

MASTER'S THESIS

Isolation and characterisation of novel ribosome-inactivating proteins from the root tubers of *Trichosanthes kirilowii*

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**Isolation And Characterisation Of Novel Ribosome-
Inactivating Proteins From The Root Tubers
Of *Trichosanthes kirilowii***

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**A thesis submitted in partial fulfilment of the
requirements for the degree of
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ABSTRACT

Two new ribosome-inactivating proteins (RIPs), probably isoforms of trichosanthin, were isolated from the root tubers of *Trichosanthes kirilowii*. The purification steps involved ion-exchange chromatography on CM-Sepharose CL-6B column followed by MPLC on Mono-S column. Three RIPs were eluted from the Mono-S column. They were designated as T₁, T₂ and T₃ according to the order of elution. T₃, the major protein, is most probably identical to trichosanthin, the extensively studied RIP from *T. kirilowii*, while T₁ and T₂ are new RIPs. Results from SDS-PAGE analysis indicated that all their molecular weights were around 26 kDa. Protein fingerprinting by chemical and enzymatic cleavage followed by SDS-PAGE indicated that T₁ is structurally distinct from T₂ and T₃ while T₂ and T₃ are very similar. The identity of T₁ was confirmed by N-terminal and partial protein sequencing of a CNBr fragment which showed sequence difference from trichosanthin while a partial sequence of T₂ (20 residues) is identical to trichosanthin. However, all the three proteins inhibited cell-free protein synthesis and exhibited N-glycosidase activity. T₁, T₂ and T₃ also expressed DNase activity by converting supercoiled double-stranded DNA to open-circular and linear DNA. The cytotoxicity assay toward fibroblasts showed T₁ and T₃ are more toxic than T₂ while T₁ and T₂ display less cytotoxicity than T₃ towards lymphocytes.

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