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Research Article

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In vitro synergistic effect of sumac in combination with cloxacillin, cephalexin, and vancomycin against clinical isolates of methicillin-resistant *staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been associated with substantially higher morbidity and mortality rates due to the acquired multidrug resistance. Accordingly, there exists a growing demand for development of natural products as alternative treatments or combination drug therapies against this infection. This study aimed to assess the synergistic effect of sumac in combination with clinically important antibiotics against ten clinical isolates of MRSA. A total of 50 staphylococci clinical isolates were screened for susceptibility to antibiotics, and 10 isolates were selected for further studies. The disc diffusion method (DDM) was used to screen the synergy effects of the sumac extract with 7 antibiotics against clinical isolates of MRSA. The broth microdilution method determined the minimum inhibitory concentration (MICs) of sumac extract and three selected antibiotics. The synergistic effect of the sumac extract and three antibiotics was assessed and evaluated using checkerboard dilution methods. The three antibiotics, i.e., cloxacillin, cephalexin, and vancomycin, showed considerable synergistic effects with sumac extract based on DDM. The MICs of sumac against ten clinical MRSA isolates ranged from 1600 to 6400 µg/ml. In the checkerboard dilution method, sumac markedly reduced the MICs of the antibiotics cloxacillin and cephalexin, and a significant synergistic effect was recorded by sumac in combination with these two antibiotics. Our results demonstrated that sumac extract enhances the antibiotic potential against MRSA in vitro conditions. This study suggested that sumac, in combination with antibiotics, could lead to the development a new combination of antibiotics against MRSA.

Keywords: *Rhus coriaria* L.; Sumac; Methicillin-resistant *Staphylococcus aureus* (MRSA); Minimum inhibitory concentration (MIC); Synergistic effects.

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1. Introduction

Staphylococcus aureus (*S. aureus*) is a major nosocomial pathogen that commonly grows on the skin, respiratory tract, and nose [1]. *S. aureus*, which can cause a variety of human infections such as skin and soft tissue abscesses [2], pyogenic infections [3], and even fatal bloodstream infection [4], demonstrated to be susceptible to the earliest antimicrobial substances [5]. However, increasing use of antibiotics has led to the rapidly developed antibiotic-resistant *S. aureus* strains. A recent meta-analysis has demonstrated that the frequency of methicillin-resistant *S. aureus* (MRSA) infections was 43.0% (95% confidence interval 36.3–50.0%) among confirmed *S. aureus* in different parts of Iran [6].

MRSA, which is mediated by the acquisition of the mecA gene, encoding for a novel penicillin-binding protein (PBP), PBP-2a, emerged in the early 1960s [7]. MRSA have become resistant to not only methicillin and other β-lactam antibacterial agents, but also to other commonly used antistaphylococcal antibiotics [8]. Therefore, emerging multidrug-resistant MRSA, one of the most important hospital and community pathogens worldwide, has led to research to identify new agents to treat MRSA.

The use of combination therapy confers several potential benefits over the antibiotic monotherapy including a broader antibacterial spectrum, enhancing the antibiotic activity (synergistic effects), preventing the emergence of resistance, and reducing the risk of infection during therapy [9]. Nowadays, natural compounds have increasingly attracted the attention of researchers as a combination therapy [10], proving to exert potent antimicrobial effects when used alone or in combination with other antimicrobial agents [11]. *Rhus coriaria* L., or sumac (spelled as sumagh in Persian), is a wild plant grown from the Mediterranean coastline to Iran, Afghanistan, and Turkey [12]. Sumac, one of the most important spices used in the Middle East, exhibits a variety of biological effects such as antimicrobial, antifungal, antioxidant, antimutagenic, antiviral, cytotoxic, antifibrogenic, hypoglycaemic, leukopenic, and anti-inflammatory as well as antitumorigenic activities [13]. Previous studies have shown that sumac extract exerts a variable degree of antimicrobial activity on the growth of some food-borne bacteria, including pathogens, and was able to interfere with the adherence of bacterial biofilm formation on abiotic and biotic surfaces [14]. However, little is known about the antimicrobial effects of Sumac on MRSA.

In the present study, we investigated the synergistic effect of Sumac (*Rhus coriaria* L.) in combination with clinically important antibiotics against ten clinical isolates of MRSA. The minimum inhibitory concentrations (MICs) of sumac extract and three selected antibiotics were determined by the broth microdilution method, and to investigate the effect of the combination of sumac extract and three antibiotics, a checkerboard dilution test was performed.

2. Materials and Methods

2.1. Plant materials

The plant materials of the current study, fruits of sumac (*Rhus coriaria* L.), were prepared from the Tehran botanical market and were authenticated in the herbarium of Faculty of Pharmacy, Alborz University of Medical Sciences, Tehran, Iran (Sample code: R01-017). The sumac fruits were powdered and maintained at 25°C until use.

Water extract of sumac: One hundred grams of the powder of sumac L. was soaked in 1 liter of distilled water at 45°C for 5 days. The extract was concentrated via a rotary evaporator (Heidolph, Germany) and was left to dry under the laboratory hood. The yield of extraction was calculated as follows:

$$\text{Yield of extraction} = \frac{\text{Weight of dry extract}}{\text{Weight of dry starting material}} \times 100$$

The final concentrations of 30 mg/ml and 6.4 mg/ml were prepared by dissolving the extract in sterile distilled water for disc diffusion assay and broth microdilution technique, respectively. The selection of dose concentration was based on our previous study [15] in which a crude extract of sumac was able to inhibit the growth of clinical isolates of *S. aureus* at 6.4 mg/ml. Before antibacterial assays, the extract was diluted at desirable concentrations in distilled water and was then filtered via 0.22 µm pore size syringe filter.

2.2. Bacterial strains and growth conditions

Staphylococcal strains used in this study were 50 clinical isolates from inpatients in Shariati and Bouali, Tehran,

Iran. They were identified by the Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. *S. aureus* ATCC 6538P was used as a standard strain resistant to methicillin. Antibiotic susceptibility of isolates was determined by disc diffusion method, according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [16]. Seven different antibiotic discs of 6 mm diameter (HiMedia, India) were used as follows: Ampicillin (AMP) 30 µg/disc, Cephalexin (CEF) 30 µg/disc, chloramphenicol (CAM) 30 µg/disc, Gentamycin (GEN) 30 µg/disc, Methicillin (MET) 30 µg/disc, Tetracycline (TET) 30 µg/disc and vancomycin (VAN) 30 µg/disc. After the cultivation of isolates on Mueller-Hinton agar (MHA) (HiMedia, India), the bacterial suspensions were adjusted to the 0.5 McFarland standards (1.5 $\times 10^8$ CFU/ml) in Mueller-Hinton broth (HiMedia, India). One hundred microliter (µl) of each bacterial suspension was spread uniformly by spreader on Mueller-Hinton agar surface. Then, antibiotic discs were placed on the inoculated Mueller-Hinton agar plates and incubated at 37 °C for 24 h. The zone of inhibition (ZOI) for each antibiotic was then recorded by the aid of caliper. The effectiveness of antibiotics is based on the ZOI that surrounds a disc. Ten MRSA isolates resistant to most antibiotic discs were selected for further study.

2.3. Screening for synergistic activity

The synergy effects of the sumac extract with antibiotics were screened by the disc diffusion method against 10 MRSA isolates and a standard *S. aureus* ATCC 6538P intermediate to methicillin. Nine antibiotic discs were used for the synergy effect analysis, including the above-mentioned antibiotics, azithromycin (AZM) 30 µg/disc, and co-trimoxazole (SXT) 30 µg/disc. These antibiotics were chosen because of their different mechanisms of action so any potential synergistic effects could be detected. Antimicrobial discs and sterile filter paper discs (Whatman No. 1, 6 mm) were placed on the surface of inoculated MHA. Then, sumac extract was carefully and slowly dispensed on disks to reach 30 mg/disc. Sumac discs (30 mg sumac extract/disc) were prepared by dispensing 60 µl of sumac extract solution (500mg/ml) on blank disc. The plates were incubated at 37ºC for 24 h. At the end of the period, the diameters of inhibition zones were measured and compared with that of the sumac extract and antibiotic discs alone. Each extract was assayed in triplicate and sterile distilled water was used as negative control.

2.4. Antimicrobial activity of sumac and antibiotics

The minimum inhibitory concentration (MIC) test of sumac extract and three selected antibiotics was measured using the microdilution broth method. Two-fold serial dilutions of sumac extract, cephalexin, vancomycin, and cloxacillin were prepared in the sterile 96-well microplates containing Mueller Hinton broth (MHB). Ten microliters of the ten MRSA suspensions were inoculated in each well to obtain a final concentration of 1.5×10^6 CFU. Sumac extract ranged from 6400 to 3.1 μg/ml, and antibiotics ranged from 320 to 0.1 μg/ml were used for testing. The growth inhibition was demonstrated via optical density at 570 nm using a microplate reader [Bio-Rad, USA] after 24 h incubation at 37 °C. Considering the 100% growth in the MHB + bacteria as the control well, the percentage of growth inhibition was attributed to the remaining wells. The sterile water solution which is used for dissolving sumac extract and antibiotics was used as control. The MIC was presented as the lowest concentration that prevent bacterial growth after 24 h of incubation at 37°C.

2.5. Antimicrobial activity of the combinations of sumac extract and antibiotics

The antimicrobial effects of combinations of the sumac extract with three antibiotics, cloxacillin, cephalexin, and vancomycin, were assessed using the checkerboard test. Synergy testing by checkerboard is a standard technique widely used to evaluate the synergistic activity of antimicrobial combinations. In brief, serial 2-fold dilutions of sumac extract with the antibiotics were mixed in equal ratios in MHB in a 96-well microplate. Therefore, each row and column included a constant amount of one antimicrobial agent and increasing amounts of the second agent. The final bacterial concentration in each well was adjusted to 1.5×10^6 CFU/ml, and the microplates were incubated for 24 h at 37ºC. The MIC was defined as the lowest concentration of sumac extract and antibiotics in combination form, inhibiting the growth of MRSA bacteria by measuring the OD at 570 nm using a microplate reader (BioRad, USA). Each experiment was performed in triplicate.

2.6. FIC testing

The fractional inhibitory concentration (FIC) was used to quantify the *in vitro* interaction, whether synergism, additive, indifference, or antagonism between the sumac extract and the used antibiotics. The FIC was derived from the MIC of sumac extract and antibiotic combination inhibiting the observable growth of the test MRSA bacteria on the microplates. The FIC value for each agent was calculated using the standard formula:
FIC (antibiotic) =MIC of antibiotic in combination/ MIC of antibiotic alone

FIC (sumac extract) = MIC of sumac extract in combination/ MIC of sumac extract alone

The interfaces between the sumac extract and the antibiotics were assessed in terms of the FIC index calculated as follows using the formula:

FIC index =∑FIC = FIC (antibiotic) + FIC (sumac extract)

The FIC index obtained was categorized as follows: synergistic was defined when the FIC indices were < 0.5; additive was defined when the FIC indices were > 0.5 but <1; indifferent when the FIC indices were > 1.

2.7. Statistical Analysis

All the experiments were performed in triplicates. Synergistic effects were defined when the FIC indices were < 0.5, additive when the FIC indices were > 0.5 but <1, and indifferent when the FIC indices were > 1.

3. Results and Discussion

3.1. Antimicrobial Susceptibility Test

The antibiotic sensitivity of 50 *S. aureus* isolates displayed various susceptibility patterns against the antibiotics used in this study. Generally, vancomycin and chloramphenicol were the most effective antibiotics to clinical isolates of *S. aureus* (98%; 49/50). A higher resistance to gentamicin (58%; 29/50), cephalexin (70%; 35/50), tetracycline (74%; 37/50), methicillin, and ampicillin (92%, 46/50) were observed. The percentage of antibiotic resistance of *S. aureus* isolates is demonstrated in **Figure 1**. Ten MRSA with the highest resistance to the antibiotics used in this study were selected to evaluate the antimicrobial effects of sumac extract alone and in combination with clinically important antibiotics.

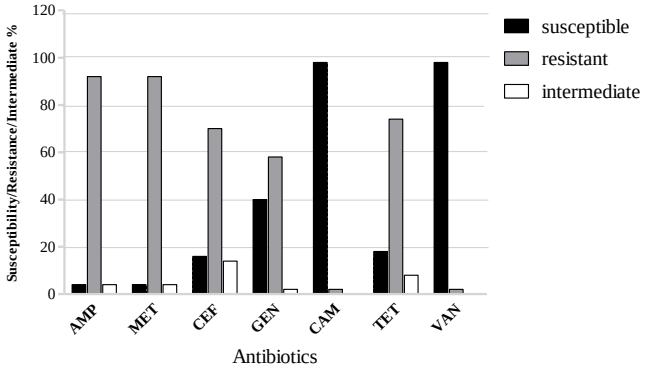

Figure 1. The percentage of antimicrobial resistance profiles of 50 *S. aureus*, isolated from Patients admitted to Shariati and Bouali hospitals, Tehran, Iran. These strains were identified by the Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The disc diffusion method determined the antibacterial activity of common antibiotics against these clinical isolates. AMP = ampicillin, MET= methicillin, CEF= Cephalexin, GEN= gentamicin, CAM= chloramphenicol, TET = tetracycline, VAN= vancomycin

3.2. Screening for synergistic activity

In a screening procedure performed by disc diffusion method, we tested the combinations of sumac extract (30mg/disc) with 7 antibiotics (30µg/disc) against ten MRSA clinical strains and an *S. aureus* ATCC 6538P as a standard strain. The results demonstrated that gentamycin, methicillin, tetracycline, cotrimoxazole, ciprofloxacin, and azithromycin showed no obvious synergy with the sumac extract against the MRSA strains. However, we identified three antibiotics, cloxacillin, cephalexin, and vancomycin, in combination with sumac extract that showed potential clinically relevant synergy (**Table 1**). The diameter of inhibition zones produced by the sumac extract and three antibiotic combinations varied in size. It was mainly wider than those obtained from the sumac extract or the antibiotics.

3.3. Antimicrobial activity of sumac extract and antibiotics

The MICs of sumac extract and three antibiotics, cloxacillin, cephalexin and vancomycin against the 10 MRSA isolates were determined using broth microdilution method. The sumac extract and the antibiotics displayed antibacterial activity against all the MRSA strains. The MIC values of sumac extract against the ten MRSA strains were 1600 and 6400 µg/ml. The MIC of vancomycin ranged from 0.31 to 80 µg/ml, the MIC of cloxacillin was 160 and > 320 µg/ml, and the MIC of cephalexin was > 320 µg/ml. The results are demonstrated in **Table 2**.

Table 1. Average zones of inhibition from sumac extract alone, antibiotics alone, and their combination concentrations.

Strains used	SE&AZM			SE&CEF			SE&CIP			SE&CXA			SE&GEN			SE&MET			SE&SXT			SE&TET			SE & VAN		
	SE	AZM	SE+AZM	SE	CEF	SE+CEF	SE	CIP	SE+CIP	SE	CXA	SE+CXA	SE	GEN	SE+GEN	SE	MET	SE+MET	SE	SXT	SE+SXT	SE	TET	SE+TET	SE	VAN	SE+VAN
MRSA1	11	17	14	11	6	8	10	24	11	7	6	11	11	10	13	10	6	13	11	15	13	9	15	17	12[a]	6	13
MRSA2	14	6	16	13	16	18	14	13	16	14	6	16	13	12	15	14	6	15	13	6	15	15	6	17	13	15	17
MRSA 3	13	20	16	14	21	19	14	32	16	14	6	16	14	23	20	14	6	16	14	6	16	15	6	17	14	17	18
MRSA4	22	30	27	20	31	31	18	25	21	21	6	21	21	21	24	21	6	21	23	30	27	18	22	25	21	20	24
MRSA5	11	16	13	11	7	14	12	6	12	11	6	13	11	9	12	11	6	12	11	6	11	11	6	12	11	8	11
MRSA6	12	6	10	12	13	16	11	6	12	11	6	13	12	6	13	12	6	14	12	6	12	11	9	14	11	10	12
MRSA7	11	18	13	11	11	16	11	6	11	11	6	13	12	18	14	11	6	15	11	6	12	11	8	13	11	7	13
MRSA8	12	12	10	12	13	15	12	6	12	12	6	14	12	13	15	11	6	11	12	6	12	11	10	12	12	8	13
MRSA9	12	8	11	13	8	14	11	7	12	11	6	13	11	9	12	11	6	13	11	6	12	12	10	13	12	11	13
MRSA10	12	7	11	11	11	16	12	6	12	12	6	14	12	16	15	12	6	14	12	6	12	12	9	14	12	7	12
ATCC 6538-p	14	17	14	13	31	31	13	31	14	14	6	15	13	15	16	13	9	15	13	6	15	13	20	17	14	17	18

[a]Diameter of the zone of inhibition (mm), including the disk's diameter (6 mm). Abbreviations: AZM, azithromycin; CEF, cephalexin; CIP, ciprofloxacin; CXA, cloxacillin; GEN, gentamicin; MET, Methicillin; SXT, cotrimoxazole; TET, tetracycline; and VAN, vancomycin.

Table 2. Synergistic effects of the sumac extract (SE) with antibiotics against clinical isolates of MRSA.

Test bacteria	Agent	MIC (μg/mL)		FIC	FICI	Outcome
		Alone	Combination[1]			
MRSA-1	SE	6400	400	0.0625	0.5625	Additive
	Cloxacillin	>320	160	0.5		
	SE	6400	400	0.0625	0.09375	Synergistic
	Cephalexin	>320	10	0.03125		
	SE	6400	400	0.0625	1.0625	Indifference
	Vancomycin	80	80	1		
MRSA-2	SE	1600	400	0.25	0.25	Synergistic
	Cloxacillin	>320	0.31	0.0009		
	SE	1600	400	0.25	0.3125	Synergistic
	Cephalexin	>320	20	0.0625		
	SE	1600	200	0.125	1.125	Indifference
	Vancomycin	0.31	0.31	1		
MRSA-3	SE	1600	400	0.25	0.2656	Synergistic
	Cloxacillin	>320	5	0.0156		
	SE	1600	400	0.25	0.3125	Synergistic
	Cephalexin	>320	20	0.0625		
	SE	1600	200	0.125	1.125	Indifference
	Vancomycin	0.31	0.31	1		
MRSA-4	SE	1600	200	0.125	0.132	Synergistic
	Cloxacillin	>320	5	0.0078		
	SE	1600	400	0.25	0.3125	Synergistic
	Cephalexin	>320	20	0.0625		
	SE	1600	200	0.125	1.125	Indifference
	Vancomycin	0.31	0.31	1		
MRSA-5	SE	1600	400	0.25	0.281	Synergistic
	Cloxacillin	>320	10	0.031		
	SE	1600	200	0.125	0.25	Synergistic
	Cephalexin	>320	40	0.125		
	SE	1600	200	0.125	1.125	Indifference
	Vancomycin	0.31	0.31	1		
MRSA-6	SE	1600	200	0.125	0.1406	Synergistic
	Cloxacillin	>320	5	0.0156		
	SE	1600	400	0.25	0.375	Synergistic
	Cephalexin	>320	40	0.125		
	SE	1600	200	0.125	1.125	Indifference
	Vancomycin	0.31	0.31	1		
MRSA-7	SE	1600	400	0.25	0.2656	Synergistic
	Cloxacillin	>320	5	0.0156		
	SE	1600	400	0.25	0.312	Synergistic
	Cephalexin	>320	20	0.0625		
	SE	1600	200	0.125	1.125	Indifference
	Vancomycin	0.31	0.31	1		
MRSA-8	SE	1600	400	0.25	0.281	Synergistic
	Cloxacillin	>320	10	0.031		
	SE	1600	400	0.25	0.375	Synergistic
	Cephalexin	>320	40	0.125		
	SE	1600	200	0.125	1.125	Indifference
	Vancomycin	0.31	0.31	1		
MRSA-9	SE	1600	400	0.25	0.253	Synergistic
	Cloxacillin	>320	1.25	0.003		
	SE	1600	200	0.125	0.25	Synergistic
	Cephalexin	>320	40	0.125		
	SE	1600	200	0.125	1.125	Indifference
	Vancomycin	0.31	0.31	1		
MRSA-10	SE	3200	800	0.25	0.28125	Synergistic
	Cloxacillin	160	5	0.03125		
	SE	3200	800	0.25	0.375	Synergistic
	Cephalexin	>320	40	0.125		
	SE	3200	800	0.25	0.55	Additive
	Vancomycin	1.25	0.4	0.32		

Abbreviation: MRSA, Methicillin-resistant *Staphylococcus aureus*; FICI, [2]The fractional inhibitory concentration index (FIC index). [1]The MIC of sumac extract (SE) with antibiotics.

3.4. Determination of MICs and evaluation of synergistic effect

The *in vitro* antibacterial activity of the combinations of sumac extract and each of the three selected antibiotics, cloxacillin, cephalexin, and vancomycin, were further evaluated on the ten MRSA bacteria based on the fractional inhibitory concentration (FIC) index, indicating the sum of the FICs (∑FICs) of each antimicrobial agent tested, where the FIC is calculated for each agent by dividing the MIC of each agent when used in combination by the MIC of each agent when used alone. Sumac extract considerably lowered the MICs of antibiotics (cephalexin and cloxacillin) against the MRSA bacteria. The results of the synergistic evaluation are presented in **Table 2** and **Figure 2;** a significant synergistic effect was recorded by sumac extract in combination with cloxacillin or cephalexin, and FICIs for this combination ranged from 0.09 to 0.374 against the MRSA test bacteria. The MIC values of the combination of sumac extract with cloxacillin or cephalexin against MRSAs showed 4- to 32-fold reduction. Our findings demonstrated that combining sumac extract with antibiotics could effectively inhibit MRSA bacteria.

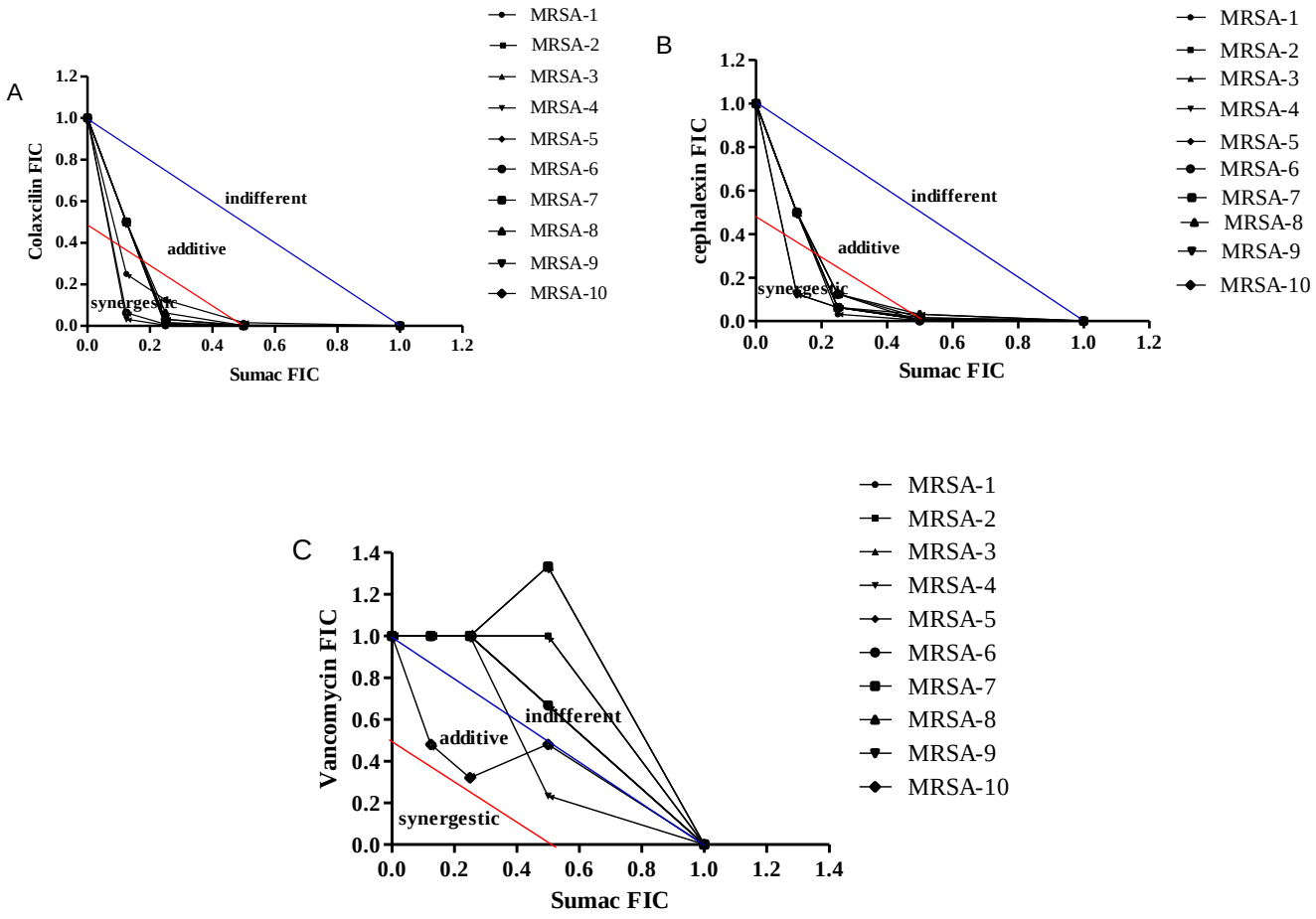

Figure 2. Graphs showing synergistic, additive, and indifferent interactions between sumac extract and antibiotics (cephalexin, vancomycin, and cloxacillin) tested in pairs. Data are the fractional inhibitory concentrations (FICs) of the combined two antimicrobial agents. Sumac extract showed synergistic interactions with antibiotics cephalexin and cloxacillin (A and B), while sumac extract displayed additive and indifferent interaction with antibiotic vancomycin against ten MRSA (C).

FIC (sumac extract) = MIC of sumac extract in combination/ MIC of sumac extract alone

FIC (antibiotic) =MIC of antibiotic in combination/ MIC of antibiotic alone

FICI = FIC (antibiotic) + FIC (sumac extract)

FICI ≤ 0.5 is defined as synergistic, >0.5 to ≤1.0 as additive, and>1.0 to ≤2.0 as indifferent.

3.5. Discussion

Multidrug-resistant (MDR) staphylococci are regarded as major causes of nosocomial infections. These infections are difficult to cure and cause serious problems to the public health [17]. There are limited effective antimicrobial drugs for treating infections caused by MDR bacteria. Therefore, finding compounds that enhance the antimicrobial activity of antibiotics on MDR bacteria seems valuable. The capability of herbal extracts to interact with antibiotics synergistically provides a potential strategy that could contribute to solving the problem of bacterial resistance [11].

Albeit the antibacterial activity of sumac extract has been previously reported [14,16], the amount of data published regarding its effectiveness against multidrug-resistant MRSA is very inadequate. Accordingly, the present study was focused on the antibacterial as well as synergistic activity of sumac extract with clinically important antibiotics. The results of the conducted experiments using the disc-diffusion method demonstrated that *in vitro* interactions between sumac extract and three antibiotics, cloxacillin, cephalexin, and vancomycin, were beyond additive effects against MRSA strains, and using microdilution and checkerboard methods showed synergistic effects between combination of two of these antibiotics and sumac extract with a significant reduction in the MICs of the antibiotics against the MRSA strains.

The MICs of above-mentioned antibiotics against studied multidrug-resistant MRSA demonstrated high level of resistance to these antibiotics. The average increase in antibiotic activity by sumac extract against resistant strains ranged from 4-32 folds for cloxacillin and cephalexin. Our findings are in agreement with previous studies which showed that some herbal extracts can enhance the *in vitro* activity of antibiotics against bacteria [18, 19, 20]. The synergistic interaction recognized between sumac extract and the tested antibiotics can be translated into useful clinical applications in multidrug-resistant MRSA infections. The synergistic effect of sumac extract may be attributed to some active compounds, such as gallic acid (GA) [21], which can disestablish the bacterial cell wall and cytoplasmic membrane and consequently facilitate the influx of other antimicrobial drugs inside bacterial cells [22]. It has been previously shown that gallic acid (GA) is one of the active components of sumac [23] and that gallic acid with a longer alkyl chain from other sources improves oxacillin synergy against MRSA [24].

The results of our checkerboard assay mostly demonstrated synergistic interactions for cloxacillin and cephalexin, which are considered beta-lactam antibiotics and work by inhibiting cell wall biosynthesis [25]. Our results are the same as those of previous studies, which show that beta-lactam antibiotics are most commonly associated with a positive interaction potential [26]. Evolving beta-lactamases and altered penicillin-binding proteins (PBPs) are the major resistance mechanisms of bacterial cells against beta-lactam antibiotics [27]. The exact mechanisms by which the natural antimicrobials reduce beta-lactam resistance of bacterial cells is unknown; however, it is believed that natural antimicrobials do this by introducing structural changes in the resistant bacteria, inhibiting penicillinase activity or inhibiting the activity of altered PBP [28].

Vancomycin is the current first-line therapy for invasive MRSA infections [29]. According to Rybak et al. (2009) [30], MRSA strains with MICs higher than 1 µg/ml are prone to clinical antibiotic failure and emergence of resistance. In the present study, most strains [8 stains] displayed a low MIC of 0.31 µg/ml, and our checkerboard results showed indifferent interaction between sumac extract and vancomycin against these 8 MRSA strains. However, two strains, MRSA1 and MRSA10, showed MICs of 1.25 and 80 µg/ml, respectively. We showed an additive effect of the sumac extract and vancomycin combination against MRSA10 with a more than 3-fold increase in antibiotic activity and indifferent activity on other MRSA strains. In particular, our finding of an *in vitro* additive interaction of vancomycin+sumac extract supports the possibility that this combination therapy could also be additive or synergistic *in vivo* against vancomycin-resistant MRSA infections. Further work in this area is urgently required.

Therefore, antibiotics and plant extract combinations introduce an interesting approach for developing a new strategy to modify the resistance of bacterial cells since the use of plant extracts displays a low risk of increasing bacterial resistance to their action [31]. This study indicated that sumac combined with cloxacillin,

cephalexin, and vancomycin could lead to a new combination of antibiotics and natural compounds against methicillin-resistant *staphylococcus aureus*.

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Conflict of interest

The authors declared no conflict of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

Athors Contributions

Meysam Khanlarbeik: Data collection, Data analysis. Samin Sheikholeslami: Writing original draft. Hossein Jamalifar: Methodology, Investigation. Solat Eslami: Data analysis, Writing original draft. Hamid Reza Monsef-Esfahani: Methodology, Supervision. Mohammad Reza Fazeli: Methodology, Supervision, Funding acquisition. Mohammad Mahdi Ahmadian-Attari: Methodology, Writing, reviewing, and editing.

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Using artificial intelligence chatbots

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