

Supporting Information

Photodynamic action of *Hypericum perforatum* hydrophilic extracts against *Staphylococcus aureus*

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HPLC method for quantitative determination of total hypericins

Sample preparation

All the operations were done protected from light. The grinded sample (1.2 g) was extracted with 25 ml of MeOH by means of an ultrasonic bath (Branson 5800 Ultrasonic Cleaner) at 35 °C. After 30 minutes the sample was centrifuged during 10 minutes at 4000 rpm. The supernatant was collected in a 50 ml volumetric flask and the pellet was extracted in the same conditions. After centrifugation the second extract was combined to the first one and the volume was made up to volume (50 ml) using the same solvent. The sample, diluted 1:2 with the same solvent, was filtered on a 0.45 µm Millipore cellulose acetate syringe filter, and was used for the acquisition of the chromatographic profile at different concentration according to the following instrumental conditions.

Instrumental conditions

The measures were carried out by an Agilent 1100 Series HPLC-DAD system consisting of a vacuum degasser, a quaternary pump, a Peltier autosampler thermostated at 20 °C, a Peltier column compartment thermostated at 20 °C, and a Diode Array Detector. The column used was from Waters (Milford, MA; Spherisorb ODS2, 250 mm x 4.6 mm, 5 µm) and the elution was performed with SDS 10 mM solution, pH 2.5 controlled with phosphoric acid (solvent A), and CH₃CN (solvent B). The gradient program used was: 0 min 25% A 75% B, 15 min 5% A 95% B. The flow rate was 1.2 ml/min. The Diode Array Detector wavelength was set at 590 nm. Hypericin (Sigma-Aldrich, Milan, Italy) was dissolved in methanol/Pyridine 18/2. The linearity of the method was found between 0.06 and 0.3 mg/ml. The working solutions were 0.061, 0.122, 0.244 mg/ml. The correlation was $r=0.999966$. Each solution was injected three times; the CV was lower than 1%.

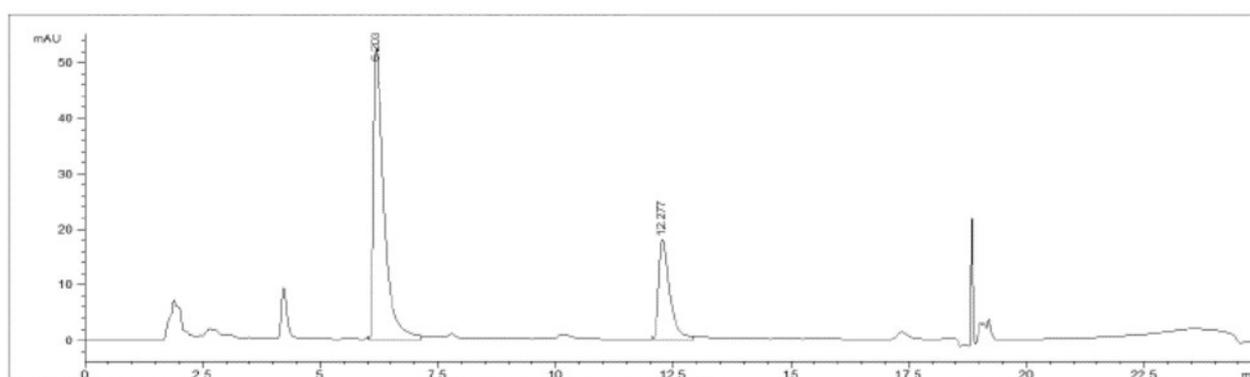


Figure S1. HPLC- Diode Array Detector fingerprint chromatogram for hypericum hydrophilic extract: pseudohypericin 6.20 min and hypericin 12.27 min.

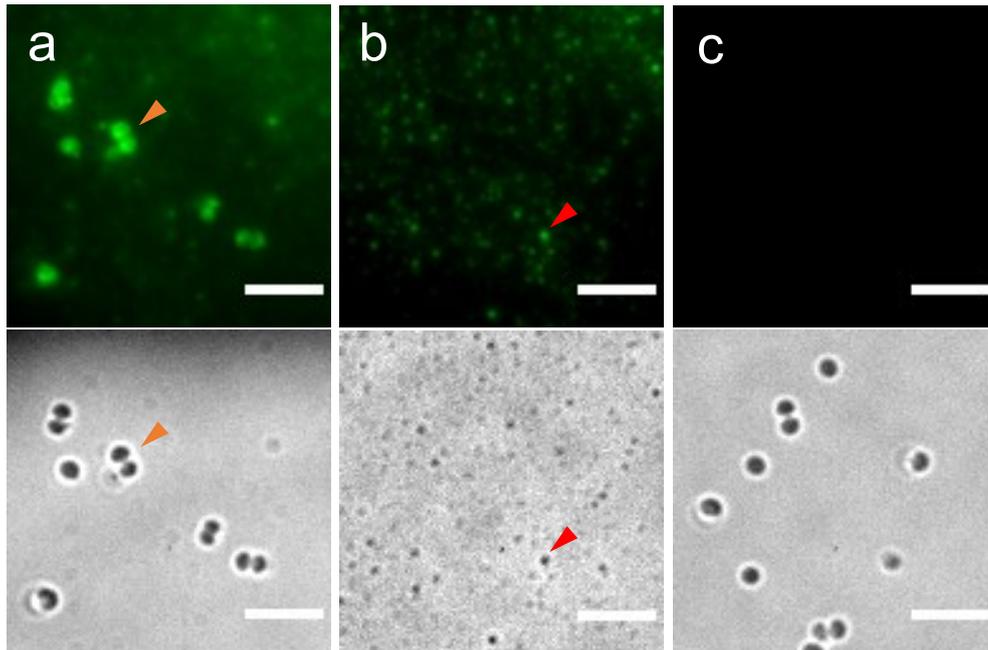


Figure S2. Fluorescence (top) and corresponding bright field (bottom) images obtained for *S. aureus* cells incubated with aHyp concentration corresponding to 3 μM Hyp (a). Representative images of the corresponding control samples obtained with aHyp in absence of bacteria (b) and with *S. aureus* cells in buffer solution (c) are provided. The orange arrow in (a) indicates the typical morphology of *S. aureus* cells, while the red arrow in (b) indicates an example of the large fluorescent aggregates found on the coverslip surface. Excitation wavelength = 561 nm; scale bars = 5 μm ; constant contrast in all fluorescence images.

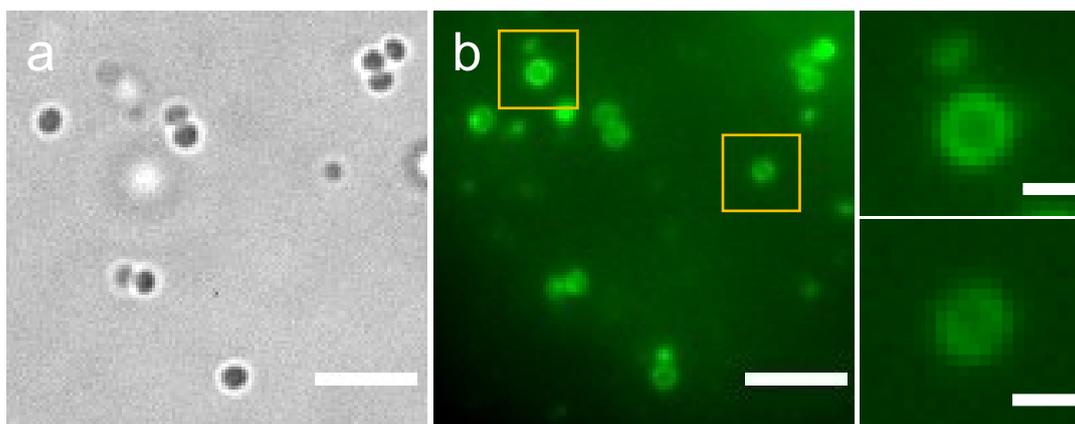


Figure S3. Bright field (a) and corresponding fluorescence (b) images of *S. aureus* cells incubated with aHyp solution, containing 3 μM Hyp (green), delivered with 6 μM of Bovine Serum Albumin (scale bars 5 μm). Zoomed-in views of the bacteria indicated by the orange squares are shown on the right (scale bars 1 μm). Excitation wavelength = 561 nm.

It is interesting to note that a similar distribution of the fluorescence emission was obtained for *S. aureus* cells exposed to aHyp solutions pre-mixed with BSA. In these conditions, a large fraction of Hyp molecules is

initially bound to this protein. As observed when delivery is performed with ethanol or DMSO solutions of aHyp, ¹ Hyp fluorescence is mainly localized on the bacterial cell wall (**Figure S3**). Moreover, in the presence of BSA, the presence of fluorescent aggregates on the coverslip surface was drastically reduced. This is in line with the decreased aggregation of hydrophobic molecules, such as Hyp, induced by the binding to the protein carrier in aqueous environment, as already demonstrated for several protein carriers.¹⁻⁶ Finally, we note that the intensity of the fluorescence emission detected on the bacteria was lower (~50%) in the presence of BSA than for cells directly exposed to the aHyp solution. This possibly indicates a different amount of cell loaded Hyp in the two modalities of delivery. However, the precise measurement of fluorescence intensity is often affected by several variables such as heterogeneous cell morphology, residual photo-bleaching, different irradiation conditions or background fluorescence and would require a more accurate characterization.

References

1. D. Pezzuoli, M. Cozzolino, C. Montali, L. Brancaleon, P. Bianchini, M. Zantedeschi, S. Bonardi, C. Viappiani and S. Abbruzzetti, *Food Control*, 2018, **94**, 254-262.
2. P. Bianchini, M. Cozzolino, M. Oneto, L. Pesce, F. Pennacchietti, M. Tognolini, C. Giorgio, S. Nonell, L. Cavanna, P. Delcanale, S. Abbruzzetti, A. Diaspro and C. Viappiani, *Biomacromolecules*, 2019, **20**, 2024–2033.
3. P. Delcanale, B. Rodríguez Amigo, J. Juárez-Jimenez, J. Luque, S. Abbruzzetti, M. Agut, S. Nonell and C. Viappiani, *J. Mat. Chem. B*, 2017, **5**, 1633--1641.
4. B. Rodríguez-Amigo, P. Delcanale, G. Rotger, J. Juárez-Jiménez, S. Abbruzzetti, A. Summer, M. Agut, F. J. Luque, S. Nonell and C. Viappiani, *J. Dairy Sci.*, 2015, **98**, 89–94.
5. P. Delcanale, F. Pennacchietti, G. Maestrini, B. Rodríguez-Amigo, P. Bianchini, A. Diaspro, A. Iagatti, B. Patrizi, P. Foggi, M. Agut, S. Nonell, S. Abbruzzetti and C. Viappiani, *Sci. Rep.*, 2015, **5**, 15564.
6. J. Comas-Barceló, B. Rodríguez-Amigo, S. Abbruzzetti, P. d. Rey-Puech, M. Agut, S. Nonell and C. Viappiani, *RSC Advances*, 2013, **3**, 17874-17879.