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In vitro antidiabetic activity of fractionated extracts of *Coccinia grandis* (L.) Voigt

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Coccinia grandis (L.) Voigt (Family; Curcurbitaceae) leaves in the therapy of diabetes mellitus has been well legendary in Sri Lankan traditional medicine since antiquity. The present study was aimed to evaluate *in vitro* antidiabetic activity by means of α-amylase, α-glucosidase and DPP-IV inhibitory potential of the selected crude extracts and the fractions of *C. grandis* leaves. The powder of the dry leaves of *C. grandis* were sequentially extracted into hexane, ethyl acetate (EA), methanol and water using maceration. The dry extracts resulted in the sequential extraction were subjected to determine the selected enzyme inhibitory activities. Enzyme inhibition assays were conducted using porcine pancreatic α-amylase, α-glucosidase form *Saccharomyces cerevisiae* and DPP-IV enzyme human recombinant, expressed in S*f*9 cells. Acarbose was used as the standard inhibitor for α-amylase and α-glucosidase inhibitory assays and diprotein A was used as the standard inhibitor for DPP-IV inhibitory assay. The hexane and EA extracts were further fractionated through vacuum liquid chromatography (VLC). The resulted VLC fractions (a total number of six VLC fractions from both extracts) were subjected to the selected *in vitro* antidiabetic assays as mentioned. All quantitative data resulted from *in vitro* assays were analyzed using SPSS software. One way – ANOVA followed by Tukey's test was done for multiple comparisons. Independent sample t-test was performed to compare the mean values between two groups. $p \leq 0.05$ was considered as statistically significant. The percentage yield of the hexane, EA, methanol and water extracts were 2.40%, 2.87%, 2.95% and 5.73% respectively. The hexane and EA extracts showed α-amylase, α-glucosidase and DPP-IV inhibitory activities in significantly lower IC_{50} values compared to the methanol and water extracts. The α-amylase activity of hexane extract (IC_{50} 6.42±0.44 mgmL^{-1}) and EA extract (IC_{50} 9.98±0.85 mgmL^{-1}) was not statistically different from acarbose. α-Glucosidase activity of hexane extract (IC_{50} 2.28±0.08 mgmL^{-1}) and EA extract (IC_{50} 5.92±0.21 mgmL^{-1}) showed no significant differences with acarbose. There were no significant differences ($p>0.05$) in DPP-IV inhibitory activity of hexane extract (IC_{50} 101.24±2.83 µgmL^{-1}), EA extract (IC_{50} 28.69±1.65 µgmL^{-1}) with respect to the diprotein A. IC_{50} values of the methanol and water extracts were significantly higher than that of the standard compounds in α-amylase, α-glucosidase and DPP-IV inhibitory assays. The fourth VLC fraction collected from the EA extract showed the highest α-amylase (IC_{50} 7.13±0.36 mgmL^{-1}), α-glucosidase (IC_{50} 0.40±0.02 mgmL^{-1}) and DPP-IV inhibitory (IC_{50} 27.71±2.37 mgmL^{-1}) activities compared to the other VLC fractions. The IC_{50} value of the fourth VLC fraction collected from the EA extract was significantly lower ($p=0.001$) than that of the acarbose (IC_{50} 0.56±0.02 mgmL^{-1}) for α-glucosidase inhibition denoting the importance of further fractionation and isolation of α-glucosidase inhibitors. In conclusion, the hexane and EA extracts resulted from the sequential extraction of *C. grandis* leaves and the fourth VLC fraction of the EA extract exerted considerably high α-amylase, α-glucosidase and DPP-IV inhibitory potential. Further, bioassay guided fractionation is warranted to isolate α-amylase, α-glucosidase and DPP-IV inhibitors from the bioactive fractions of *C. grandis* with an aim of developing new antidiabetic drug leads/pharmaceutical agents in the near future.

Keywords: α-Amylase, *Coccinia grandis*, DPP-IV, α-Glucosidase, Sequential extraction

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