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SYNERGISTIC EFFECT OF NARINGENIN WITH CONVENTIONAL ANTIBIOTICS AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

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Abstract:

Overcoming resistance to Methicillin resistant Staphylococcus aureus (MRSA) is attainable by inhibition of penicillin-binding protein (PBP-2a). Nowadays, natural products are used to enhance the therapeutic potential of drugs. Naringenin, a bioactive flavonoid exhibits potential antibacterial activity. Hence, present study demonstrates it's potential alone and in combination with β-lactam antibiotics against MRSA.

Individual Minimum inhibitory concentration (MIC) of Naringenin and selected antibiotics was determined by agar dilution method in 7clinical isolates. The anti-bacterial potential of naringenin in combination with the β-lactam drugs was evaluated using checkerboard method and FIC-index was calculated. Effect of naringenin on the expression of PBP-2a was studied using immunoblotting. MIC of naringenin against MRSA was 125µg/ml. On combination of naringenin with oxacillin and cloxacillin, 5 and 4 strains showed synergistic effect respectively. All the strains showed indifference effect when naringenin was treated with amoxycillin. Hence, it was found that the susceptibility of MRSA positive strains was augmented in the presence of naringenin. Combination of different drugs with naringenin showed suppression of PBP-2a protein. It is found that combination of naringenin and β-lactam antibiotics intensified the antibacterial property of these drugs. The present study also demonstrates that the mechanism of action of naringenin is dependent on the inhibition of PBP-2a.

Keywords: MRSA, β-Lactam, Antibiotics, Naringenin, Linezolid, PBP-2a, Western blot

Introduction:

Drug susceptibility to *Staphylococcus aureus* (*S. aureus*) remains a public health challenge globally due to complex resistance mechanisms **(Munita*et al*., 2016)**. The unending progression of antibiotic-resistance is presently a dire threat, leading to a rise in morbidity attributable to antibiotics with limited effective span **(Ferri*et al*., 2017)**. Methicillin-resistant *Staphylococcus aureus* (MRSA), in particular, remains a challenge to eradicate effectively (**Rossolini*et al*., 2014)**. MRSA term came in existence when the MSSA organism acquired mecA gene (i.e. the structural geneforthepenicillin-bindingprotein,PBP2a),whichcausessustenanceofcell-wallsynthesis

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thereby decreasing its affinity to β-lactams **(Poulsen*et al*., 2014)**. Vancomycin has been the drug of choice against MRSA; however, emergence of *S. aureus* with reduced susceptibility to vancomycin is also complicating the current treatment **(Howden*et al*., 2010; van Hal and Paterson, 2011).**

A conceivable approach to achieving a therapeutic impact caused by deadly pathogens and handling the complicated drug resistance issues is by using synergism. Phytomedicine is a safe alternative for applying synergism, and it essentially comprises combining a phytochemical with commercially available antibiotics. In this context, flavonoids have been shown to possess diverse beneficial activities, including antimicrobial properties, and may be utilized for this purpose. Origin from natural sources makes these agents safer than chemically synthesized drugs. In addition to this, natural compounds can act by various mechanisms on the cell, thereby reducing the chances to be resistant. **(Wright, 2016)**.

Naringenin, a flavonoid obtained from fruits of citrus species, tomatoes and figs (*Ficuscarica*) has been demonstrated to possess broad positive effects on human health, including a decrease in lipid peroxidation biomarkers and protein carbonylation**(Wang *et al*., 2015)**, increasing antioxidant defences**(Erlund*et al*., 2001)**, scavenging of reactive oxygen species as anti-inflammatory effects**(Pinho-Ribeiro*et al*., 2016)**, modulation of immune system activity **(Ke*et al*., 2017)**, and also the exertion of anti-atherogenic effect **(Karim*et al*., 2018)**. This flavonoid has been reported to possess anti-cancer as well as anti-proliferative activities **(Erlund*et al*., 2001)**. Naringenin also shows dose-dependent inhibitory effect against dengue virus **(Frabasile*et al*., 2017)**, prevents intracellular replication of chikungunya virus **(Ahmadi*et al*., 2016)** and also shows inhibitory effects against hepatitis-C virus in a dose-dependent manner **(Gonçalves*et al*., 2017)**.

Given the benefits, as mentioned earlier of flavonoids and extensive research carried out in naringenin's investigation as a potential antimicrobial, we hypothesized a study to explore the effect of naringenin on MRSA isolates as a potential anti-bacterial agent in combination with other commonly used antibiotics.

Materials and Methods:

Materials

Drugs

Table 1: Concentration ranges of drugs according to CLSI 2020

S. No.	Drugs	Resistant (R)	Sensitive (S)
1.	Amoxicillin	≥ 16μg/ml	≤ 4μg/ml
2.	Oxacillin	≥ 4μg/ml	≤ 2μg/ml
3.	Cloxacillin	≥ 4μg/ml	≤ 2μg/ml
4.	Linezolid	≥ 8μg/ml	≤ 4μg/ml

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Amoxycillin (CAS 26787-78-0) and Oxacillin (CAS 1173-88-2): Titan Biotech Ltd., India. Cloxacillin (Cat. No. 68636): SRL, India. Standard drug, Linezolid (Linid): Zydus Synovia (Zydus Healthcare Ltd., India).Naringenin (test flavonoid): Sigma-aldrich Co., St. Louis, USA.

Chemicals and Reagents:
Mueller Hinton Agar (Cat. No. 24756): SRL, India; Sodium Chloride (Cat No. 194848): MP Biomedicals Pvt. Ltd., India; Blood agar plates: Titan Biotech Ltd., India.

Bacteria:
7 MRSA isolates were collected from Bacteriology laboratory, Shree Guru Gobind Singh Tricentenary (SGT) University, Gurugram, Haryana, India and the reference strains MRSA (ATCC 43300) and MSSA (ATCC 11632) were purchased from ATCC, Manassas, USA.

Culture:
Reference MRSA and MSSA strains were obtained in lyophilized form. They were reconstituted and grow in nutrient broth. All the bacterial isolates and reference strains were sub-cultured on blood agar plates (overnight at 37^{o}C) for further use in the experiment.

Coagulase test:
Coagulase test was performed to confirm the identity of *Staphylococcus aureu*s in all clinical isolates.

Procedure:
A colony of clinical isolate was added to a drop of human plasma and mixed gently after mounting on a slide. After about 10 sec. slides were observed for clumping or no clumping.

Bacterial concentration adjustment using McFarland:
Inoculum of the MRSA positive strains were prepared and mixed well. The suspensions were then placed under the instrument (densiCHEK) to evaluate and adjust density of bacterial suspension. The test tube containing the inoculum was rotated slowly upto one full rotation. The instrument displayed a reading between 0.44 and 0.56 indicating a Mcf value of 0.5.

Preparation of drug stock solutions and calculation of MIC:
After sub-culturing of the bacterial strains, 10X stock solutions of different drugs used in the study were prepared and MIC was calculated after overnight incubation of the agar plates with desired amount of selected clinical isolates of MRSA (0.2µl of 0.5McF concentration of bacterial inoculum i.e. $2X10^{4}$ CFU/ml).

MIC evaluation using agar-dilution method:
Concentration ranges of the antibiotics were selected according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2020 **(CLSI M100, 2020) (Table 1)**.Thefinal concentrations of antibiotics were 256-1µg/ml, 64-0.25µg/ml, 64-0.25µg/ml and 128-0.5µg/ml for amoxicillin, cloxacillin, oxacillin and linezolid respectively. Concentration range for test flavonoid Naringenin was2000-0.12µg/ml**.**

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Fractional inhibitory concentration (FIC) and FIC index interpretation –Checkerboard method:

Checkerboard method is an experiment in which the right combination of two different chemicals/drugs can be measured **(Santiago *et al*., 2015).**

FIC was evaluated using Checkerboard method. The antibiotics and Naringenin showed different MIC values for different clinical isolates. Dilutions of antibiotics and Naringenin at their respective MIC concentrations were prepared along with 5 subsequent serial dilutions for each clinical isolate and standard strain of MRSA and MSSA. MHA plates were prepared at different dilutions for each bacterial strain separately. After spotting of bacteria following above method, the plates were incubated overnight at 37°C. Thereafter, MIC of combination wascalculated.

FIC and FIC Index (FICI) were evaluated using following formula:

$$FIC\ of\ Antibiotic = \frac{MIC\ of\ Antibiotic\ used\ in\ combination\ with\ Naringenin}{MIC\ of\ Antibiotic\ alone}$$

$$FIC\ of\ Naringenin = \frac{MIC\ of\ Naringenin\ used\ in\ combination\ with\ Antibiotic}{MIC\ of\ Naringenin\ alone}$$

$$FIC\ Index = FIC\ of\ Antibiotic + FIC\ of\ Naringenin$$

The effect of test drug and antibiotics in combination was evaluated for individual strains and were reported following standard range of FIC index **(Hu *et al*.,2002)**.

Penicillin Binding Protein 2a (PBP2a) expression analysis:

For estimation of PBP2a protein expression in clinical isolates along with standard reference strains were evaluated through Immuno-blot (Western blot) analysis.

Sample preparation:

The individual bacteria were inoculated in nutrient broth and culture overnight at 37˚C. 1-2 colonies were collected after centrifugation (13,000 rpm; 4°C; 20 min) of the inoculum. The pellets obtained were washed with PBS twice. The pellets were then agitated on an ultrasonic processor (750watt; pulse duration: 9s on and 9s off; 15 min; 4˚C). Lysozyme (1% w/v) and protease inhibitor (1% v/v in PBS; pH 7.4) were added to the lysate solution. The lysed cells were centrifuged (13,000 rpm at 4˚C; 20 min) once more to remove cellular debris and aggregates. The supernatants containing the protein were collected in separate micro-centrifuge tube. Quantification of protein was done using the standard method (Bradford protein assay) following manufacturer's instructions **(Sianglum*et al*.,2011)**.

After quantification, equal amount of protein (40µg) was separated on 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto the nitrocellulose membrane (Bio-TraceTM NT, Pall Corporation, USA). The unoccupied sites on membrane were blocked using 3% bovine serum albumin for 1 hour. Then membrane was incubated (12h; 4°C) using respective antibodies (primary antibody incubation).

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Different primary antibodies used for the study included GAPDH (mouse; cat. No. A01020) and PBP-2a (mouse; MBS530221). These were then detected further using HRP-conjugated (Goat anti-mouse; 10004302) secondary antibody. The membranes were then evaluated for protein quantification using chemiluminescence kit (Thermo Fischer Scientific Inc., USA) under the FluorChem M Protein Imaging System (ProteinSimple, USA) and were quantified using Image-J software.

Statistical Analysis:

The analysis of the data was performed using ANOVA followed by Tukey's multiple comparison post hoc test which was done using GraphPadInStat 3 software (Graph-Pad Software Inc., San Diego, USA). All the data was reported as mean ± SD. P-value ≤0.05 was considered significant statistically.

Results:

All clinical isolates of MRSA showed different MIC values on treatment with different β-lactam drugs and Naringenin. These MIC values were used to evaluate the fractional inhibitory concentrations of the drugs when they were combined with naringenin.

Effect of drugs alone on Clinical isolates (MIC determination)

Amoxicillin:

For reference MRSA, MIC of Amoxicillin was found to be 256µg/ml whereas for MSSA reference strain, it showed an MIC value of 2µg/ml. MIC for all other strains were also evaluated and reported **(Table 2).**

Table 2: MICs, FIC and FICI of amoxicillin and test drug naringenin against MRSA reference strain, MSSA reference strain and MRSA clinical isolates. S: Synergy; A: Additive; I: Indifference.

S. No.	Strain No.	MIC (µg/ml)		MIC of combination (µg/ml)		FIC (µg/ml)		FICI
		Naringenin (2000-0.12 µg/ml)	Amoxicillin (256-1µg/ml)	Naringenin	Amoxicillin	Naringenin	Amoxicillin	N+A
*	MRSA Ref.	125	256	125	16	1	0.0625	1.0625 (I)
**	MSSA Ref.	15.6	2	3.9	0.25	0.25	0.125	0.375 (S)
1	138	31.25	64	31.25	4	1	0.062	1.062 (I)

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2	180	125	32	125	2	1	0.0625	1.0625 (I)
3	134	31.25	32	31.25	2	1	0.062	1.062 (I)
4	39	7.8	64	7.8	4	1	0.062	1.062 (I)
5	61	0.12	16	0.12	1	1	0.0625	1.0625 (I)
6	170	15.6	16	15.6	1	1	0.062	1.062 (I)
7	130	2000	32	2000	2	1	0.0625	1.0625 (I)

Cloxacillin:

For reference MRSA, MIC of cloxacillin was found to be 64µg/ml whereas for MSSA reference strain, it was found to be 0.25µg/ml. MIC of cloxacillin against MRSA positive clinical isolates was also evaluated **(Table 3).**

Table 3: MICs, FIC and FICI of cloxacillin and test drug naringenin against MRSA reference strain, MSSA reference strain and MRSA clinical isolates. S: Synergy; A: Additive; I: Indifference.

S. No.	Strain No.	MIC (µg/ml)		MIC of combination (µg/ml)		FIC (µg/ml)		FIC I
		Naringenin (2000-0.12µg/ ml)	Cloxacillin (64-0.25 µg/ml)	Naringenin	Cloxacillin	Naringenin	Cloxacillin	N+C
*	MRSA Ref.	125	64	62.5	32	0.5	0.5	1 (A)
**	MSSA Ref.	15.6	0.25	1.95	0.031	0.125	0.125	0.25 (S)
1	138	31.25	64	15.62	32	0.49	0.5	0.99 (A)
2	180	125	64	62.5	32	0.5	0.5	1 (A)

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3	134	31.25	2	7.81	0.5	0.24	0.25	0.49 (S)
4	39	7.8	8	1.95	2	0.25	0.25	0.5 (S)
5	61	0.12	2	0.015	0.125	0.125	0.0625	0.187 (S)
6	170	15.6	64	3.9	16	0.25	0.25	0.5 (S)
7	130	2000	0.5	1000	0.25	0.5	0.5	1 (A)

Oxacillin:

MIC of oxacillin was found to be 64µg/ml for MRSA reference strain and 1µg/ml for MSSA reference strain. Different MRSA positive strains showed different MIC values on treatment with the drug **(Table 4).**

Table 4: MICs, FIC and FICI of oxacillin and test drug naringenin against MRSA reference strain, MSSA reference strain and MRSA clinical isolates. S: Synergy; A: Additive; I:Indifference.

S. No.	Strain No.	MIC (µg/ml)		MIC of combination (µg/ml)		FIC (µg/ml)		FICI
		Naringenin (2000-0.12 µg/ml)	Oxacillin (64-0.25 µg/ml)	Naringenin	Oxacillin	Naringenin	Oxacillin	N+O
*	MRSA Ref.	125	64	62.5	16	0.5	0.25	0.75 (A)
**	MSSA Ref.	15.6	1	1.95	0.125	0.125	0.125	0.25 (S)
1	138	31.25	8	7.8	2	0.24	0.25	0.49 (S)
2	180	125	32	62.5	16	0.5	0.5	1 (A)
3	134	31.25	4	7.81	1	0.24	0.25	0.49 (S)
4	39	7.8	8	0.975	1	0.125	0.125	0.25 (S)
5	61	0.12	0.25	0.015	0.031	0.125	0.125	0.25 (S)
6	170	15.6	64	7.8	32	0.5	0.5	1 (A)
7	130	2000	0.5	500	0.125	0.25	0.25	0.5 (S)

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Linezolid:
Linezolid was found to inhibit the growth of MRSA reference strain at 128µg/ml whereas for MSSA it inhibited the growth at 0.5µg/ml. For all other clinical isolates, the observed MIC was reported in **(Table 5)**.

Table 5: MICs, FICs and FICI of Linezolid and test drug naringenin against MRSA reference strain, MSSA reference strain and MRSA clinical isolates

S. No.	Strain No.	MIC (µg/ml)		MIC of combination (µg/ml)		FIC (µg/ml)		FICI
		Naringenin (2000-0.12 µg/ml)	Linezolid (128-0.5 µg/ml)	Naringenin	Linezolid	Naringenin	Linezolid	N+L
*	MRSA Ref.	125	128	62.5	64	0.5	0.5	1 (A)
**	MSSA Ref.	15.6	0.5	1.95	0.125	0.125	0.25	0.375 (S)
1	138	31.25	2	15.62	0.5	0.49	0.25	0.74 (A)
2	180	125	8	31.25	0.5	0.25	0.0625	0.312 (S)
3	134	31.25	0.5	15.62	0.125	0.49	0.25	0.64 (A)
4	39	7.8	0.5	1.95	0.062	0.25	0.125	0.375 (S)
5	61	0.12	0.5	0.06	0.0625	0.5	0.125	0.625 (A)
6	170	15.6	2	3.9	0.125	0.25	0.062	0.312 (S)
7	130	2000	1	1000	0.25	0.5	0.25	0.75 (A)

Naringenin:
MIC of Naringenin was reported to be 125µg/ml for MRSA and 15.6µg/ml for MSSA reference strain respectively. The MIC values for all other strains were observed and reported **(Table 2-5)**.

Effect of antibiotics in combination with naringenin on clinical isolates (FIC determination)
Naringenin + Amoxicillin (N+A)

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Growth of MRSA (reference strain) was found to be inhibited at an MIC of 125µg/ml (N) and 16µg/ml (A) for the drugs Naringenin and Amoxicillin used in the combination respectively. So, after applying the formula for FIC, FIC of Naringenin (N) was reported as 1µg/ml and that of Amoxicillin (A) was reported as 0.0625µg/ml. FIC Index (FICI) was calculated for the combination which was found to be 1.0625. Hence, the combination of the two drugs showed an Indifference effect against reference MRSA strain. All the 7 strains showed indifference effect(I)(Table 2)**.**

Naringenin + Cloxacillin (N+C)

Growth of MRSA (reference strain) was found to be inhibited at an MIC of 62.5µg/ml (N) and 32µg/ml (C) for the drugs naringenin and cloxacillin used in the combination respectively. FIC of naringenin (N) was reported as 0.5µg/ml and that of Cloxacillin (C) it was reported as 0.5µg/ml. FIC Index (FICI) was calculated for the combination which was found to be 1. Hence, the combination of the two drugs showed an additive effect against reference MRSA strain.

Out of 7 strains tested other than the reference strains, 4strains have shown synergistic effect whereas 3 strains of MRSA showed additive effect **(Table 3)**.

Naringenin + Oxacillin (N+O)

Growth of the micro-organism MRSA (reference) was found to be inhibited at an MIC of 62.5µg/ml (N) and 16µg/ml (O) for the drugs naringenin and oxacillin used in the combination respectively. FIC of naringenin (N) was reported as 0.5µg/ml and that of Oxacillin (O) it was 0.25µg/ml. FIC Index (FICI) was calculated for the combination which was found to be 0.75. Hence, the combination of the two drugs showed an additive effect against the reference MRSA strain.

Out of 7strains tested other than the reference strains, 5 strains have shown synergistic effect whereas 2 strains of MRSA showed additive effect **(Table 4)**.

Naringenin + Linezolid (N+L)

Growth of MRSA (reference strain) was found to be inhibited at an MIC of 62.5µg/ml (N) and 64µg/ml (L) for the drugs naringenin and Linezolid used in the combination respectively. FIC of naringenin (N) and Linezolid (L) was reported as 0.5µg/ml and 0.5µg/ml respectively. FIC Index (FICI) was calculated for the combination which was found to be 1. Hence, the combination of the two drugs showed an additive effect against reference MRSA strain.

Out of 7 strains tested other than the reference strains, 3 strains have shown synergistic effect whereas 4 strains of MRSA showed additive effect **(Table 5)**.

Western blotting analysis:

PBP-2a was expressed in high concentration by MRSA standard strains despite treatment with any of the conventional drugs **(Figure 1, 2 and 3)**.

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Figure 1: Protein expression of PBP-2a after treatment with Amoxicillin MRSA (ATCC43300), MSSA (ATCC11632) and selected clinical isolates

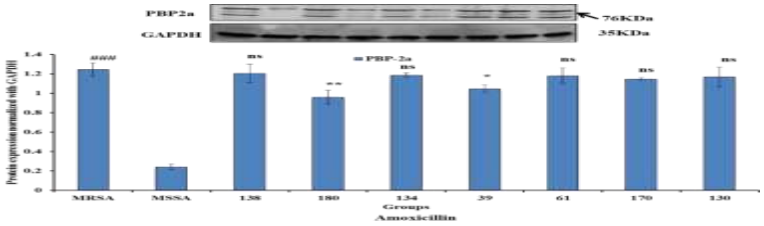


Figure 2: Protein expression of PBP-2a after treatment with Cloxacillin MRSA (ATCC43300), MSSA (ATCC11632) and selected clinical isolates

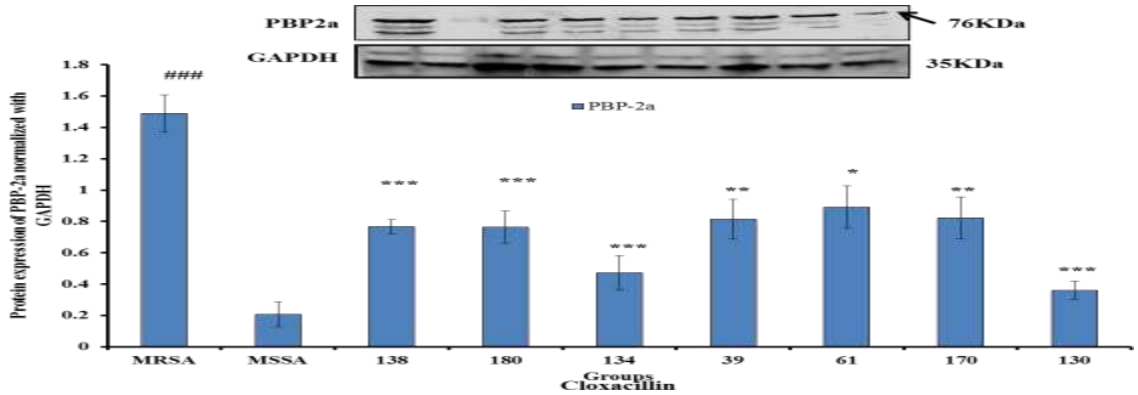
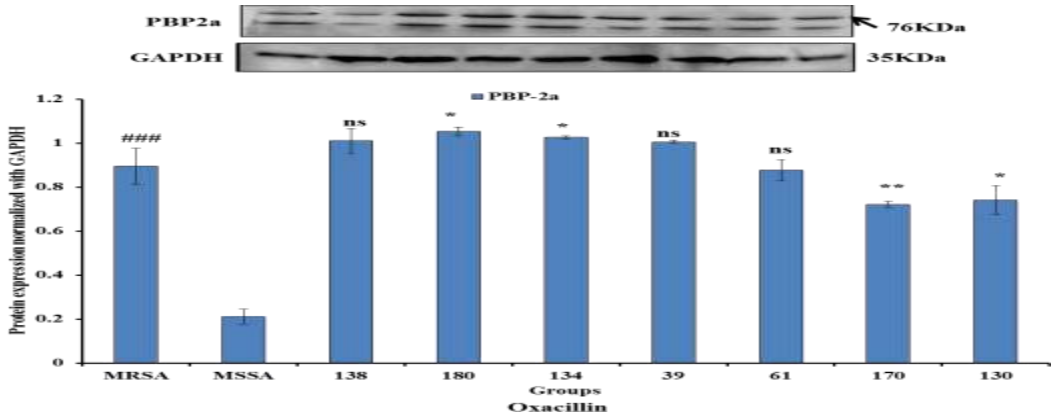


Figure 3: Protein expression of PBP-2a after treatment with Oxacillin MRSA (ATCC43300), MSSA (ATCC11632) and selected clinical isolates



Strain no. 61 was found to express the protein PBP-2a significantly. So, it was further evaluated using different drugs alone **(Figure 4)** and in combination with the test drug naringenin. PBP-2a

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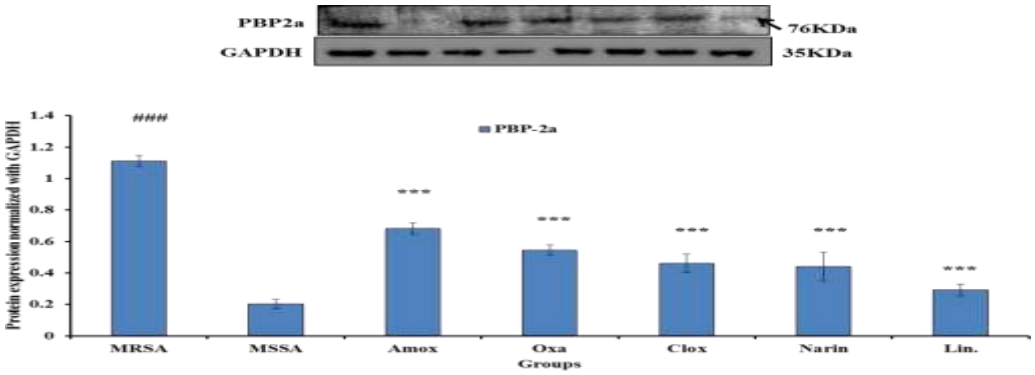
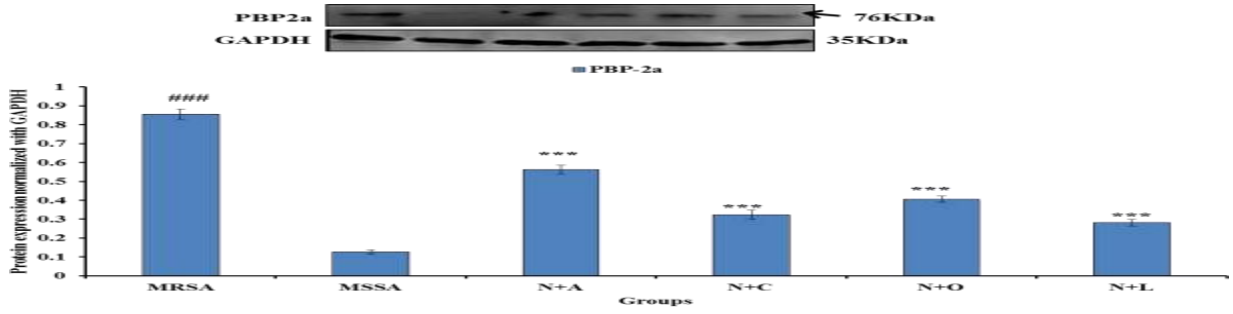
expression was not reduced on treatment with any of the conventional antibiotics i.e. Amoxicillin, Cloxacillin and Oxacillin, **(Figure 1, 2 and 3)**individually.

Figure 4: Differential Protein expression of PBP-2a in Clinical isolate (61) after treatment with Amoxicillin (A), Cloxacillin (C), Oxacillin (O), Linezolid (L) and Naringenin (N).



But, when the strains were being treated with the antibiotics in combination with naringenin, the expression of the protein was reduced significantly **(Figure 5)**. It was observed that the combination of cloxacillin and oxacillin with naringenin showed much better anti-bacterial effects as compared to amoxicillin. Treatment with combination of amoxicillin and naringenin resulted in indifference effect for all the MRSA strains which was further proved by evaluating expression of the protein PBP-2a.

Figure 5: Differential Protein expression of PBP-2a in Clinical isolate (61) after treatment with Amoxicillin (A), Cloxacillin (C), Oxacillin (O), Linezolid (L) and Naringenin (N).



Discussion:

The mechanism by which β-lactam antibiotic kill gram-positive organisms has been investigated extensively in the past and pieces of evidence have linked this to inhibition of the cell-wall synthesis through PBP-binding to the cell wall and thus the trans-peptidase activity was inactivated **(Brudzynski and Sjaarda, 2014; Mun*et al*., 2015).** Over time, some of the gram-positive organisms like *Staphylococcus aureus* evolved to produce the enzyme β-lactamase, which breaks the β-lactam ring leading to the annulment of the antibiotic's activity **(Al-Habib*et al*., 2010).** Hence, we designed a study to investigate an alternative anti-MRSA drug that could cut down the antibiotic resistance and might be utilized to prevent and treat diseases caused by MRSA. In the present study, naringenin's anti-microbial capability alone and in combination

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with amoxicillin, oxacillin and cloxacillin against MRSA was explored.
The MIC assay results showed higher MIC values for reference MRSA and MRSA-isolates when conventional antibiotics were used, thereby authenticating the resistance of the strains used in the study. Interestingly, naringenin showed enhanced anti-bacterial activity against reference MRSA strain, MSSA strain, andMRSA-isolates.
Naringenin's ability to suppress the growth of *S. aureus* has been demonstrated recently by Kozłowska and colleagues where they showed that naringenin had a higher susceptibility to inhibit gram positive organisms like *S. aureus* in comparison to gram-negative organisms like *E. coli***(Kozłowska*et al*., 2019).**The lower susceptibility of Gram-negative strains to flavonoids has been extensively studied in the past and is attributable to the presence of a bacterial outer membrane, especially the lipopolysaccharides portion. On further evaluation using the checkerboard method, the FIC-index interpretation indicated synergistic activity of oxacillin-naringenin (O+N) and cloxacillin-naringenin (C+N) for most of the clinical isolates, whereas indifference activity was reported for the combination of amoxicillin-naringenin (A+N). Naringenin's valuable antimicrobial properties have been ascribed to the active groups primarily attached to the C-7 and C-40 positions of the molecule, as evidenced by various studies **(Sakoda*et al*., 2016;Nobakht *et al*., 2014).**One such derivative, 7-O-butyl-naringenin has been described as a promising anti-bacterial agent in the therapy of infections caused by MRSA **(Lee *et al*., 2014).**
By conducting this study, we evaluated the mechanism by which naringenin exerts its anti-MRSA effect by checking its effect on PBP-2a. Interestingly, we found the expression of PBP-2a to be diminished with the naringenin combination group when compared to the individual antibiotics. So, it is confirmed from the current study that the effect of β-lactam antibiotics against MRSA can be potentiated on treatment with naringenin. Several studies in the past have shown various flavonoids with PBP-2a suppressing activity, including quercetin**(Rani *et al*., 2016),**epicatechingallate**(Rosado *et al*., 2015)**and morin**(Mun*et al*., 2015)**. However, we have unravelled the PBP-2a suppressing activity of naringenin for the first time through our research. From our study, it is evident that naringenin has a good anti-MRSA potential. One of the advantages of adding it to conventional therapy may be reducing the dose requirement of synthetic effects, therefore decreasing adverse effects and increasing compliance. There is still a need for investigating the *in-vivo* effects of combining naringenin with antibiotics in terms of pharmacokinetics and pharmacodynamics. Naringenin can be a valuable addition to the conventional chemotherapy of MRSA infections.
In conclusion, our study revealed that naringenin had anti-MRSA properties, which were further enhanced and may have synergetic or additive effect when combined with amoxicillin, cloxacillin and oxacillin in the *in-vitro* assays. The synergistic and additive property of naringenin's anti-microbial effect in this study was attributed to the inhibition of PBP-2a expression. Hence, naringenin is a potential molecule for the development of a novel therapeutic regimen for MRSA. However, further studies are required to evaluate naringenin's translatability for humanuse.

Authors' contributions:
All authors participated in the design, interpretation and analysis of the manuscript. AT and SK conducted the experiments. SK wrote the manuscript; SB, TB and DSA supervised the experimental work.

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Declaration of interest:
The author(s) declare no conflict of interest with anyone.

Ethical Approval:
Ethical approval was obtained from the ethics committee of SGT University, Gurugram, Haryana, India and the Ethics approval no. is SGTU/FMHS/MICRO/341.

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