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HEAT STABILITY AND PROTEASE INHIBITORY PROPERTIES OF 28KDa GLYCOPROTEIN FROM TURMERIC (*CURCUMA LONGA)*

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ABSTRACT

Turmeric (*Curcuma longa*) belongs to Zingiberaceae family extensively used as food colourant, flavour, spice and as medicine in Middle Asia and South East Asian countries. Herein we are reporting the property of heat stability and proteases like trypsin and chymotrypsin inhibitory property of glycoprotein of 28kDa from *Curcuma longa*. It exerts inhibitory activity protease Trypsin and Chymotrypsin up to 53 and 57% at 100µM dosage respectively. The protein alone shows 67% inhibition and standard antioxidant Ascorbic acid shows 65%. This protein was stable up to a temperature of 100^0C, active over a wide range of pH from 2 to 12, specifically more at weak acidic and neutral pH and less at basic pH. This is the first report on protease inhibitor activity and heat stability activity of glycoprotein of 28kDa from Turmeric (Curcuma longa).

KEYWORDS: Turmeric, *Curcuma longa*, Heat stability, Protease inhibitors, trypsin and chymotrypsin.

INTRODUCTION

Turmeric is widely used as a spice, food colorant, medicine and its characteristic flavor in many South East Asian countries(Kocaadam B, 2017). The importance of Turmeric explained in traditional Chinese medicine and Indian Ayurvedic medicine (Ishita C, 2004). Lot of studies has been done on the yellow pigment of Turmeric called Curcumin and reported for its anti-carcinogen property (Soleimani V et.al, 2018 and Horie S 2012). Meanwhile, in the recent years, researchers concentrating on the other part of Turmeric like Polysaccharides, proteins etc. Research studies has shown that turmeric has antibacterial, antiviral, antifungal, antioxidant, and anticancer activities and also has a potential to reduce the risk of various malignant disease, arthritis, Alzheimer's disease, and other chronic illness including rheumatoid arthritis (Tuba Ak. 2008, Moghadamtousi SZ et.al, 2014 and Taty Anna K et.al, 2011). Earlier from laboratory reported potent proteins from Turmeric called Turmerin (14kDa)(Chethan Kumar and Leela Srinivas 2006), BGS-Haridrin (28kDa) (Dinesha and Leela Srinivas, 2010) and one more glycoprotein Beta Turmerin (34kDa)(Smitha et al., 2009) from Turmeric industrial waste. The above proteins showed their antioxidant, antimicrobial, anti-inflammatory, DNA damage protectant activities. It was reported that, pulses are the rich source of proteins, but their poor digestibility is due to presence of protease enzymes on their coats. This coat will reduce the nutritional potential of the legume protein leading to stomach distension and flatulence (Prabhu M. S et.al 2015).

The legume seed protease inhibitors normally contain enzymes like trypsin/chymotrypsin (Neurath H. 1984). These proteolytic enzymes of digestive tract reduce the ability of body to utilize food proteins, causing defect in protein efficiency ratio (Koiwa H, 1997). However, these seeds coat exhibits lots of useful applications in medicine, agriculture and food technology (Guillamon E, 2008).

The present study is to highlight the protease inhibitor action and heat stability nature of a glycoprotein from Turmeric (*Curcuma longa*).

MATERIALS AND METHODS

1.1. Chemicals

Trypsin, Chymotrypsin, Ammonium sulphate and all other chemicals unless otherwise mentioned were of analytical grade procured from Merck (Germany). Solvents were distilled before use.

1.2. Isolation of glycoprotein from *Curcuma longa*

The glycoprotein was isolated from *Curcuma longa* by 55% ammonium sulphate precipitation (Dinesha and Leela Srinivas, 2011). In brief, 10g of powder of Turmeric powder vortexed with 250 ml of double distilled water for two hours at 20°C. Later the extract centrifuged at 10000 rpm for 20 min at 4°C. The supernatant collected was subjected to 55% ammonium sulphate precipitation, kept for votexing at 4°C overnight. Further the precipitated crude protein was separated by centrifugation. The obtained crude precipitate of protein was subjected to dialysis against double distilled was for 72 hours with an interval of six hours. The dialyzed sample was examined and confirmed is free of unwanted salts using silver nitrate. The crude protein was subjected to column chromatography using Sephadex G-25 at the flow rate of 1ml/5 min using distilled water as eluent. The fractions collected were read at 280nm using spectrophotometer. The selected fractions were analyzed through SDS PAGE and followed with MS MALDI.

1.3. Protease inhibitory activity

The protease inhibitory activity was assayed according to the method of *Satakee M et al* 1963. 50µL aliquot of trypsin and chymotrypsin was pre incubated separately with different concentrations of protease inhibitor. To the above denatured casein was added as substrate of 0.4 mL (2%) in a final volume of 1 mL using 0.2 M Tris-HCl buffer of pH 8.5 for 2 h at 37°C. After incubation, the reaction was stopped by adding 1.5 mL of 0.44 M TCA and the mixture was allowed to stand for 30 min. The reaction mixture was centrifuged at 1500g for 15 min. An aliquot (1 mL) of the supernatant was mixed with 2.5 mL of 0.4 M sodium carbonate and 0.5 mL of Folin–Ciocalteu reagent (1:2 v/v). The colour developed was read at 660 nm. Activity was expressed as units/hr. Protease inhibitor activity of the enzyme is finally expressed in terms of percent inhibition.

1.4 DPPH radical scavenging activity along with protease enzymes

DPPH is a stable purple coloured nitrogen-centered free radical that gets reduced to a yellow coloured diphenylpicryl hydrazine by the fractions in a concentration-dependant manner. DPPH radical scavenging activity was assessed according to the method described by *Aquino et al*. 2001. Different doses of glycoprotein of *Curcuma longa and* constant amount of proteases like trypsin or chymotrypsin was mixed with 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer. The resulting reaction mixtures were incubated at 37°C for 30 min, and the absorbance was measured at 517 nm. The % DPPH radical scavenging activity was calculated using the following formula,

$$\% \text{ Inhibition of DPPH radical scavenging activity} = \frac{(\text{Abs of control} - \text{Abs of samples})}{\text{Abs of control}} \times 100$$

1.5. Thermal and pH stability of Protease inhibitor

The effect of temperature on trypsin inhibitory activity of glycoprotein from *Curcuma longa* were tested by incubating at different temperatures 37, 40, 50, 60, 70, 80, 90, 100°C for 30 min. after cooling the samples to room temperature the residual trypsin inhibitory activity was determined as described earlier.

The effect of pH on the trypsin inhibitory activity was examined at pH ranging between 2-12 for 30 min at room temperature using the buffers: glycine-HCl (pH 2 to 3), sodium acetate-acetic acid (4 to 5), Sodium phosphate buffer (pH 6), Tris-HCl (pH 7 to 9) and glycine-NaOH (pH 10 to 12). The residual inhibitory activity was measured as described earlier and the final concentration of used buffers is of 50mM.

1.6. Salt stability

The glycoprotein from *Curcuma longa* were incubated at room temperature for 30 min in the presence of NaCl ranging from 0% to 3% and were tested for inhibitory activity against trypsin and chymotrypsin, the residual inhibitory activity was measured.

1.7. Statistical analysis

All experiments were carried out in triplicate to check the reproducibility of results. The data presented here are the averages of triplicate determinations and the standard deviations for all the values were $< \pm 5\%$.

RESULTS AND DISCUSSION

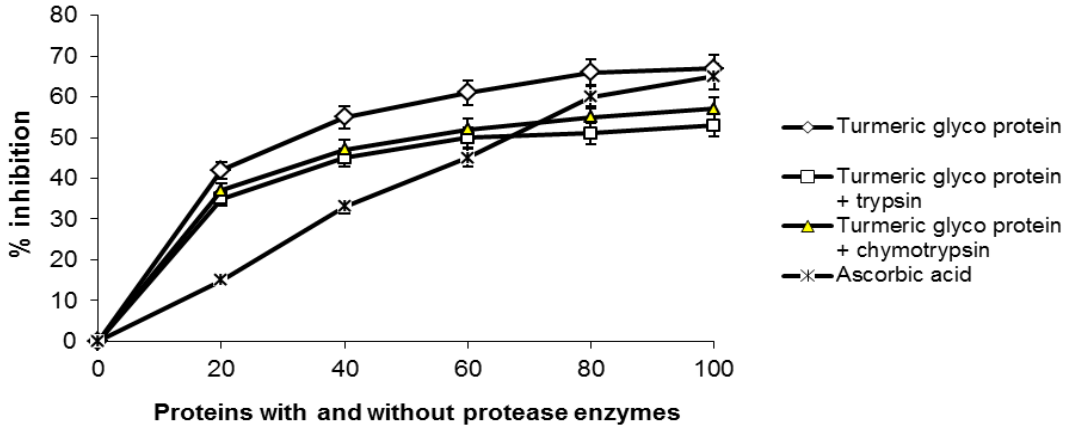

Figure 1: DPPH radical scavenging activity of Turmeric (*Curcuma longa*) glycoprotein with and without protease enzymes.

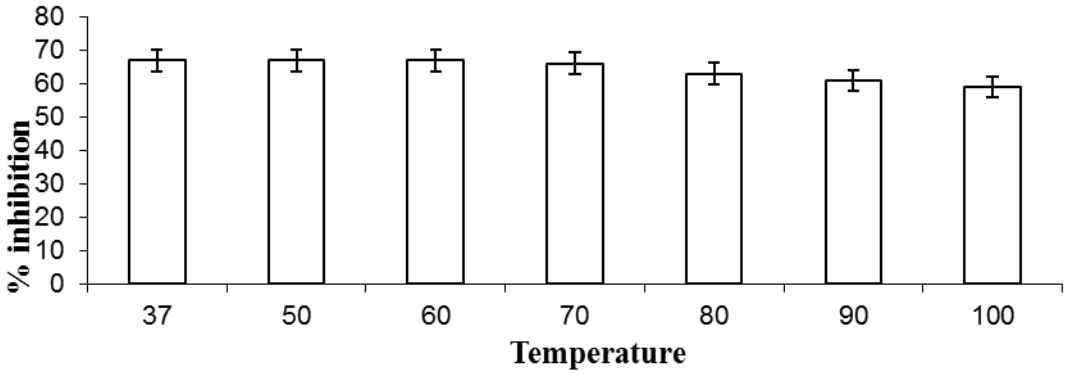

Figure 2: Effect of temperature on DPPH radical scavenging activity of Turmeric glycoproteins.

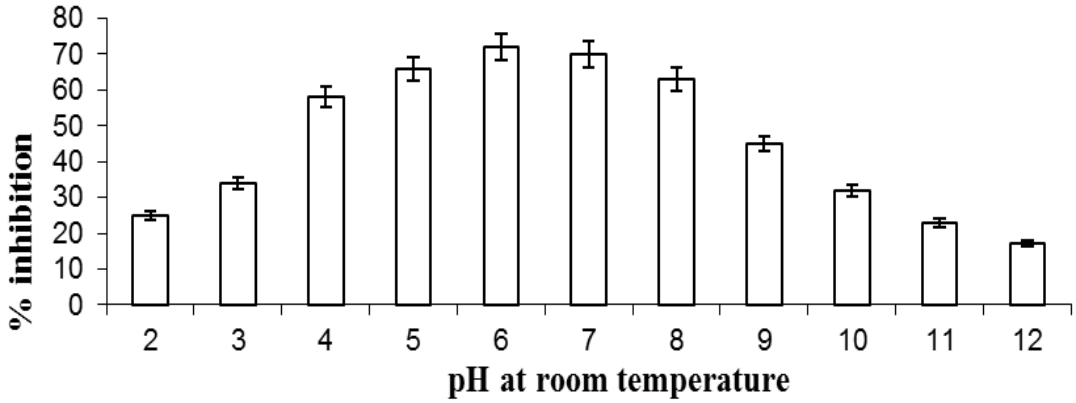

Figure 3: Effect of pH on DPPH radical scavenging activity of Piper longum crude proteins.

The extracted 28kDa glycoprotein from Turmeric (*Curcuma longa)* showed strong serine protease (Trypsin and Chymotrypsin) inhibitory activity.

To analyze the protease inhibitor activity of glycoprotein a fixed interval dose dependent DPPH radical scavenging activity was done. Where different doses of protein enzymes are mixed with glycoprotein incubated at room temperature and subjected to antioxidant analysis. Figure-1 shows that, no effect of protease enzymes on crude proteins, when compared to crude protein alone, bar indicates standard deviation from triplicate determination.

The protease inhibitor of glycoprotein was stable up to 100^0C without loss or negligible amount of loss in its activity, as the temperature increased from 75^0C to

100^0C the decreased inhibitory activity not much shown (Fig. 2).

The protease inhibitory activity of Glyco protein was tested at different pH between 2.0 and 12.0 (Fig. 3), the crude protein is stable over a broad range of pH. However there was some decrease in activity at more acidic but stable at weak acidic and at neutral pH. At basic pH, one can observe that, decrease in its antioxidant ability. This may due to the acidic nature of glycoprotein of Turmeric.

CONCLUSION

This is the first report a protease inhibitory activity from Turmeric (*Curcuma longa)* glycoprotein, and showed a potent inhibitory activity against both trypsin and chymotrypsin. Therefore, future studies in this direction have to be performed to completely elucidate the characteristic features of Serine type protease inhibitor of the Turmeric protein.

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