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PROTEASE INHIBITORY AND THERMAL STABILITY PROPERTIES OF TURMERIN PROTEIN FROM TURMERIC (*CURCUMA LONGA LINN.)* RHIZOME

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ABSTRACT

Turmeric – a golden spice widely used for its medicinal and colourant property. Herein we are reporting the property proteases like trypsin and chymotrypsin inhibition and heat stable nature of a protein of 14kDa called Turmerin from *Curcuma longa*. It exerts inhibitory activity of protease like Trypsin and Chymotrypsin up to 51 and 57 % at 100µM dosage respectively. The protein alone shows 77% inhibition and standard antioxidant Ascorbic acid shows 65%. The 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 inhibitory and thermal stability properties of Turmerin protein from Turmeric (*Curcuma longa linn*).

KEYWORDS: Turmerin, Turmeric, *Curcuma longa*, Thermal stability, Protease inhibitors.

INTRODUCTION

The Indian golden spice Turmeric rhizome is widely used as a spice, food colourant, medicinal source and its characteristic flavor in many South East Asian countries especially in India (**Monika Nagpal 2013, Toda S, Miyase T 1985)**. Lot of literature based on Ayurveda and in Chinese folk medicine says the importance and usage of Turmeric (**WHO 1999 and Chen YH 1983**).

Decades of years back, Western researcher's believed that, the yellow coloured polyphenol content, lipid soluble portion of Turmeric called Curcumin is only the active principle of Turmeric (**Louay Labban 2014**). But Indian researchers proved, and showed that, not only the Curcumin is responsible for the medicinal property of Turmeric, whereas, other components like proteins, flavonoids, polysaccharides are also equally responsible for this type of activities (**Srinivas L et.al 1992, Lekshmi PC.2012, Chinedum E. 2015, Ramanaiah Illuri et.al 2015, Rajeshwari Sahu. 2013**). Research studies have shown that the usage of Turmeric can reduce /prevent the chances of infections or diseases like Cancer, arthritis, heart ailment etc (**Abhishek Niranjan 2008**). It was also has a potential to reduce the risk of various malignant disease, Alzheimer's disease, and other chronic illness (**Ramadasan Kuttan and Ahmed 2013**).

Earlier from our laboratory reported potent proteins from Turmeric grits called Turmerin (14kDa) (**Chethan Kumar and Leela Srinivas 2006**), BGS-Haridrin (28kDa) (**Dinesha and Leela Srinivas, 2010**) and glycoprotein Beta Turmerin (34kDa) (**Smitha et al., 2009**) from Turmeric industrial waste. The above proteins had shown their antioxidant, antimicrobial, anti-inflammatory, DNA damage protectant activities (**Shalini V.K et.al 1987 and Srinivas, L 1991**). Turmerin is a 14kDa protein isolated, purified and characterized in our laboratory and reported its powerful antioxidant, anti-inflammatory properties both in invitro and invivo model systems (**Leela Srinivas and Chethan Kumar 2006**) and also the amino acid sequence has been done and registered in Uniprot with no. P85278.

The protein Turmerin from Turmeric has protease inhibitor activity, which can inhibit enzymes like trypsin/chymotrypsin. These proteolytic enzymes of stomach and digestive tract reduce the ability of body to utilize proteins from food, reduce protein efficiency ratio (**Prabhu M. S et.al 2015**). Present study report is to highlight the protease inhibitory action and thermal stability nature of Turmerin protein 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 14kDa protein from Turmeric (*Curcuma longa*)

The Turmerin protein was isolated from Turmeric (*Curcuma longa*) by 55% ammonium sulphate precipitation (**Srinivas L & Shylaja M 1992**). In brief, 10g of powder of Turmeric powder vortexed with 250 ml of double distilled water for two hours at 20^{o}C. Later the extract centrifuged at 10000 rpm for 20 min at 4^{o}C. The supernatant collected was subjected to 55% ammonium sulphate precipitation, kept for votexing at 4^{o}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^{0}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 Turmerin protein 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 Turmerin protein of *Curcuma longa* were tested by incubating at different temperatures 37, 40, 50, 60, 70, 80, 90, 100^{0}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

Turmerin protein of *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 < ± 5%.

RESULTS AND DISCUSSION

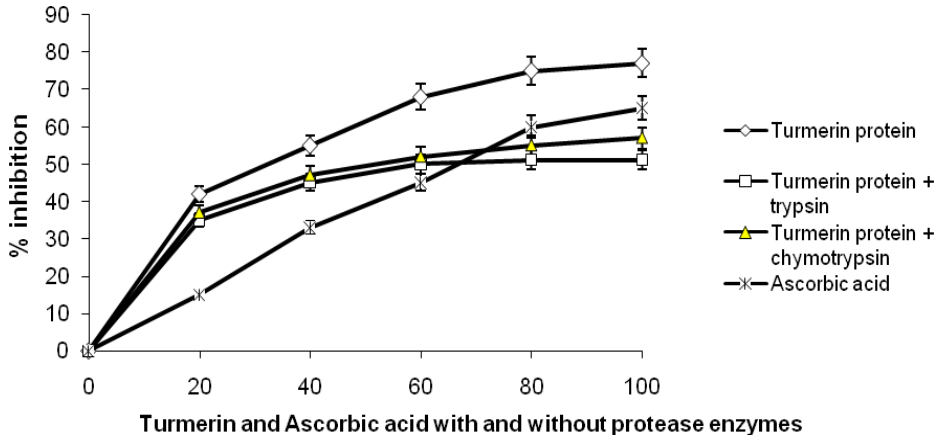

Figure-1: DPPH radical scavenging activity of Turmerin protein and Ascorbic acid with and without protease enzymes.

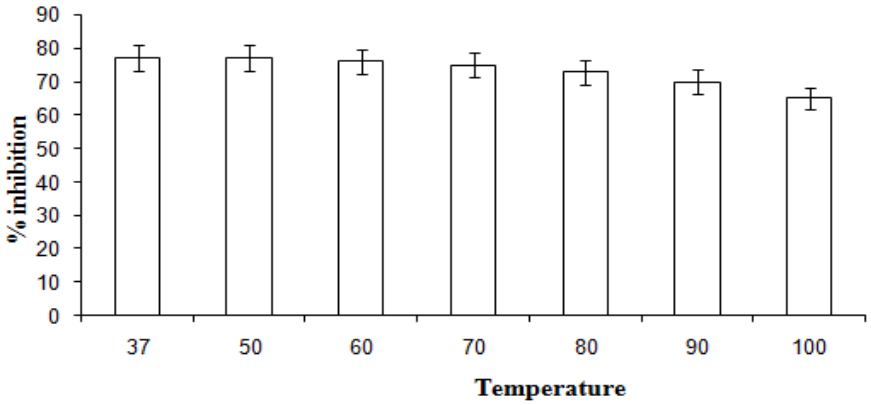

Figure-2: Effect of temperature on DPPH radical scavenging activity of Turmerin protein.

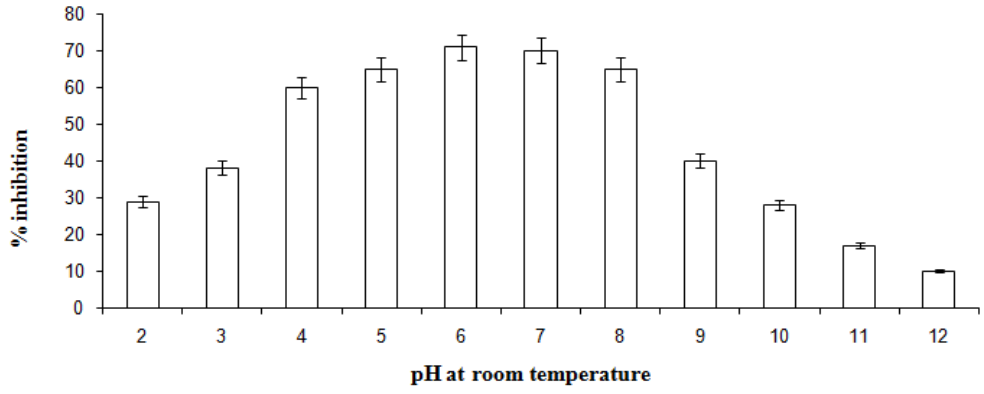

Figure-3: Effect of pH on DPPH radical scavenging activity of Turmerin protein from *Curcuma longa*.

The extracted 14kDa protein Turmerin from Turmeric showed strong serine protease (Trypsin and Chymotrypsin) inhibitory activity. To analyze the protease inhibitor activity of Turmerin a fixed interval dose dependent DPPH radical scavenging activity was done. Where different doses of protein enzymes are mixed with Turmerin protein incubated at room temperature and subjected to antioxidant analysis. **"Fig. 1"** shows that, no effect of protease enzymes on Turmerin when compared to Turmerin protein alone, bar indicates standard deviation from triplicate determination.

The protease inhibitor Turmerin 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"**. During the process of Turmeric raw rhizome, farmers going to boil the rhizomes in hot water for hours together. Further while powdering, also dried rhizomes are crushed and rubbed together, where high temperature is generated, even though this special spice retains its medicinal property.

The inhibitory activity of enzymes trypsin and chymotrypsin was tested at different pH between 2.0 and 12.0 "**Fig. 3"**, protein Turmerin from Turmeric 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 Turmerin protein of Turmeric.

CONCLUSION

This is the first report a protease inhibitor Turmerin from Turmeric 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 Turmerin.

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