Journal of Animal & Plant Sciences, 35(4): 2025, Page: 1112-1126
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.4.0095

EFFLUX PUMP INHIBITORY ACTIVITY OF BERBERINE AND PALMATINE AND THEIR SYNERGISTIC ACTIVITY WITH CIPROFLOXACIN IN MULTIDRUG-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM INFECTED WOUNDS IN SHAHROUD, IRAN

S. S. Khoramrooz[1], S. S. Aghayan[2], D. Darban-Sarokhalil[3], M. T. Rahimi[4], H. Tahmasebi[4] and M. Mirzaii[4] *

[1]Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[2]Department of Basic Sciences, Islamic Azad University, Damghan Branch, Damghan, Iran
[3]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[4]School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
Corresponding Author's E-mail: mirzaii1386@gmail.com
ORCID: https://orcid.org orcid.org/0000-0002-6215-1619
ORCID: https://orcid.org/0000-0002-9749-0389
ORCID: https://orcid.org/0000-0001-8081-3906
ORCID: https://orcid.org/0000-0002-9543-7779
ORCID: https://orcid.org/0000-0002-9257-4479
ORCID: https://orcid.org/0000-0002-2509-0300

ABSTRACT

Increased efflux due to over-expression of efflux pump is a common mechanism of multidrug resistance in *Staphylococcus aureus*. More recognized resistance mechanisms have eclipsed the contribution of efflux pumps to resistance in S. aureus. This research aimed to assess the efficacy of combining berberine and palmatine with ciprofloxacin in treating *S. aureus* infections obtained from wounds. The microtiter plate tests were used to study the antimicrobial susceptibility pattern, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of ciprofloxacin, berberine, and palmatine. The EP inhibition and accumulation assays were performed with a combination of plant extract and ciprofloxacin. Eighty (80) *S. aureus* isolates were collected from the burn infections. Out of 80 isolates, 76.25 % were resistant to cloxacillin and erythromycin. Also, 1.25 % of *S. aureus* isolates were resistant to clindamycin and teicoplanin. Likewise, 65%, 21.25%, and 1.25% were considered MDR, XDR, and PDR strains, and 90% of *S. aureus* isolates were MRSA. The *norA*, *norB*, *norC,* and *mecA* were reported in 67.5%, 91.25 %, and 90 % of isolates, respectively. Based on the ciprofloxacin-resistant strains (72.5%), the MIC range of berberine and palmatine extracts in 27 isolates (33.7%) was 16 µg/mL to 64 µg/mL. After the combination, the MIC of ciprofloxacin was reduced more than 10-fold in resistant strains. A significant relationship was reported between the combination of plant extract and ciprofloxacin in resistant isolates ($p<0.05$). The best results of combining berberine, palmatine extract, and ciprofloxacin were observed in MDR, XDR, and PDR strains ($p<0.001$). Antimicrobial results of berberine and palmatine extracts against *S. aureus* were observed; however, clinical trials are necessary to confirm the efficiency of these extracts. We reported that combining berberine and palmatine with ciprofloxacin might reduce treatment failure due to EP overexpression.

Keywords: *Staphylococcus aureus*, Anti-MRSA plants, Multi-Drug Resistant, Antibiotic resistance.

Published first online June 18, 2025 Published final July 29, 2025

INTRODUCTION

Staphylococcus aureus has adapted multiple methods to counteract the damaging effects of antibacterial treatments (Heydari *et al*., 2017). Over-expression of efflux pump (EP) is one of the key factors contributing to multidrug resistance (MDR) (Bokaeian and Tahmasebi, 2017). Treatment costs rise, and fatalities increase due to infections caused by bacteria resistant to multiple drugs. Therefore, identifying new EPIs (EPI) presents a significant hurdle for drug development. For the empirical treatment of infections due to methicillin-resistant *S. aureus* (MRSA), a bacterium that is becoming increasingly common, ciprofloxacin is no longer an acceptable choice.*S. aureus*. Despite this, there has been a swift increase in reports of quinolone resistance (Arabestani *et al*., 2017; Heydari *et al*., 2018; Tahmasebi *et al*., 2025).

Interactions between bacteria and other organisms play an important role in the spread of drug-resistant strains, and some minerals also play a role in the spread of these strains. EPs are an assembly of proteins that removes toxic molecules from bacterial cells(Jahantigh M and Tahmasebi H, 2024; Khazaei *et al*., 2025). Activation of the chromosomally encoded *norA* gene is associated with low-level fluoroquinolone resistance. Increased expression levels of the *norC* gene, an MDR-EP from the major facilitator superfamily, are observed during infection. The murine subcutaneous abscess model shows that *norB* in *S. aureus* leads to fitness advantages (Costa *et al*., 2013; Kwak *et al*., 2013). Several compounds that alter the EP's function have been identified from natural sources during the last few years. These EPI have been discovered against gram-positive and gram-negative bacteria. These EP inhibitors' source was natural and synthetic (Wasaznik *et al*., 2009; Eslami *et al*., 2025).

Plant extracts having synergistic activity with different antibiotics have been known. Different plants showed synergistic activity when combined with different antibiotics, and they contain some EP inhibitor-like compounds that inhibit the EP of *P. aeruginosa*. Berberine and palmatine are the plant alkaloids present in the *Berberis vulgaris*. These alkaloids can be found in the plant's roots, rhizomes, and stem bark (Imanshahidi and Hosseinzadeh, 2008). Significant inhibitory activity of berberine and palmatine against some bacteria was reported in studies(You Gan, 2012; Jung *et al*., 2014). Evidence indicates that plant extracts have been considered the potential inhibitory effect on *norA* mutants (Hsieh *et al*., 1998; Eslami *et al*., 2025). The inhibitory effect of plant alkaloids was reported in *norA* mutants of *S. aureus* (Wasaznik *et al*., 2009). Numerous earlier investigations have demonstrated the combined effects of antibiotics and bioactive chemical substances found in natural products.

Furthermore, numerous plant-derived extracts and essential oils have been identified as agents that inhibit the drug Eps (You Gan, 2012; Jung *et al*., 2014). It has been identified that capsaicin, a significant element of chili peppers, functions as a blocker of the *norA* drug efflux pump in *S. aureus*. A molecular docking simulation study found that carvacrol and thymol are associated with the *norA* drug efflux pump (Seo *et al*., 2024). Nevertheless, information on the EPI activity associated with berberine and palmatine is insufficient. Also, there is limited clear evidence for the synergistic effects of these extracts with ciprofloxacin(de Sousa Andrade *et al*., 2020; Seo *et al*., 2024). Hence, this study aimed to determine berberine and palmatine EPI activity and their synergistic activity with ciprofloxacin in *S. aureus* collected from wound infection.

MATERIALS AND METHODS

Sample collection and identification: From March 2017 to 2019, 690 clinical samples were collected from wounded patients in Tehran hospitals, Iran. Wound swabs were enriched in trypticase soy broth (with 10% NaCl and 1% sodium pyruvate) (MERCK, Germany). Then, loop culture was streaked on the mannitol salt agar (MERCK, Germany) and incubated for 24 hours at 37°C. Typical single round yellow colonies with the yellowish zone on MSA agar plates were picked up for further identification. Colonies were tested for Gram's staining and biochemical tests such as catalase, oxidase, and coagulase. All steps of identification of *S. aureus* were performed according to Mahon *et al*. (Connie R. Mahon, 2014). Also, the polymerase chain reaction (PCR) method was used to confirm *S. aureus* isolates.

Antimicrobial susceptibility testing: The antimicrobial susceptibility pattern was determined based on the disk diffusion (Kirby-Bauer) method (Porbaran *et al*., 2021). This study used 11 antibiotic disks from MAST, a UK company. Those disks include cloxacillin, mupirocin, teicoplanin, ceftriaxone, ciprofloxacin, clindamycin, cotrimoxazole, erythromycin, gentamycin, imipenem, kanamycin, and tigecycline. Antibiotic profiles of each isolate were determined according to the recommendation of the CLSI 2019 (Anon.). The assessment of antibiotic susceptibility for MDR and XDR isolates was carried out following the approval from the European Center for Disease Prevention and Control (ECDC). *S. aureus* ATCC 29213 was used as a control strain.

MIC determination of berberine, palmatine, and ciprofloxacin: The broth microdilution technique was utilized to determine the MIC of berberine, palmatine, and ciprofloxacin (Sigma-Aldrich). We followed the guidelines outlined in CLSI 2019 (14) for each step (Anon.). Briefly, bacterial strains were incubated overnight in 5 ml of Mueller-Hinton broth (MERCK, Germany) at 37◦C. The 0.5 McFarland (1.5×10^8 CFU/ml) was then further diluted in saline solution to generate an inoculum of 1.5×10^5 CFU/well in a final volume of 100 ml. Following incubation at 37◦C, the MIC results were documented after 18-24 hours. The synergistic antibacterial activity of berberine and palmatine with ciprofloxacin was evaluated for *S. aureus* ATCC 33593 by modifying a previous study's method. The minimum concentration of the substance at which *S. aureus* cells did not grow after 24 h was determined as the MIC. The MIC experiments were performed in triplicate. (Seo *et al*., 2024)

DNA extraction and PCR assay: The phenol-chloroform method was used for DNA extraction according to the Porbaran *et al*. study(Porbaran *et al*., 2021). Briefly, the organism was grown in a 37°C

shaking incubator for 18 hours of inappropriate media; 2ml of this bacterial suspension was then centrifuged for 10 minutes at 16,000 x g. Then, 100 µl of 1 M TE (pH 6.8) was added to the pellet. Subsequently, suspensions were incubated at 100ºC for 30 minutes, followed by 5 minutes on ice. After adding 200 µl of NaOH (50 mM), suspensions were centrifuged at 14 000 rpm for 5 minutes, and the supernatant was collected to new Eppendorf tubes and stored at -20ºC until further use. The DNA concentration was measured by spectrometry recurring to Nanodrop® (Thermo Scientific Nano-Drop 2000C Spectrophotometer).

For the detection of *nucA*, *mecA*, *norA*, *norB,* and *norC*, Reaction mixes for the amplification of *nucA*, *mecA*, *norA*, *norB,* and *norC* genes 1 µL of each primer (10 pmol), 2 µL of template DNA (25 ng.µL $^{-1}$), 12.5 µL of Master Mix 2x (Amplicon, Denmark). The final volume of 25 µl was made using 8.5 µL of ultrapure water (Promega, USA). All the reactants were thoroughly mixed and flash-spun in a spinner. The mixtures were placed in an MJ Research® PTC 100 using the program shown in Table 1. The PCR products were stored at -20ºC until confirmation by agarose gel electrophoresis. 8µl of each PCR product was tested for positive amplification by 1% (W/V) agarose gel electrophoresis and visualized in UV light.

Checkerboard assay: The antimicrobial combinations were assessed using checkerboard determinations according to the procedure outlined by Cokol-Cakmak *et al*. (Cokol-Cakmak and Cokol, 2019). Each concentration was diluted in microtiter plates from 512 to 0.25 µg/ml, with a volume of 10 µl for each dilution. To create a final inoculum of 1.5×105 CFU/ml, 90 µl of bacterial suspension in BHI medium (Hi-medium, India) was applied to the microtiter plate. Incubation at 37 °C was maintained for 24 hours for the plates. According to the study, FIC was calculated to combine two antimicrobial agents (Cokol-Cakmak and Cokol, 2019).

Real-time accumulation assay of ethidium bromide (EtBr): According to Paixão *et al*. (Paixão *et al*., 2009), an accumulation test was used to identify EtBr accumulation in *S. aureus* strains. In summary, 20 mL of fresh TSB was mixed with 10 mL of overnight culture, and the mixture was incubated for an hour at 37 °Cin an orbital shaking incubator. The culture was centrifuged at 2860 g for 20 minutes, and the supernatant was discarded. The pellet of cells was reconstituted by combining it with a 30 ml solution of 1 mM MgCl2 in 0.01 M PBS. The particle was centrifuged again and resuspended in 1195 milliliters of the same buffer. The cell solution was divided into four equal portions (2.5 ml each), and 250µl of 1 mg/ml EtBr and 2.5 ml of the drug's double working concentration were added to each quarter. Then, 0.05 mL of the cleaned cell suspension was mixed with 0.05 mL of PBS solutions, including ciprofloxacin, berberine, palmatine, ciprofloxacin/berberine, and ciprofloxacin/palmatine, with and without EtBr. The FICA is the MIC of ciprofloxacin/berberine in the combination/MIC of ciprofloxacin alone; likewise, the FICB is the MIC of ciprofloxacin/palmatine in the combination/MIC of ciprofloxacin alone. The FIC index (FICI) is FICA + FICB. A FICI $\leq$ 0.5 indicates synergistic antibacterial activity, 0.5 < FICI $\leq$ 1 indicates partial synergistic antibacterial activity, 1 < FICI $\leq$ 4 indicates indifference, and a FICI > 4 indicates antagonistic antibacterial activity. Each synergistic antibacterial activity experiment was performed in triplicate(Seo *et al*., 2024).

By using real-time data, the relative fluorescence index (RFI) at the final time point (minute 30) of the EB accumulation assay was calculated to assess the activity of the compounds according to the formula:

$$RFI = \frac{RFI\ treated - RFI\ untreated}{RFI\ untreated}$$

Statistical Analysis Techniques: Statistical analysis of all data was performed using a student's t-test or Kruskal-Wallis test followed by Dunn's posthoc multiple comparison test, two-way ANOVA, and χ^2 test (GraphPad Prism® 8.00, GraphPad Software, Inc., CA, USA). A significance level of $P < 0.05$ denotes significance in all cases.

RESULTS

This study collected 80 S. aureus isolates from burn infections using standard biochemical methods.

Prevalence of antibiotic resistance: According to Figure 1, the resistance rate was high for cloxacillin and erythromycin, with 61 (76.25 %) isolates resistant. The lowest resistance level was against clindamycin and teicoplanin (1.25 %). Also, 52 isolates (65%) were considered MDR, 17 isolates (21.25%) were considered XDR, and one isolate (1.25%) was considered PDR. However, 72 isolates (90%) were MRSA, and all isolates were sensitive to tigecycline.

The MIC and MBC of ciprofloxacin, berberine, and palmatine: According to Table 2, 58 isolates were resistant, and 22 isolates were susceptible to ciprofloxacin. Also, The MIC of berberine against five *S. aureus* isolates was more than 128 µg/ml. The MIC of the Palmatine also against 27 *S. aureus* isolates ranged from 64 mg to 128 µg/ml and against MRSA strains was 32 µg/ml.

Prevalence of mecA, norA, norB, and norC genes. As shown in Figure 2, the frequency of *norA*, *norB,* and *norC* genes was reported to be 54 strains of *norA* (67.5 %), 73 strains of *norB* (91.25 %), and 72 strains of *norC*

(90 %). Resistance to ciprofloxacin in strains containing *norA*, *norB*, and *norC* genes was positive in 83.33%, 73.81%, and 75.61% of isolates, respectively. Besides, resistance to ciprofloxacin in strains containing all three genes was 86.66%. Further, 72 isolates (90%) carried the *mecA* gene and were considered MRSA strains.

Fractional inhibitory concentration (FIC): *of berberine and palmatine*. According to Table 3, berberine has potent synergistic activity with ciprofloxacin for MDR strains. In contrast, the XDR and PDR strains have not shown any synergistic effects of ciprofloxacin with palmatine extract.

The FIC results in Tables 4 and 5 showed a synergistic effect of ciprofloxacin + berberine against MRSA strains. The synergistic effect of ciprofloxacin + palmatine against MRSA strains was also observed. EP inhibitors' efficacy was gauged by identifying a substantial 4-fold or greater rise in susceptibility following the introduction of berberine (Table 5). A 4- to 64-fold decrease in MICs was seen in 75.2% of the 89 S. aureus isolates. Both berberine and palmatine were shown to decrease the MIC of ciprofloxacin from 128µg/ml to 8 µg/ml and 4 µg/ml, respectively.

Accumulation Assays of EtBr: As shown in Figure 3, after 30 min, ciprofloxacin-berberine (RFI: 39.13) and ciprofloxacin-palmatine (RFI: 18.09) had EPI activity compared to ciprofloxacin (RFI: 6.13) on the MRSA strain. However, ciprofloxacin had no EPI activity on the XDR and PDR strain at the concentration applied in the assay. Ciprofloxacin-berberine was the most effective EPI activity on the strain of the XDR (RFI: 16.13) and PDR (RFI: 7.33). Based on the real-time accumulation data, ciprofloxacin-palmatine did not affect the XDR and PDR strain.

Statical analysis: Based on Table 4, there was a significant difference between the antibiotic resistance pattern and the berberine and palmatine's inhibitory effect ($p<0.05$). In MRSA strains, berberine and palmatine had more inhibitory effects than MSSA strains. Also, the synergistic effect of berberine and palmatine with ciprofloxacin was higher in MDR strains than in XDR and PDR strains ($p<0.001$). However, no clear significant difference was observed between the frequency of *norA*, *norB,* and *norC* genes and the inhibitory effect of berberine and palmatine extracts ($p>0.001$) (For more detailed data, please see Figure S1 and Table S1).

Table 2. Clinico-demographic characteristics of patients with *S. aureus* infections and the prevalence of MRSA at Shahroud Hospital, in Northern Iran.

Clinico -demographic characteristics	No. of patients with *S. aureus* (n = 80)	No. of patients with MRSA (%)	P-value	Prevalence ratio (95%CI)
Total	80	72(90%)		
Sex			-	
Female	25 (31.2%)	11(15.2%)	<0.0001	Reference
Male	55(68.7%)	44(61.1%)		1.65 (1.1 -2.0)
Age groups (years)				
< 1	1(1.1%)	0	0.65	0.75 (0.09 -2.35)
1-5	1(1.1%)	0	0. 75	0.85 (0.44 -1.95)
6-18	14(17.5%)	10(13.8%)	0.80	0.99 (0.33 -1.51)
19 -35	22(27.5%)	18(25.0%)		Reference
36 – 50	19(23.7%)	15(20.8%)	0.50	1.29 (0.95 -1.68)
51 - 65	20(25.0%)	11(15.2%)	0.10	1.44 (0.55 -1.79)
> 65	3(3.7%)	1(1.3%)	0.45	1.06 (0.81-1.28)
Hospitalization				
Inpatient	74(92.5%)	66 (91.6%)		
Outpatient	6(7.5%)	6 (8.3%)	0.001	1.39 (0.62 -1.61)

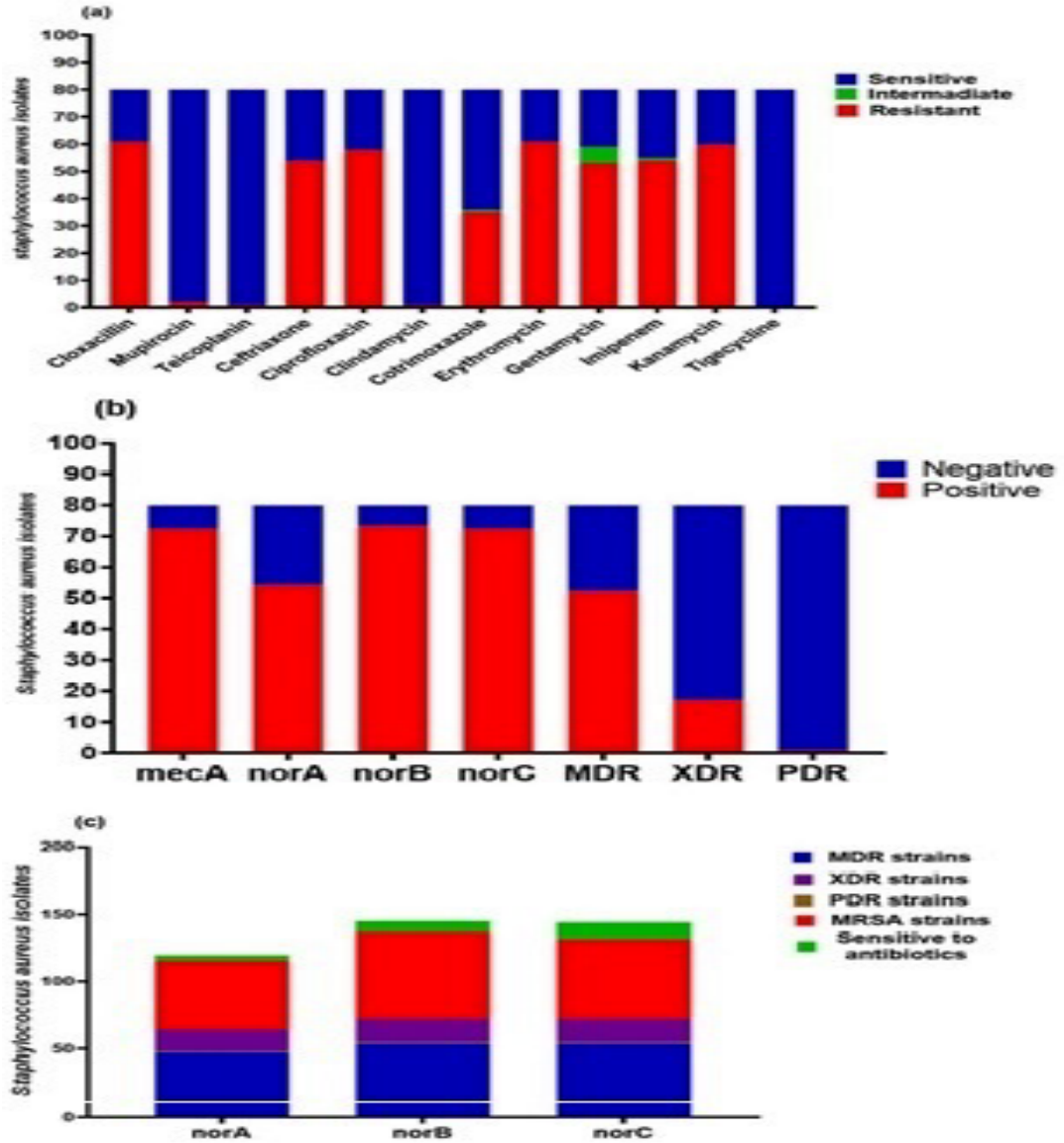


Figure 1. Prevalence of EPgenes and antibiotic resistance profile in wound collection of *S. aureus* isolates. (a) Antibiotic resistance profile in wound collection of *S. aureus* isolates. (b) Efflux pump and *mecA* gene prevalence in wound collection of *S. aureus* isolates. (c) Efflux pump genes in wound collection of *S. aureus* isolates.

Table 3. MICs of ciprofloxacin, berberine and palmatine against *S. aureus* isolates.

S. aureus isolates No (%)	MIC CIP (µg/ml)	MIC Berberine (µg/ml)	MIC (CIP + Berberine) (µg/ml)	MIC Palmatine (µg/ml)	MIC (CIP + Palmatine) (µg/ml)
5 (5.6)	256	128	9	128	10
11 (12.3)	8	6	2	4	2.5
3 (3.3)	16	2	2	4	4
19 (21.3)	32	18	4	8	4
13 (14.6)	4	2	4	4	2
22 (24.7)	128	64	8	128	16
16 (17.9)	6	2	4	4	1.5

Table 4. Minimum inhibitory/bactericidal concentration of ciprofloxacin, berberine, and palmatine with and without (in mg/ml) tested in different ratios (1:1, 1:2, 2:1; mean ± SD).

S. aureus isolate No (%)	1:1		1:2		2:1	
	MIC	MBC	MIC	MBC	MIC	MBC
5 (5.6)	< 0.09:0.09	< 0.09:0.09	0.9:1.8 ± 0.3:0.7	0.9:1.7 ± 0.1:0.1	2.0:1.0 ± 0.0:0.0	24.0:12.0 ± 9.9.3:4.7
11 (12.3)	6.9:6.9 ± 1.8:1.8	11.8:11.8 ± 4.1:4.1	1.8:3.1 ± 0.7:0.9	2.7:3.9 ± 0.7:1.0	16.0:8.0 ± 0.0:0.0	26.7:13.3 ± 8.5:4.8
3 (3.3)	4.0:4.0 ± 1.9:1.9	6.6:6.6 ± 1.9:1.9	2.2:3.3 ± 0.2:0.6	2.8:3.9 ± 1.1:2.1	16.0:8.0 ± 0.0:0.0	>32.0:16.0
19 (21.3)	5.3:5.3 ± 2.8:2.8	14.1:14.1 ± 3.8:3.8	0.5:1.2 ± 0.2:0.4	4.3:7.1 ± 1.7:3.9	9.0:8.0 ± 1.0:1.0	18.7:9.3 ± 6.0:3.0
13 (14.6)	7.0:7.0 ± 1.9:1,9	7.5:7.5 ± 0.3:0.3	1.7:2.9 ± 0.2:0.5	3.0:6.0 ± 0.9:1.9	9.0:5.0 ± 1.0:1.0	29.3:14.7 ± 5.0:2.0
22 (24.7)	4.2:4.2 ± 2.0:2.0	7.2:7.2 ± 2.2:2.2	1.9:3.1 ± 0.2:0.5	2.8:4.7 ± 1.1:2.1	5.0:7.0 ± 0.1:0.1	12.0:7.0 ± 8.1:4.2
16 (17.9)	1.9:1.9 ± 1.0:1.0	3.9:3.9 ± 0.9:0.9	0.5:1.0 ± 0.2:0.5	3.7:5.3 ± 1.4:2.7	< 0.02:0.07	2.2:1.1 ± 1.9:0.7

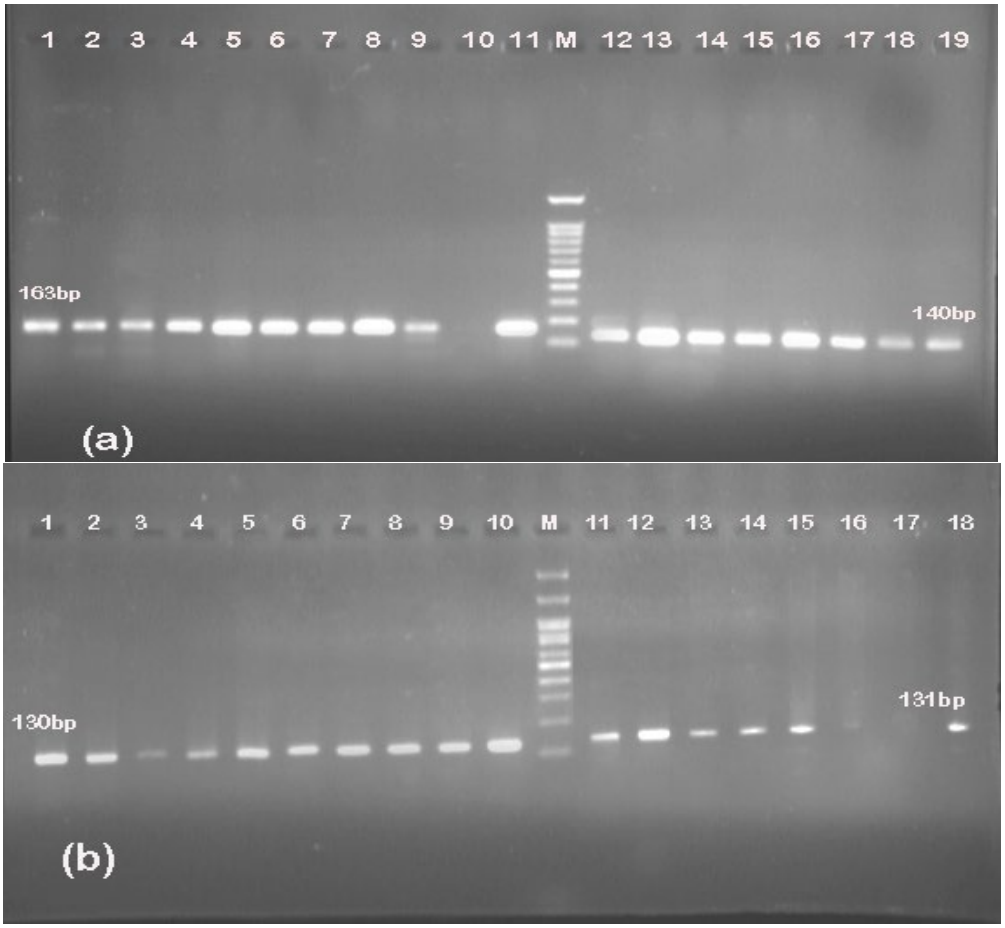


Figure 2. The amplification results of EP and *mecA* genes in *S. aureus*. (a) *mecA* gene with 163bp, and *norA* gene with 140bp; Wells 1: Positive control, well 2 to 11: Positive strains with *mecA* gene; Wells 12: Positive control, well 13 to 19: Positive strains with *norA* gene. (b) *norB* gene with 130bp, *norC* gene with 131p; Wells 1: Positive control, well 2 to 10: Positive strains with *norB*; Wells 11: Positive control, well 12 to 18: Positive strains with *norC*. M: Ladder 100bp.

Table 5. Synergistic activity of berberine and palmatine extract with ciprofloxacin for wound collection of *S. aureus* isolates

Name of the strain of *S. aureus*	FIC value with ciprofloxacin	Synergistic activity of berberine with ciprofloxacin	Synergistic activity of palmatine with ciprofloxacin	*p*
Antibiotic sensitive strains	2	Indifferent	Indifferent	0.4
MDR strains	0.35	Synergistic	Synergistic	0.036
XDR strains	0.75	Synergistic	Synergistic	0.011
PDR strains	0.23	Synergistic	Synergistic	0.007
Efflux gene positive strains	0.19	Synergistic	Synergistic	0.029
Efflux gene-negative strains	1.5	Indifferent	Indifferent	0.7

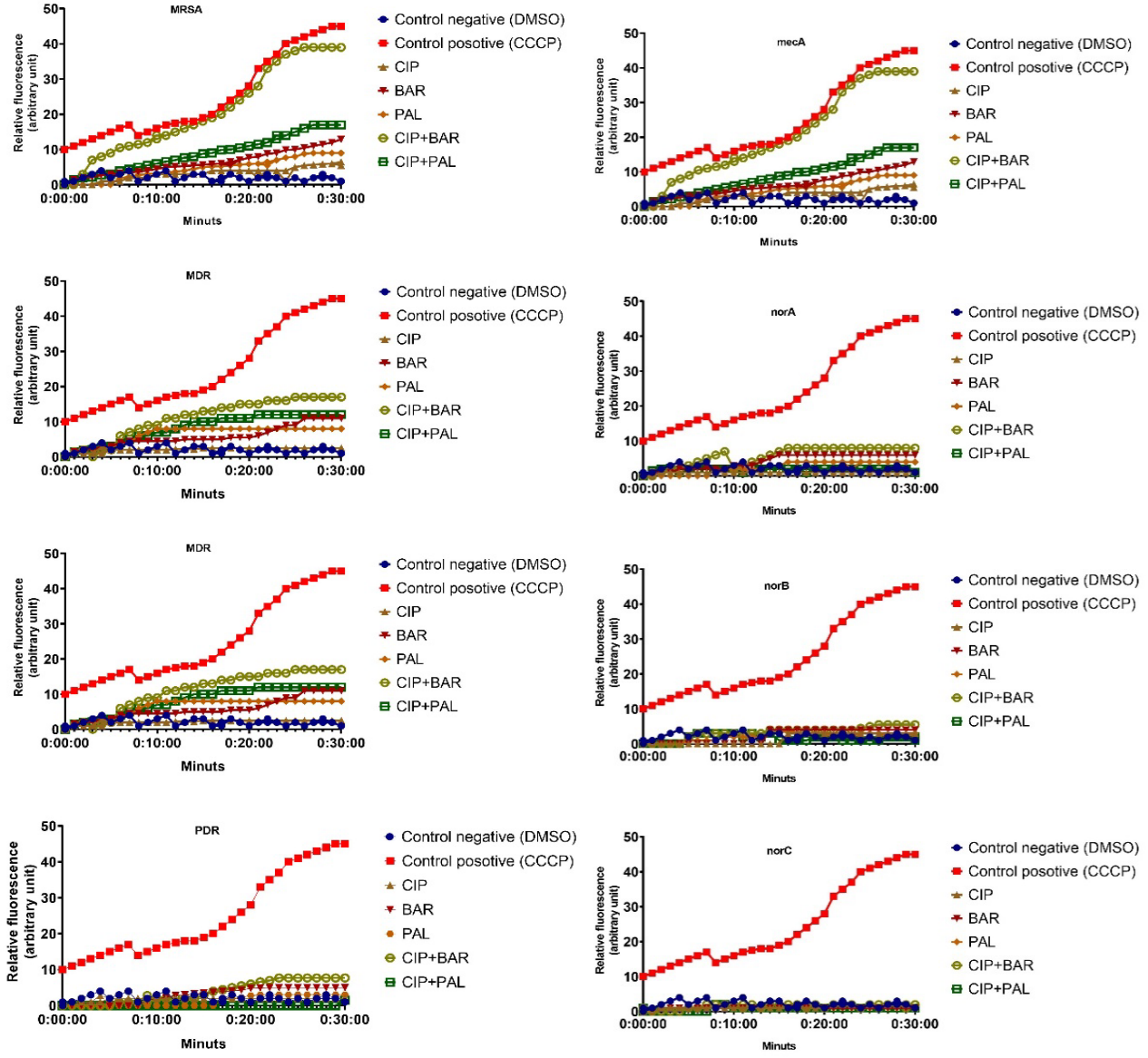


Figure 3.Effect of ciprofloxacin/berberine and ciprofloxacin/palmatine on accumulation of EtBr on *S. aureus* strains. *S. aureus*CIP: Ciprofloxacin; Bar: Berberine; Pal: Palmatine; MDR: Multi-drug resistant; XDR: Extensively drug resistant; PDR: Pan drug resistance.

DISCUSSION

This study collected 80 S. aureus isolates from 690 burn infection samples. In the study of Khaledifar *et al.*, the analysis showed no important connection between the frequency of *S. aureus* isolates and the age ranges examined (P>0.05)(Khaledifar *et al*., 2023). Among the samples analyzed, *S. aureus* isolates were more commonly detected in inpatient cases, representing 68.7% (55 out of 80), as opposed to 31.2% (25 out of 80) from outpatient cases (Table 2). This may be because sex steroid hormones, particularly estrogens, significantly influence skin homeostasis. Research indicates that the individual's gender plays a role in the recovery process of skin injuries(Thomason *et al*., 2015).

According to Figure 1, the resistance rate was high for cloxacillin and erythromycin, with 61 (76.25 %) isolates resistant. The results from determining antibiotic sensitivity were inconsistent with other studies(Fomda *et al*., 2014; Mohammadi *et al*., 2014; Tahmasebi *et al*., 2019). The prevalence rate of *S. aureus* and MRSA in wound infection in different countries varies depending on the size, geographical area, and management practices of the herd under study. MDR, XDR, and PDR were found in 65%, 21.25%, and 1.25% isolates, respectively. Similar results were achieved by Kot *et al*. and Gurung *et al*.,(Gurung *et al*., 2020; Kot *et al*., 2020). *S. aureus* Several factors contribute to the observed differences in outcomes, such as the characteristics and site of the injury, the specific antibiotics administered to mitigate infection, the quality of medical treatment provided, and the strategies implemented to reduce the risk of hospital-acquired infections.

In the present study, the prevalence of MRSA strains was reported in 90% of the cases, representing a higher range than in other studies (Pournajaf *et al*., 2014; Shahmohammadi *et al*., 2015; Heydari *et al*., 2017; Tahmasebi *et al*., 2025). The study suggested that drug resistance, even against effective antibiotics, is increasing in *S. aureus*, an alarming feature and attention to control the increasing drug resistance problem.

Table 3 shows that 58 isolates were resistant, and 22 were susceptible to ciprofloxacin. Also, The MIC of berberine against five *S. aureus* isolates was more than 128 µg/ml. Berberine exhibited significant antibacterial properties against MRSA and MDR in the study, with a MIC of 32 mg/ml for both strains that differed from the results obtained by Guo Ying Zuo *et al*., (Zuo *et al*., 2012). The MIC of the Palmatine also against 27 *S. aureus* isolates ranged from 64 mg to 128 µg/ml and against MRSA strains was 32 µg/ml. It has been documented that berberine can block the efflux pumps found in E. coli and *Pseudomonas aeruginosa*(Seo *et al*., 2024). The results obtained from this study affirm a similar cooperative effect in the Gram-positive bacterium *S. aureus*. *S. aureus*. However, in 2014, Upadhyay *et al*., Aparna *et al*., and Medeiros *et al*. confirmed that some compounds can communicate with EP proteins, causing bacterial sensitivity to return to antibiotics and inhibiting the EP. Recently, new products, particularly bio-active phytochemical compounds, have been reported to act as EP inhibitors for *S. aureus*; they can be used as antibiotic complements(Aparna *et al*., 2014; Medeiros Barreto *et al*., 2014; Upadhyay *et al*., 2014).

As illustrated in Figure 2, resistance to ciprofloxacin in strains containing *norA*, *norB*, and *norC* genes was positive in 83.33%, 73.81%, and 75.61% of isolates, respectively. Besides, resistance to ciprofloxacin in strains containing all three genes was 86.66%. Previous research indicates that variations in percentage outcomes may stem from differences in sample sizes, the locations and timing of sample collection, the isolates' antibiotic resistance profiles, and the patient's overall health status(Hassanzadeh *et al*., 2020)(Kwak *et al*., 2013). Those studies confirmed that the EP is among the factors creating multiple antibiotic resistance in *S. aureus* by inducing antibiotic exodus and reducing intracellular concentration in the antibiotic cells.

Concerning the details outlined in Tables 3 and 4, berberine has potent synergistic activity with ciprofloxacin for MDR strains. In contrast, the XDR and PDR strains have not shown any synergistic effects of ciprofloxacin with palmatine extract. It has been widely documented that berberine functions as a substrate for pumps that expel multiple drugs. Various efflux pumps associated with the resistance-nodulation-division (RND) superfamily exist in bacteria that exhibit multidrug resistance(Zhou *et al*., 2016).

The evidence we gathered shows that berberine improves the antibacterial properties of ciprofloxacin when targeting MRSA, and their combined use could allow for a reduced ciprofloxacin dosage in therapy, offering the added advantage of decreasing the risk of drug resistance and adverse reactions. There is currently no information available regarding how berberine and ciprofloxacin interact and their influence on MRSA. A 4- to 64-fold decrease in MICs was seen in 75.2% of the 89 *S. aureus* isolates. Both berberine and palmatine were shown to decrease the MIC of ciprofloxacin from 128µg/ml to 8 µg/ml and 4 µg/ml, respectively. Research on the combined influence of active natural ingredients, including berberine/ palmatine and ciprofloxacin, on *S. aureus* strains, is rare(Wojtyczka *et al*., 2014). There is a lack of extensive reports concerning the synergistic interactions of various compounds with *S. aureus*; however, the available evidence hints at the possibility of enhancing the antibacterial performance of common antibiotics by integrating specific natural compounds(Wojtyczka *et al*., 2014; Seo *et al*., 2024).

Berberine's MIC value was 8 times lower than MRSA strains when tested against XDR and PDR.

Berberine has the same MIC value for both *mecA* and EP-positive strains. Palmatine had no significant effect on strains carrying *mecA*, *norA*, *norB,* and *norC* genes. The close agreement of the results with the Tan *et al*. study suggested the antibacterial effect of berberine in treating MRSA(Tan *et al*., 2019). It is worth noting that berberine emerged as the most powerful major against all strains. The findings of Wojtyczka *et al.,* indicate that berberine has notable antimicrobial efficacy against MRSA and that its use alongside ciprofloxacin creates a synergistic response. The relationship between berberine and various antimicrobial agents may stem from its potential to obstruct different mechanisms of bacterial resistance, especially through its inhibitory action on efflux pumps in bacteria(Wojtyczka *et al*., 2014; Wultańska *et al*., 2020).

The current study observed Synergistic effects in the ciprofloxacin + berberine combinations, as indicated by FIC 0<5. Barbarian reduced the MIC levels in 43.75% and 18.75% of the MRSA and MDR strains, respectively. Palmatine also condensed the MIC levels in 34.37% and 12.5% of the MRSA and MDR strains, respectively. Consistent with Seukep *et al*. and Álvarez *et al*., findings, we found the bacteriostatic effect of berberine and palmatine on MRSA and MDR strains(Álvarez-Martínez *et al*., 2020; Seukep *et al*., 2020). Therefore, these bioactive phytochemical compounds can be used as supplementary medicine to increase antibiotics' synergetic effect. It is widely thought that medications, along with berberine and palmatine, generally exhibit few side effects. Findings from a study suggested that using berberine in combination with macrolide medications could pose risks of drug toxicity, especially concerning heart-related issues(Khaledifar *et al*., 2023). Consequently, clinicians should think about administering this in conjunction with Azithromycin. However, clear information regarding ciprofloxacin and berberine is unavailable, and further clinical work is needed.

Based on the real-time accumulation data, ciprofloxacin-palmatine did not affect the XDR and PDR strain. However, no synergistic interaction or antimicrobial action occurred when both agents were at the lowest concentration. These findings have been echoed in the more recent research conducted by Li *et al*., and Zhang *et al*., (Li *et al*., 2020; Zhang *et al*., 2020). The results indicate that consistently documenting EPs could help uncover the defensive strategies employed by bacteria. It is possible that some of these systems were triggered to eliminate as much berberine as they could from the cells. Berberine might influence the growth of bacteria by increasing the transcription levels of EP gene activity (Wojtyczka *et al*., 2014).

This study indicates that EtBr was primarily expelled within the initial 20 minutes when ciprofloxacin + berberine and ciprofloxacin + palmatine were not included. That being said, the presence of ciprofloxacin had no impact on the EtBr efflux. These results were essentially confirmed in the study by Wojtyczka *et al.,* (Wojtyczka *et al*., 2014). Most studies have demonstrated that these concentrations of berberine are sufficiently antibacterial for the MRSA and MDR strains to inhibit the EP defense mechanism that involves antibiotic resistance, consistent with our reported findings(Zhang *et al*., 2020; Li *et al*., 2021).

Berberine was decided to encourage a more significant buildup of EtBr in cells, which is essential for initiating efflux assessments. Li *et al*., and Ning Sun *et al*., demonstrated a potential combination therapy of berberine in inhibiting MDR EP(Sun *et al*., 2014; Li *et al*., 2021). The effect of berberine on EtBr accumulation in MDR strains is similarly demonstrated and confirmed by Mangiaterra *et al*. and Lu *et al*., (Mangiaterra *et al*., 2017; Lu *et al*., 2019).

Based on Tables 4 and 5, there was a significant difference between the antibiotic resistance pattern and the berberine and palmatine's inhibitory effect ($p<0.05$). In MRSA strains, berberine and palmatine had more inhibitory effects than in MSSA strains. Also, the synergistic effect of berberine and palmatine with ciprofloxacin was higher in MDR strains than in XDR and PDR strains ($p<0.001$). Researchers hypothesize that the synergistic effect of berberine in combination with antibiotics arises from its ability to obstruct the NorA pump, amplifying the antibiotics' efficacy. The accumulation of berberine within the cell, influenced by the membrane potential, could inhibit the NorA pump from removing certain antibiotics (Stermitz *et al*., 2000; Ettefagh *et al*., 2011). These findings also are consistent with the study of Kotani *et al*., (Kotani *et al*., 2019).

Based on Supplementary 1 and 2, variability can be observed in the efflux time of the substrate among MRSA, MDR, XDR, and PDR strains via the accumulation method. The first 10 to 15 minutes, the highest efflux in MRSA and MDR strains is seen. The extension of efflux time in XDR strains is most apparent in the first 25 minutes, a trend seen in PDR strains. Earlier findings indicate that berberine's ability to kill bacteria may stem from several processes, including preventing DNA replication, RNA transcription interference, and protein production disruption. It might also alter or inhibit enzyme activities and damage the structure of bacterial cell surfaces, causing the release of $Ca2+$ and $K+$ ions(JIN; *et al*., 2010; Peng *et al*., 2015). The combined action of antibiotics and berberine could offer a promising strategy in clinical settings for effectively eliminating infectious MRSA strains, particularly in situations that necessitate an unconventional treatment method. Good agreement was also found when comparing results from this work against published data by some scientists(Wojtyczka *et al*., 2014; Zhang *et al*., 2020; Li *et al*., 2021).

In conclusion, this research reveals that the synergistic effect of ciprofloxacin and berberine/ palmatine has notable antibacterial capabilities, potentially enabling a decrease in ciprofloxacin dosage, curbing the development of ciprofloxacin-resistant MRSA, and lessening the rates of drug resistance. The bactericidal properties of berberine work together in a complementary manner, potentially aiding in treating infections in clinical settings. Berberine/ palmatine could play a crucial role in advancing antibiotics targeting MRSA and EP activity, particularly in multidrug-resistant and extensively drug-resistant infections and wound care. Despite its effectiveness, the specific molecular processes through which berberine enhances the antimicrobial properties of ciprofloxacin against multidrug-resistant and MRSA strains remain unclear. *S. aureus.*

Data Availability: The data supporting this study's findings are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no competing interests.

Compliance with ethical standards: Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

Acknowledgments: The authors gratefully thank Islamic Azad University, Damghan, Iran, for providing facilities to conduct the research.

REFERENCES

Alam, M.M., M.N. Islam, M.D. Hossain Hawlader, S. Ahmed, A. Wahab, M. Islam, K.M.R. Uddin and A. Hossain (2021). Prevalence of multidrug resistance bacterial isolates from infected wound patients in Dhaka, Bangladesh: A cross-sectional study. Intr. J. Surgery Open. 28, 56-62. http://doi.org/10.1016/j.ijso.2020.12.010.

Álvarez-Martínez, F.J., E. Barrajón-Catalán and V. Micol (2020). Tackling antibiotic resistance with compounds of natural origin: a comprehensive review. Biomedicines. 8 (10), 405. http://doi.org/10.3390/biomedicines8100405.

Anon. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100- S22. Wayne, PA: Clinical and Laboratory Standard Institute; 2012.

Aparna, V., K. Dineshkuma, N. Mohanalakshmi, D.Velmurugan and W. Hopper, W. (2014). Identification of natural compound inhibitors for multidrug efflux pumps of Escherichia coli and Pseudomonas aeruginosa using in silico high-throughput virtual screening and in vitro validation. PLoS One. 9 (7), e101840. http://doi.org/10.1371/journal.pone.0101840.

Arabestani, M.R., H. Tahmasebi and B. Zeyni (2017). Diagnostic value of melting curve analysis based on multiplex-real time PCR in identification of Enterococci species. J. Mazandaran Univ. Med. Sci. 26 (145), 234-247.

Bokaeian, M. and H. Tahmasebi (2017). Molecular identification of genes responsible for resistance to aminoglycosides and methicillin in clinical samples of Staphylococcus Aureus. J Babol Univ Med Sci. 19 (3), 38-46. http://doi.org/10.22088/jbums.19.3.38

Cokol-Cakmak, M. and M. Cokol (2019). Miniaturized checkerboard assays to measure antibiotic interactions. Methods Mol. Biol. 1939, 3-9. http://doi.org/10.1007/978-1-4939-9089-4_1.

Connie, R., D.C.L. Mahon and George Manuselis Jr. (2014) Textbook of Diagnostic Microbiology (5th Edition). USA: Saunders.

Costa, S.S., M. Viveiros, L. Amaral and I. Couto (2013). Multidrug efflux pumps in staphylococcus aureus: an update. The open microbiology journal. 7, 59-71. http://doi.org/10.2174/1874285801307010059.

de Sousa Andrade, L.M., A.B.M. de Oliveira, A.L.A.B. Leal, F.A. de Alcântara Oliveira, A.L. Portela, J. de Sousa Lima Neto, J.P. de Siqueira-Júnior, G.W. Kaatz, C.Q. da Rocha and H.M. Barreto (2020). Antimicrobial activity and inhibition of the NorA efflux pump of Staphylococcus aureus by extract and isolated compounds from Arrabidaea brachypoda. Microbial Pathogenesis. 140, 103935. http://doi.org/10.1016/j.micpath.2019.103935.

Eslami, M., R. Naderian and A. Bahar (2025). Microbiota as diagnostic biomarkers: advancing early cancer detection and personalized therapeutic approaches through microbiome profiling. Frontiers in Immunology, Volume 16 - 2025. http://doi.org/10.3389/fimmu.2025.1559480

Ettefagh, K.A., J.T. Burns, H.A. Junio, G.W. Kaatz and N.B. Cech (2011) .Goldenseal (Hydrastis canadensis L.) extracts synergistically enhance the antibacterial activity of berberine via efflux pump inhibition. Planta Med. 77 (8), 835-840. http://doi.org/10.1055/s-0030-1250606.

Fomda, B.A., M.A. Thokar, G. Bashir, A. Khan, A. Kour, D. Zahoor and P. Ray (2014). Prevalence and genotypic relatedness of methicillin resistant Staphylococcus aureus in a tertiary care hospital. J Postgrad Med. 60 (4), 386-389. http://doi.org/10.4103/0022-3859.143964.

Gurung, R.R., P. Maharjan and G.G. Chhetri (2020). Antibiotic resistance pattern of Staphylococcus aureus with reference to MRSA isolates from

pediatric patients. Future science OA. 6 (4), FSO464-FSO464. http://doi.org/10.2144/fsoa-2019-0122.

Hassanzadeh, S., Ganjloo, S., Pourmand, M.R., Mashhadi, R. and Ghazvini, K. (2020). Epidemiology of efflux pumps genes mediating resistance among Staphylococcus aureus; A systematic review. Microb Pathog. 139, 103850. http://doi.org/10.1016/j.micpath.2019.103850.

Heydari, N., M.Y. Alikhani, F.A. Jalilian, H. Tahmasebi and M.R. Arabestani (2017). Evaluation of real time PCR for detection of clinical isolates of Staphylococcus aureus and methicillin-resistance strains based on melting curve analysis method. Koomesh. 19 (4), 877-886.

Heydari, N., M.Y. Alikhani and H. Tahmasebi (2019). Design of Melting Curve Analysis (MCA) by Real-Time Polymerase Chain Reaction Assay for rapid distinction of Staphylococci and antibiotic resistance. Arch. Clin. Infect Dis, 14(2), e81604. http://doi.org/10.5812/archcid.81604.

Hsieh, P.C., S.A. Siegel, B. Rogers, D. Davis and K. Lewis (1998). Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. Proc Natl Acad Sci U S A. 95 (12), 6602-6606. http://doi.org/10.1073/pnas.95.12.6602.

Imanshahidi, M. and Hosseinzadeh, H. (2008). Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. Phytother. Res. 22 (8), 999-1012. http://doi.org/10.1002/ptr.2399.

Jahantigh, M. and H. Tahmasebi (2024). Correlation between efflux pumps activity and frequency of pslABD genes in clinical isolates of *Pseudomonas aeruginosa* collected from diabetic foot infections. J. Knowledge & Health in Basic Medical Sciences 19 (2), 22-40. http://doi.org/10.22100/jkh.v19i2.3219.

JIN;J., G. Hua, Z. Meng and P. Gao .(2010) . Antibacterial mechanisms of Berberine and reasons for little resistance of bacteria chinese herbal medicines. 4, 8. http://doi.org/10.3969/j.issn.1674-6384.2011.01.007.

Jung, J., Choi, J.S. and Jeong, C.S. (2014). Inhibitory Activities of Palmatine from Coptis chinensis Against Helicobactor pylori and Gastric Damage. Toxicol Res. 30 (1), 45-48. http://doi.org/10.5487/tr.2014.30.1.045.

Khaledifar, A., M.R. Khosravi and E. Raeisi (2023). Berberine efficacy against doxorubicin-induced cardiotoxicity: A systematic review. J Herbmed Pharmacol. 12 (2), 187-193. http://doi.org/10.34172/jhp.2023.19.

Khazaei, M., M. Parsasefat and A. Bahar (2025). Behavioral cooperation or conflict of human intestinal roundworms and microbiomes: a bio-activity perspective. Cells, 14(7). Retrieved from. http://doi.org/10.3390/cells14070556.

Kot, B., K. Wierzchowska, M. Piechota and A. Grużewska (2020). Antimicrobial Resistance patterns in methicillin-resistant Staphylococcus aureus from patients hospitalized during 2015-2017 in hospitals in Poland. Med Princ Pract. 29 (1), 61-68. http://doi.org/10.1159/000501788.

Kotani, K., M. Matsumura, Y. Morita, J. Tomida, R. Kutsuna, K. Nishino, S. Yasuike and Y. Kawamura (2019). 13-(2-Methylbenzyl) berberine is a more potent inhibitor of MexXY-dependent aminoglycoside resistance than Berberine. Antibiotics. 8 (4), 212. http://doi.org/10.3390/antibiotics8040212.

Kwak, Y.G., Q.C. Truong-Bolduc, H. Bin Kim, K.H. Song, E.S. Kim and D.C. Hooper (2013). Association of norB overexpression and fluoroquinolone resistance in clinical isolates of Staphylococcus aureus from Korea. The J. antimicrobial chemotherapy. 68 (12), 2766-2772. http://doi.org/10.1093/jac/dkt286.

Li, X., Y. Song, L. Wang, G. Kang, P. Wang, H. Yin and H. Huang (2021). A potential combination therapy of berberine hydrochloride with antibiotics against multidrug-resistant Acinetobacter baumannii. Front Cell Infect Microbiol. 11, 660431. http://doi.org/10.3389/fcimb.2021.660431.

Li, X., P. Wang, X. Hu, Y. Zhang, X. Lu,C. Li, T. Nie, G. Li, X. Wang, J. Pang, Y. Lu, X. Yang and X. You (2020). The combined antibacterial effects of sodium new houttuyfonate and berberine chloride against growing and persistent methicillin-resistant and vancomycin-intermediate Staphylococcus aureus. BMC Microbiol. 20 (1), 317. http://doi.org/10.1186/s12866-020-02003-2.

Lu, W.J., H.J. Lin, P.H. Hsu, M. Lai, J.Y. Chiu and H.T.V. Lin (2019). Brown and Red seaweeds serve as potential efflux pump inhibitors for drug-resistant Escherichia coli. Evidence-Based Complementary and Alternative Medicine. 2019, 1836982. http://doi.org/10.1155/2019/1836982.

Mangiaterra, G., Laudadio, E., Cometti, M., Mobbili, G., Minnelli, C., Massaccesi, L., Citterio, B., Biavasco, F. and Galeazzi, R. (2017). Inhibitors of multidrug efflux pumps of *Pseudomonas aeruginosa* from natural sources: An in silico high-throughput virtual screening and in vitro validation. Medicinal Chemistry Research. 26

(2), 414-430. http://doi.org/10.1007/s00044-016-1761-1.

Medeiros Barreto, H., F. Cerqueira Fontinele, A. Pereira de Oliveira, D.D. Arcanjo, B.H. Cavalcanti Dos Santo, A.P. de Abreu, H. Douglas Melo Coutinho, R. Alves Carvalho da Silva, T. Oliveira de Sousa, M. Freire de Medeiros, A.M. Lopes Cito and J.A. Dantas Lopes (2014). Phytochemical prospection and modulation of antibiotic activity in vitro by Lippia origanoides H.B.K. in methicillin resistant Staphylococcus aureus. Biomed Res Int. 2014, 305610. http://doi.org/10.1155/2014/305610.

Mohammadi, S., Z. Sekawi, A. Monjezi, M.H. Maleki, S. Soroush, N. Sadeghifard, I. Pakzad, F. Azizi-Jalilian, M. Emaneini, K. Asadollahi, F. Pourahmad, R. Zarrilli and M. Taherikalani (2014). Emergence of SCCmec type III with variable antimicrobial resistance profiles and spa types among methicillin-resistant Staphylococcus aureus isolated from healthcare- and community-acquired infections in the west of Iran. Int J Infect Dis. 25, 152-158. http://doi.org/10.1016/j.ijid.2014.02.018.

Paixão, L., L. Rodrigues, I. Couto, M. Martins, P. Fernandes, C.C. de Carvalho, G.A. Monteiro, F. Sansonetty, L. Amaral and M. Viveiros (2009). Fluorometric determination of ethidium bromide efflux kinetics in Escherichia coli. J Biol Eng. 3, 18. http://doi.org/10.1186/1754-1611-3-18.

Peng, L., S. Kang, Z. Yin, R. Jia, X. Song, L. Li, Z. Li, Y. Zou, X. Liang, L. Li, C. He, G. Ye, L. Yin, F. Shi, C. Lv and B. Jing (2015). Antibacterial activity and mechanism of berberine against Streptococcus agalactiae. Int J Clin Exp Pathol. 8 (5), 5217-5223.

Porbaran, M., H. Tahmasebi and M. Arabestani (2021). A comprehensive study of the relationship between the production of β-lactamase enzymes and iron/siderophore uptake regulatory genes in clinical isolates of acinetobacter baumannii. Int. J. Microbiol. 2021, 5565537. http://doi.org/10.1155/2021/5565537.

Pournajaf, A., A. Ardebili, L. Goudarzi, M. Khodaban, T. Narimani and H. Abbaszadeh (2014). PCR-based identification of methicillin-resistant Staphylococcus aureus strains and their antibiotic resistance profiles. Asian Pac. J. Trop. Biomed. 4 (Suppl 1), S293-297. http://doi.org/10.12980/apjtb.4.2014c423.

Seo, Y., M. Kim and T.J. Kim (2024). Enhanced efficacy of ciprofloxacin and tobramycin against staphylococcus aureus when combined with corydalis tuber and berberine through efflux pump inhibition. Antibiotics. 13 (5), 469. http://doi.org/10.3390/antibiotics13050469.

Seukep, A.J., V. Kuete, L. Nahar, S.D. Sarker and M. Guo (2020). Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. J. Pharmaceutical Analysis. 10 (4), 277-290. http://doi.org/doi.org/10.1016/j.jpha.2019.11.002.

Shahmohammadi, M.R., M.R. Nahaei, A. Akbarzadeh and M. Milani (2015). Clinical test to detect mecA and antibiotic resistance in Staphylococcus aureus, based on novel biotechnological methods. Artif Cells Nanomed Biotechnol. 1-5. http://doi.org/10.3109/21691401.2015.1041639.

Stermitz, F.R., P. Lorenz, J.N. Tawara, L.A. Zenewicz and K. Lewis (2000). Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. Proc Natl Acad Sci U S A. 97 (4), 1433-1437. http://doi.org/10.1073/pnas.030540597.

Sun, N., F.Y. Chan, Y.J. Lu, M.A.C. Neves, H.K. Lui, Y. Wang, K.Y. Chow, K.F. Chan, S.C. Yan, Y.C. Leung, R. Abagyan, T.H. Chan and K.Y. Wong (2014). Rational design of berberine-based FtsZ Inhibitors with Broad-spectrum antibacterial activity. PLOS ONE. 9 (5), e97514. http://doi.org/10.1371/journal.pone.0097514.

Tahmasebi, H., S. Dehbashi and M.R. Arabestani (2019). Association between the accessory gene regulator (agr) locus and the presence of superantigen genes in clinical isolates of methicillin-resistant Staphylococcus aureus. BMC Res Notes. 12 (1). http://doi.org/10.1186/s13104-019-4166-7.

Tahmasebi, H., N. Arjmand and M. Monemi (2025). From Cure to crisis: understanding the evolution of antibiotic-resistant bacteria in human microbiota. Biomolecules, 15(1), 93. http://doi.org/10.3390/biom15010093.

Tan, J., J. Wang, C. Yang, C. Zhu, G. Guo, J. Tang and H. Shen (2019). Antimicrobial characteristics of berberine against prosthetic joint infection-related Staphylococcus aureus of different multi-locus sequence types. BMC Complementary and Alternative Medicine. 19 (1), 218. http://doi.org/10.1186/s12906-019-2558-9.

Thomason, H., H. Williams and M. Hardman (2015) Sex and sex hormones mediate wound healing. pp.31-48.

Upadhyay, A., I. Upadhyaya, A. Kollanoor-Johny and K. Venkitanarayanan (2014). Combating pathogenic microorganisms using plant-derived antimicrobials: a minireview of the mechanistic basis2014, 761741. http://doi.org/10.1155/2014/761741.

Wasaznik, A., M. Grinholc and K.P. Bielawski (2009). Active efflux as the multidrug resistance mechanism]. Postepy Hig Med Dosw (Online). 63, 123-133.

Wojtyczka, R.D., A. Dziedzic, M. Kępa, R. Kubina, A. Kabała-Dzik, T. Mularz and D. Idzik (2014). Berberine Enhances the antibacterial activity of selected antibiotics against coagulase-negative staphylococcus strains in vitro. molecules. 19 (5), 6583-6596. http://doi.org/10.3390/molecules19056583.

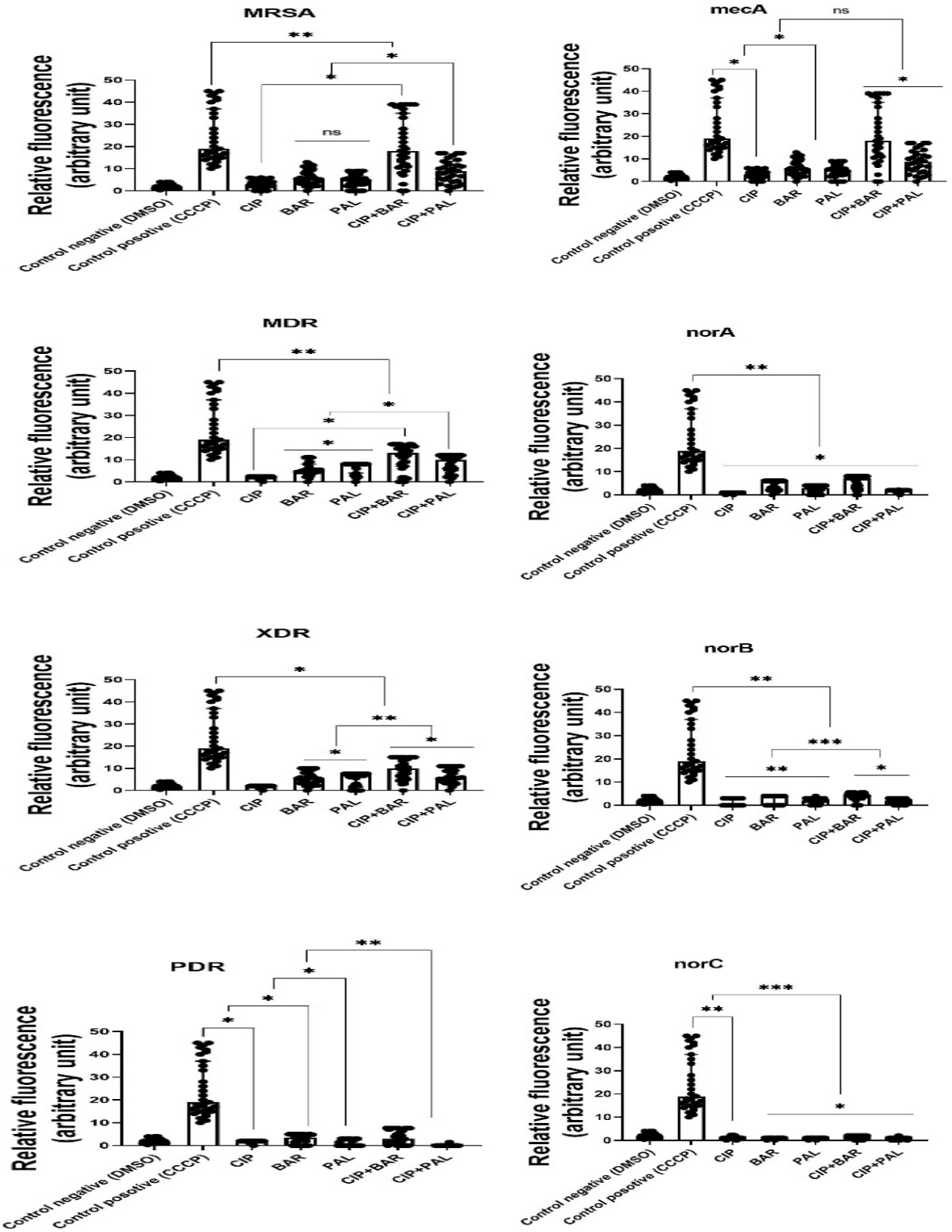
Wultańska, D., M. Piotrowski and H. Pituch (2020) .The effect of berberine chloride and/or its combination with vancomycin on the growth, biofilm formation, and motility of Clostridioides difficile. European J. Clinical Microbiology & Infectious Diseases. 39 (7), 1391-1399. http://doi.org/10.1007/s10096-020-03857-0.

You Gan, R. (2012). Bioactivities of Berberine: An Update. Int. J. Modern Biol. Med. 1 (1), 48-81.

Zhang, X., X. Sun, J. Wu, Y. Wu, Y. Wang, Y. Hu and X. Wang (2020). Berberine damages the cell surface of methicillin-resistant staphylococcus aureus. Front Microbiol. 11, 621-621. http://doi.org/10.3389/fmicb.2020.00621.

Zhou, X.Y., X.G. Ye, L.T. He, S.R. Zhang, R.L. Wang, J. Zhou and Z.S. He (2016). In vitro characterization and inhibition of the interaction between ciprofloxacin and berberine against multidrug-resistant Klebsiella pneumonia e. The J. Antibiotics. 69 (10), 741-746. http://doi.org/10.1038/ja.2016.15.

Zuo, G.Y., Y. Li, J. Han, G.C. Wang, Y.L. Zhang and Z.Q. Bian (2012). Antibacterial and synergy of berberines with antibacterial agents against clinical multi-drug resistant isolates of methicillin-resistant Staphylococcus aureus (MRSA). Molecules. 17 (9), 10322-10330. http://doi.org/10.3390/molecules170910322.



Supplementary 1. Correlation of ciprofloxacin/berberine and ciprofloxacin/palmatine on accumulation of EtBr on *S. aureus* strains. Each data set was analyzed using the student's t-test, the two-way ANOVA, and turkey's post hoc and was presented as Mean+SEM. * p-value <0.05; ** p-value <0.01; *** p-value <0.001; **** p-value <0.0001. ns: non-sense.

Supplementary 2.Relationship between distribution of ciprofloxacin, Berberine, and Palmatine MIC and MBC, antibiotic resistance profile, and Efflux pump genes in wound collection of *S. aureus* isolates based on p-values.

Antibiotic-resistant strains	P.value of ciprofloxacin, berberine and palmatine MIC and MBC										MRSA strains
	Cip* MIC	Cip MBC	Ber* MIC	Ber MBC	Pal* MIC	Pal MBC	Cip+BerMIC	Cip+Pal MBC	Cip+BerMBC	Cip+Pal MIC	
Cloxacillin	<0.05**	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.001	<0.001	<0.001	0.034
Mupirocin	-	-	-	-	-	-	-	-	-	-	-
Teicoplanin	-	-	-	-	-	-	-	-	-	-	-
Ceftriaxone	<0.001***	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				0.007
Ciprofloxacin	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.001	<0.001	<0.001	0.049
Clindamycin	-	-	-	-	-	-	-	-	-	-	-
Cotrimoxazole	>0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				0.019
Erythromycin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				0.082
Gentamycin	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.001	<0.001	<0.001	0.060
Imipenem	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				0.002
Kanamycin	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.001	<0.001	<0.001	0.003
Tigecycline	-	-	-	-	-	-	-	-	-	-	-
Cloxacillin	<0.001	<0.001	<0.001	>0.001	<0.001	<0.001	<0.001				0.038
MDR*	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.001	<0.001	<0.001	0.040
XDR*	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.001	<0.001	<0.001	0.055
PDR*	-	-	-	-	-	-	-	-	-	-	-
Efflux pump genes											
norA	0.18	0.8	0. 3	0.67	0.82	0.9	0.1	0.9	0.73	0.19	0.017
norB	0.16	0.4	0.6	0.27	0.46	0.20	0.53	0.11	0.4	0.85	0.049
norC	0.2	0.8	0.22	0.61	0.09	0.073	0.29	0.19	0.45	0.57	0.004

* CIP: Ciprofloxacin; Bar: Berberine; Pal: Palmatine; MDR: Multi-drug resistant; XDR: Extensively drug resistant; PDR: Pandrug resistance;

** Each data set was analyzed using $\chi 2$ and was presented as Mean + SEM. * p-value <0.05; ** p-value <0.01; *** p-value <0.001; ****

*** Each data set was analyzed using the student's t-test and the two-way ANOVA and was presented as Mean + SEM. p-value <0.001;