Dear Editorials

Regarding manuscript "A synergistic nanoformulation of propolis and chlorhexidine against Acanthamoeba: encapsulation efficiency, release kinetics, and safety evaluation", with thanks for inviting me as a reviewer, my general recommendation is reject/major revision based on serious falls in methods. Comments are as below for specific sections.

Bests

Zahra Hesari

Encapsulation of molecular weight-variant propolis and CHX into CS-NPs

- How was the molecular weight of the propolis samples calculated?
- What is the concentration of propolis extract in ethanol? And what is the concentration of CS-NPs in water?
- How was CHX added to the CS-NPs? The procedure is not clearly mentioned. (The solvent? Concentration? Incubation duration?)
- For calculation of EE, how was the amount of encapsulated propolis and CHX in CS-NPs measured? There is no explanation!

Transmission electron microscopy (TEM) analysis of chitosan nanoparticles

- As is claimed in the text: "After encapsulation, the chitosan nanoparticles' surface morphology and structural characteristics were examined using Transmission electron microscopy (TEM)" TEM does not provide the surface morphology of Nps.

pH-responsive drug release kinetics of propolis-loaded chitosan nanoparticles

- Were NPs directly dispersed in release medium (phosphate buffer)?
- At the time of sampling, how were the particles separated from release medium?
- Did the separated particles return to the medium for the rest of release test?
- Was the release of propolis and CHX measured separately?