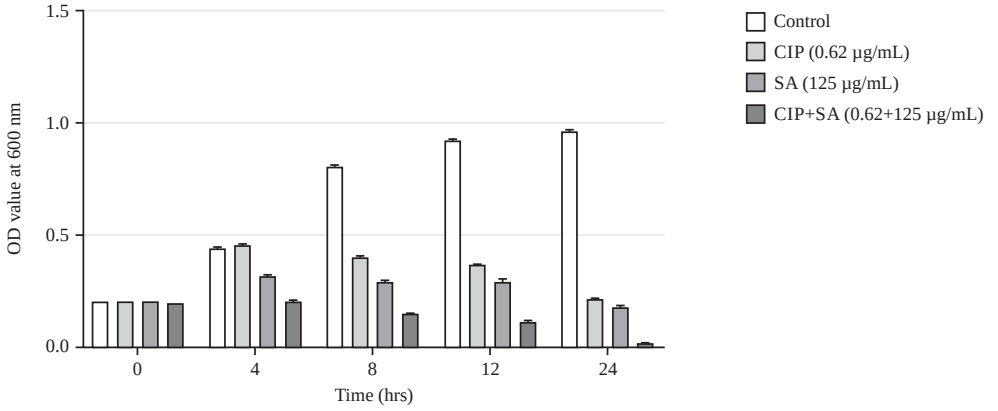


Fig. 13: Time-kill kinetics of *S. marcescens*

Findings are depicted as mean and standard deviation and subjected to one way ANOVA (Tukey's multiple comparisons test) which indicated noticeable differences between control and treated groups (p<0.0001)

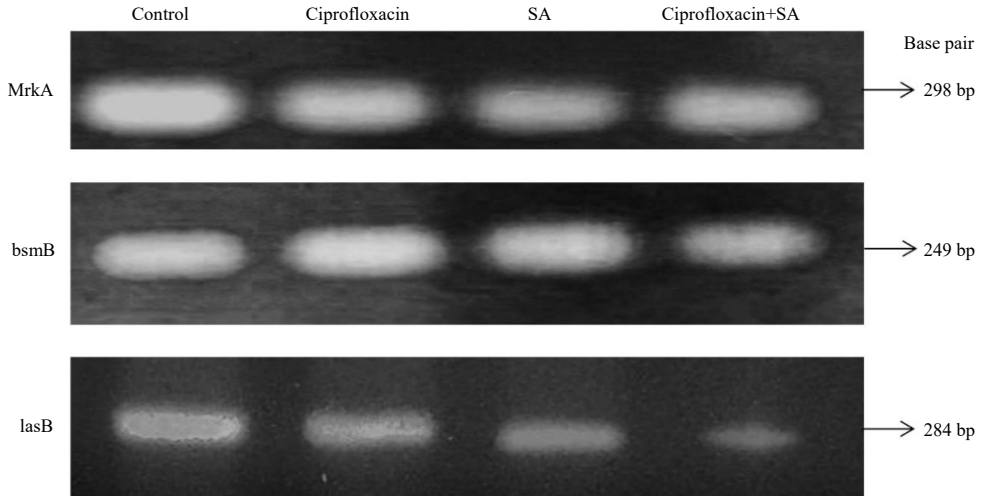


Fig. 14: PCR products in agarose gel electrophoresis

MrkA, bsmB and lasB were negatively regulated after treatment with the combination of CIP and SA than control, CIP and SA alone

surfaces. The expression of bsmB was downregulated by the combination of CIP and SA which was consistent with other studies[21-23]. In a summarised form, phenolic acid compound, SA and CIP act synergistically and downregulated the virulence factors and quorum sensing regulatory genes in all the tested microbes.

The outcomes of the aforementioned assays and tests imply that SA in combination with CIP possesses greater antibacterial potential than SA alone. In a similar vein, even though CIP was broad spectrum antibiotic, its antibacterial potential was significantly increased when combined with SA. As it is generally known, phenolic compounds possess multifaceted antibacterial action. By causing the production of ROS, phenolic substances can cause endogenous oxidative stress in bacterial cells. Polyphenols have the potential to alter bacterial cell metabolism and protein production. It has been observed phenolic compounds inhibit ATP and DNA synthesis via inhibiting gyrase activity[24,25]. All these strategies could have been the reason for the antibacterial action of SA towards tested microbes. Conversely, CIP primarily acts by inhibiting the bacterial DNA gyrase enzyme. Briefly, CIP blocks bacterial topoisomerase II and DNA topoisomerase IV, as well as further stopping bacterial DNA from unwinding and duplicating, to impede DNA replication and transcription[5]. According to current study, at lower doses, the combined effects of CIP and SA unambiguously exhibited remarkable antibacterial efficacy against the tested microbes.

Finally, results demonstrated synergistic interaction for the combination of CIP and SA for *P. aeruginosa*, *K. pneumoniae* and *S. marcescens*. In closing, antibacterial capability against *P. aeruginosa* was more effective than compared to *K. pneumoniae* and *S. marcescens*. By decreasing the dosage of both drugs (CIP and SA), this combination at adequate dosage may be helpful in medical use and lessen the probability of an adverse impact on humans.

CONCLUSION

To conclude, in the present investigation the antibacterial efficacy of SA was assessed alone and in combination with CIP against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* but *E. coli* and *S. aureus* showed resistance to SA. The outcomes demonstrated the combination of CIP and SA exerts antibacterial potential at much lower concentrations against *P. aeruginosa*, *K. pneumoniae* and *S. marcescens* which could be beneficial for future use to minimize the detrimental effects on humans. To fully explore and comprehend the appropriate mode of action for the combination of CIP and SA, more research is required.

SIGNIFICANCE STATEMENT

The present work explored the synergistic effect of salicylic acid and ciprofloxacin, against microbial strains. *Escherichia coli* and *S. aureus* were resistant to SA. The combination of CIP and SA demonstrated less antibacterial activity against these bacteria, which might be useful for future application.

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