Supporting information

Nanoparticles of lignin and saccharides from fishery wastes as sustainable UV-shielding, antioxidant and antimicrobial bio-fillers

Eliana Capecchi[1,], Elisabetta Tomaino[1], Davide Piccinino[1], Peter Elias Kidibule[2], Maria Fernández-Lobato[2], Daniele Spinelli[3], Rebecca Pogni[4], Ana Garcia Cabado[5], Jorge Lago[5], Raffaele Saladino[1,*].*

Supporting information S1 Production of Chit 33

Supporting information S2 DSC curves of KLNPs and OLNPs (Figure S2.1), **CH/KLNPs** and **CH/OLNPs** (Figure S2.2), and **COS/OLNPs** (Figure S2.3), respectively.

Supporting information S3 FT-IR of original CH 1-7 (Panel A) and OL (Panel B)

Supporting information S4 Morphological analysis of **CH/KLNPs** (Figure S4.1) and dimensional analysis of **CH/KLNPs** (Table S4.2).

Supporting information S5 UV-absorbing spectra of **CH/KLNPs**

Supporting information S6 Antimicrobial activity for **CH/OLNPs** and **CH/KLNPs**

Supporting information S7 UV-visible absorption spectra of **COS3/OLNPs** and OLNPs (Panel A), and translation of the UV-visible absorption spectra into Tauc's plots to calculate the HOMO-LUMO gap of **COS3/OLNPs** and OLNPs

Supporting information S1 Production of Chit 33

The gene *chit33* from *Trichoderma harzianum* CECT2413 fused to the *Saccharomyces cerevisiae* MF1 secretion signal was previously cloned in plasmid pIB4 and expressed in *Pichia pastoris* as previously reported[1]. The *P. pastoris* fermentation was obtained in a 5 L bioreactor (Biostart BPluss Sartorius Ltd., Gottingen, Germany) containing 3.5 L of medium (40 g/L glycerol, 26.7 mL H_3PO_4 85%, 0.93 g/L $CaSO_4$, 18.2 g/L K_2SO_4, 14.9 g/L $MgSO_4$, 4.13 g/L KOH, 2 mL biotin (0.2 g/L), and 4.35 mL of PTM1 trace salts). The fermentation parameters were maintained at 30 ˚C, 600 rpm agitation, 20% dissolved oxygen and pH was controlled at 5.0 units with NH_4OH 28% (v/v) during 24 h (~40 OD_{600} units). Then 100% methanol was added continuously during 4 days at 20 μL/ min L of fermentation volume to induce the expression of protein Chit33 (final ~300 OD_{600} units). Culture growth were monitored spectrophotometrically at 600 nm (OD_{600}) and protein concentration using NanoDrop at 280 nm. The cells were removed by centrifuging at 6000 × *g* for 15 min, then the extracellular fraction was concentrated using 10000 MWCO PES membranes in a Vivaflow 50 system (Sartorius, Gottingen, Germany).

Supporting information S2 DSC curves of KLNPs, and OLNPs (Figure S2.1), **CH/KLNPs** and **CH/OLNPs** (Figure S2.2) and **COS/OLNPs** (Figure S2.3), respectively.

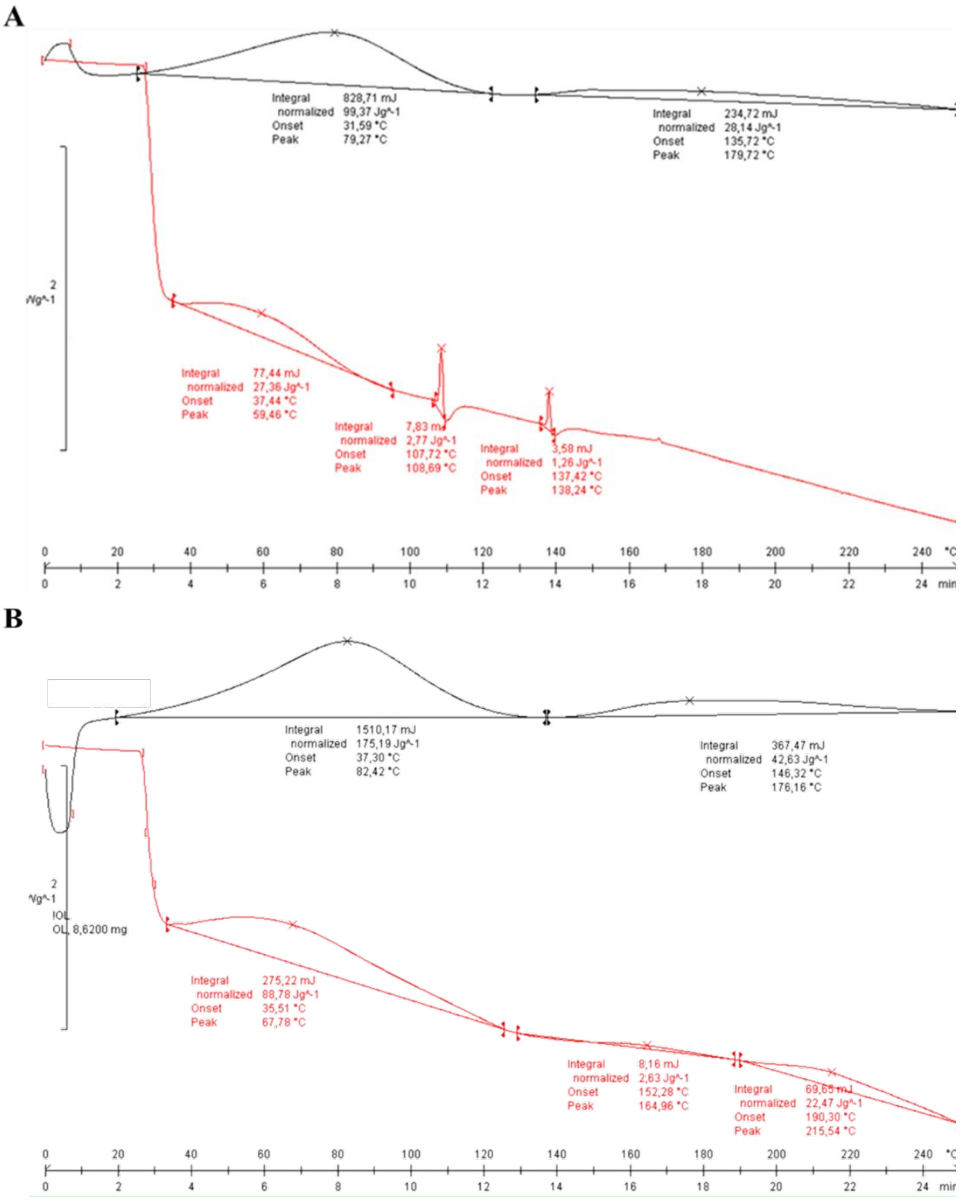


Figure S2.1 Panel A: DSC curves of KLNPs (red line) *versus* KL (black line). Panel B: DSC curves of OLNPs (red line) *versus* OL (black line).

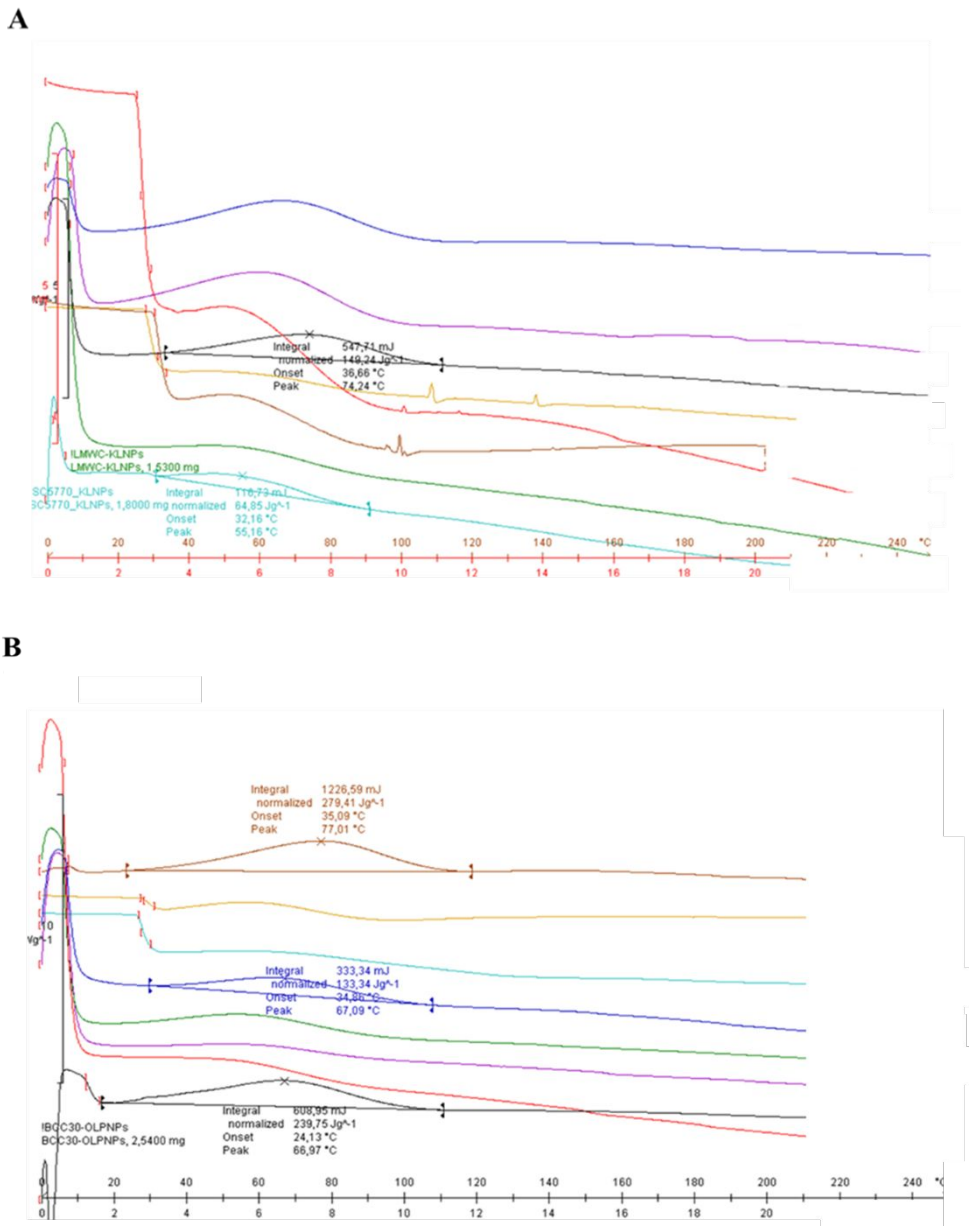
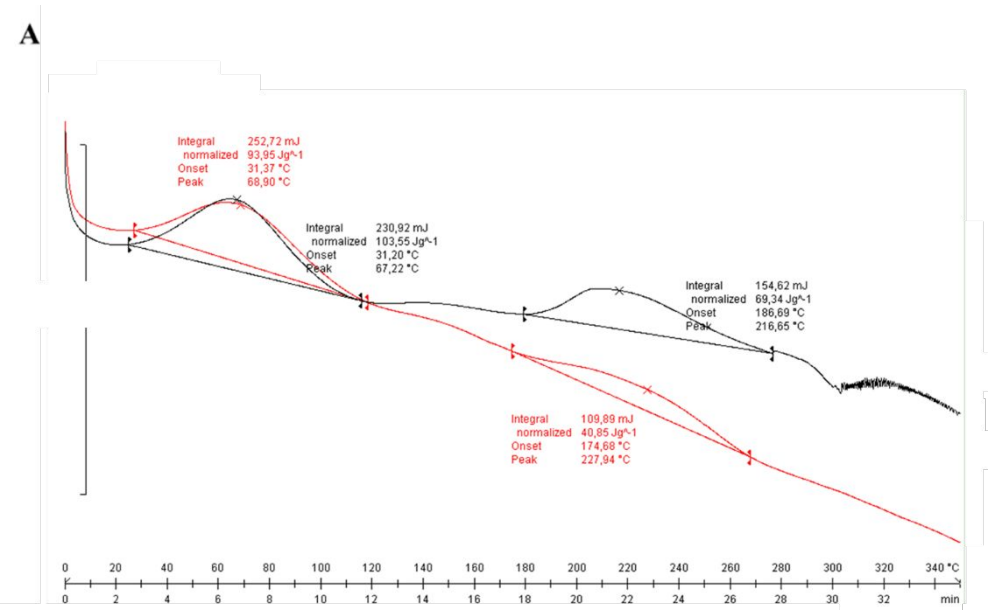
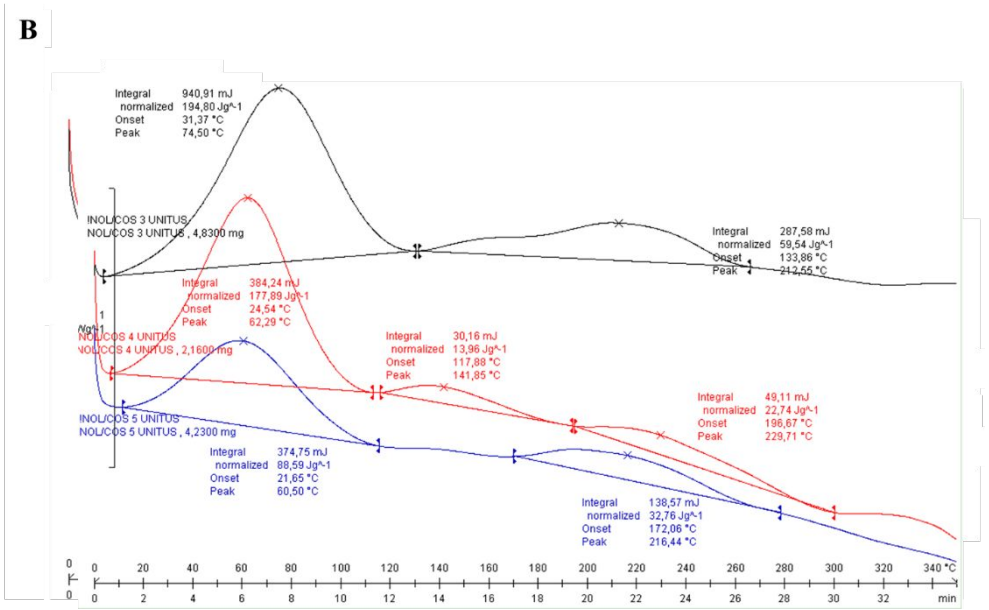


Figure S2.2 Panel A: DSC curves of **CH/KLNPs** *versus* KLNPs assigned with the following color code: KLNPs (yellow line), **CH1/KLNPs** (brown line), **CH2/KLNPs** (azure line), **CH3/KLNPs** (red line), **CH4/KLNPs** (black line), **CH5/KLNPs** (purple line), **CH6/KLNPs** (green line), **CH7/KLNPs** (blue line). Panel B: DSC curves of **CH/OLNPs** *versus* OLNPs assigned with the following color code: OLNPs (azure line), **CH1/OLNPs** (brown line), **CH2/KLNPs** (yellow line), **CH3/KLNPs** (red line), **CH4/KLNPs** (black line), **CH5/KLNPs** (purple line), **CH6/KLNPs** (green line), **CH7/KLNPs** (blue line).

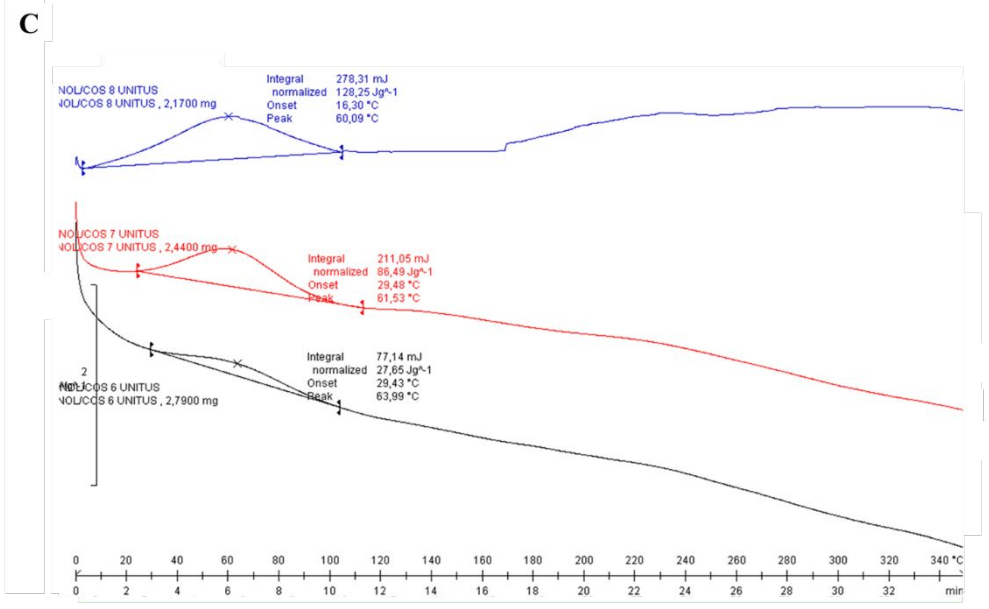
A


Integral 252,72 mJ
normalized 93,95 Jg^-1
Onset 31,37 °C
Peak 68,90 °C
Integral 230,92 mJ
normalized 103,55 Jg^-1
Onset 31,20 °C
Peak 67,22 °C
Integral 154,62 mJ
normalized 69,34 Jg^-1
Onset 186,69 °C
Peak 216,65 °C
Integral 109,89 mJ
normalized 40,85 Jg^-1
Onset 174,68 °C
Peak 227,94 °C
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 min

B


Integral 940,91 mJ
normalized 194,80 Jg^-1
Onset 31,37 °C
Peak 74,50 °C
INOLICOS 3 UNITUS
NOLICOS 3 UNITUS , 4,8300 mg
Integral 287,58 mJ
normalized 59,54 Jg^-1
Onset 133,86 °C
Peak 212,55 °C
Integral 384,24 mJ
normalized 177,89 Jg^-1
Onset 24,54 °C
Peak 62,29 °C
NOLICOS 4 UNITUS
NOLICOS 4 UNITUS , 2,1600 mg
Integral 30,16 mJ
normalized 13,96 Jg^-1
Onset 117,88 °C
Peak 141,85 °C
Integral 49,11 mJ
normalized 22,74 Jg^-1
Onset 196,67 °C
Peak 229,71 °C
INOLICOS 5 UNITUS
NOLICOS 5 UNITUS , 4,2300 mg
Integral 374,75 mJ
normalized 88,59 Jg^-1
Onset 21,65 °C
Peak 60,50 °C
Integral 138,57 mJ
normalized 32,76 Jg^-1
Onset 172,06 °C
Peak 216,44 °C
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 min

C


NOLICOS 8 UNITUS
NOLICOS 8 UNITUS , 2,1700 mg
Integral 278,31 mJ
normalized 128,25 Jg^-1
Onset 16,30 °C
Peak 60,09 °C
NOLICOS 7 UNITUS
NOLICOS 7 UNITUS , 2,4400 mg
Integral 211,05 mJ
normalized 86,49 Jg^-1
Onset 29,48 °C
Peak 61,53 °C
Integral 77,14 mJ
normalized 27,65 Jg^-1
Onset 29,43 °C
Peak 63,99 °C
NOLICOS 6 UNITUS
NOLICOS 6 UNITUS , 2,7900 mg
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 °C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 min

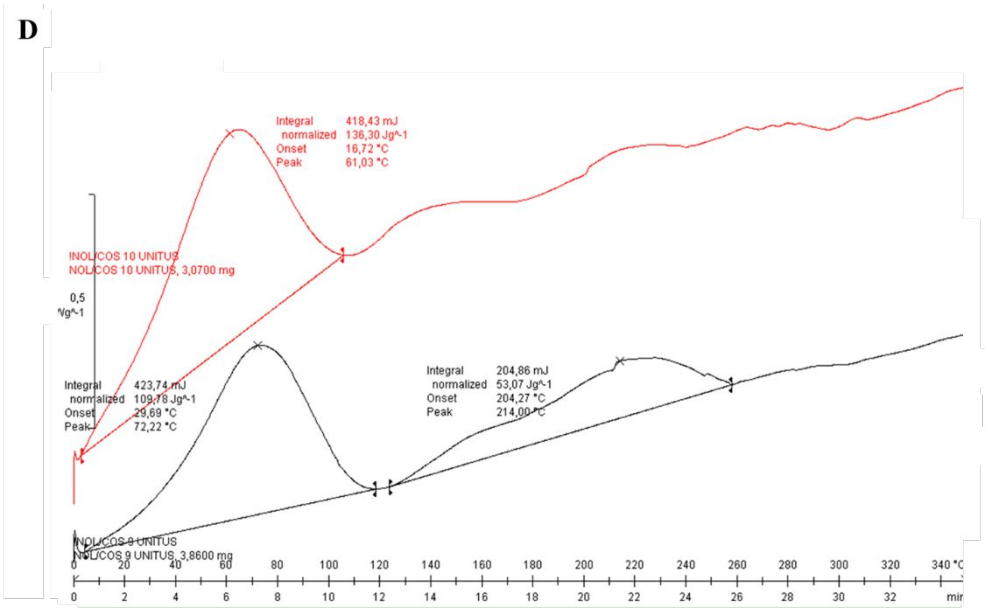
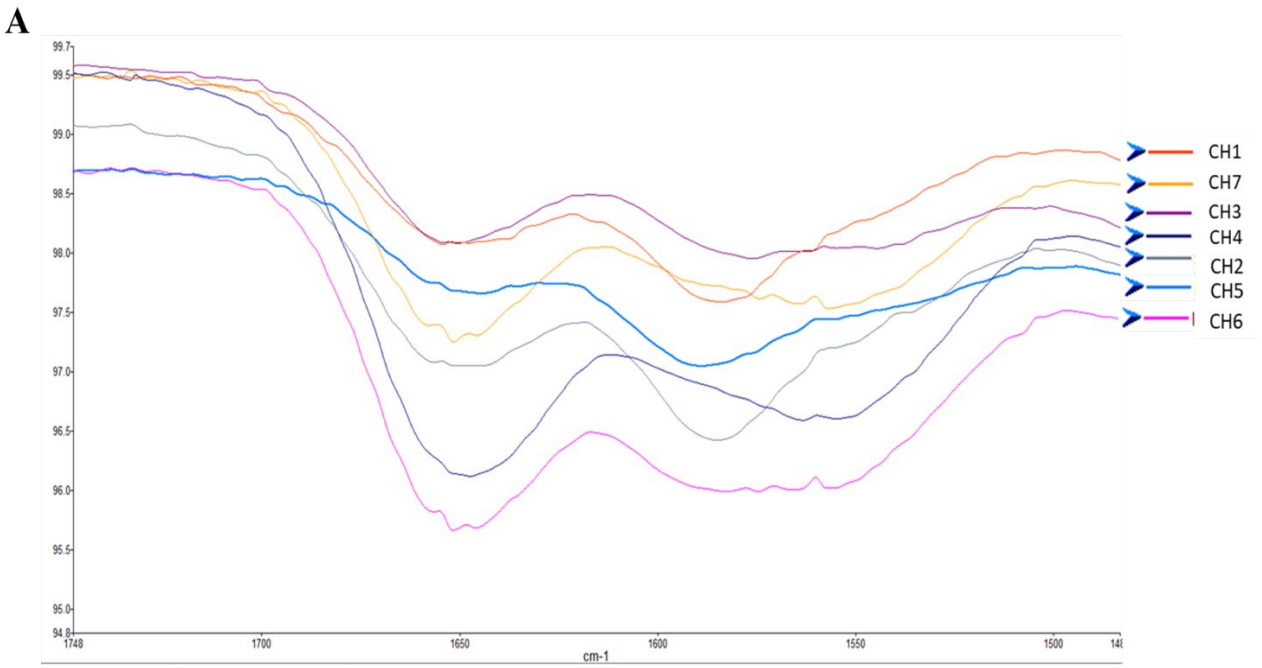


Figure S2.3 Panel A: DSC curves of **COS1/OLNPs** (black line) and **COS2/OLNPs** (red line). Panel B: DSC curves of **COS3/OLNPs** (black line)**, COS4/OLNPs** (red line) and **COS5/OLNPs** (blue line). Panel C: DSC curves of **COS6/OLNPs** (black line)**, COS7/OLNPs** (red line) and **COS8/OLNPs** (blue line). Panel D: DSC curves of **COS9/OLNPs** (black line) and **COS10/OLNPs** (red line).

Supporting information S3 FT-IR of original CH 1-7 (Panel A) and OL (Panel B)



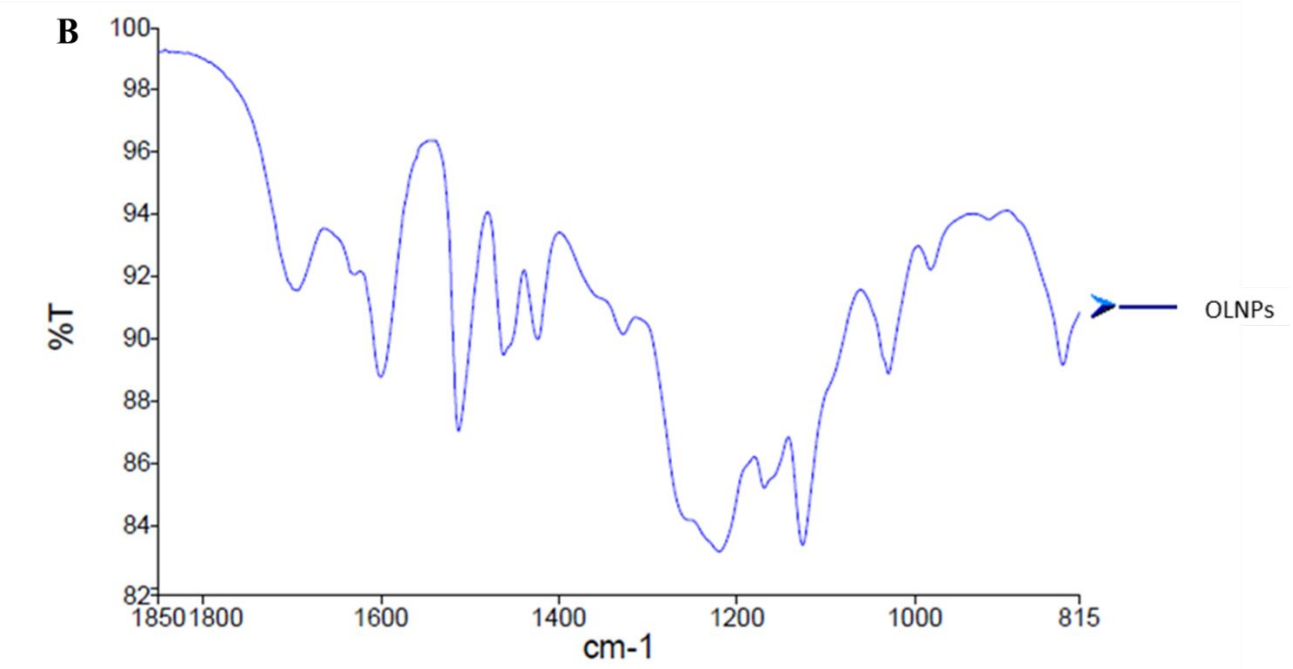


Figure S3. FT-IR spectra of original CH 1-7 and OL. Panel A: amide I band (1649 cm-1) and amide II band (1585 cm-1) of CH. Panel B: 1614 cm^{-1} (C=C Aromatic bending vibration), 1462 cm^{-1} (C–H deformations in $–CH_2–$ and $–CH_3$ vibration band), 1332 cm^{-1} (C=O stretching of syringyl unit), and 1116 cm^{-1} (aromatic C–H in plane deformation of syringyl unit), respectively.

Supporting information S4 Morphological analysis of **CH/KLNPs** (Figure S4.1) and dimensional analysis of **CH/KLNPs** (Table S4.2).

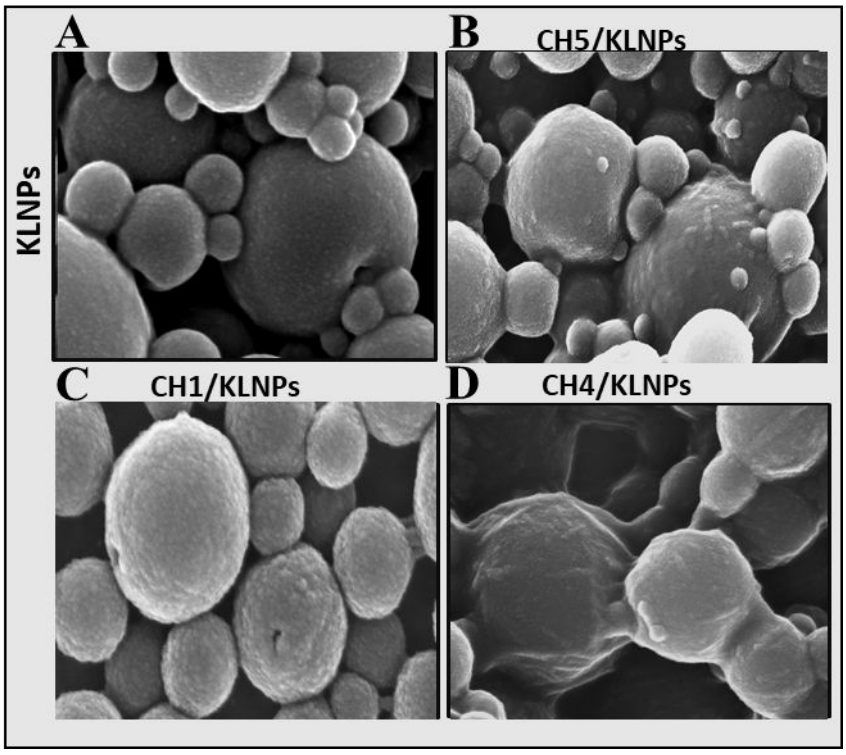


Figure S4.1. SEM images of **CH/KLNPs**. Panel A: **CH/KLNPs** bearing CH with high DD (**CH5/KLNPs** and **CH1/KLNPs**) and low DD (**CH4/KLNPs**), respectively.

Table S4.2 DLS and ζ-potential of CH/OLNPs and COS/OLNPs

Entry	Sample	Size(nm)	PDI[a]	ζ-Potential (mV)
1	**KLNPs**	210	0,35	-42
2	**CH1/KLNPs**	586	0,40	+69
3	**CH4/KLNPs**	551	0,54	+50
4	**CH5/KLNPs**	160	0,23	+30

[a]Polydispersion index.

Supporting information S5 UV-absorbing spectra of **CH/KLNPs**

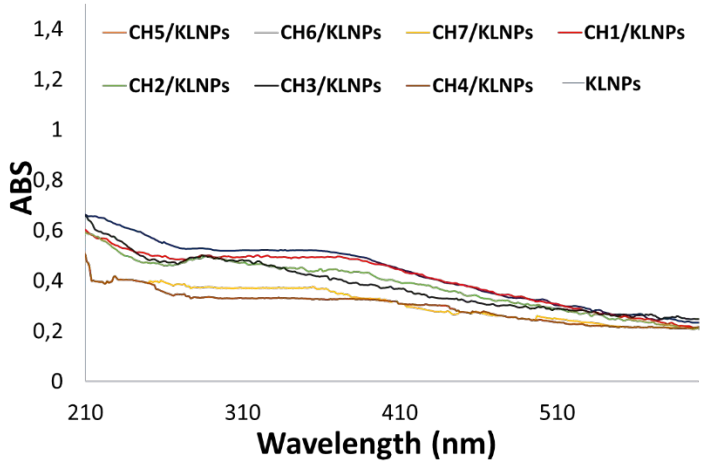


Figure S5. UV-absorbing capacity of **CH/KLNPs**

Supporting information S6 Antimicrobial activity of **CH/OLNPs** and **CH/KLNPs**

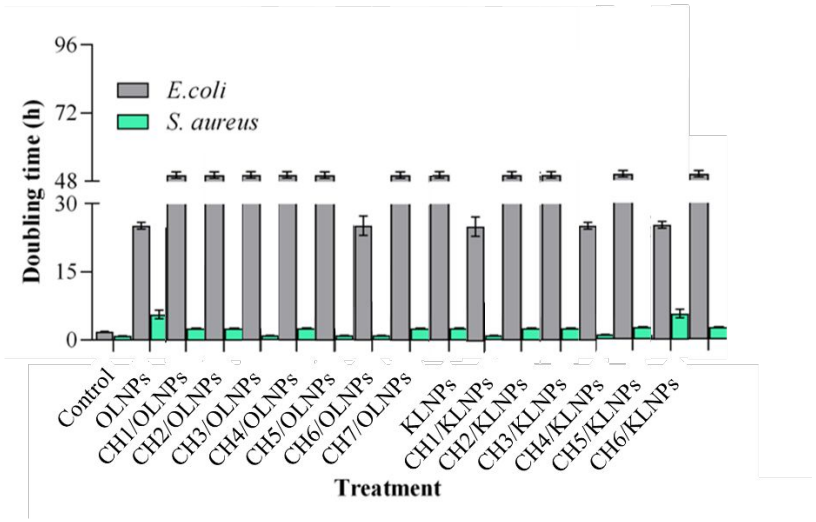
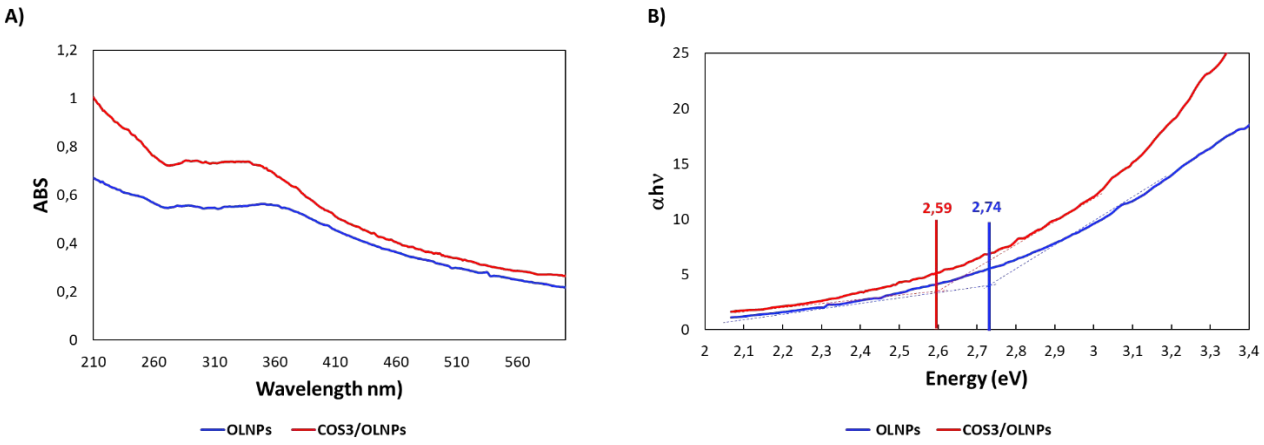


Figure S6. The doubling time for the referred Gram-negative and Gram-positive bacteria of CH/OLNPs and CH/KLNPs

Supporting information S7 UV-visible absorption spectra of **COS3/OLNPs**, and OLNPs (Panel A) and translation of the the UV-visible absorption spectra into Tauc's plots to calculate the HOMO-LUMO gap of **COS3/OLNPs**, and OLNPs



1. Kidibule, P. E.; Santos-Moriano, P.; Plou, F. J.; Fernández-Lobato, M. Endo-chitinase Chit33 specificity on different chitinolytic materials allows the production of unexplored chitooligosaccharides with antioxidant activity. J. Appl. Biotechnol. Rep., 2020, 27, 500.