Biochemical and spectroscopic characterization of a copper induced peroxidase, CCPP from Caribbean copper plant: *Euphorbia cotinifolia*

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Objective for purification and biochemical and biophysical characterization

*Euphorbia cotinifolia* plant with medicinal implications

- Latex extracted from stem
- Using anion exchange chromatography
- Purification of Peroxidase (CCPP)
  - pH, Temperature optima and Stability
  - Effect of inhibitors
  - Effect of metal ions
  - Effect of substrate concentration on Reaction Velocity
  - Effect of Chaotrops, Organic Solvents, detergents
  - Autodigestion study
  - Spectroscopic Studies: Absorbance, Fluorescence and Circular Dichroism
Purification of Peroxidase, CCPP

Protein content (●) and activity (○)

<table>
<thead>
<tr>
<th>Steps</th>
<th>Total Protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>315</td>
<td>3455</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>DEAE Sepharose</td>
<td>10.5</td>
<td>294</td>
<td>28</td>
<td>3.3</td>
</tr>
</tbody>
</table>
Effects of pH  Activity (●)  
pH Optima 6.0

Effects of Temperature  Activity (○)  
Temperature Optima 50 °C
Effect of substrates on the activity of CCPP

Effect of additives on Peroxidase
**Activity measured toward Guaiacol as a substrate**

(□) CaCl₂  (●) NaCl
(■) Na₂SO₃ (○) MgCl₂

**Visible absorption spectra of CCPP, 50 mM Tris buffer, pH 8.4.**

- 2 µM (dashed line)
- 5 µM (solid line)
- 10 µM (dotted line)
Spectroscopic studies of Peroxidase, CCPP

Intrinsic fluorescence spectra

Circular dichroism spectrum
Summery

• Adequate amount of latex

• Easy economic purification

• Broad substrate specificity

• A copper induced peroxidase

• Stability against different temperature, pH

• Stability against different salts and additives

• Excellent model system to study structure-function relationship of other peroxidase

• Crucial for food and biotechnological industries as well as protein folding studies.
Thank you !!