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Supplementary Materials

A Sustainable Multistage Process for Immobilizing Bioactive Compounds on Layered Double Hydroxides

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Table S1. Physico-chemical data of commercial FA-H, RA-H, and GA-H

Bioactive molecule [1]	Molecular weight (mol/g)	Physical state	Color	Melting point (°C)	Purity (%) [analysis]	FT-IR [2]
FA-H	194.18	powder	off white	168-172	99 [GC]	Conforms to structure
RA-H	360.31	powder	light beige	171-175	97 [HPLC]	Conforms to structure
GA-H	470.68	powder	white	292-295	97 [TLC]	Conforms to structure

[1] All bioactive molecules were purchased from Sigma-Aldrich, and the corresponding SDS sheets can be downloaded from the Sigma-Aldrich website. [2] The FT-IR spectra of the three acids, FA-H, RA-H, and GA-H, are reported below.

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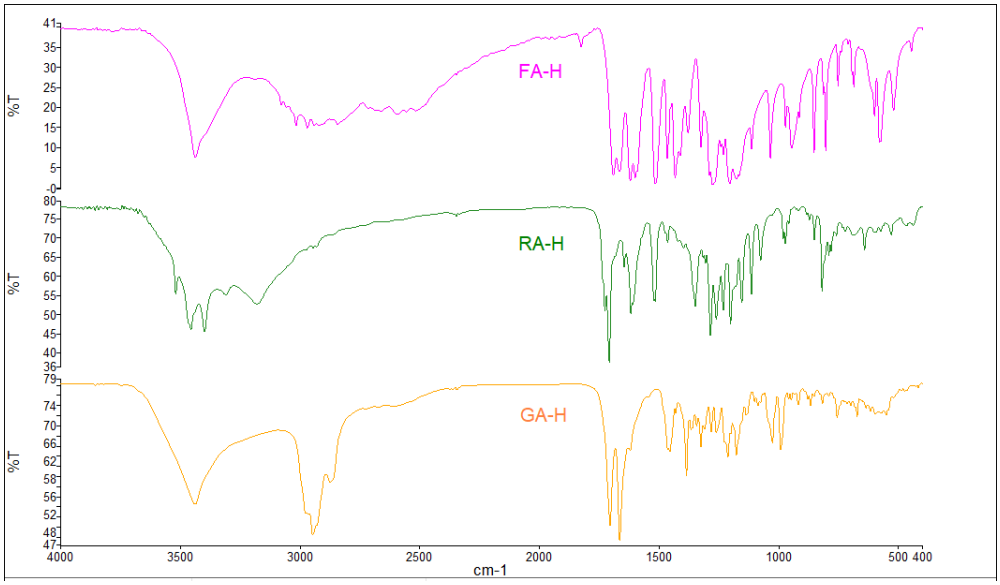


Figure S1. FT-IR spectra of commercial FA-H, RA-H, and GA-H.

Characterizations

X-ray diffraction (XRD) analysis was conducted at room temperature using an X'PERT PRO (PANalytical, Malvern, UK) powder diffractometer equipped with Cu Kα radiation (wavelength of 1.541874 Å), a nickel filter with a thickness of 0.02 nm, and a fast detector (PIXcel) with an active range of 3.347°. Spectra were acquired in the 2θ range from 1.5 to 30°, employing a step size of 0.0131° and a counting time of 207.5 s. The basal distance of the LDH was determined from the diffraction signal (003) using Bragg's law.

Infrared spectra were collected with a Fourier Transform Spectrometer (Spectrum Two, PerkinElmer, Waltham, MA, USA) in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}, averaging 16 scans. The FT-IR spectra of LDHs and organic anions were obtained by mixing the samples with potassium bromide powder. Data processing was performed using the Spectrum software (version 10.4.2, 2014, PerkinElmer, Waltham, MA, USA).

UV-Vis absorption spectra were collected at room temperature using a Jasco V-750 UV-Visible spectrophotometer equipped with an integrating sphere (Jasco International Co., Ltd. in Tokyo, Japan). To obtain calibration curves for FA-H, Na-FA, RA-H, GA-H, and Na-GA, the absorbance of solutions at different concentrations was measured, and data were fitted as a function of molar concentration. The absorbance was measured at the maximum wavelength for each molecule: 323 nm for FA-H, 311 nm for Na-FA, 333 nm for RA-H, 250 nm for GA-H, and 250 nm for Na-GA. The molar extinction coefficients were calculated from the linear fitting of each analysis: FA-H ε_{323} = 15,700 $M^{-1}\cdot cm^{-1}$, Na-FA ε_{311} = 14,600 $M^{-1}\cdot cm^{-1}$, RA-H ε_{333} = 18,800 $M^{-1}\cdot cm^{-1}$, GA-H ε_{250} = 10,700 $M^{-1}\cdot cm^{-1}$, and Na-GA ε_{250} = 10,400 $M^{-1}\cdot cm^{-1}$. To quantify the organic anions FA and RA in LDH-FA and LDH-RA, a known amount of each modified LDH (approximately 5 mg) was dissolved in 25 mL of a 1 M HCl aqueous solution. The GA content was determined by dissolving a known amount (2–3 mg) of LDH-GA in a few drops of concentrated HCl and then diluting it in EtOH. After appropriate dilution, the UV-Vis spectra of the solutions were recorded, and the amount of organic fraction present in the modified LDHs was determined. The solid-state UV-Vis spectrum of LDH-RA_1.1 was recorded in reflection mode with $BaSO_4$ background.

Thermogravimetric analysis (TGA) was performed using a Seiko SII TG/DTA 7200 EXSTAR instrument (Chiba, Japan). For each LDH, 5-10 mg was placed in 70 μL alumina crucibles and analyzed under a 200 mL/min airflow at a heating rate of 10 °C/min, with a temperature range of 30 to 900 °C.

Scanning electron microscopy (SEM) analyses were carried out at the "Centro per l'Integrazione della Strumentazione Scientifica - Università di Pisa (CISUP)" using a FEI Quanta 450 FEG-SEM.

DPPH assay

DPPH method was used to evaluate the antioxidant activity of FA-H, RA-H, LDH-FA, LDH-RA, and Tolox as a reference. The method was similar to that previously reported [23], in particular, methanol solutions of DPPH (about 6×10^{-5} M), RA-H (about 1×10^{-4} M), FA-H (about 1.4×10^{-3}) and Trolox (about 2×10^{-3} M) were prepared. Suspensions of LDH-RA and LDH-FA (0.5 mg/mL) were also prepared and sonicated for 10 min to promote delamination of LDH-XA. 3 mL of the DPPH solution were mixed with different aliquots of FA-H or RA-H solutions. A blank DPPH solution was prepared by adding MeOH to the DPPH solution. In the case of LDH-FA and LDH-RA, 3 mL of the DPPH solution were mixed with different aliquots of LDH-FA or LDH-RA suspension and MeOH was also added to reach a final volume of 3.18 mL. All solutions were kept in the dark for 24 h, and absorbance at 515 nm of all samples was recorded after 24 h. Each sample was analyzed three times, and the average values of the parameters were reported. The percentage of DPPH reduction (I%) (Eq. 1) was calculated as a function of the antioxidant concentration, and linear fitting of the experimental data was performed to obtain the EC_{50} value defined as the antioxidant concentration corresponding to I% = 50.

$$I\% = \left[\frac{(A_0 - A_t)}{A_0}\right] \times 100 \quad \text{Eq. (1)}$$

where: A_0 is the absorbance of the DPPH solution in the absence of the antioxidant at t = 24 h; A_t is the absorbance of the DPPH solution in the presence of the antioxidant at t = 24 h.

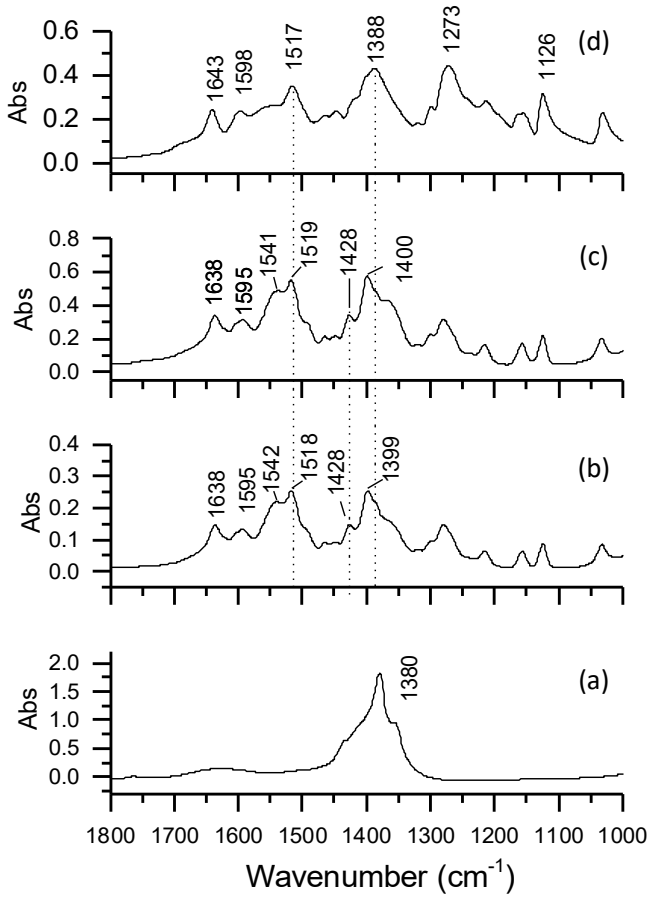


Figure S2. FT-IR spectra in the region 1800-1000 cm^{-1} of (a) LDH-NO_3, (b) LDH-FA_1.1, (c) LDH-FA_1.2, and (d) NaFA.

Table S2. Frequencies of the carboxylate asymmetric and symmetric stretching modes and frequency difference values ($\Delta\nu = \nu_{as} - \nu_s$) between carboxylate stretching modes of NaFA and LDH-FA samples

Sample	ν_{as} (cm^{-1})	ν_s (cm^{-1})	$\Delta\nu = \nu_{as} - \nu_s$ (cm^{-1})
NaFA	1517	1388	128
LDH-FA_1.1	1518	1399	119
	1542	1428	114
LDH-FA_1.2	1519	1400	119
	1541	1428	113

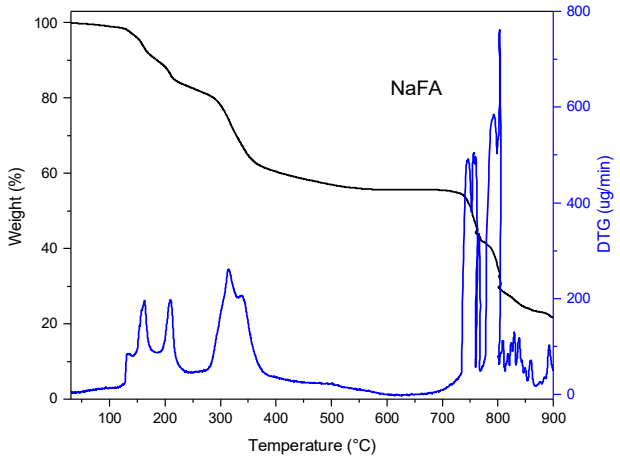


Figure S3. TG/DTG thermogram of NaFA.

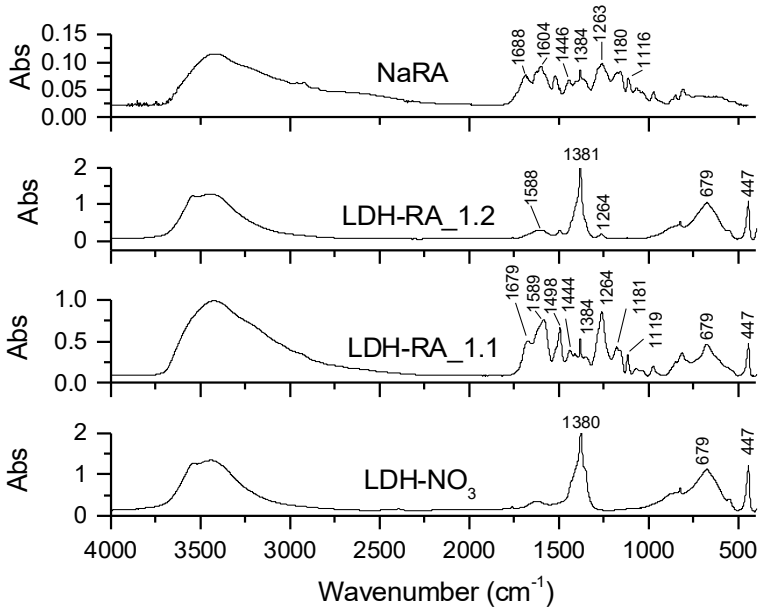


Figure S4. Representative FT-IR spectra of LDH-NO_3, LDH-RA_1.1, LDH-RA_1.2, and NaRA.

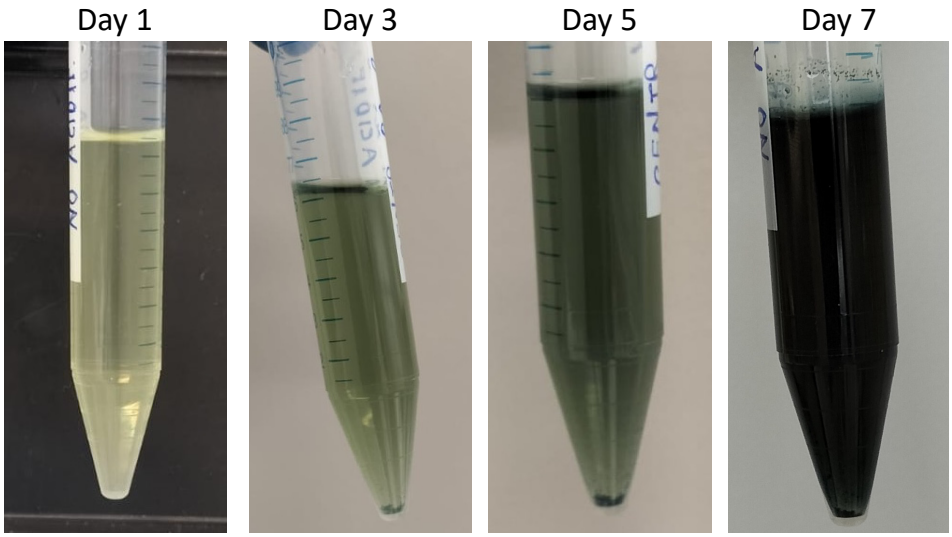


Figure S5. Images of the centrifugate-RA_1.1 acquired over 7 days (unprotected storage).

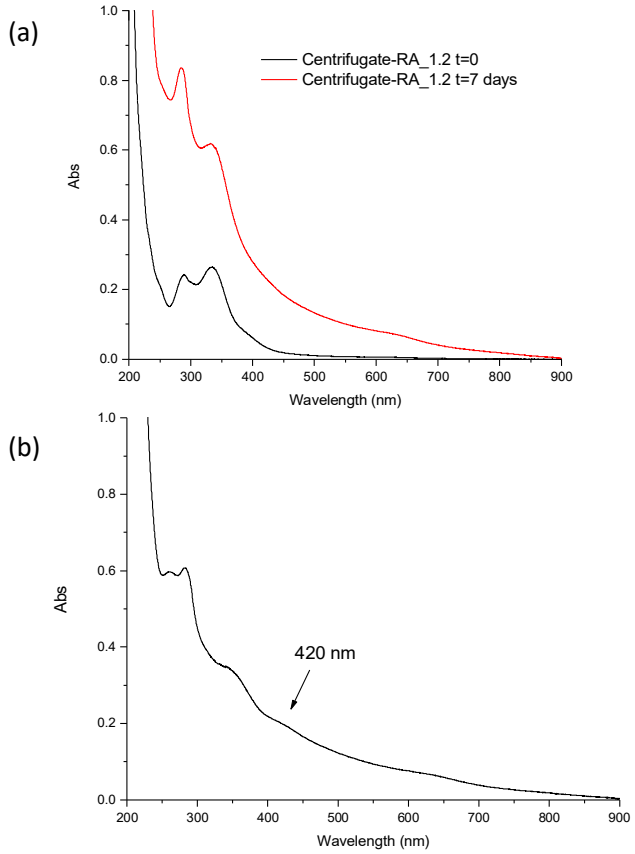


Figure S6. UV-Vis spectra of centrifugate-RA_1.1 at time zero and after seven days of storage in a non-inert atmosphere; difference between the two spectra (b).

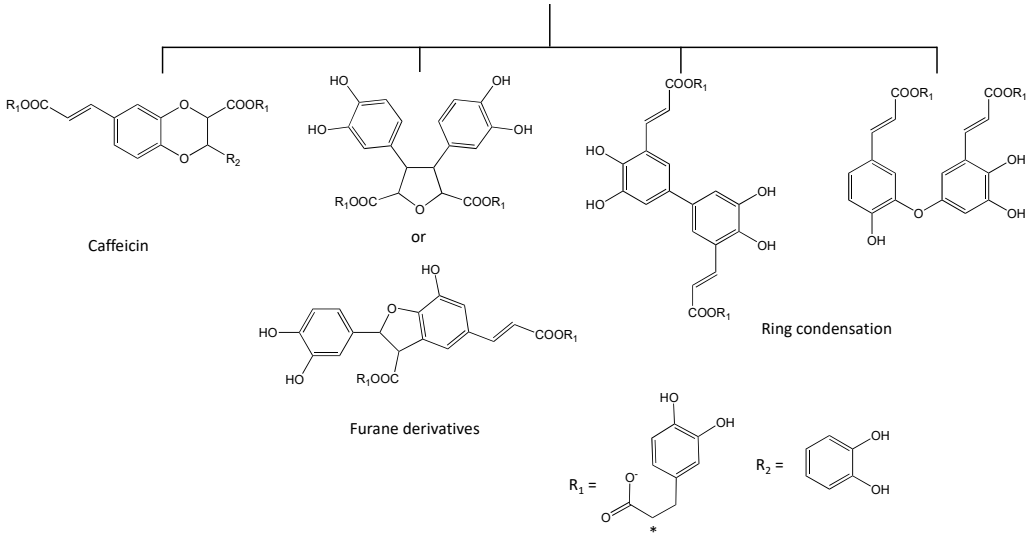


Figure S7. Possible autoxidation mechanisms of RA-H and derived products; other isomers are possible. *Position of bond with the other carboxylic group of RA.

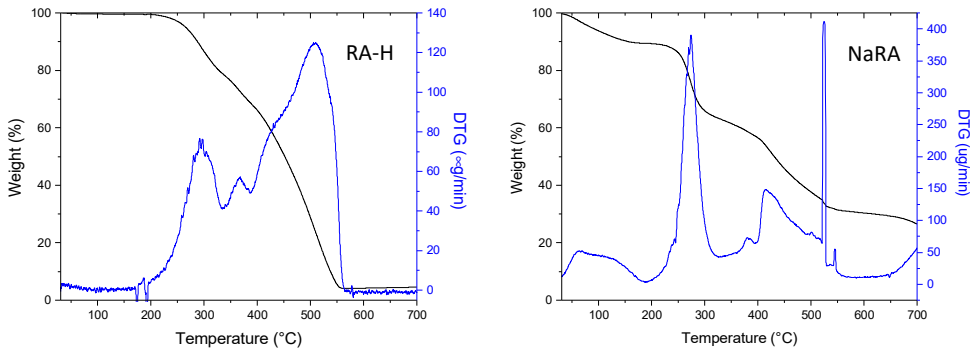


Figure S8. TG/DTG thermograms of RA-H and NaRA.

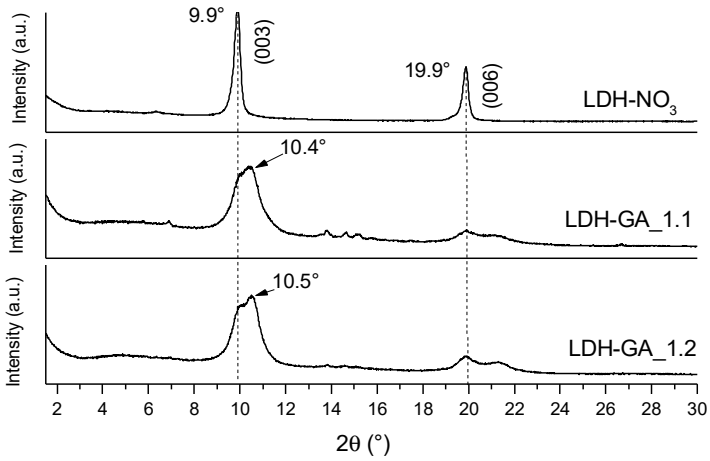
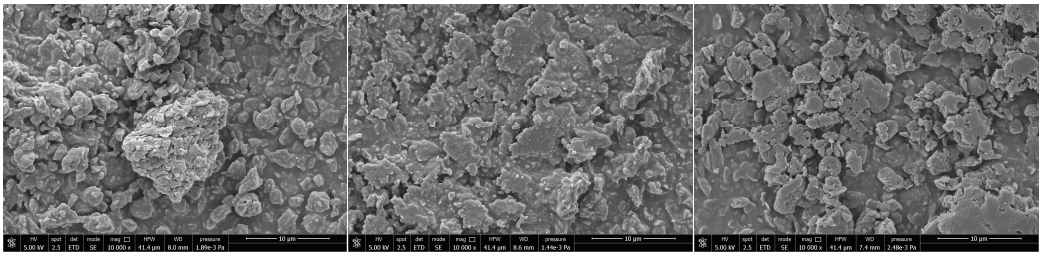


Figure S9. XRD patterns of LDH-NO_3, LDH-GA_1.1, and LDH-GA_1.2 in the 2Θ interval 1.5-30°.



(a) (b) (c)

Figure S10. Representative SEM micrographs of LDH-FA (a), LDH-RA (b), and LDH-GA (c).

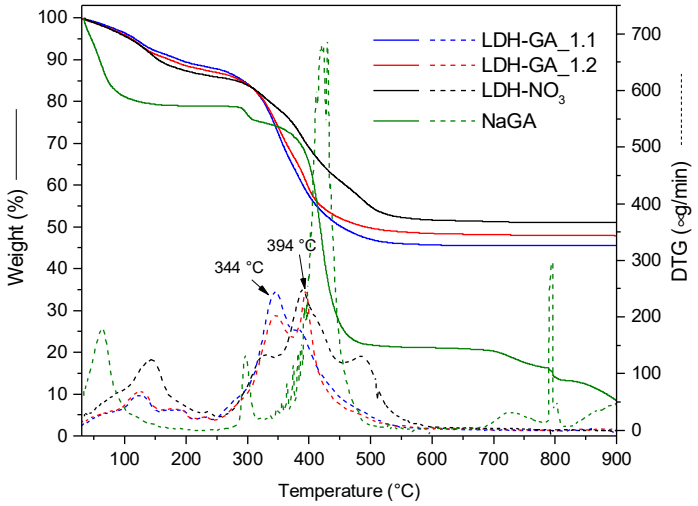


Figure S11. TG/DTG thermograms of LDH-GA_1.1, LDH-GA_1.2, LDH-NO_3, and NaGA.