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Antidiabetic efficacy of two major compounds isolated from *Coccinia grandis* (L.) Voigt and their effect on insulin-producing cell line RINm5F *in vitro*

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Coccinia grandis (L.) Voigt (Cucurbitaceae family) is widely used in traditional treatment of diabetes. The present study aimed to isolate and characterize major bioactive compounds from mature unripe fruits of *Coccinia grandis* (L.) Voigt and evaluate their insulinotropic properties in insulin producing rat RINm5F cells *in vitro*. The *n*-butanolic concentrate of the fruit extract was subjected to thin layer chromatography (TLC) and repeated silica gel column chromatography followed by elution with various solvents. The compounds were identified based on observed spectral (IR, ¹H NMR, ¹³C NMR and mass spectrometry) data. The structure of two major compounds isolated from *Coccinia grandis* was elucidated as β -sitosterol (C₂₉H₅₀O) and lupeol (C₃₀H₅₀O) by extensive spectroscopic studies. Furthermore, the isolated compounds *viz.* β -sitosterol and lupeol were assessed for insulinotropic properties using RINm5F cells *in vitro*. Insulin secretion activity of β -sitosterol/lupeol was determined after 60 min incubation with RINm5F cells. Glucose-free Krebs-Ringer bicarbonate (KRB) buffer was used as Vehicle and Glibenclamide (10 μ M) dissolved in DMSO, further diluted with Kreb's Ringer buffer was used as positive control. Aliquots (10 μ L) were removed from each well, centrifuged (2000 rpm for 5 min, at 4 °C), and assayed for insulin with Mercodia Rat Insulin ELISA kit as per Manufacturer's protocol. A concentration dependent increase in insulin secretion was observed for both the compounds. The isolated compound β -sitosterol significantly ($p < 0.05$) increased the insulin secretion from 0.0198 ± 0.0065 (vehicle control) to 0.098 ± 0.0058 μ g/10⁵ cells at the concentration of 15 μ g/mL. Likewise, lupeol increased the insulin secretion from 0.0187 ± 0.0054 (vehicle control) to 0.0652 ± 0.0043 μ g/10⁵ cells at the same concentration. The anti-diabetic compounds isolated from mature unripe fruits of *Coccinia grandis* (L.) Voigt validates the use of this plant in traditional medicine for diabetes. However, as this is only a preliminary study, further studies are in progress to evaluate the possible molecular mechanisms underlying the insulin-producing action of β -sitosterol and lupeol and *in vivo* oral rodent efficacy studies in a disease model.

Keywords: β -sitosterol, *Coccinia grandis*, diabetes mellitus, glibenclamide, lupeol, RINm5F cell line