ABSTRACT

Centella asiatica has been used as medicinal plant to treat various types of symptoms and diseases as well as to improve memory recognition. C. asiatica crude extracts showed excellent potential In-vitro but poor In-vivo bioavailability and drug delivery system resulting from its poor lipid solubility and undesired molecular size. The most economical and readily material used for generating the nanoparticles is gelatin. It can act as the carrier and primary protection for crude extracts to be able to increase bioavailability and drug delivery system. Therefore, this study was aimed to develop C. asiatica crude extract-loaded Gelatin Nanoparticles (CGNP) to improve bioavailability and drug delivery system. CGNPs were prepared by using 2 methods, gelatin one-step desolvation and gelatin two-step desolvation methods, on three different ratio of 95% ethanolic C. asiatica crude extracts: Gelatin (1:2, 1:3, and 1:4 w/w). Entrapment and loading efficiencies are parameters used to measure the ability for the bioactive compounds to be trapped into the carrier system and the quantity of bioactive compounds loaded into carrier, respectively. As the result, entrapment efficiencies in all concentration of CGNPs showed no significant difference. Whereas, loading efficiencies were varied depending on the concentration of C. asiatica crude extract that has been used. The highest loading efficiencies were 18.39±2.08 % and 16.47±4.89 % from one-step gelatin desolvation nanoparticle and two-step gelatin desolvation nanoparticle, respectively. The solubility showed that CGNPs were barely dissolved in water comparing to C. asiatica crude extract at 31.10 to 45.40 µg/ml whereas the solubility of crude extract was 216.53±32.46 µg/ml. Therefore, gelatin desolvation nanoparticles could help to deliver hydrophilic bioactive compounds to attach and penetrate cell membranes of human and pathogenic bacteria in which, their cell membranes allow only hydrophobic compounds to access. C. asiatica extract-loaded gelatin nanoparticles were investigated for stability in phosphate buffer solution pH 7.4 with an hour interval for 6 hours. One-step and two-step desolvation gelatin nanoparticles were very stable over 6 hours of study. The well agar diffusion method was used for evaluating antibacterial activity of CGNPs with different concentrations (100, 200, and 300 µg/ml) against seven foodborne pathogens (Escherichia coli ATCC25822, Bacillus cereus, B. subtilis, Staphylococcus aureus, Salmonella enterica Typhimurium U302 (DT104b), S. enterica Enteritidis (human), and S. enterica 4,5,12:i:- (human) US clone). The results showed that the highest inhibition zone of CGNP was 1.65±0.57 cm against S. aureus using gelatin one-step desolvation
method on ratio 1:4, 200 μg/ml. There were no significant difference of CGNP's antibacterial activity using different preparation method and ratio. The antibacterial activity of CGNP gave almost 3 times higher than 95% ethanolic *C. asiatica* crude extracts. It was found out that antibacterial activity of CGNP was not concentration-dependent against all tested foodborne pathogens. One-step CGNP (OSCGNP) was tested on antibacterial activity by using well agar diffusion method with different concentrations (100, 200, and 300 μg/ml) against seven foodborne pathogens and antioxidant activity by using DPPH method. The inhibition zones of OSCGNP showed highly significant effective at concentration of 300 μg/ml in oesophagus-stomach section against *E. coli* ATCC25822 and *B. subtilis* respectively. In addition, *S. aureus, S. enterica* Enteritidis (human), and *S. enterica* 4,5,12:i:- (human) US clone were strongly inhibited by OSCGNP at concentration of 100 μg/ml. The highest inhibition zone of OSCGNP was 1.00±0.17 cm at pH 2.0 using gelatin one-step desolvation method. The highest antioxidant activity was 22.70±4.69 µg GAE/ml per 10 mg of OSCGNP with ratio of 1:2 occurred in stomach at pH 2.0. Moreover, antioxidant activity of OSCGNP was dropped when they reached duodenum section. The results indicated that OSCGNP gave lower antioxidant activity than crude extract. The kinetic release of *C. asiatica* crude extract-loaded gelatin nanoparticles promised to regulate the release rate of bioactive compounds in phosphate buffer at pH 7.4 at constant rate for up to 12 hours for both one-step and two-step gelatin nanoparticles. However, the acidic condition in gastric juice at pH 2.0 could denature the protein structure of gelatin which caused the structure to unfold and bioactive compounds were released at higher rate comparing to the release rate of CGNPs in PBS pH 7.4. *C. asiatica* crude ethanolic extracts showed significantly greater ferric reducing antioxidant power with 1.33±0.31 mmol Fe²⁺/mg dried weight than both one-step and two-step CGNPs at all ratios (p<0.05). CGNPs prepared with one-step desolvation method at ratio 1:2 showed highest FRAP 0.97±0.10 mmol Fe²⁺/mg dried weight among CGNPs. Moreover, *C. asiatica* crude ethanolic extracts also showed higher radical scavenging than both one-step and two-step CGNPs in DPPH radical scavenging activity (p<0.05). CGNPs prepared with two-step desolvation method at ratio 1:2 showed highest DPPH radical scavenging activity with 1.22±0.16 µg GAE/mg sample among CGNPs. In addition, there are no significantly difference between CGNPs preparation methods, gelatin one-step and two-step desolvation methods (p<0.05). Therefore, CGNPs prepared by using one-step desolvation method at ratio 1:4 is the most effective in an economical solution to produce CGNPs because it consumed the least operating time and
materials. Moreover, antioxidant activity for both DPPH and FRAP of 1:4 one-step gelatin desolvation nanoparticle showed no significant difference comparing with others and antibacterial activity showed the highest inhibition zone at 1.65±0.57 cm against *S. aureus*.