TITLE: MYRICETIN PROTECTS *Galleria mellonella* AGAINST *Staphylococcus aureus* INFECTION AND INHIBITS MULTIPLE VIRULENCE FACTORS

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ABSTRACT: *Staphylococcus aureus* is an opportunistic pathogen related to a high number and variety of life-threatening infections but for which antimicrobial resistance is limiting the treatment options. The pathogenicity of *S. aureus* is associated with the production of an impressive collection of virulence factors such as exotoxins, enzymes, biofilm formation, and staphyloxanthin pigment production. Hence, alternative therapeutic strategies involving antivirulence compounds have attracted great attention. Unlike antibacterials that aim to inhibit cell growth, antivirulence therapies are based on the inhibition of bacterial virulence. Myricetin, 3,5,7,3',4',5'-hexahydroxyflavone, is a flavonoid commonly ingested through human diets such as fruits, vegetables, tea, berries and red wine. The aim of this study was to investigate the effect of myricetin and of its glycosylated form, myricitrin, on several virulence factors produced by *S. aureus* and the potencial of the flavonol to protect the host during infection using the *in vivo Galleria mellonella* model. By appling *in vitro* (phenotypic and genotipic assays), *in vivo* and *in silico* evaluations we show that myrictein exhibits: (i) antibiofilm properties, likely due to the direct binding to sortase A enzyme and alteration in expression of cell-wall proteins involved in bacterial adhesion process, allied to the downregulation of the global virulence regulator *sae*R gene; (ii) anti-hemolytic activity due to the direct binding to α-toxin, hence preventing the oligomerization process that lead to the formation of the heptameric transmembrane pore in host cells; and (iii) anti-staphyloxanthin activity making *S. aureus* more susceptible to H_2O_2 killing. Adittionally, we demonstrate that glycosylated compound exhibits lower or absence of these activities, pointing to the importance of the hydroxyl group in position 3 of the flavonol C-ring for the antivirulence activity of myricetin. All effects observed *in vitro* culminated with an attenuated pathogenicity of *S. aureus in vivo*, indicating that agents mitigating virulence are able to control infectious process. The present findings reveal the potential of myricetin as an prototype multi-target antivirulence candidate to control *S. aureus* pathogenicity.

KEYWORDS: *Staphylococcus aureus*, virulence, antivirulence, myricetin, *Galleria mellonella*

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