

## Supplementary material

### Unraveling the Efficacy of Verbascoside in Thwarting MRSA Pathogenicity by Targeting Sortase A

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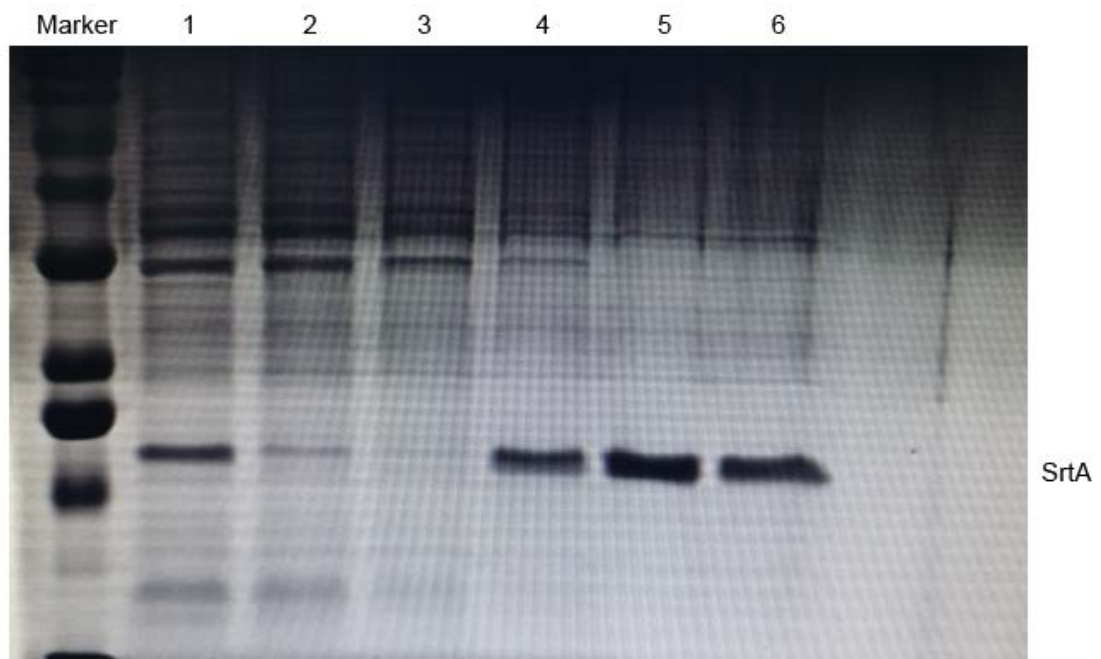
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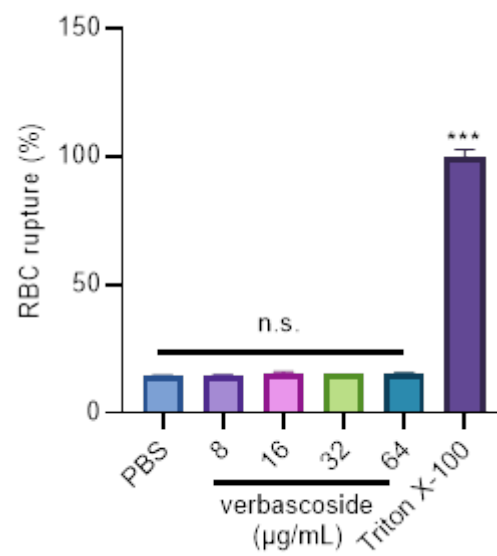
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**Supplementary Figure S1. Electrophoretic profiling of the effect of the purified SrtA protein via SDS–PAGE.**



Supplementary Figure S1. Electrophoretic profiling of the effect of the purified SrtA protein via SDS–PAGE. Lanes 1 and 2 show the BL21 (DE3) pET28a::SrtA protein expression postinduction and postinduction with IPTG, respectively. Lane 3 highlights nonspecific proteins eluted at a low imidazole concentration (10 mM). Proteins collected at a moderate imidazole concentration (50 mM) are shown in lane 4, while lanes 5 and 6 display the target SrtA protein purified under stringent conditions using 100 and 200 mM imidazole, respectively.

**Supplementary Figure S2. The impact of different concentrations of verbascoside on RBC integrity.**



**Supplementary Figure S3. The MIC of verbascoside against standard and clinical strains of *S. aureus* stained with resazurin.**

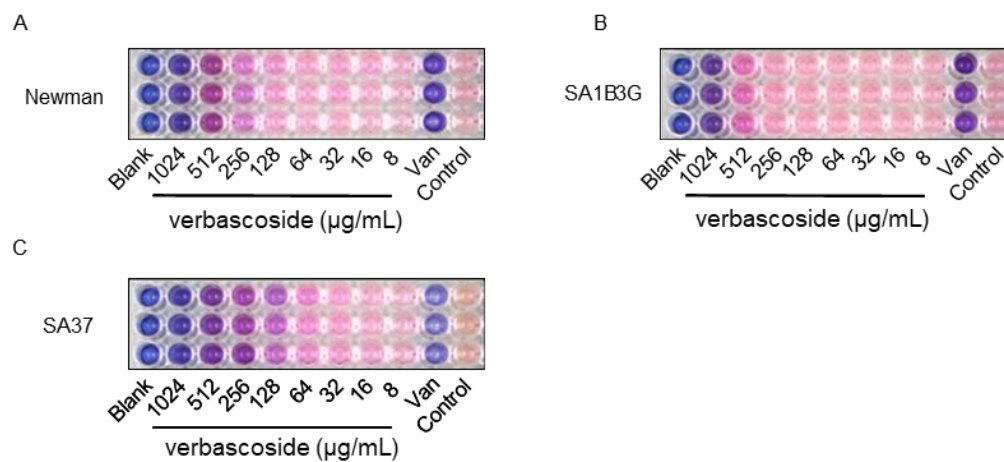


Fig S1. The MIC of verbascoside against standard and clinical strains of *S. aureus* stained with Resazurin. (A) The MIC of verbascoside against the standard strain of *S. aureus* Newman. (B) The MICs of verbascoside against clinical strains of *S. aureus*, including the SA1B3G strain (C) and SA37.

**Supplementary Figure S4. Quantitative assessment of *S. aureus* biofilm formation under verbascoside exposure using crystal violet staining.**

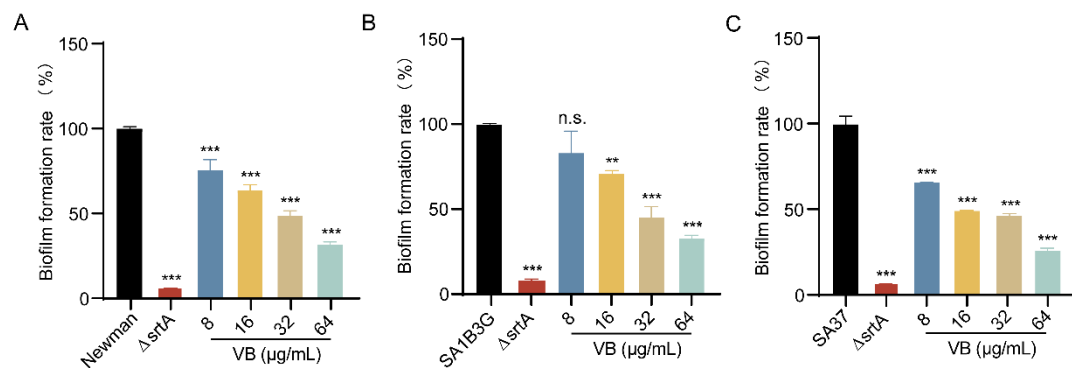


Figure S4. Quantitative assessment of *S. aureus* biofilm formation under verbascoside exposure using crystal violet staining. (A) Impact of verbascoside on biofilm formation by *S. aureus* Newman, (B) SA1B3G, and (C) SA37.

**Supplementary Figure S5. Impact of verbascoside on the survival rate of *S. aureus* USA300  $\Delta srtA$  infected with *Galleria mellonella*.**

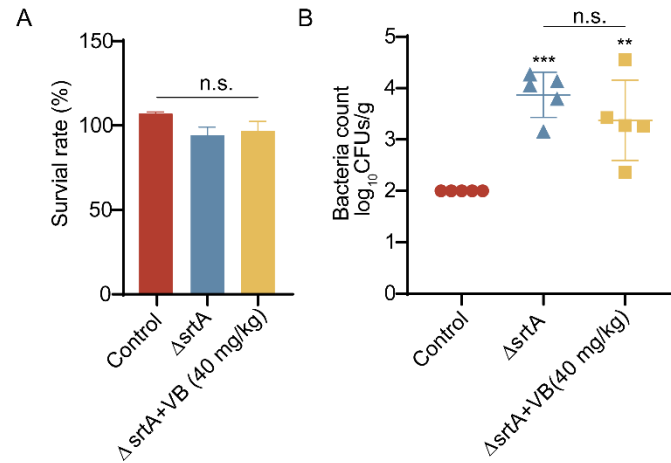


Figure S5. Impact of verbascoside on the survival rate of *S. aureus* USA300  $\Delta srtA$  infected with *Galleria mellonella*. (A) A comparative investigation revealed distinct cohorts of *Galleria mellonella* ( $n = 10$ ), comprising an untreated control group ( $\Delta srtA$ ) and a treated group ( $\Delta srtA+VB$ ) administered verbascoside (40 mg/kg). The survival rates of these cohorts were documented at 120 hours postinfection. (B) Enumeration of colony-forming units (CFUs) in *Galleria mellonella* larvae following infection ( $n = 5$ ), determined using the agar dilution method.

**Supplementary Figure S6. Treatment of mice with verbascoside-induced *S. aureus* USA300  $\Delta$ srtA strain-infected pneumonia.**

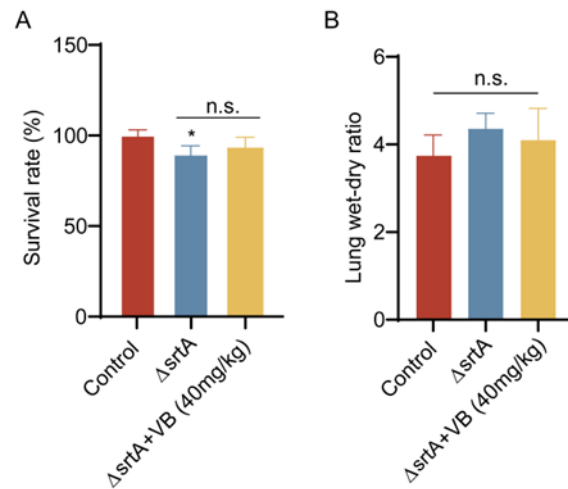


Figure S6. Treatment of mice with verbascoside-induced *S. aureus* USA300  $\Delta$ srtA-infected pneumonia. (A) Assessment of the impact of verbascoside on the survival rate of mice (n = 10) exposed to a lethal dose of the *S. aureus* USA300  $\Delta$ srtA. There was no significant difference in survival between the verbascoside-treated  $\Delta$ srtA group and the untreated  $\Delta$ srtA group. (B) Evaluation of the lung dry-to-wet weight ratio as an indicator of the therapeutic efficacy of verbascoside in *S. aureus* USA300  $\Delta$ srtA-induced pneumonia in mice (n=6).

**Supplementary Table S1. Survival rates of the mice.**

<b>Mice survival (%)</b>							
<b>Groups</b>	<b>Day 1</b>	<b>Day2</b>	<b>Day 3</b>	<b>Day 4</b>	<b>Day 5</b>	<b>Day 6</b>	<b>Day 7</b>
Noninjected	100	100	100	100	100	100	100
Control (PBS)	100	100	100	100	100	100	100
Verbascoside 20 mg/kg	100	100	100	100	100	100	100
Verbascoside 40 mg/kg	100	100	100	100	100	100	100