ESI for:

Microwave assisted one-pot green synthesis of cinnoline derivatives inside natural sporopollenin microcapsules

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Liquid chromatography-mass spectrometry (LCMS) technique for determination of m/z of the extracted cinnoline 3 compound:

The Agilent 6420 Triple Quadrupole LC/MS is a liquid chromatograph triple quadrupole mass spectrometer that performs MS/MS using three sets of parallel rods (quadrupole, hexapole and quadrupole). The first quadrupole separates ions into precursor ions that are fragmented in the hexapole into product ions, which are separated by the second quadrupole. Usually, two or more precursor ions and their product ions are monitored in sequence in MRM (multiple reaction monitoring) mode. The Agilent 6420 LC/MS is connected to an Agilent 1200 series LC module (binary pump, column compartment/oven and auto sampler). Samples can be introduced to the spray chamber either through the LC column or by flow injection. The LC column installed is an Agilent ZORBAX SB-C18, RRHT; 2.1 x 50 mm, 1.8 μm. The mass range of the instrument is 5 to 3,000 m/z and its resolution is 0.5 Da or 0.7 Da.

Since the mass spectrometer is very sensitive to contamination, precautions have to be taken concerning sample preparation, extraction and detection parameters. For measuring the m/z of the encapsulated material, the delivery carrier (LCS microcapsules) should be separated and non-covalent interactions between cinnoline **3** and the microcapsules should be broken using a suitable high-grade solvent. For our extraction and separation protocol, the dried cinn-loaded LCS microcapsules were vortexed in pure ethanol for 5 min to allow the release of cinnoline **3**, then the first filtrate was discarded. This step was repeated once more with the dried microcapsules left from the first step but with HLPC grade methanol and the second filtrate was tested for the relative mass of cinnoline 3. We have used Chromacol 4 mm Syringe Filter 0.45 μm Nylon 4-SF-45(N) having a pore size of 0.45 μm. Notice that the filter was purged with HLPC grade methanol before sample filtration. Generally, it is

important to make sure that the encapsulated material can be adequately separated from the release formulation, thus the interferences during the ionisation process are negligible. The electrospray ionisation mass spectrometry (ESI-MS) in a negative ionisation mode was applied in our protocol to obtain the m/z of the released cinnoline **3** compound. A 20 µl of the separated cinnoline 3 solution in HLPC grade methanol was introduced to the spray chamber through the LC column and the retention time was 8.8 seconds with a flow rate of 1ml/min. The ESI draws the solution of the sample to the tip of a capillary tube, where a high voltage of about 3 to 5 kV was applied. A nebuliser gas flows from outside the capillary to spray the sample, creating charged droplets with the same polarity as the applied voltage. ESI generates suitable molecular fragments for MS/MS verification and a mass spectrum of the sample was obtained.

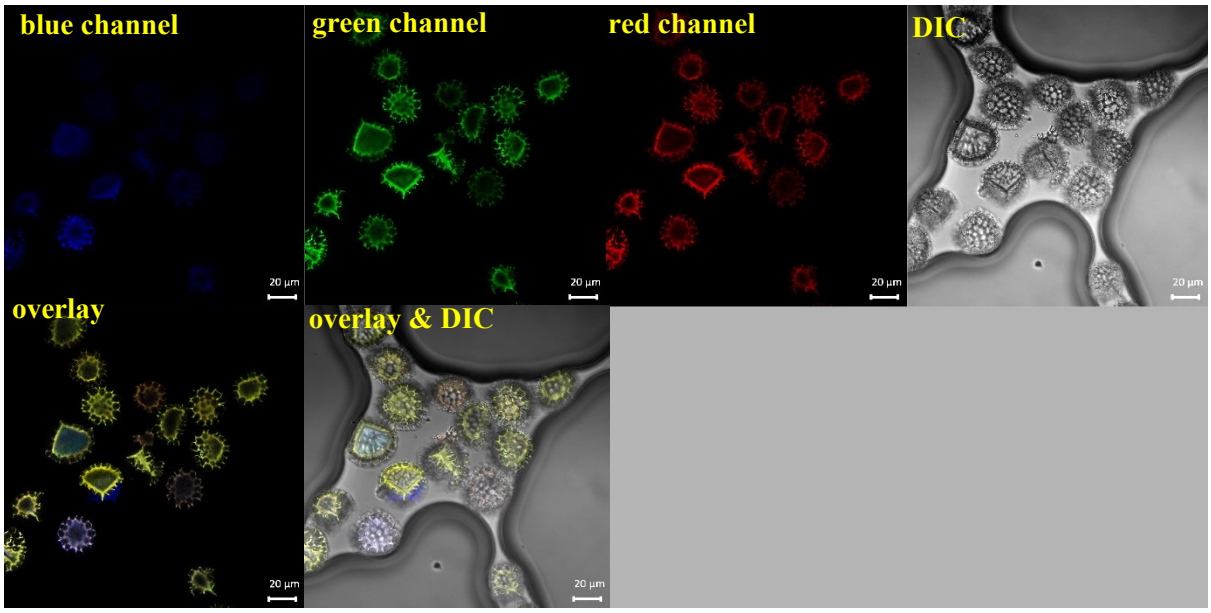

Figure S1. CLSM images of empty LCS microparticles before the MW synthesis reaction. The microparticle was scanned with three laser excitation wavelengths represented by the blue, green and red channels. Images were captured at the middle slice of the microparticles.

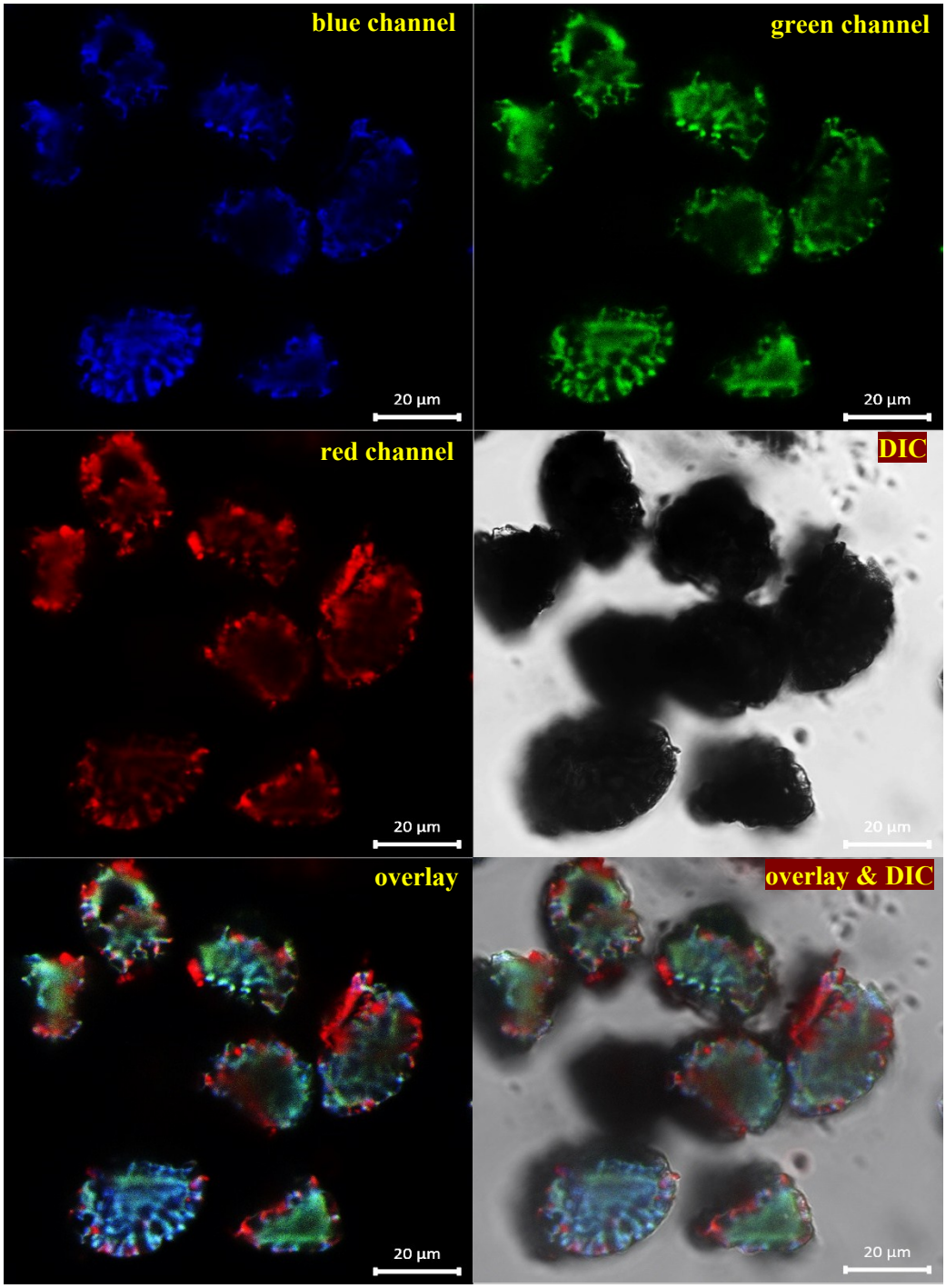

Figure S2. CLSM images of cinn-loaded LCS microcapsules after the MW reaction for the cinnoline 3 formation inside the sporopollenin. Images were recorded at different channels showing the successful loading of the cinnoline 3. Images were captured at the middle slice of the microcapsules (14 µm depth).

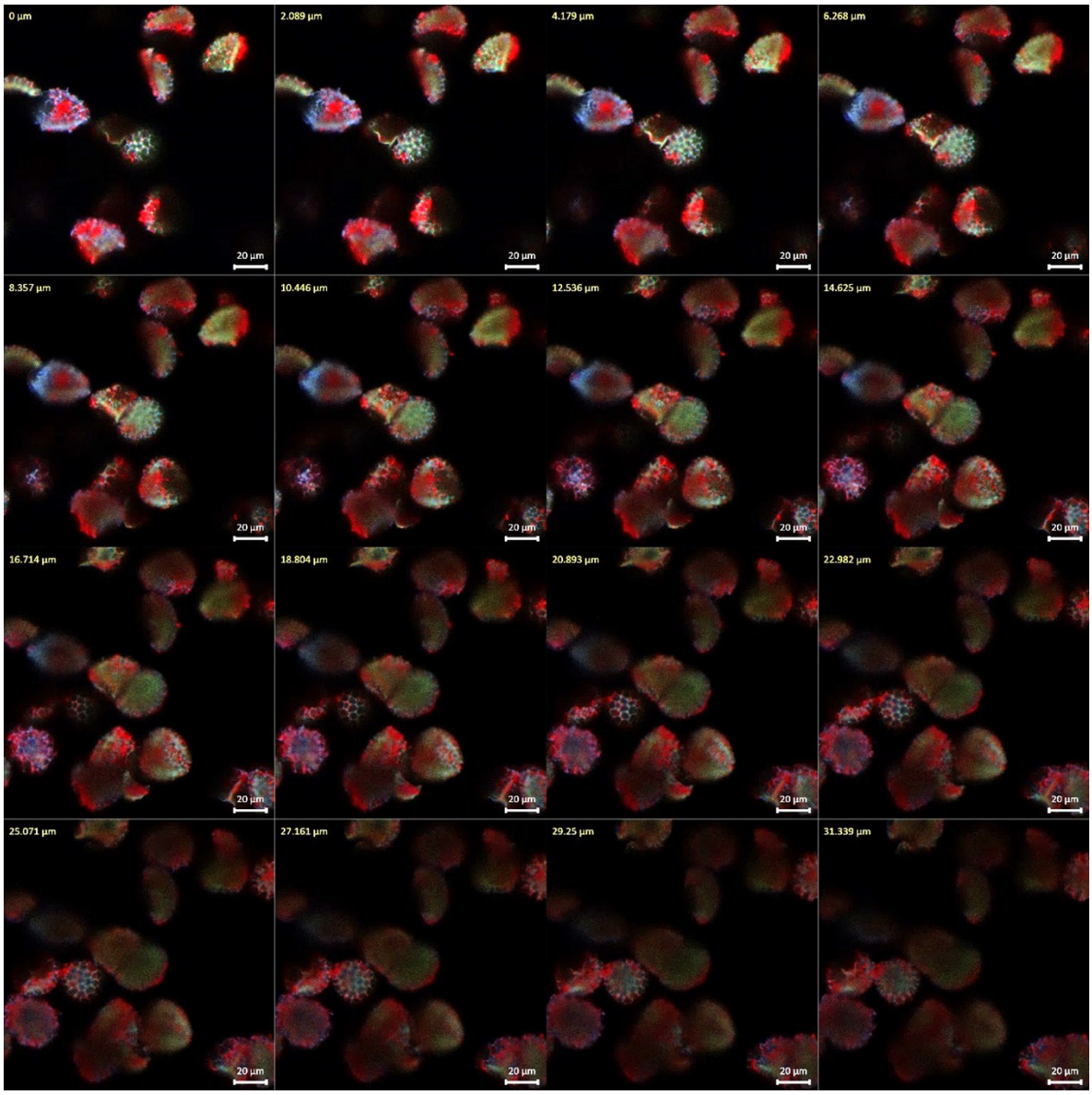

Figure S3. Overlay CLSM images of cinn-loaded LSC formed via microwave irradiation for 20 min at 100 °C. Images were captured in Z-stack mode, showing the focus position of the scanned slice (in µm given) at upper left of each image.

Figure S4. Digital image cinnoline 3 compound under a UV lamp of long wavelength of 365 nm. (A) The filtrate left after the MW synthesis reaction. (B) Diluted cinnoline 3 released from the cinn-loaded LCS in ethanol.

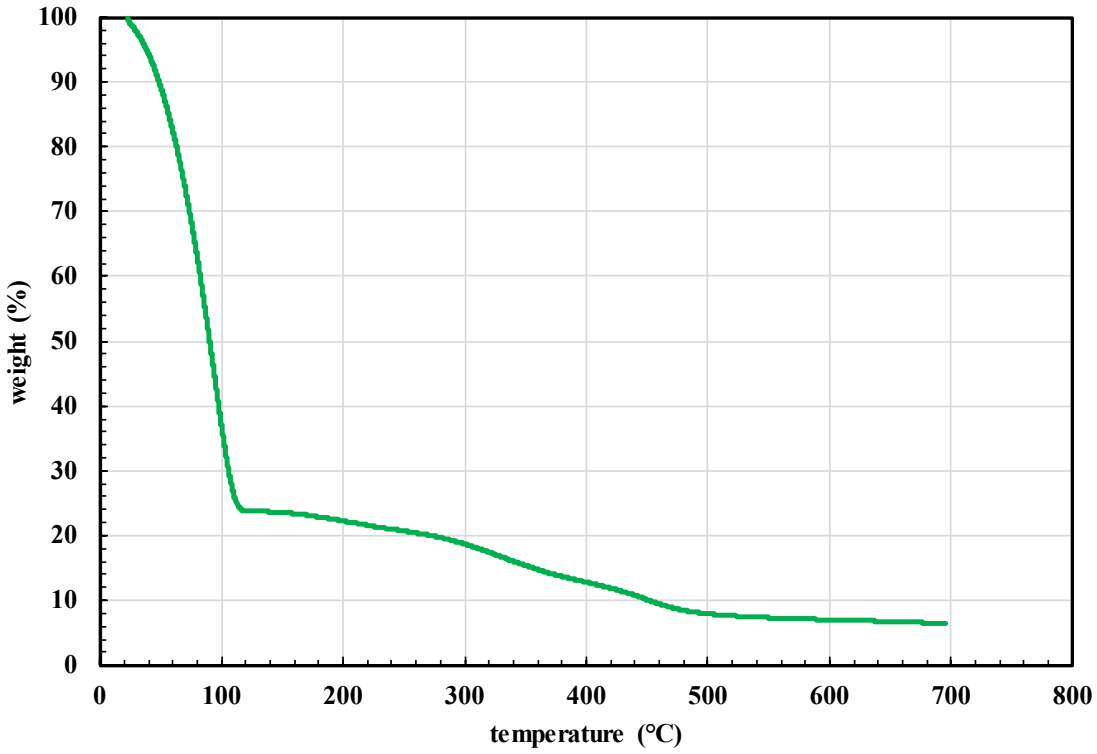

Figure S5. TGA thermograme of partially dried cinn-loaded LSC formed via microwave irradiation for 20 min at 100 °C.

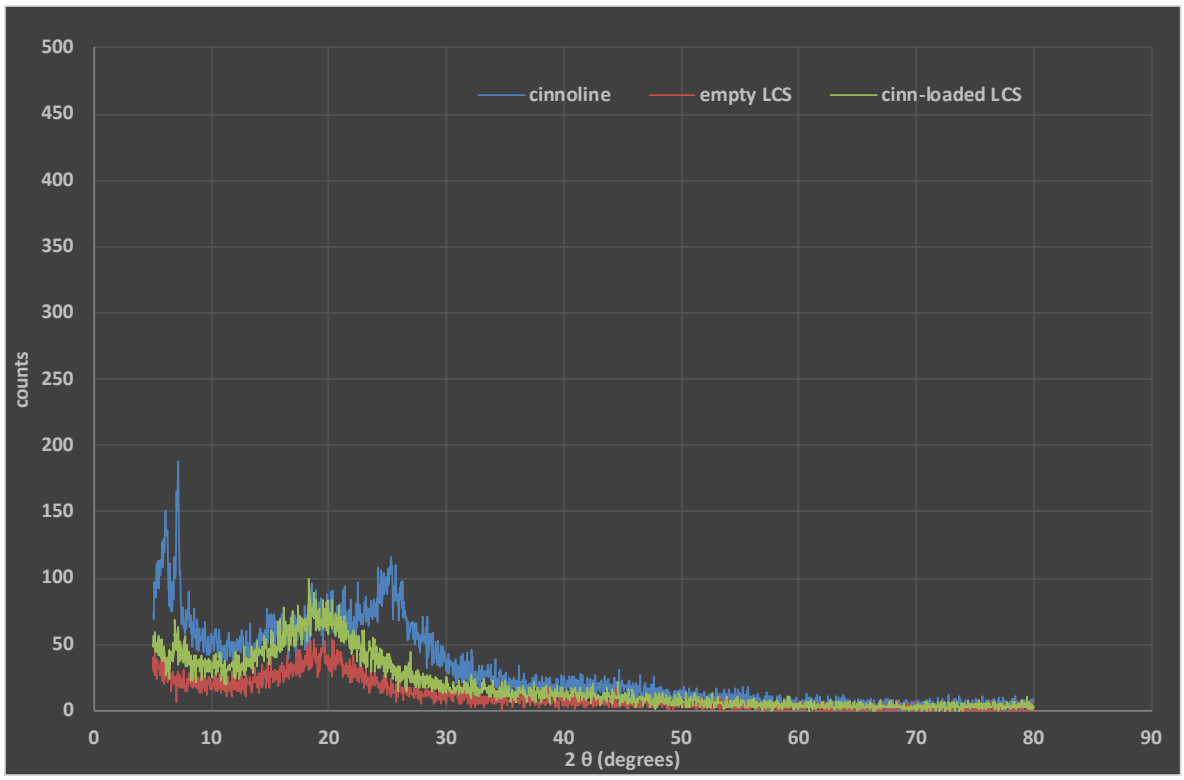

Figure S6. Observed XRD profile of pure cinnoline 3, empty LCS and cinn-loaded LCS microcapsules at 25 °C.

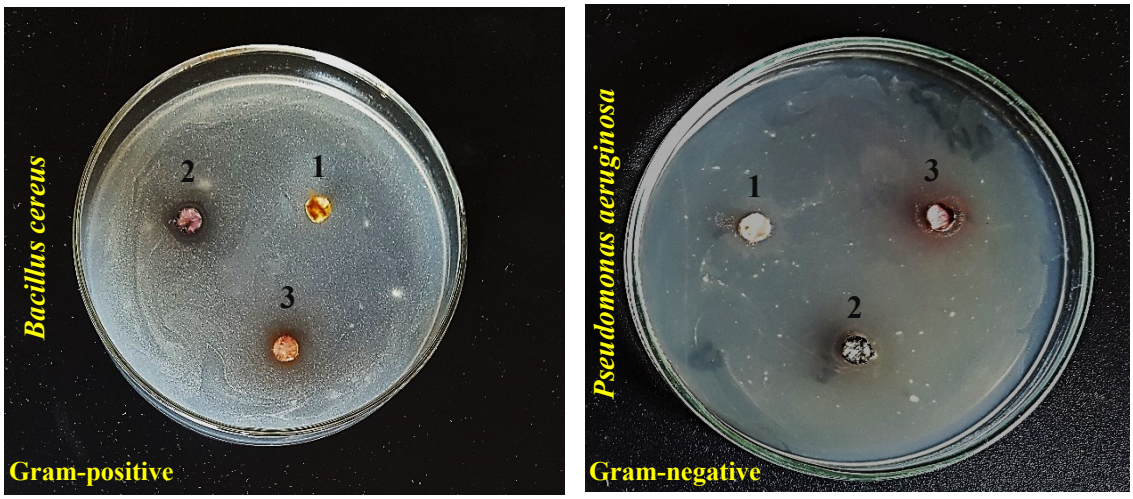

Figure S7. Antibacterial activity obtained using the disc diffusion method for different bio-agents against different bacteria strains (given). Numbers given above the different inhibition zones represent: (1) Empty LCS; (2) Cinnoline 3; (3) Cinn-loaded LCS.