

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



**Reetesh Kumar**

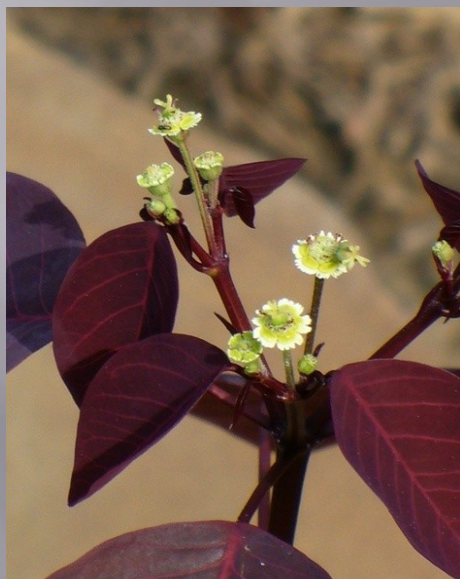
**PhD Scholar**

**Molecular Biology Unit**

**Institute of Medical Sciences**

**Banaras Hindu University**

# Objective for purification and biochemical and biophysical characterization



*Euphorbia cotinifolia* plant with medicinal implications

↓  
Latex extracted from stem

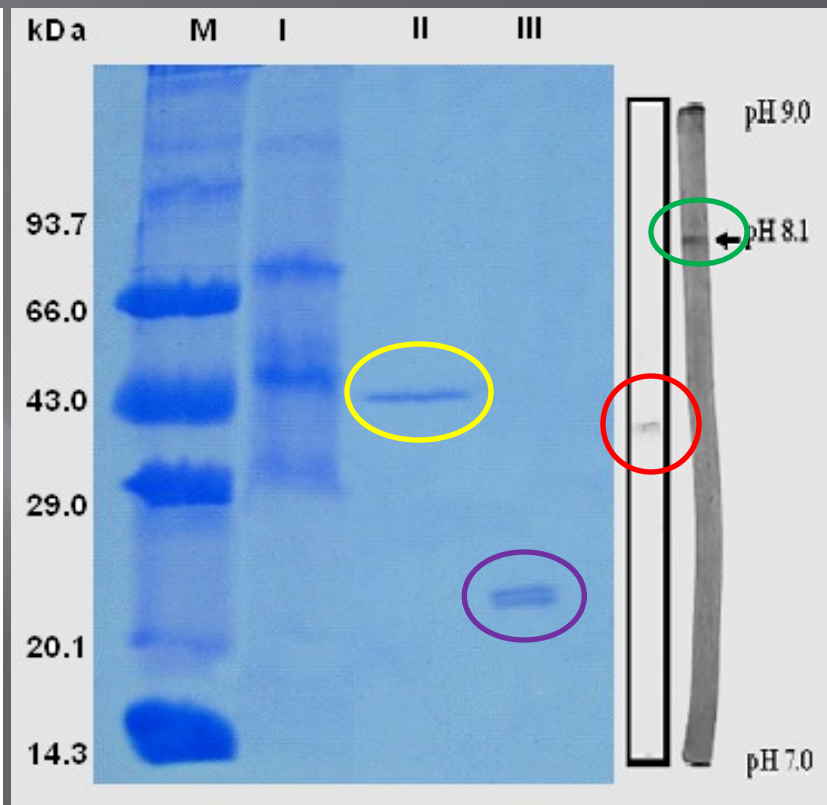
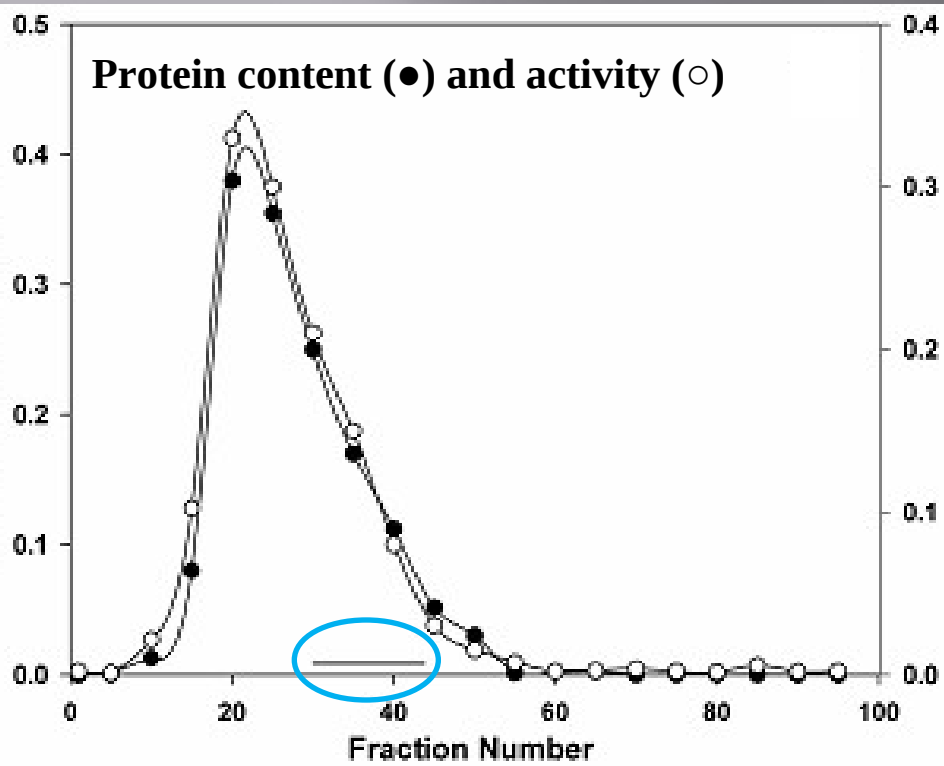
↓  
Using anion exchange chromatography

↓  
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

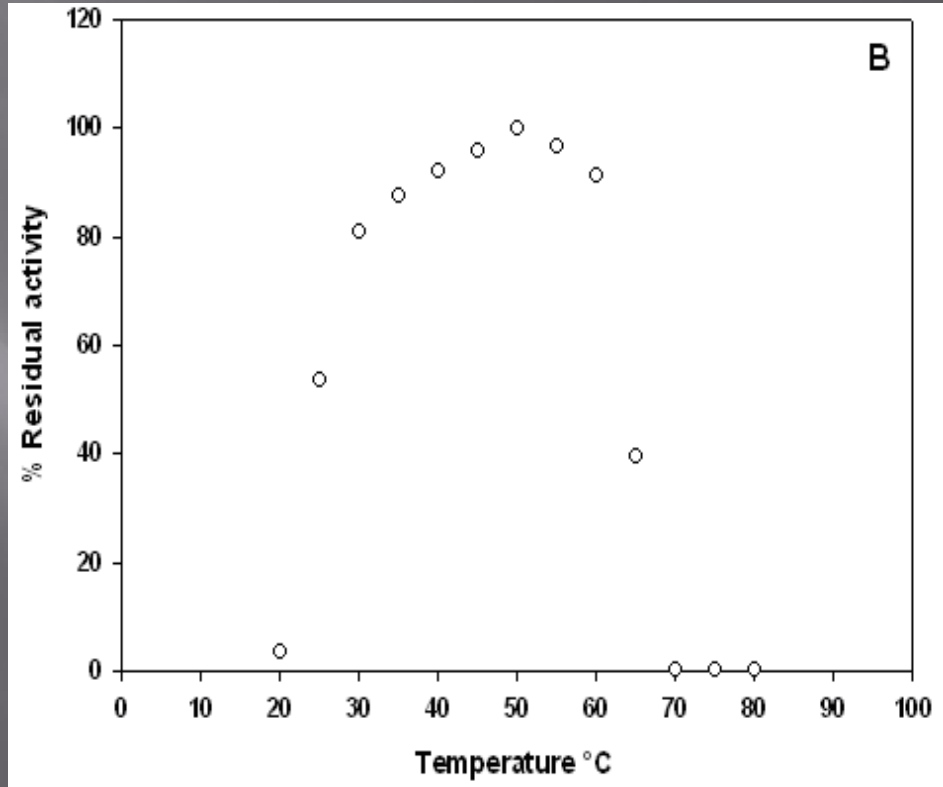
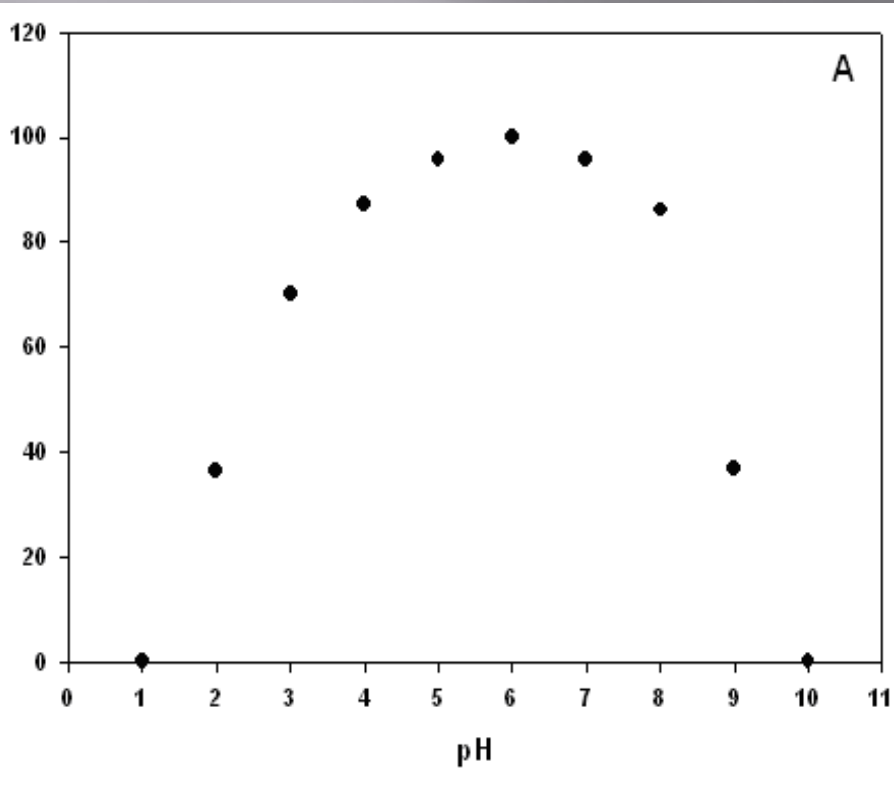
Nature Precedings : doi:10.1038/npre.2011.6410.1 : Posted 14 Sep 2011



Steps	Total Protein	Total activity <sup>a</sup>	Specific activity	Yield
Crude extract	315	3455	11	100
DEAE Sepharose	10.5	294	28	3.3

# pH, Temperature optima

Nature Precedings : doi:10.1038/npre2011104101v1 Posted 14 Sep 2011



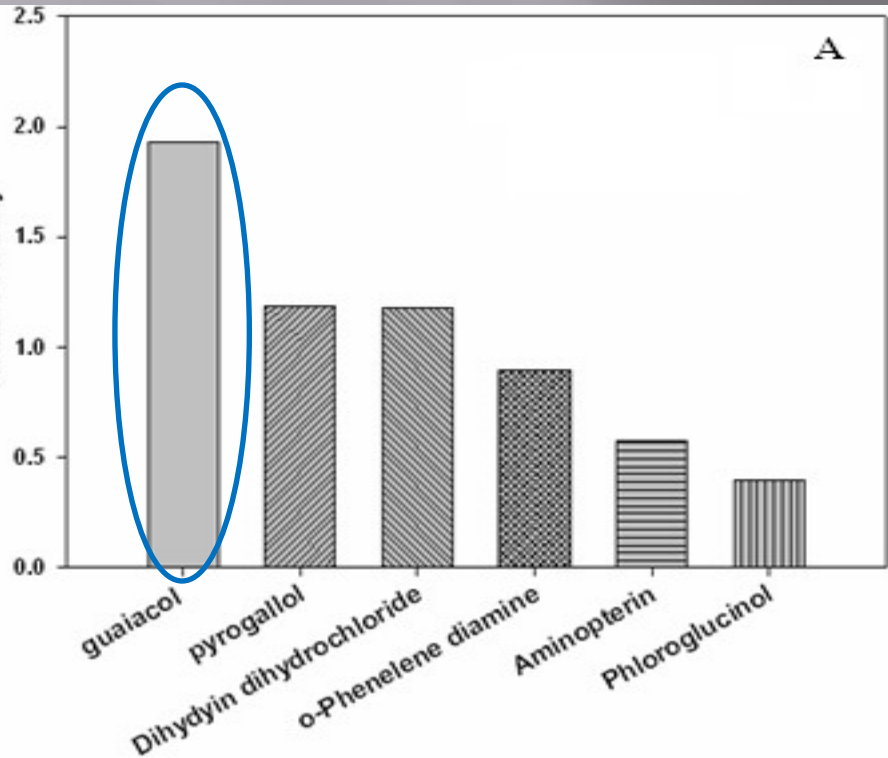
Effects of pH Activity (●)

pH Optima 6.0

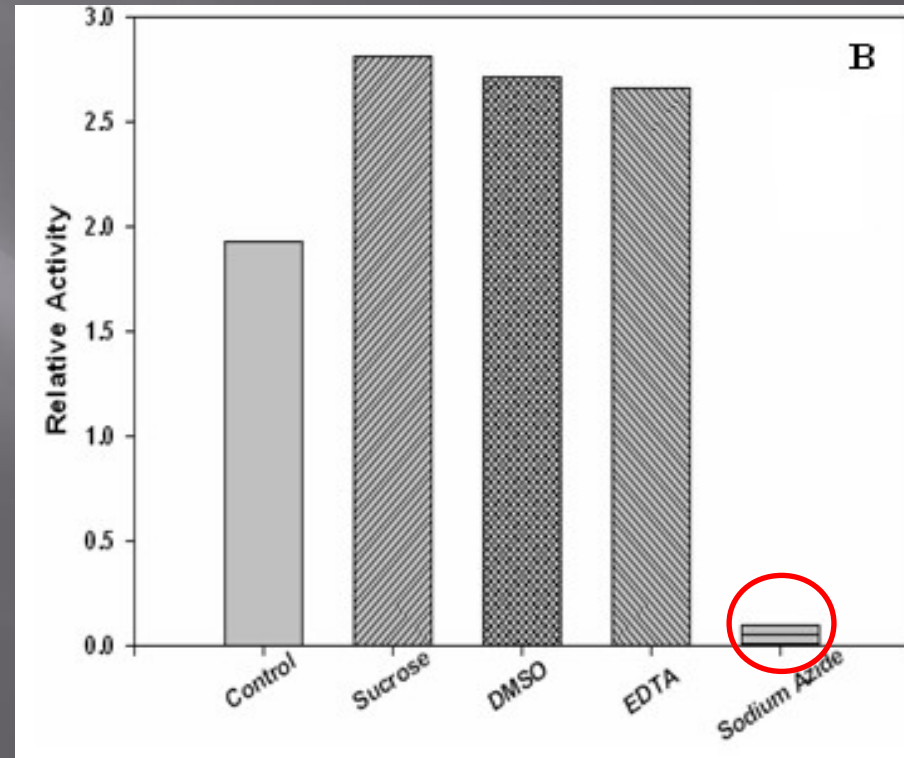
Effects of Temperature Activity (○)

Temperature Optima 50 °C

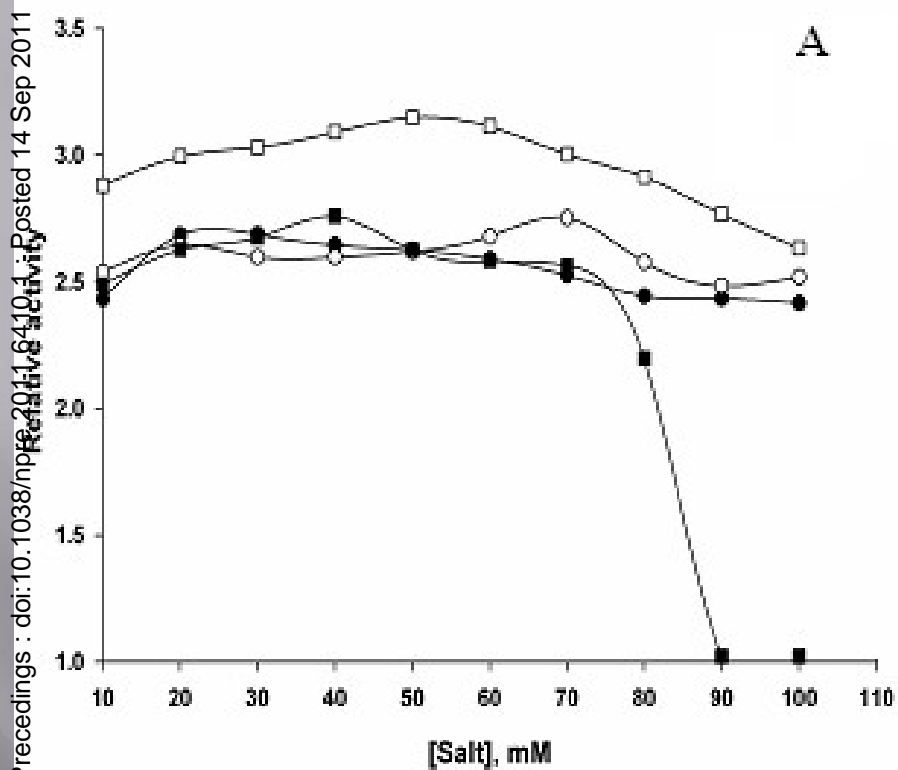
## Effect of substrates on the activity of CCPP



## Effect of additives on Peroxidase



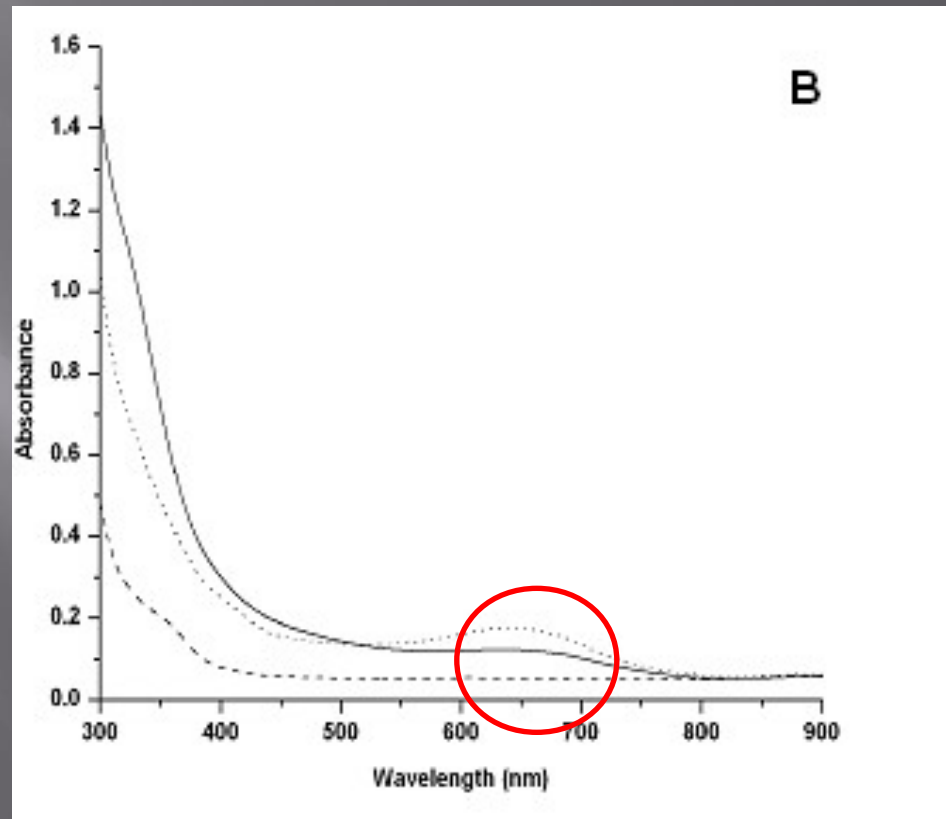
## Effect of concentration of salts



Activity measured toward  
Guaiacol as a substrate

(□) CaCl<sub>2</sub> (●) NaCl  
(■) Na<sub>2</sub>SO<sub>3</sub> (○) MgCl<sub>2</sub>

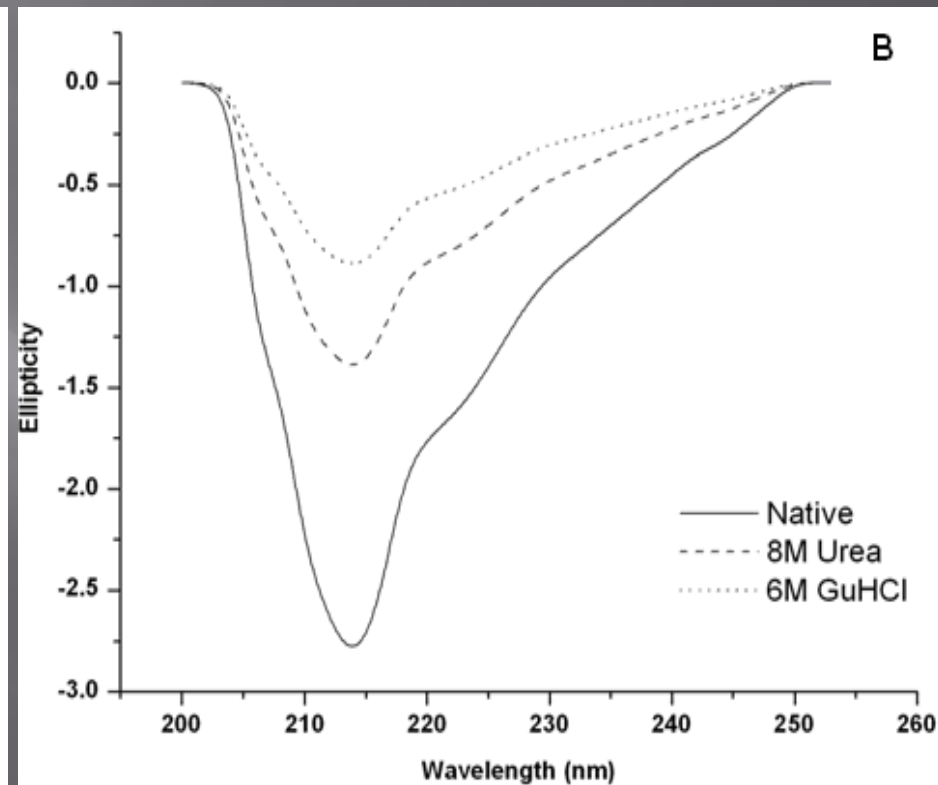
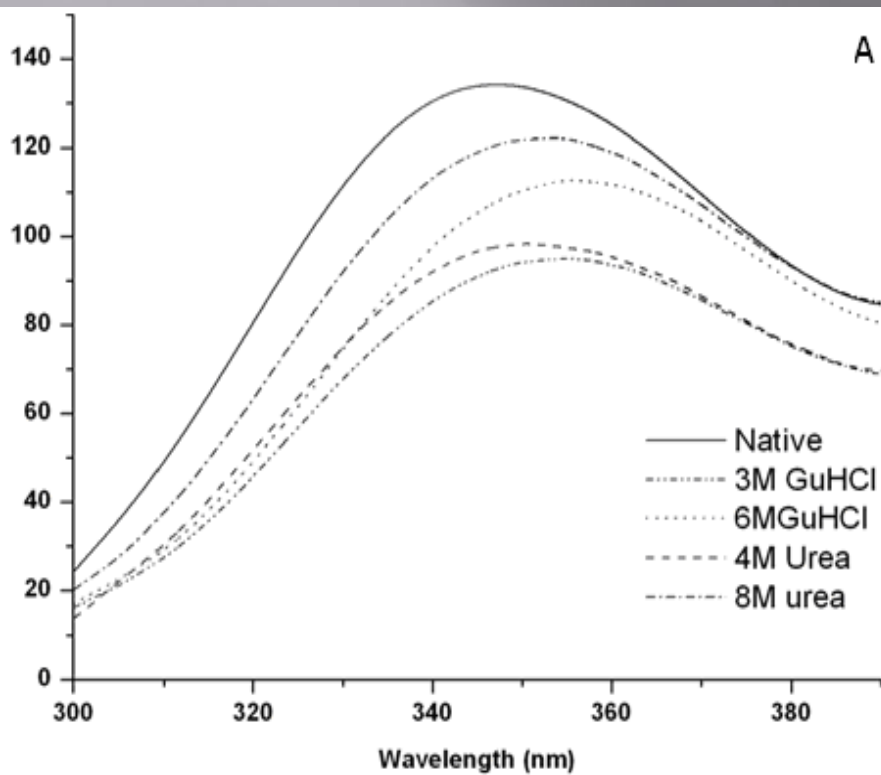
## Effect of absorbance spectra



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 !!**