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# Antimicrobial Activity of Mandelic Acid Against Methicillin-Resistant *Staphylococcus aureus*: a Novel Finding with Important Practical Implications

[1,2]*Mohammad Motamedifar,* [1]*Abdollah Bazargani,*
[3]*Mohammad Reza Namazi and* [1]*Hadi Sedigh Ebrahim Sarai*

[1]Department of Bacteriology and Virology, School of Medicine,
Shiraz University of Medical Sciences Shiraz, Iran
[2]Shiraz HIV/AIDS Research Center, Shiraz University of Medical Science, Shiraz, Iran
[3] Shiraz Skin Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

**Abstract:** *Background*: *Staphylococcus aureus* is a major health concern worldwide especially in cutaneous diseases. The aim of this study was to determine the in vitro antimicrobial activity of mandelic acid against *Staphylococcus aureus* isolates. *Methods*: In this study, the susceptibility of mandelic acid against two type strains and nineteen clinical isolates of *Staphylococcus aureus* was assessed *in vitro*. *Results*: Mandelic acid in different concentrations of 40, 80 and 160 mg/ml showed in vitro antibacterial activity against all tested clinical isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) as well as the tested type strains in disk diffusion method with inhibitory zones of 11-20 mm. The minimum inhibitory concentration and minimum bactericidal concentration (MIC/MBC) for methicillin-sensitive *staphylococcus aureus* (MSSA) and MRSA type strains were 20/20 and 40/40 mg/ml respectively. For the nineteen clinical MRSA isolates, $MIC_{50}$ and $MIC_{90}$ were 20 and ≤40 (mg/ml) respectively and $MBC_{50}$ and $MBC_{90}$ were 20 and ≤80 (mg/ml) respectively. *Conclusion*: Our results suggest that mandelic acid has an antibacterial activity against MRSA and might be a useful addition to anti-MRSA armamentarium. Further investigations regarding the use of mandelic acid in a suitable moisturizer for atopic skin, to exert the dual effects of lubrication and MRSA eradication is recommended.

**Key words:** Staphylococcus aureus · Mandelic acid · Atopic dermatitis · MIC · MBC

## INTRODUCTION

The skin of children with atopic dermatitis (AD) may represent a significant reservoir of methicillin resistant *Staphylococcus aureus* (MRSA) colonization in the community [1]. It is recently reported that 80% of atopic dermatitis patients are colonized with *S. aureus* and that of these patients, 16% are colonized with MRSA [2]. Children with atopic dermatitis (AD) are more frequently colonized with *S. aureus* than children without atopic dermatitis [1].

*S. aureus* infection is frequently associated with disease severity in children with atopic dermatitis. Some *S. aureus* toxins have been shown to function as 'superantigens' [3]. Superantigens can bypass the normal control of T-cell activation and activate all T-cell clones bearing certain types of variable chain on the T-cell receptor, this lead to vigorous T-cell activation and cytokine release. So, these bacterial superantigens may involved in induction and aggravation of atopic dermatitis [3] and elimination of staphylococcus colonization can significantly contribute to the curing and prevention of acute exacerbations of atopic dermatitis.

Moreover, there is significantly higher prevalence of chloramphenicol resistant *S. aureus* in patients with AD than in nasal carriers. Similar rates of resistance are expressed to tetracycline, erythromycin, clindamycin, penicillin and mupirocin [4]. The emergence of mupirocin resistant MRSA represents a serious threat to our capacity to manage MRSA carriage and suitable alternatives to mupirocin are required.

**Corresponding Author:** Hadi Sedigh Ebrahim Sarai, Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Zand St, Imam Hossein Sq, Shiraz, Iran.
Tel: +989368829763, Fax: +987112304356.

Mandelic acid (MA) is an alpha-hydroxy acid with a molecular weight more than those of lactic and glycolic acids. It is a nontoxic substance that, if being ingested orally, is excreted in the urine [5, 6]. Mandelic acid has a long history of use in the medicine as an antibacterial; particularly in the treatment of urinary tract infections [5]. Nowadays dermatologists suggest mandelic acid as a useful agent for wide variety of skin problems, from acne to wrinkles. Mandelic aicd, named after the German *mandel* (almond) and derived from the hydrolysis of an extract of bitter almonds has been studied extensively for its possible uses in treating common skin problems such as photoaging, irregular pigmentation and acne. Some studies has been shown that mandelic acid is useful in suppressing pigmentation, treating inflammatory noncystic acne and rejuvenating photoaged skin. Also it has proven useful in preparing the skin of patients for laser peeling and in the skin healing after laser surgery [5].

In this study we aimed to examine the susceptibility of mandelic acid against two type strains and nineteen clinical isolates of *Staphylococcus aureus* by disc diffusion and broth microdilution methods.

## MATERIALS AND METHODS

**Bacteria:** Two type strains of *Staphylococcus aureus*, MRSA ATCC 700698 and methicillin-sensitive *staphylococcus aureus* (MSSA) ATCC 25923 were used in the study. Also Nineteen MRSA clinical isolates obtained from different specimens (blood, sputum, abscess) of critically ill patients referring to Faghihi Hospital, Shiraz, Iran were used. Faghihi Hospital in Shiraz serves as a main referral center in Southern Iran with more than 10,000 admissions per year. The *S. aureus* isolates were identified by colonial morphology, Gram staining (Gram positive cocci in clusters), positive reaction in the following tests: catalase, DNase, coagulase, clumping factor reaction with rabbit plasma on microscope slides and Mannitol fermentation. MRSA isolates were then confirmed based on culture in a plate containing 6 µg/ml of oxacillin in Mueller-Hinton agar supplemented with NaCl (4% w/v; 0.68 mol/l) as a method of testing for MRSA following Clinical and Laboratory Standards Institute (CLSI) standard procedures [7].

**Mandelic acid:** Mandelic acid (2-Hydroxy-2-phenylacetic acid) (Merck Company) was obtained from a local supplier and a stock of 160 mg/ml solution was made in Muller Hinton broth.

***In vitro* Activity of Mandelic Acid Against MRSA:** Antimicrobial activity of MA was determined by disc diffusion method [8]. Overnight broth cultures of the *S. aureus* type strains (ATCC 700698 and ATCC 25923) and 19 isolates of the MRSA were prepared in Mueller–Hinton broth media (Himedia, India), adjusted to $10^8$ cfu/ml and used to inoculate Mueller-Hinton agar (Himedia, India) by flooding the plates, draining the excess and allowing the plates to dry. Six blank paper discs (6 mm) (Padtan-Teb, Iran) were placed on the inoculated agar surface and impregnated with 10 µl of 160, 80, 40, 30, 20 and 10 mg/ml of MA in Muller Hinton broth. One blank paper was also used as a control and impregnated with 10 µl of Muller Hinton broth only. The plates were incubated aerobically for 24 h at 37°C and the zones of inhibition were measured. Vancomycin disc (30 µg/disc, Mast Diagnostics, England) was used as a control of anti-MRSA agent according to CLSI standards (CLSI, 2006; CLSI, 2007) [7, 9]. To quantitate the antibacterial activity of MA against the tested bacteria, Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined using a serial broth microdilution method to arrive at concentrations between 10-160 mg/ml of MA in a 96-well tray (Falcon, USA). Overnight broth cultures were prepared as mentioned above and adjusted so that, upon inoculation, each well contained approximately $5x10^5$ CFU/mL. Positive and negative growth controls were included in every test. The concentration of each inoculum was confirmed using viable counts on blood agar plates (Himedia, India). Trays were incubated aerobically at 37°C for 24 h and the MICs and MBCs were determined. The growth of the microorganisms was determined by turbidity. To confirm MICs and to establish MBCs, 10 µl of broth was removed from each well and inoculated onto blood agar. After aerobic incubation at 37°C overnight, the number of surviving organisms was determined. The MIC was the lowest concentration which resulted in a significant decrease (>90%) in inoculum viability while the MBC was the point where 0-1% of the initial inoculum survived [10, 11]. All experiments were performed in triplicate.

## RESULTS

The inhibitory zones of six concentrations of MA were determined for 19 MRSA clinical isolates and MRSA ATCC 700698 and MSSA ATCC 25923 strains. Both type strains and the most clinical isolates were

Table 1: Mandelic acid Inhibitory zone (mm) by disc diffusion method for clinical MRSA isolates and *Staphylococcus aureus* type strains

| | | Disk content (mg/mL) | | |
|---|---|---|---|---|
| Clinical isolate or type strain | Source | 160 | 80 | 40 |
| MRSA1 | Burn | 18 | 15 | 12 |
| MRSA2 | Burn | 18 | 15 | 12 |
| MRSA3 | wound | 16 | 15 | 11 |
| MRSA4 | Burn | 18 | 14 | 12 |
| MRSA5 | wound | 18 | 12 | 0 |
| MRSA6 | Burn | 18 | 14 | 0 |
| MRSA7 | Burn | 14 | 0 | 0 |
| MRSA8 | Burn | 19 | 15 | 0 |
| MRSA9 | Septicemia | 15 | 0 | 0 |
| MRSA10 | Pneumonia | 19 | 13 | 0 |
| MRSA11 | Pneumonia | 15 | 0 | 0 |
| MRSA12 | wound | 16 | 0 | 0 |
| MRSA13 | wound | 18 | 13 | 0 |
| MRSA14 | wound | 17 | 15 | 11 |
| MRSA15 | Pneumonia | 20 | 15 | 0 |
| MRSA16 | wound | 18 | 13 | 0 |
| MRSA17 | Septicemia | 16 | 0 | 0 |
| MRSA18 | Septicemia | 17 | 12 | 0 |
| MRSA19 | wound | 15 | 0 | 0 |
| MRSA (ATCC 700698) | Type strain | 19 | 14 | 11 |
| MSSA (ATCC 25923) | Type strain | 18 | 15 | 0 |

Table 2: Mandelic acid MIC and MBC (mg/ml) for clinical MRSA isolates and *Staphylococcus aureus* type strains

| Clinical isolate or type strain | Source | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|---|
| MRSA1 | Burn | 40 | 40 |
| MRSA2 | Burn | 40 | 40 |
| MRSA3 | wound | 40 | 40 |
| MRSA4 | Burn | 40 | 40 |
| MRSA5 | wound | 20 | 20 |
| MRSA6 | Burn | 20 | 20 |
| MRSA7 | Burn | 20 | 20 |
| MRSA8 | Burn | 20 | 20 |
| MRSA9 | Septicemia | 20 | 20 |
| MRSA10 | Pneumonia | 20 | 80 |
| MRSA11 | Pneumonia | 20 | 20 |
| MRSA12 | wound | 20 | 20 |
| MRSA13 | wound | 20 | 20 |
| MRSA14 | wound | 20 | 20 |
| MRSA15 | Pneumonia | 40 | 80 |
| MRSA16 | wound | 20 | 20 |
| MRSA17 | Septicemia | 20 | 20 |
| MRSA18 | Septicemia | 40 | 80 |
| MRSA19 | wound | 20 | 20 |
| MRSA (ATCC 700698) | Type strain | 40 | 40 |
| MSSA (ATCC 25923) | Type strain | 20 | 20 |

susceptible to 80-160 mg/ml concentrations of MA (Table 1), with an inhibitory zone of 12-20 mm. Five clinical isolates and MRSA type strain showed inhibitory zone of 11-12 mm at the 40 mg/ml and no inhibitory zone was seen at the lower concentrations. The MIC/MBC of the tested MSSA and MRSA type strains, as determined by the broth micro dilution method were 20/20 and 40/40 (mg/ml) respectively. Concentrations required to inhibit 50 and 90% of the clinical isolates (MIC50 and MIC90) were 20 and ≤40 (mg/ml) respectively and those required to kill 50 and 90% of the strains (MBC50 and MBC90) were 20 and ≤80 (mg/ml) respectively. Except for 3 isolates MIC and MBC were the same. All tested bacteria were susceptible to vancomycin disc showing 15 mm growth inhibition zone. More details of anti-MRSA activity of MA are presented in Table 2.

## DISCUSSION

In this study we found that mandelic acid in different concentration of 20-160 mg/ml has in vitro antimicrobial activity against clinical isolates of MRSA as well as the tested type strains. Previous reports had shown some in vitro antimicrobial properties of the mandelic acid and their derived compounds. Stickler *et al*. proved effectiveness of mandelic acid and mandelic acid (0.5% w/v)/lactic acid (0.5% v/v) in eliminating the biofilm forming organisms related to urinary tract infections [12]. Also Fuursted *et al*. demonstrated Lag of regrowth in some bacterial pathogens after exposure with mandelic–lactic acid [13].

MIC and MBC determination by broth microdilution method revealed that $MIC_{50}$ for clinical MRSA isolates was 20 mg/ml of MA and MA in concentration of 40 mg/ml had bactericidal effect on MRSA type strain. In a study conducted by Alves *et al*. some mandelic acid derived compounds were tested for their antimicrobial activity against few Gram positive and Gram negative bacteria by using disc diffuision method, which shown inhibited more MRSA isolates [14].

Based on the disc diffusion susceptibility testing results, except for 6 clinical MRSA isolates that showed susceptibility only to 160 mg/ml discs of MA, all other clinical isolates along with standard MSSA and MRSA strains could be considered susceptible to ≤80 mg/ml concentration of MA. These in-vitro results suggest that MA, can be considered for the topical treatment of MRSA skin infection or carriage.

In our study MIC and MBC of MA for the tested bacteria were the same or just one or two serial dilution away. This finding shows that MA maybe considered as a bactericidal biocide. The mechanism of bactericidal action of MA needs to be more studied. Biocidal activity of Sodium chlorite which was a two-component system

containing mandelic acid previously showed [15]. Also it is known that MA in the form of methenamine deraivative as a urinary antiseptic may lead to urine acidification [6]. So it is reasonable to assume that this acid exert its bactericidal effect through lowering the pH. In this regard, it may be suggested that other organic acids may have the same effect as MA. The fact that MA is used as a skin care preparation for a wide variety of skin concerns [16,17], make it sensible to assign MA as a better candidate to any other organic or fatty acid for the treatment of skin staphylococcal infections. It deserves noting that as MA has a greater molecular weight than lactic acid [5], it has a lower potential for skin irritation compared to it.

Based on our study which shows efficacy of MA against MRSA, we suggest to use of MA in production of a novel moisturizer for atopic skin, exerting the dual effects of lubrication and MRSA eradication. We also recommend the usage of MA in combination with urea in the treatment of xerotic skin of patients with mycosis fungoides and Sézary syndrome, which are supposed to arise from antigen-driven clonal expansion and accumulation of helper-memory T cells. Recent studies show staphylococcal carriage in nares and skin lesions of patients with mycosis fungoides is similar to that in atopic dermatitis [18].

## CONCLUSION

In sum, for the first time, we showed the antimicrobial effect of MA, in concentrations of 20-160 mg/ml, against MRSA and encourage the conduction of clinical studies on this agent for treatment of MRSA and other bacterial skin infections.

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**Conflict of Interest:** None declared

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