Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

A sustainable porous composite material based on Loofah-Halloysite for gas adsorption and drug delivery

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Experimental Procedures

General information

Loofah sponge was purchased from Ibérica de Esponjas Vegetales, SL. All the required chemicals were purchased from Merck and Aldrich Chemical Company. Pre-coated aluminum sheets (silica gel 60 F254, Merck) were used for thin-layer chromatography (TLC), visualizing spots under UV light. UV-vis measures were carried out with a Jasco V-730 spectrophotometer using quartz cuvettes.

Synthesis of LHCx and LHNx composites

100 mg of loofah and 500 mg of halloysite ($Al_2Si_2O_5(OH)_4$ 2 H_2O, MW: 294.19) were suspended in 10 mL of DMF and sonicated for 10 minutes at room temperature. Subsequently, the quantity of crosslinker C (1,1'-carbonyldiimidazole) or N (1,4,5,8-naphthalenetetracarboxylic dianhydride) was added with respect to halloysite moles, depending on the composite to be obtained: for the composites LHC2 and LHN2, 2 eq. of crosslinker were added; for the composites LHC10 and LHN10, 10 eq. of crosslinker were added; for the composites LHC20 and LHN20, 20 eq of crosslinker were added. In all cases, such a quantity of DMF was added to solubilize the added crosslinkers. The reaction mixture was stirred at 80 °C. Once the reaction was completed, the raw product was washed and sonicated with acetone several times until the solution was clear and dried under vacuum.

Characterizations

Infrared spectroscopy

The FT-IR ATR spectra were recorded in absorbance mode (64 scans and 4 cm^{-1} resolution) using a Varian 640-IR by Agilent Technologies, with a ZnSe crystal fixed at the incident angle of 45°. A 10 mm fibers of each sample were mounted on top of ATR crystal and pressed gently by a pre-mounted sample clamp.

Thermogravimetric analysis (TGA)

Thermogravimetric analyses were conducted on a TA Instruments Q5000 IR. The tests were conducted with a temperature ramp of 10 °C/min from 25 °C to 900 °C under nitrogen flow of 25 cm^3 min^{-1} atmosphere, followed by 15 min isotherm at 900 °C in air flux. About 10 mg of each sample was collected from different points in order to analyze the sample homogeneously.

High-resolution transmission electron microscopy (HRTEM)

HRTEM micrographs on L, H, and LH samples taken from the sonicated suspensions, were carried out with a Philips CM 200 field emission gun microscope operating at an accelerating voltage of 200 kV. Few drops of the suspension were deposited on a 200-mesh lacey carbon-coated copper grid and air-dried for several hours before analysis. During the acquisition of HRTEM images, the samples did not undergo structural transformation. Low beam current densities and short acquisition times were adopted. The Gatan Digital Micrograph software was used to estimate the dimensions of the halloysite nanotubes.

Scanning electron microscopy (SEM)

The morphology of the loofah and LHNx composites was analyzed by scanning electron microscopy (SEM). The specimens, mounted onto aluminum stubs, were sputter-coated with gold and examined with a StereoScan 360 Cambridge microscope at 10 kV. Samples were observed at 2000× and 10000× magnification, respectively.

Tensile Tests

The tensile tests were performed on a TA Instruments Rheometric Series RSA III analyzer. A sample of 30 mm length was clamped into the two jaws of the machine. Each end of the jaws covered 4 mm of the sample. Reading of the tensile strength test instrument for Newton force and extension were initially set to zero. Tensile stress was applied until the failure of the sample was obtained. Four (4) specimens of each sample have been used to measure the above mechanical properties at ambient laboratory environment, and average results are reported.

CO_2 adsorption and CO_2-TPD measurements

The carbon dioxide adsorption experiments were carried out in a quartz U-shape reactor, utilizing 150 mg of the investigated sample and a CO_2 (99.999%) flow of 30 mL/min. The CO_2 was detected by a quadrupole mass spectrometer (Sensorlab VG Quadrupoles) following the m/z = 44 signal. The breakthrough curves of CO_2 were determined by measuring the ratio between the concentration of CO_2 after the achievement of saturation in the sample and the initial carbon dioxide concentration (*i.e.*, without the sample). Before the measurements, the materials were pre-treated in He flow (50 mL/min) at 100 °C for 1 h to remove eventual impurities from the sample's surface. The amount of CO_2 adsorbed was calculated considering the following formula q (mg/g) = $[(C_{in} - C_f) \times t \times Q]/w$, where w is the weight of the examined sample (g), Q is the CO_2 flow rate (mL/min), t is the time (min) at which the saturation was

achieved, and C_{in} and C_f are the initial and final (at the saturation) CO_2 concentrations (mg/mL), respectively.[1] All the measurements were performed at room temperature and atmospheric pressure.

The adsorption kinetics of CO_2 was determined with a thermogravimetric analyzer (Linseis STA PT 1600 instrument). The examined sample was degassed under a He stream (50 mL/min) at 100 °C for 1 h. When the experimental temperature was stable at 25 °C, the CO_2 gas was fed into the test chamber with a flow rate of 30 mL/min, and the weight variation with time was recorded. The measurement's error is within 3%.

The CO_2 TPD (Temperature Programmed Desorption) measurements were carried out in the same type of reactor utilizing 150 mg of sample. The CO_2 flow (30 mL/min) was stopped for these determinations after adsorption and surface saturation processes. Subsequently, the reactor was heated from 30 °C to 400 °C (10 °C/min). After desorption, the products were analyzed with the mass spectrometer. Also, in this case, the samples were pre-treated with a He flow (50 mL/min) for 1 h at 100 °C.

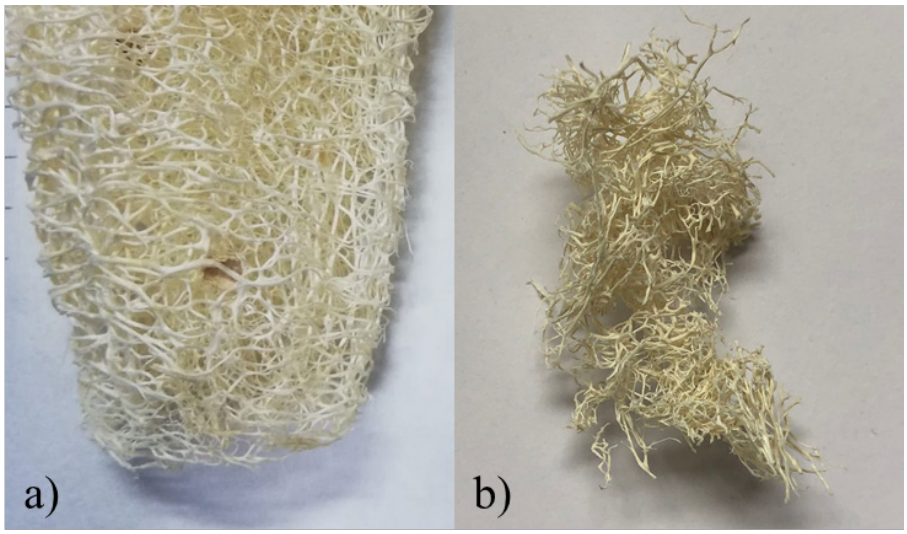

Fig. S1 Loofah sponge (a) before of the functionalization and (b) after.

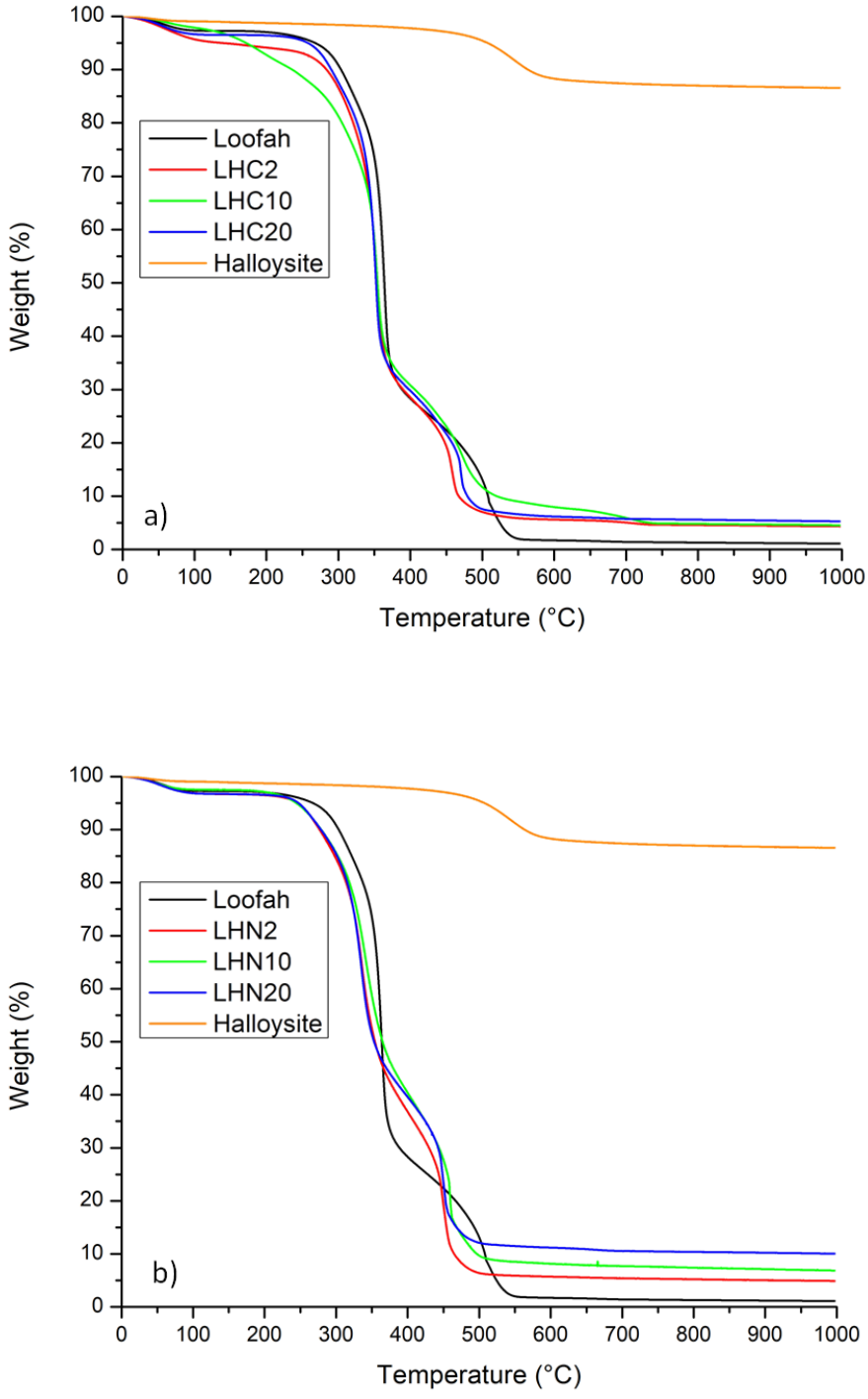

Fig. S2 TGA traces of H (orange line), L (black line) and LH composites: a) LHC2 (red line), LHC10 (green line) and LHC20 (blue line); b) LHN2 (red line), LHN10 (green line) and LHN20 (blue line).

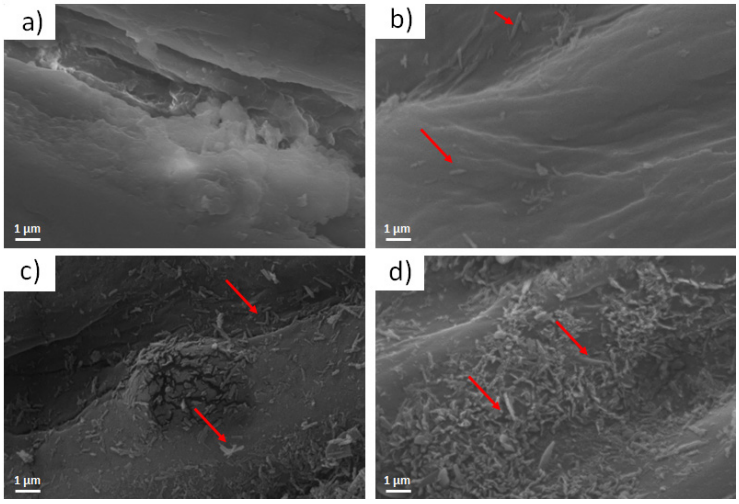

Fig. S3 SEM micrographs of Loofah (a), LHN2 (b), LHN10 (c) and LHN20 (d) composites. Red arrows indicate halloysite nanotubes on the loofah surface.

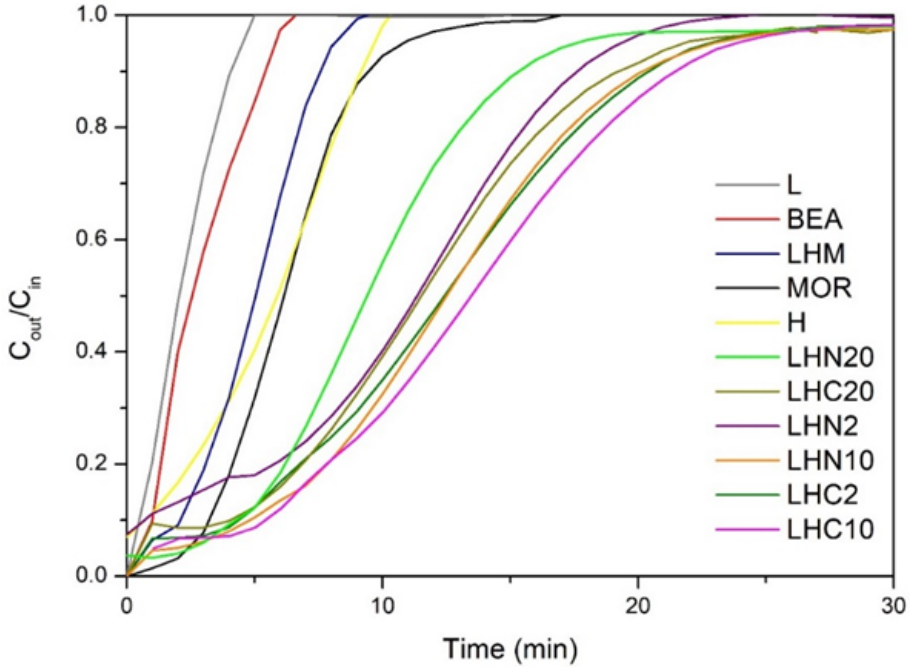

Fig. S4 Breakthrough curves of CO_2 for the investigated samples.

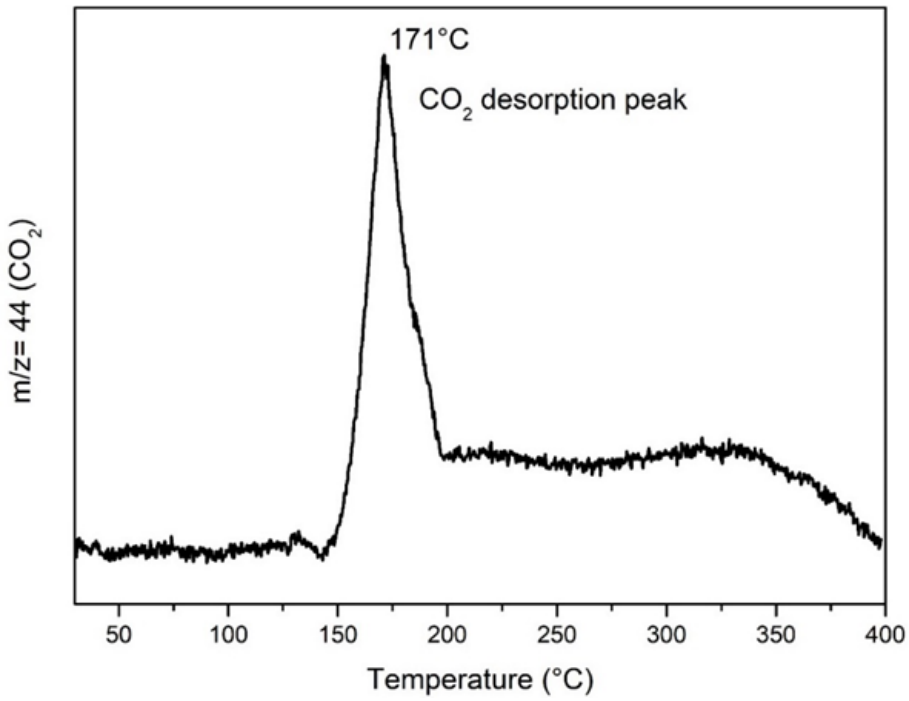

Fig. S5 CO_2-TPD profile of LHC2 composite.

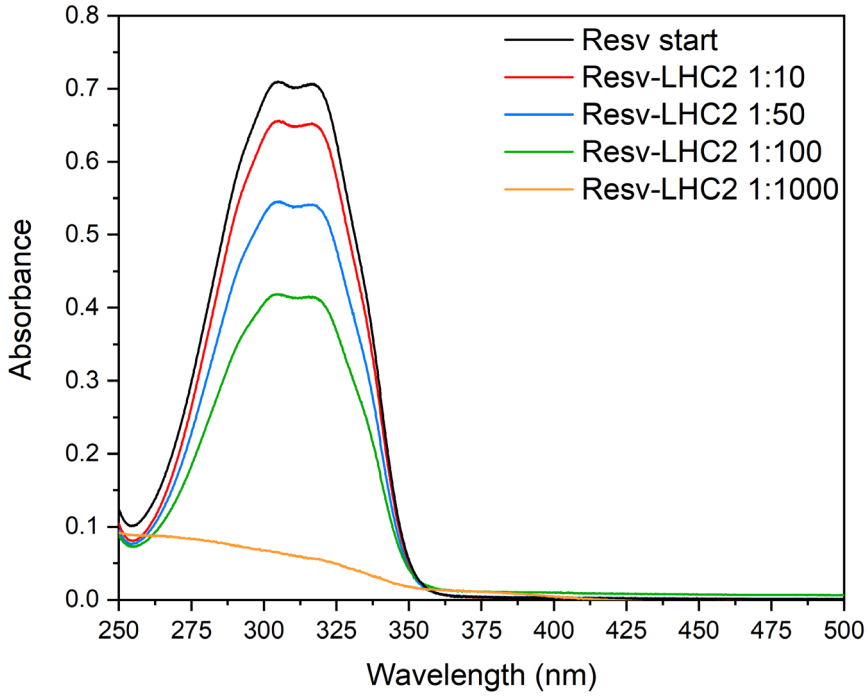

Fig. S6 UV spectra of the loading capacity of resveratrol on the LHC2 composite with different ratios.

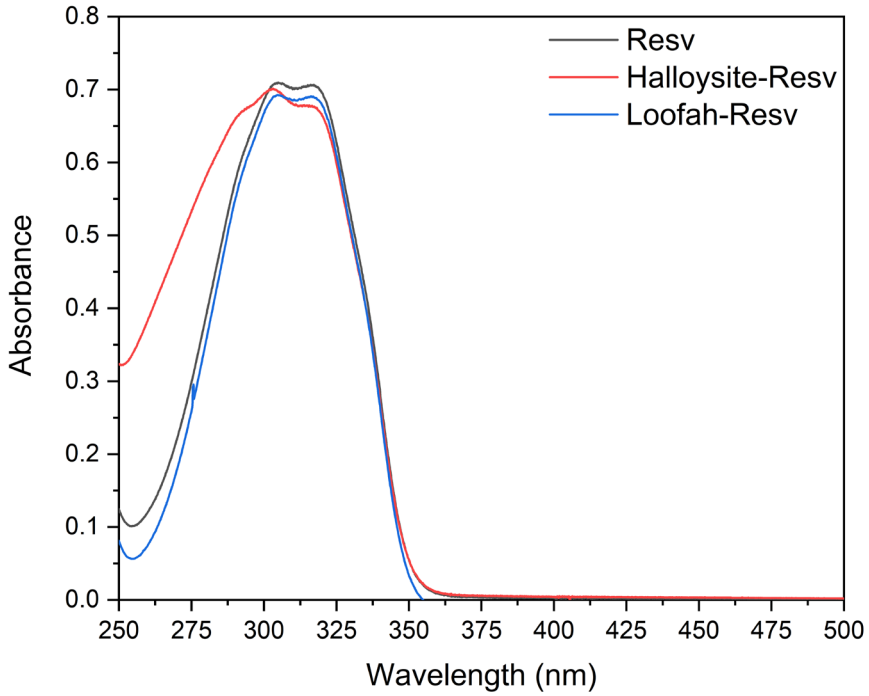

Fig. S7 UV spectra of the loading capacity of resveratrol on the individual starting materials.

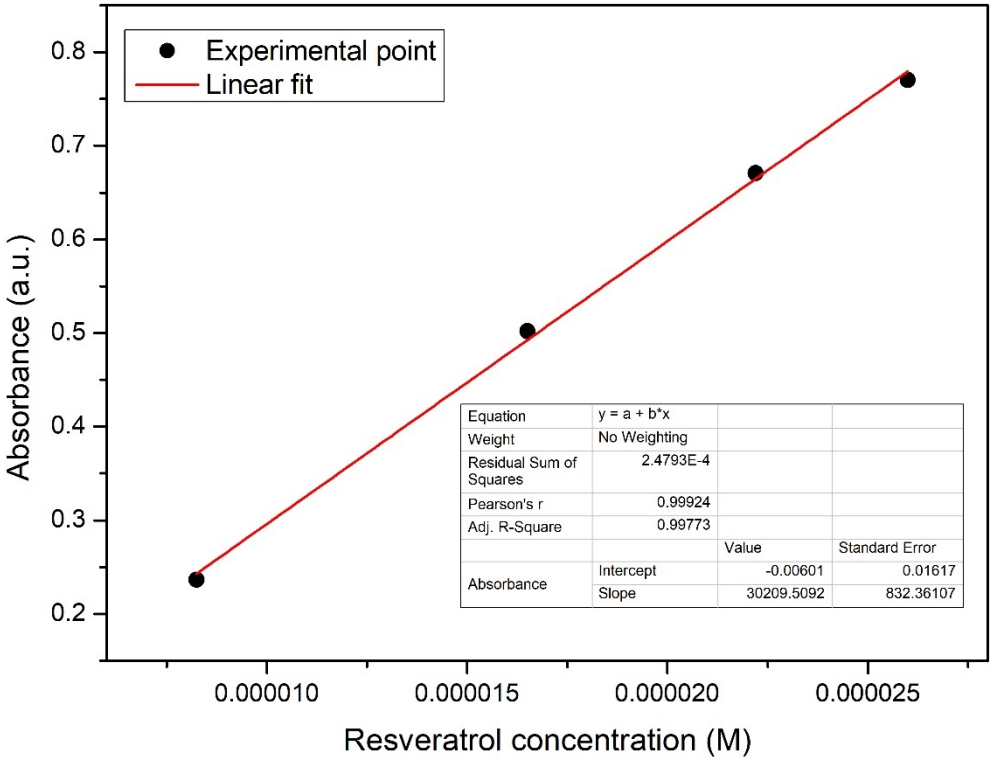

Fig. S8 UV-Vis calibration curve of resveratrol.

Table S1 Method adopted for the evaluation of tensile properties of Loofah and LH composites

Sample	Stress at break (N)	Elongation at break (%)
Loofah	4.16	9.46
LNH2	5.77	16.47
LNH10	7.96	18.50
LNH20	8.24	17.41
LNC2	5.80	14.10
LNC10	8.31	16.22
LNC20	9.48	13.71

Biological assay

The HCT 116 cells were plated in a 24-well plate at a density of 5×10^4/well. Cell viability was measured through the colorimetric tetrazolium salts assay. This assumption assumes total cellular integrity and assesses the ability of cells to reduce, employing the mitochondrial succinate dehydrogenase, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The tetrazolium salts enter the cells and are transformed into violet-colored formazan crystals. The level of formazan is used as an indirect index of cell density. After incubating the cells for 3 hours in 5% CO_2 at 37 °C, with 500 μL of the tetrazolium salt solution (5 mg/mL), the supernatant was removed, and 500 μL of DMSO per well was added for the elution of the crystals allowing the measurement of the optical density (O.D.) at a wavelength of 570 nm with the use of a spectrophotometer for microplates (Titertek Multiscan, Flow Laboratories).

Briefly, HCT 116 cells were seeded in the 24-well plate at a density of 5×10^4 cells in RPMI 1640 (Gibco) medium supplemented with 10% FBS, 1% Pen/Strep antibiotics, and 2 mM glutamine. After 24 hours from when they were sown, the treatments [LHC2-A (alone or associated to 50 μM of resveratrol) 1.63 mg/mL; LHC2-B (alone or associated to 100 μM of resveratrol) 3.26 mg/mL] were carried out according to the protocol. Cell viability was determined by MTT testing, following the supplier's instructions. Each test was conducted in triplicate, and the results were expressed as mean ± SD.

All experiments were conducted in triplicate, and the results were expressed as mean ± SD. The data was processed via one-way ANOVA (Tukey's multiple comparisons test), using a p-value <0.05 as threshold for statistical significance.

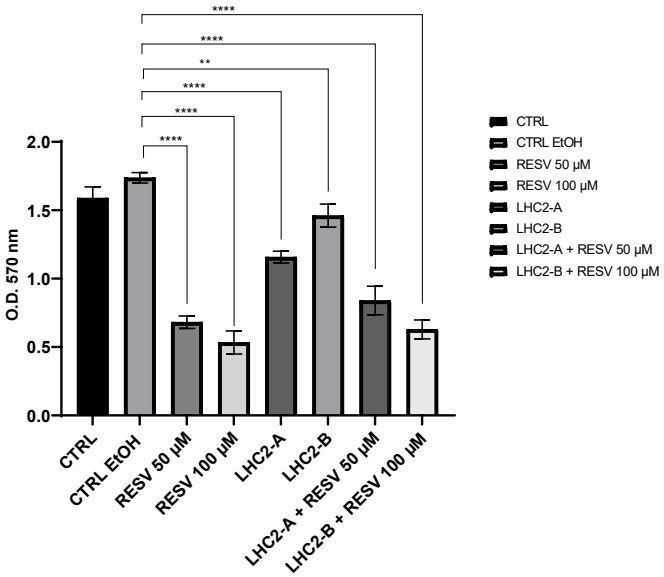

Fig. S9 Statistical analysis Control + EtOH versus drugs. The * is correlated to p-value <0.05; a major significance has been related to an increasing number of *.

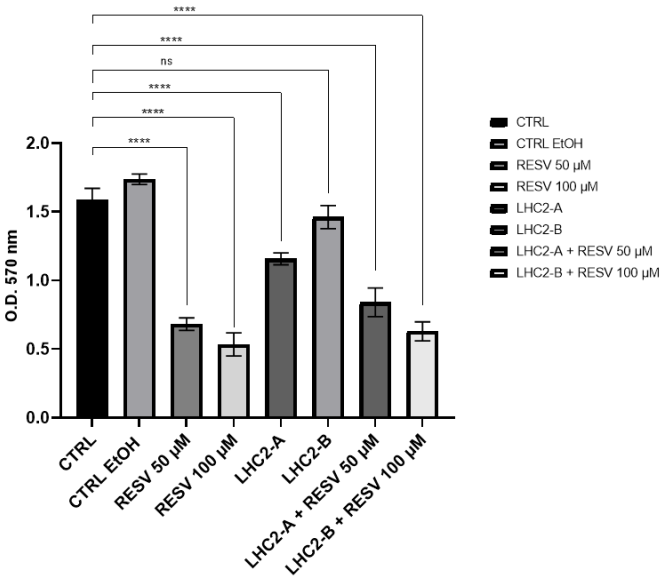

Fig. S10 Statistical analysis Control versus drugs. The * is correlated to p-value <0.05; a major significance has been related to an increasing number of *.

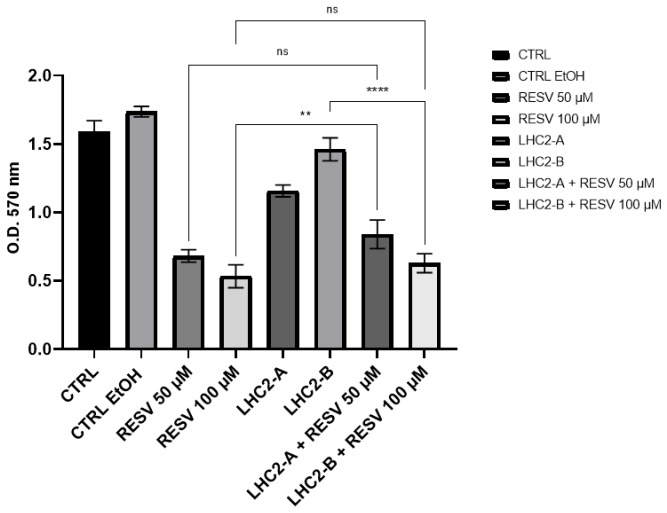

Fig S11 Statistical analysis of loaded LHC2 versus resveratrol and loaded LHC2 versus LHC2. The * is correlated to p-value <0.05; a major significance has been related to an increasing number of *.

Reference

1. C. Y. Lu, H. L. Bai, B. L. Wu, F. S. Su and J. Fen-Hwang, Comparative study of CO_2 capture by carbon nanotubes, activated carbons, and zeolites, *Energ Fuel*, 2008, **22**, 3050-3056.