Antiprotozoal Activity of Trypsin Inhibitor from *Moringa oleifera* Flowers against *Trypanosoma cruzi* Trypomastigotes

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The protozoan *Trypanosoma cruzi* is the etiologic agent of Chagas disease and the trypomastigotes are the infective form of this parasite. Drugs used to treat the disease are highly toxic and do not act during the chronic phase. This work reports the isolation of *M. oleifera* flower trypsin inhibitor (MoFTI) and its antiprotozoal activity against *T. cruzi* trypomastigotes. Flowers (50 g) were added to distilled water (100 mL) and homogenized in a blender. After filtration and centrifugation (9,000 g, 15 min, 4 °C), the supernatant (flower extract) was evaluated for trypsin inhibitor activity using bovine trypsin and N-α-benzoil-DL-arginine-ρ-nitroanilide (BApNA) as substrate. The substrate hydrolysis was followed by measurement of absorbance at 405 nm and inhibitor activity was determined by remaining hydrolytic activity towards BApNA. The flower extract was also evaluated by SDS-PAGE and reverse zymography using bovine trypsin. The extract was dialyzed against 0.1 M Tris-HCl pH 8.0 buffer and chromatographed on a Trypsin-Agarose column. The adsorbed fractions were eluted with 0.1 M KCl-HCl pH 2.0 and pooled (MoFTI). The inhibitor was assayed for trypanocidal activity and the concentration able to kill 50% of trypomastigotes (IC₅₀) was determined. Flower extract strongly inhibited (98 %) trypsin activity and SDS-PAGE revealed a single polypeptide band of 170 kDa, which was proved to be a trypsin inhibitor by reverse zymography. MoFTI killed *T. cruzi* trypomastigotes with IC₅₀ of 38 μg/mL. In conclusion, a trypsin inhibitor isolated from *M. oleifera* flower extract is an effective agent against *T. cruzi* trypomastigotes.
