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In vitro antidiabetic activity of *Spondias pinnata* aqueous extract and encapsulated chitosan-TPP nanoparticles

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Spondias pinnata (L. f.) Kurz is a medicinal plant used in complementary medicine. Decoctions prepared using stem-bark of *S. pinnata* find applications in treating diabetes mellitus. However, low bioavailability of bioactive metabolites (polyphenols and flavonoids) and lack of appropriate release of metabolites delimit the antidiabetic activity of *S. pinnata* aqueous extract (SAE). Encapsulation of SAE with chitosan-tripolyphosphate (CS-TPP) could enhance its therapeutic potential and provide controlled release. The objective of this work to determine *in vitro* antidiabetic activity of *S. pinnata* stem-bark extracts and SAE-encapsulated CS-TPP nanoparticles using α-amylase inhibitory, α-glucosidase inhibitory, glucose uptake and glucose adsorption assay. The extracts were prepared by extracting dried and powdered stem-bark of *S. pinnata* into distilled water, acetone, ethyl acetate, dichloromethane under ultrasonication (40 kHz, 37 °C, 30 min) separately. The total phenol content (TPC) and flavonoid content (TFC) of the extracts were determined using Folin-Ciocalteu and aluminium chloride methods, respectively. Based on the results of α-amylase inhibitory assay, SAE was selected for the encapsulation with CS-TPP. The SAE had TPC of 4.18±0.02 mg gallic acid equivalents per gram of dry weight (GAE/g DW) and TFC of 0.37±0.01 mg quercetin equivalents per gram of dry weight (QE/g DW) and showed the highest α-amylase inhibitory activity (IC_{50} 53.34±7.43 µg/mL). The acetone extract had TPC of 34.43±0.35 mg GAE/g DW and TFC of 4.06±0.05 mg QE/g DW and showed the highest α-glucosidase inhibitory activity (IC_{50} 8.82±1.42 µg/mL). The highest glucose uptake and glucose adsorption were shown by acetone extract and aqueous extract, respectively. SAE-encapsulated nanoparticles were prepared from CS-TPP at varying concentrations (0.250, 0.375, 0.500 and 0.625% w/v) of SAE using ionic gelation method under magnetic stirring; the highest encapsulation efficiency (68.21% ± 0.66%) and loading capacity (0.79% ± 0.17%) were obtained at 0.625% w/v of SAE. Loaded nanoparticles were separated by centrifugation and free polyphenols were determined by Folin-Ciocalteu method. The Z-average particle diameter of SAE-encapsulated CS-TPP nanoformulations was 417±86 nm with polydispersity index of 0.574 and zeta potential of +20.63 mV. The IC_{50} values corresponding to α-amylase inhibitory activity and α-glucosidase inhibitory activity of SAE-encapsulated CS-TPP nanoparticles were 1.10±0.03 mg/mL and 3.16±0.15 mg/mL, respectively. Although the percentage of glucose uptake and adsorption in SAE encapsulated CS-TPP nanoparticles is lower than the crude extract, it had shown 11.59±1.03 % glucose uptake at 5 mM glucose concentration and 1.47 mmol/g glucose adsorption at 100 mM glucose concentration. The SAE, acetone extract and SAE-encapsulated CS-TPP nanoparticles showed higher antidiabetic activity than the positive control, acarbose. Further investigations on the releasing profiles of SAE-encapsulated CS-TPP nanoparticles would reveal the rates at which the active metabolites are released to the media during the timeframes of the conducted assays.

Keywords: *S. pinnata*, Antidiabetic activity, CS-TPP, Particle size, Zeta potential

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