

SUPPLEMENTARY INFORMATION

Experimental

Antioxidant activity of longan seed extract (LE)

The antioxidant activity of LE was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity using DPPH method. The stock solutions of both LE and ascorbic acid in distilled water were serially diluted to obtain the solutions with final concentrations of 2500, 1250, 625, 313, 156, 78, 39, 20, 10 and 5 $\mu\text{g}\cdot\text{mL}^{-1}$. Exactly 1.0 mL of a methanolic solution of DPPH (100 μM) was added to 1.0 mL of each diluted solution, and the obtained mixtures were incubated for 30 min at room temperature in darkness. The absorbance of the final solution was determined spectrophotometrically at the wavelength of 517 nm. The antioxidant activity (%AA) of LE and ascorbic acid was expressed as the percentage decrease in DPPH compared with the level in the control solution (i.e., the testing solution without the presence of LE or ascorbic acid), according to the following equation:

$$\%AA = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (1)$$

Where A_{control} and A_{sample} are the absorbance values of the testing solution without and with the presence of LE or ascorbic acid.

In vitro cytotoxicity study of longan seed extract

The NCTC clone 929 cell line (ATCC® CCL-1™) was isolated from mouse subcutaneous connective tissue; areola and adipose. Cells were grown and maintained in a complete medium (Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 3.7 $\text{g}\cdot\text{L}^{-1}$ sodium bicarbonate, and 100 $\text{unit}\cdot\text{mL}^{-1}$ penicillin and 100 $\mu\text{g}\cdot\text{mL}^{-1}$ streptomycin) and incubated at 37 °C humidified incubator with 5% CO_2 . Cells at a logarithmic growth were harvested and diluted to 1×10^4 cells/mL in complete medium prior to assay. This assay was performed in four replicate wells in 96-well plate. First, plates were seeded with 200 μL of cell suspension or blank medium into well and incubated at 37 °C in a humidified incubator containing 5% CO_2 for 48 h. Subsequently, the culture medium was replaced with 200 μL of fresh medium containing test-compounds or 1% DMSO, and plates were further incubated for 24 h. After the incubation period, the plates were added with 50 μL of 125 $\mu\text{g}\cdot\text{mL}^{-1}$ resazurin solution and incubated at 37 °C in a humidified incubator containing 5% CO_2 for 4 h. Fluorescence was measured at 530 nm excitation and 590 nm emission wavelengths using the bottom-reading mode of a fluorometer. The signal was subtracted with the blank before calculation.

The percentage of cytotoxicity was calculated by the following equation:

$$\% \text{Cytotoxicity} = \left[1 - \left(\frac{\text{FUT}}{\text{FUC}} \right) \right] \times 100 \quad (2)$$

Where FUT and FUC are the mean fluorescent unit from cells treated with test compound and that treated with 1% DMSO, respectively. A threshold of 50% cytotoxicity was used as a cut off for cytotoxic activity of the compound, which can be classified by these criteria:

If % cytotoxicity was < 50%, the activity was reported as “Non-cytotoxic”

If % cytotoxicity was \geq 50%, the activity was reported as “Cytotoxic”

The IC₅₀ value was derived from dose-response-curve that was plotted between % cytotoxicity versus the sample concentrations by SOFTMax Pro software.

MTT assay

The culture medium in each plate was first removed and washed with 100 μ L of phosphate buffered saline (GIBCO, USA) solution. After that, 100 μ L of MTT solution (0.5 mg·mL⁻¹, AMRESCO, USA) was added to wells of 96-well TCPS. The plates were then incubated for 2.5 h at 37 °C. After incubation, the solution was removed. 100 μ L of DMSO was added to dissolve the formazan crystals. After 10 min of agitation, the absorbance of the solution was measured at the wavelength of 570 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments, USA). The indirect cytotoxicity results were expressed as the percentage of viable cells according to the following equation:

$$\text{Cell viability (\%)} = \frac{\text{OD}_{570(\text{Sample})}}{\text{OD}_{570(\text{control})}} \times 100 \quad (3)$$

Where OD_{570(Sample)} represents the absorbance obtained from the wells containing hydrogels and OD_{570(Control)} corresponds to the absorbance obtained from the wells containing fresh SFM (without hydrogels). All data are presented as the mean of three measurements.

Stability of thermosensitive chitosan/ β -glycerolphosphate (CS/ β -GP) hydrogels

The stability of the thermosensitive hydrogels at different pH values of media at 37 °C was evaluated using the gelation time. The hydrogels were prepared using CS solutions at a fixed concentration of 3.5% w/v, 50% w/v of β -GP solution at three different pH values (i.e., 4, 7, and 10), and the volume ratio of CS and β -GP solutions was equal to 0.5 and 0.5 mL.

Results

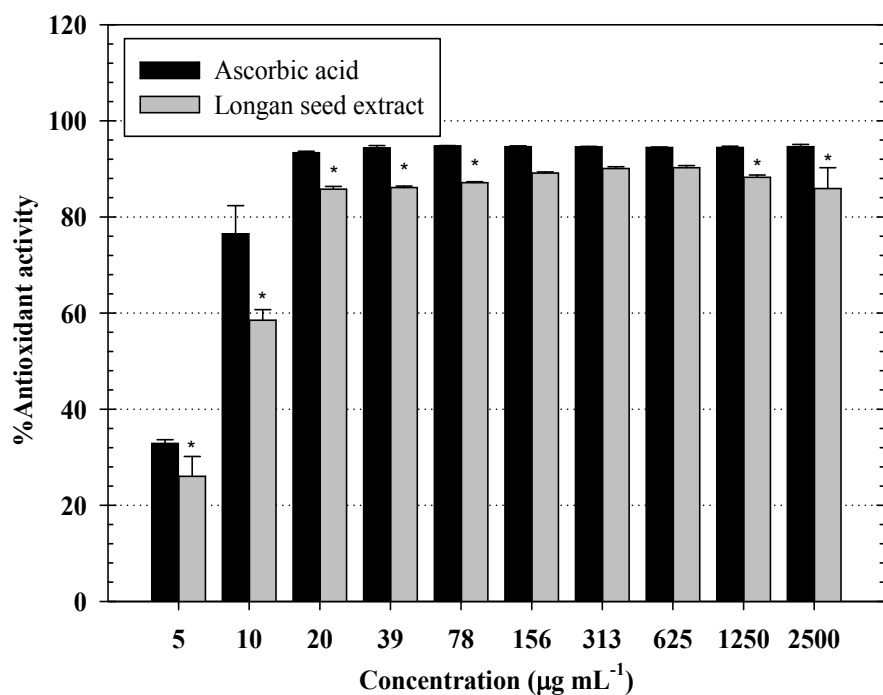


Fig. S1 Antioxidant activity of LE and ascorbic acid (n=3) * $p < 0.05$ compared with ascorbic acid.

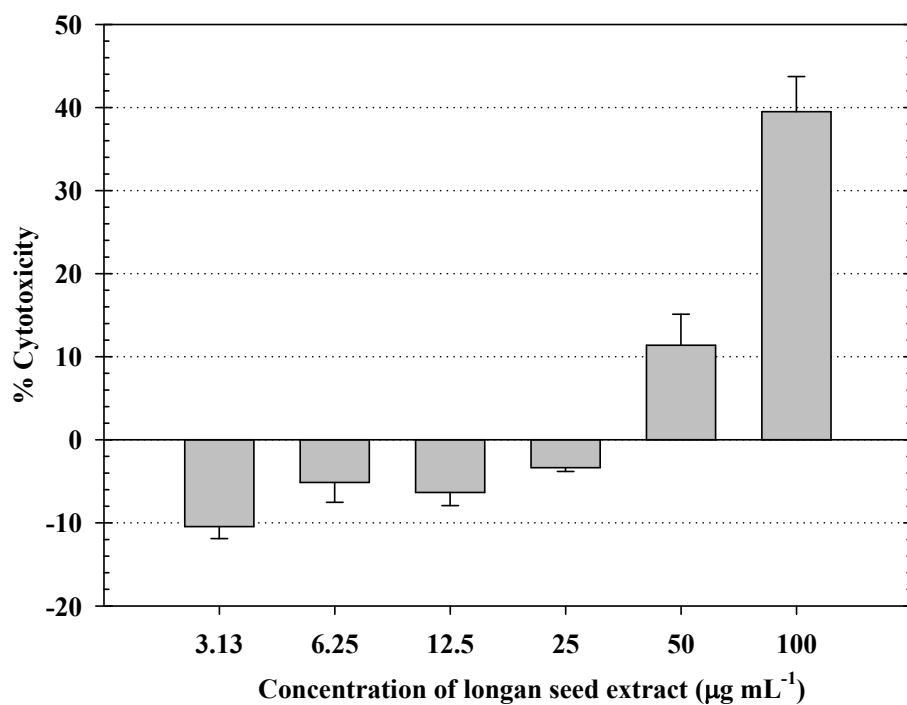


Fig. S2 Viability of NCTC clone 929 cells via direct culture by the REMA assay.

Table S1 Stability of the thermosensitive CS/ β -GP hydrogels in media at different pH values at 37 °C

Sample code	pH of media	Gelation time at 37 °C (min)
CS/ β -GP-pH4	4	31.52 \pm 2.66
CS/ β -GP-pH7	7	9.85 \pm 0.57
CS/ β -GP-pH10	10	4.94 \pm 0.17