

## Kallikrein Inhibitor from *Caesalpinia echinata* (Pau-Brasil) Seeds: Production and Purification of the Antibody

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Leguminous seeds contain large amounts of proteins, including proteases and inhibitors. *Caesalpinia echinata* is a Brazilian native specie of the Leguminosae family. We have already purified proteases and inhibitors of different proteases (kallikrein, trypsin, plasmin, factor XII and factor X - CeKI, pancreatic and neutrophil elastases – CeEI and cathepsin B - CeCB) from *C. echinata* seeds. Now, we are interested to detect the distribution of these inhibitors in *C. echinata* tissues by immunolocalization. Balb-C mice were immunized with a subcutaneous injection of a solution of purified CeKI (10 µg) in complete Freund's adjuvant. This procedure was repeated three times, using incomplete Freund's adjuvant, each ever two weeks. The antibody production was confirmed by Western Blotting - CeKI band from SDS-PAGE was electrotransferred onto a Hybond-P PVDF membrane and further treated to block unspecific sites. After exposition to mouse anti-CeKI IgG, as primary antibody, the membrane was incubated with anti-mouse IgG conjugated to horseradish peroxidase, treated with an enhanced chemiluminescence solution and exposed to an X-ray film. The antiserum titration with CeKI as antigen was performed in ELISA. The midpoint titration occurred at a dilution of 1:1,000 and the best dilution was 1:200. The immunoglobulin G fraction was purified by affinity chromatography in a protein G-Sepharose column. For futures immunohistochemical assays with purified anti-CeKI, *C. echinata* tissues were dehydrated in ethanol, fixed in formaldehyde and kept in -80°C (FAPESP, CAPES and CNPq).