Supplementary Information

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Materials and methods

Chemicals

Sodium hydroxide sinapinic acid (SA) and sodium carbonate were purchased from sigma Aldrich, USA and used without any further purification. Hydrochloric acid was purchased from Scharlau, Spain. *E. coli* (BCRC 12570) and *Staphylococcus aurius* were purchased from Bioresource Collection and Research Center (BCRC), Hsin-Chu, Taiwan and cultured as per the instruction provided by the company.

Chemical Processing of the Shrimp shells

Shrimp shells were collected manually from the local market in Taiwan. The collected shrimp shells were washed with deionized water (4 to 5 times) to remove residual impurities. The shells were kept for drying at 80 ^{o}C for 48 hours. The dried shells were crushed manually using motor and pestle into fine solid powder. Dried shell powder was treated with 1% NaOH solution for 6 hours to remove unwanted molecules. The treated power was washed and pH was adjusted to neutrality. It was further incubated at 60 ^{o}C for removing water content.

Instrumentation

TEM images of nanomaterials were obtained by using transmission electron microscope (TEM) (TEM-3010, JEOL, Tokyo, Japan). The UV-visible spectra of nanomaterials were recorded by using double beam UV–visible spectrophotometer (Hitachi U-3501, Tokyo, Japan). The elemental composition of nanomaterials was observed by energy-dispersive X-ray spectroscopy (EDXS). X-ray diffraction (XRD) patterns were obtained on XRD spectroscopy (Bruker D8 advance, Germany). Nano-materials functional groups were confirmed by Fourier transform infrared spectrometer (Bruker FT-IR IFS-48, Germany). Bacterial protein profile mass spectra were obtained using a MALDI-time-of-flight mass spectrometer (Microflex, Daltonics Bruker, Bremen Germany) equipped with a nitrogen laser (337 nm).

Synthesis of calcium oxide nano-plates

3.0 gm of dried prawn shells powder was treated with 1% hydrochloric acid solution for 6 h at room temperature. The solution was filtered using Whatman filter paper and liquid filtrate was collected in beaker. To this solution, 20 ml of 2N Na_2CO_3 was added drop wise till the solution turns white. Then the solution was stirred for 30 minutes make the homogeneous solution. The solution was transferred to Teflon coated stainless steel bench-top autoclave and incubated at 200 °C for 80 min. Finally, the resulting white precipitate was calcinated at 900 °C for 2 hrs.

Optical density (OD_{600}) measurements

First, all glassware and nano material were sterilized in autoclave at 121°C for 20 min. To investigate the bacterial kinetics of growth rate, *E. coli* (~10^6 cfu mL^{-1}) and *S. aureus* (~10^6 cfu mL^{-1}) cells were grown in 20 mL liquid LB medium which contain various concentrations of CaO NPs such as 5, 10, 20, 30, and 50 μg mL^{-1}. The bacteria cells growth rate was acquired by measuring the optical density at 600 nm (OD_{600}) of each sample at different time intervals.

Disk diffusion method

Agar disk diffusion method was used to further investigate the CaO NPs antibacterial activities qualitatively. *E.coli* (~10^6 cfu mL^{-1}) and *S.aures* (~10^6 cfu mL^{-1}) suspension were spread onto LB agar plates. 0.5 cm filter paper was dipped in 30 µg/mL of CaO NPs suspension and then placed on the surface of bacteria inoculated agar plates. For control experiment, filter paper was dipped into deionized water and then placed on the surface of bacteria inoculated agar plates. After incubating at 37 °C for 24 h, the zones of inhibition area was observed, measured and optical images of the plates were taken.

MALDI-MS Analysis

Sinapinic acid (50 mM) matrix solution was prepared in acetonitrile/deionized water (2: 1, v/v). To investigate the antibacterial activity by MALDI-MS, *E. coli* (~10^6 cfu mL^{-1}) and *S. aureus* (~10^6 cfu mL^{-1}) cells were grown in 20 mL liquid LB medium which contain various concentrations of CaO NPs such as 5, 10, 25, and 50 µg mL^{-1}. Then the samples were collected after 12 h and centrifuged at 36,220 g, 4 ^{0}C for 20 min using a high speed centrifuge (Mikro 22R, Hettich, Zentrifugen, Germany). The precipitate was collected and diluted with 50 µL of deionized water. 2 µL of these samples was spotted on the MALDI target plate and overlaid with the sinapinic acid. Finally samples were allowed to dry at room temperature and analyzed by MALDI-MS.

Supporting figures

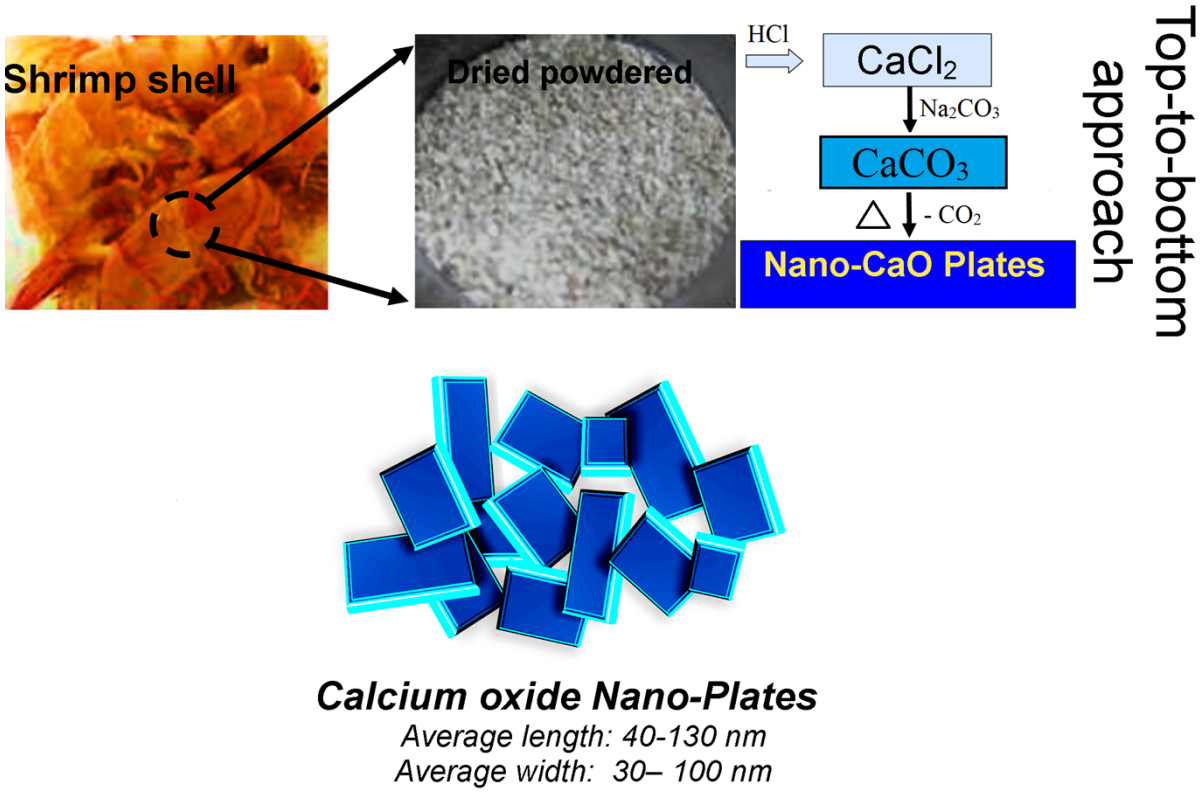

Figure S1. Schematic illustration of CaO nano-plates fabricated from Shrimp shells

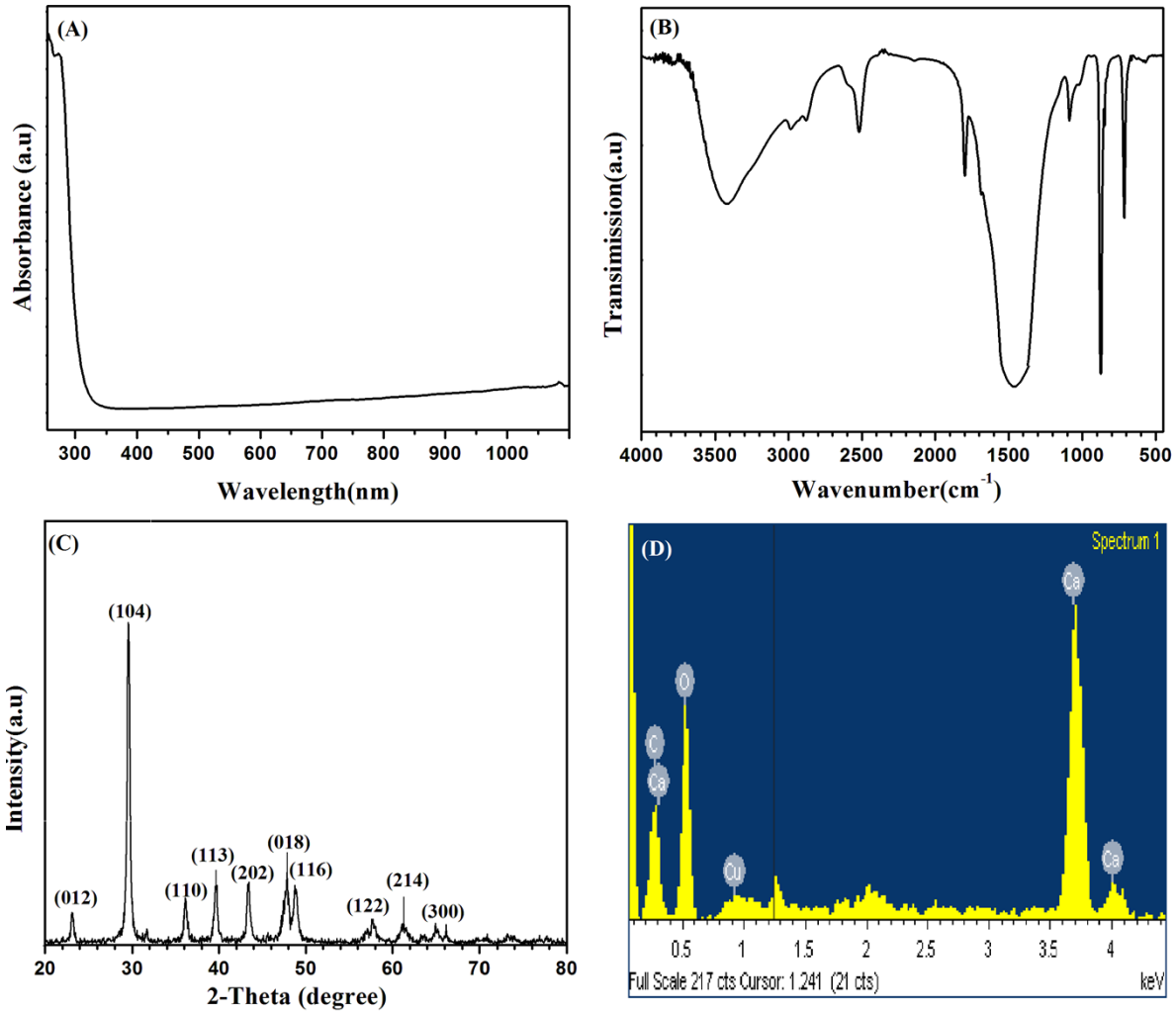

Figure S2. Characterization of $CaCO_3$ (A) UV-Visible spectrum (B) FT-IR Spectrum (C) XRD pattern (D) EDX pattern

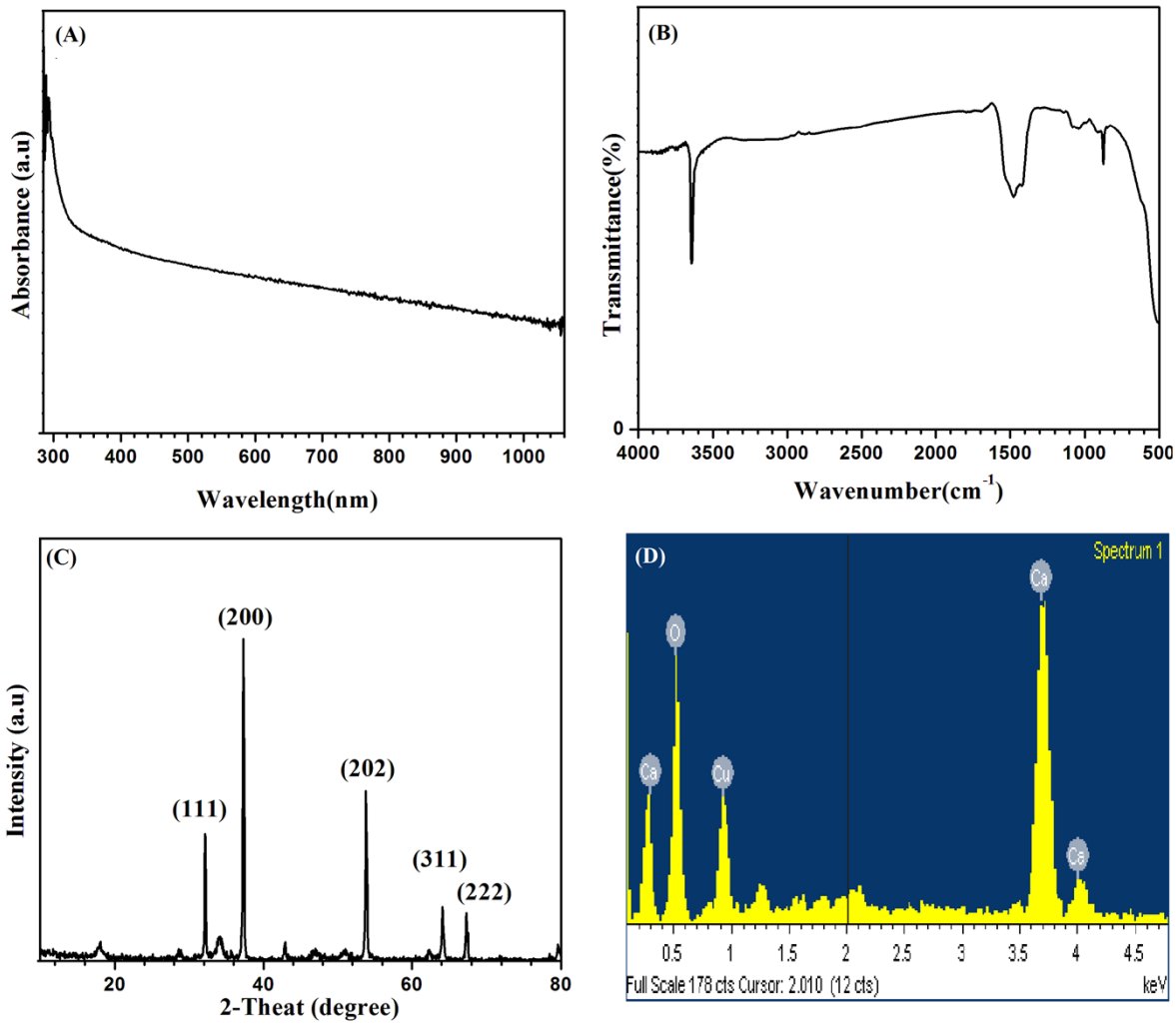

Figure S3. Characterization of CaO (A) UV-Visible spectrum (B) FT-IR Spectrum (C) XRD pattern (D) EDXS pattern

Table S1a: Red shift with respect to number of days explaining the optical stability of CaO nano-plates

No. of days	λ_{max}	Red shift (from 270 nm)
1	270.00	0.00
5	270.05	0.05
10	270.10	0.10
15	270.15	0.15

Table S1a: Red shift with respect to concentration of NaCl explaining the optical stability of CaO nano-plates

Concentration of NaCl(mM)*	λ_{max}	Red shift (from 270 nm)
100	270.1	0.1
200	270.5	0.5
300	270.8	0.8
500	272	2

*Total volume was adjusted to 3 ml

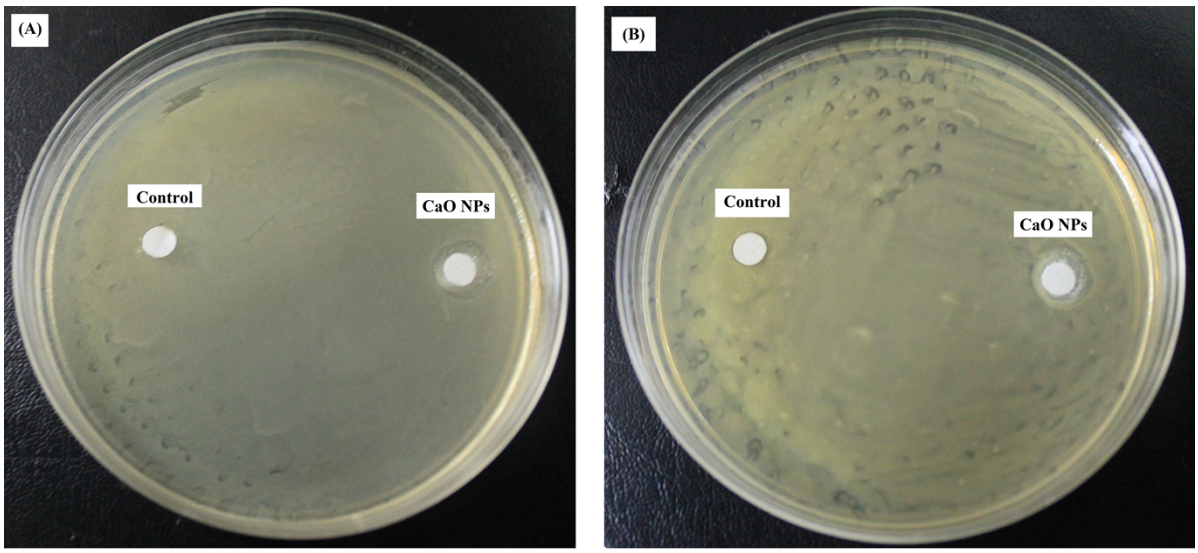

Figure S4. Zone inhibition of CaO nano-plates against (A) E.coli and (B) S.aureus

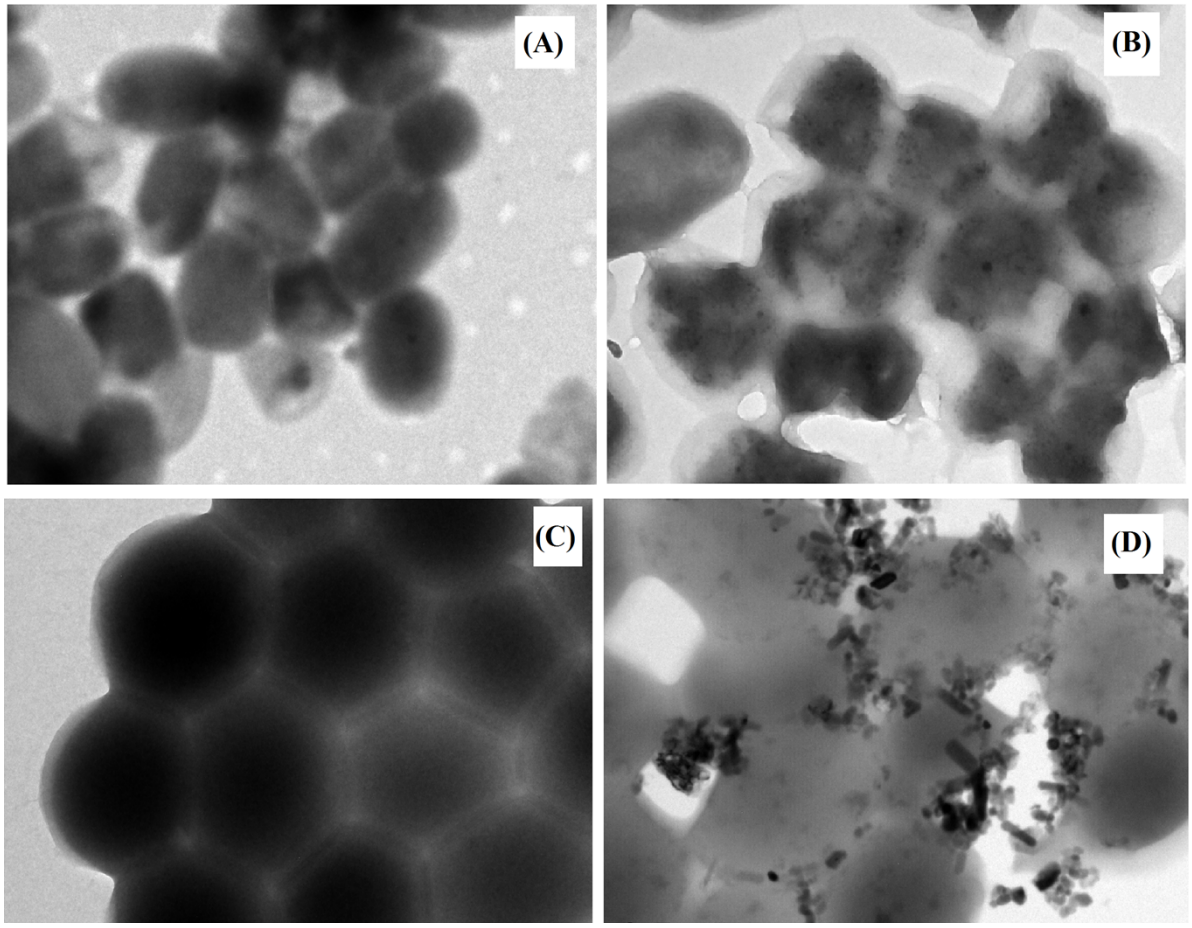

Figure S5. TEM showing the interactions between nanoparticles and bacteria (a) *E.coli* without nanoparticles (b) killed *E.coli* cells in presence of nanoparticles (c) *S.aureus* without nanoparticles and (d) killed bacterial cells in presence of nanoparticles