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## ACCEPTED MANUSCRIPT

# Purification, biochemical characterization and antioxidant property of ZCPG, a cysteine protease from *Zingiber montanum* rhizome

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#### Highlights

- 1. ZCPG was purified to homogeneity to sixteen fold with a total activity of 39.4U/mg.
- 2. HPLC chromatogram presented a prominent sharp peak.
- SDS-PAGE resolved into ~24.3 and ~24.6kDa subunits indicating the heterodimeric nature.
- 4. The pH and temperature optimum were 9 and 60°C respectively.
- 5. Km and Vmax were 0.5 $\pm$ 0.03 µg and 13.73 $\pm$ 2.07 U/ml respectively.
- 6. It exhibited strong antioxidant activity and can be effective in therapeutic and food applications.

#### Abstract

*Zingiber montanum* cysteine protease glycoprotein (ZCPG) was purified to homogeneity by DEAE- cellulose and Sephadex G50 resulting in sixteen fold purification and total activity of 39.4U/mg. ZCPG presented a prominent single peak in HPLC chromatogram with an estimated molecular weight of 48kDa on native PAGE. SDS-PAGE gave two subunits of ~24.3 and ~24.6 kDa showing its heterodimeric form. Protein sequencing was studied by MALDI-TOF MS/MS. Isoelectrofocusing exhibited two isoforms with pI values of 4.8 and 5.1. Analysis of the total carbohydrate by GC-MS/MS showed the presence of glucose,

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