

***ISOLATION AND BIOCHEMICAL  
CHARACTERIZATION OF ANTIMICROBIAL  
TRYPSIN INHIBITOR FROM *Pongamia pinnata* OIL  
SEED CAKE***

**SUBMITTED TO:**

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## ABSTRACT

*Pongamia pinnata* seed cake is a low value by-product resulting from the bio-diesel production. The seed cake is highly toxic, but it has great potential for biotechnology applications that could be important in agriculture, medicine and industry (Helen P.S Costa et.al, 2014). To explore this potential, the trypsin inhibitor was isolated from the seed cake by defatting and extraction with water (crude) and buffer (Thiol reducing agent- $\beta$  mercaptoethanol, metal chelating compound-EDTA). The percentage of inhibition caused by the inhibitor in the extracts was obtained by performing an assay. Since the crude extract had a shown a considerable amount of inhibition than the buffer extract, it was further purified partially by ammonium sulfate precipitation and completely purified using affinity chromatography (Trypsin agarose matrix).

The ammonium sulfate purified sample was used to check the antimicrobial activity of the trypsin inhibitor on the laboratory organisms by agar diffusion technique. It was seen that there was no antimicrobial activity by trypsin inhibitor up to the concentration of 150 $\mu$ l (300-645 $\mu$ grams).

The molecular weight of the protein (trypsin inhibitor) was determined (approximately 24kDa) by performing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a marker.