

PURIFICATION OF A KUNITZ-TYPE INHIBITOR FROM *PITHECOLOBIUM DUMOSUM* SEEDS AND ITS EFFECT TOWARDS INSECTS

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Proteins that are inhibitors of proteinases are ubiquitous in nature. In legume seeds, two families of serine proteinase inhibitors are found: Bowman-Birk-type and Kunitz-type-inhibitors. Some these inhibitors are bifunctional inhibiting serine and cysteine proteinases. These inhibitors display important role in the defense against insect pests. The trypsin-inhibitor, named JB1, was purified from *Pithecolobium dumosum* seeds by TCA precipitation, trypsin-Sepharose chromatography and reversed-phase-HPLC. JB1 was purified 161 fold and recovered 4.7%. SDS-PAGE showed that JB1 is a single polypeptide chain of 20 kDa and 19.7 kDa by MALDI-TOF. The inhibition on trypsin was stable at pH range 2-10, and temperature of 50<sup>o</sup>C. The Ki-values were 3.56 x 10<sup>-8</sup> and 7.61 x 10<sup>-7</sup> M with a competitive and noncompetitive inhibition mechanism for trypsin and papain, respectively. The N-terminal sequence had identity with members of Kunitz-type inhibitors from Mimosoideae and Caesalpinoideae subfamilies. JB1 was effective against digestive proteinase from *Zabrotes subfasciatus*, *Ceratitis capitata*, *Plodia interpunctella*, *Alabama argillacea* and *Callosobruchus maculatus*, with 69, 66, 44, 38 and 29% Inhibition, respectively. Results support that JB1 is a member of Kunitz-inhibitor family and its insecticidal properties indicate a potent insect antifeedant.

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