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Research Article

# Quantitative Analysis of Proteins and Studies on Serine Protease Inhibitory Activity from Aqueous Testa Extracts in Some Cucurbitaceae Members

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**Abstract**

Proteins play a vital role in seed germination and dormancy. Role of proteins present in testa of some Cucurbitaceae members (*Citrullus lanatus, Cucurbita maxima* and *Cucumis sativus*) was to be analyzed for understanding better health products. *Citrullus lanatus, Cucurbita maxima* and *Cucumis sativus* testa crude extract was shown as 6.0±0.07, 4.8±0.04 and 5.8 ±0.04 mg/ml of protein respectively. The high protein content was shown in 90% isolated buffer extracts from Cucurbitaceae members. Cucurbitaceae members were shown the inhibitory activities of Trypsin, Chymotrypsin and Proteinase K on crude testa extracts. Trypsin inhibitory activity is more in *Citrullus lanatus* (65%) followed by *Cucumis sativus* (40%) and *Cucurbita maxima* (58%). Trypsin specific activity is more in *Citrullus lanatus* (10.8) followed by *Cucurbita maxima* (8.3) and *Cucumis sativus* (10). Chymotrypsin inhibitory activity is more in *Cucumis sativus* (53%) followed by *Citrullus lanatus* (47%) and *Cucurbita maxima* (46%). Chymotrypsin specific activity is more in *Cucurbita maxima* (9.3) followed by *Citrullus lanatus* (9.5) and *Cucumis sativus* (8.1). Proteinase K inhibitory activity is more in *Citrullus lanatus* (15%) followed by *Cucurbita maxima* (5%) and *Cucumis sativus* (13%). Proteinase K specific activity is more in *Citrullus lanatus* (2.5) followed by *Cucurbita maxima* (1.04) and *Cucumis sativus* (2.24).

**Key words:** Cucurbitaceae, testa, specific activity, inhibitory activity

## 1. INTRODUCTION

Polymers of amino acids are linked to each other with peptide bond are termed as proteins. The properties of the protein are governed by various R groups or amino acids. The backbone of a protein is made up of the peptide linkage and is common in all proteins within the living cellular system [1,2,3]. The interaction between the R groups in a peptide/protein helps in giving a 3D (three dimensional) structure or conformation of the peptide/protein.

The α-helical structure of proteins is proposed by Pauling is stabilized by intrachain hydrogen bonds. Most of the bonds that help in the formation of 3D structure are weak forces and are easily disturbed by changes in pH, temperature, surface tension, etc [4]. The bonds subjected to the drastic forces makes to lose 3D structure and the protein is denatured. Hence proteins or peptides have to be handled gently and should always be kept in a buffered solution and at low temperature range from 0 - 4 ˚C.

A protein solution without a buffer should not even be allowed to keep at very low temperatures that stand below 10 ºC [4,5,6]. The proteins become more rigid implying the enhancing flexibility that can restore function to show denaturation with changes in conformation of the protein molecules. The amino acids like glutamic acid and lysine have extra ionizable groups make a protein unstable. The response of glutamic acid and lysine to pH changes confers electrical charges to proteins [7,8].

*Citrullus lanatus* is cultivated in northern and western parts of India. The fruits of watermelon are available during summer season [9]. *Citrullus lanatus* is used in the treatment of various diseases like diabetes diarrhea, dysuria, jaundice, beri-beri and rheumatism

[10]. The seeds of *Citrullus lanatus* contain a biochemical compound that shows medicinal properties in the treatment for kidney stones, demulcent, diuretic, pectoral urethral problems and tonic [11]. *Cucurbita maxima and Cucumis sativus* are also belongs to Cucurbitaceae members that are extensive using as food and medicinal sources in several countries like India, Japan, China, US, etc.

## 2. MATERIALS AND METHODS

The materials and the methods used in the present experimentation are given below.

### 2.1. Collection of Testa Powder from Cucurbitaceae Members

In the present experiment, *Citrullus lanatus*, *Cucurbita maxima* and *Cucumis sativus* fruits was collected from the fields of Visakhapatnam district, AP, India during March to June 2011. The plants are authenticated by Dr. P.V. Arjun Rao, Ethanobotanist, Dept. of Botany, Phytopharma Technology Laboratory, Visakhapatnam (No. Res/2 dated 21-09-2010). *Citrullus lanatus*., *Cucurbita maxima* and *Cucumis sativus* respectively on comparison with the details given in "FLORA OF THE PRESIDENCY OF MADRAS" by J.S. Gamble, Volume i, Page nos.534-536, Bishen Singh Mahendra Pal Singh publishers, India (2004) and "Flowering plants from Chittoor district, Andhra Pradesh, India" by K. Madhava Chetty, K. Sivaji and K. TulasiRao, First edition published by students offset printers, Thirupathi, India, pp. 137-139 (2008).

#### 2.1.1. Preparation of Crude Extract

The seeds present in the fruits are collected and dried for two days. The testa is separated and crushed to a fine powder using mortar and pestle. The fine testa powder is selected for the present experimentation.

The testa powder was depigmented, dehydrated, and defatted by washing with acetone for several times, followed by hexane and Folch's mixture (chloroform: methanol, 2:1) and with 1% PVP. The solvents were removed by filtration and the powdery air-dried. About 15g of testa powder was homogenized in 100 ml of 0.1M phosphate buffer at pH 7.0 and the extract was prepared in 500 ml conical flask. The homogenate was mixed by incubating the extract in a rotary shaker at 120 rpm for 30 minutes at room temperature. Then the cell debris was removed by the slurry filtered through cheesecloth. Filtrate was collected and centrifuged at 10,000 rpm for 15 minutes at 4°C [12]. The crude extract appears as a clear supernatant was selected for further studies. The crude extracts were quantified by Lowry's method (1951) using BSA as a standard [13].

### 2.2 Quantitative Analysis of Proteins by Lowry Method

Quantitative analysis of proteins by Lowry method is conducted by the procedure given below.

#### 2.2.1. Reagents

(1) Reagent A: 2% $Na_2CO_3$ in 0.1N NaOH.
(2) Reagent B: 0.5% $CuSO_4$ in 1% Sodium potassium tartarate.
(3) Alkaline cupper solution: 50 ml of reagent A was mixed with 1ml of reagent B (just before use).
(4) Fc Reagent (Folin-ciocalteau reagent): Dilute this reagent 1 in 3 (1:3) with distilled water (just before use).
(5) Standard protein solution (for 1mg/ml Concentration): Dissolve 50mg of BSA (Bovine serum albumin) in 50 ml of distilled water in a volumetric flask.

#### 2.2.2. Quantitative Analysis of Proteins

The BSA (Bovine serum albumin1mg/ml) as standard was prepared with dilutions of 0.2, 0.4, 0.6, 0.8 and 1ml of working standard protein solution into five different test tubes with water to bring the volume to 1ml in each test tube. Add 5ml cupper solution (Reagent A: 2% $Na_2CO_3$ in 0.1N NaOH and Reagent B: 0.5% $CuSO_4$ in 1% Sodium potassium tartarate) to all the test tubes, mix well and allowed to stand for 10 minutes. Then add 0.5ml dilute Fc (Folin-ciocalteau reagent (1:3) and stand for 30 minutes in dark at room temperature to develop blue color. Measure the optical density in a photoelectric calorimeter at 660nm using a red filter. Simultaneously prepare a blank with 1ml of water and 1ml of unknown protein solution (extract) and proceed as per standards.

Plot a graph with the concentration of protein in mg/0.1 ml on X-axis and optical density at 660nm on Y-axis. Determine the amount of protein content present in the given samples (sample size = 3) using standard calibration curve (as Mean ± SE) using formulae:

Mean (or $\bar{x}$) are calculated using formulae $\bar{x} = \frac{(\Sigma x)}{n}$ where $(\Sigma x)$ is the sum of x variables and n is the sample size.

Standard deviation or SD is calculated using formulae

$$SD = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n \quad 1}}$$

Standard Error or SE is calculated using formulae

**SE = SD / sqrt(n)** **Note:** sqrt is square root

**2.3. Isolation of Extract by Ammonium Sulphate Precipitation Method**

*Citrullus lanatus, Cucurbita maxima* and *Cucumis sativus* testa was collected from the fields of Visakhapatnam district, AP, India and the extracts are prepared as described above as crude extract preparation. The protein was isolated by the Ammonium sulphate precipitation method. The protein isolation was done by the method of Englard and Seifter, 1990 [14]. The unwanted proteins are removed by ammonium sulphate precipitation and at the same time the protein of interest could be concentrated. Varying concentrations of ammonium sulphate (30%, 50%, 70% and 90%) to the crude extract was kept at 4˚C for about one day precipitation to optimize the selected protease inhibitor. The precipitated protease inhibitor was collected by centrifugation of extract at 10,000 rpm at 4˚C for 15 minutes.

The precipitated protein was further dialyzed against 0.01M phosphate buffer at pH 7.0 to remove the ammonium sulphate present in the precipitate as details given below.

The dialysis tube (Sigma-Aldrich) was washed in running water for about 3-4 hrs. The tube was rinsed with the 0.3% *(w/v)* solution sodium sulfide at 80°C for 1 minute. After washing with hot water (60°C) for 2 minutes, the solution was acidified with 0.2% sulphuric acid (v /v) and rinsed with hot water (60°C).The process is done to build the pores of the tube more clear. A tube will be opened then pack the sample solution and this packed solution was kept in 0.01M phosphate buffer. The method was useful for the removal of salts in the sample solution. Finally the protein was lyophilized, subjected to various analytical techniques and also used in the further purification.

**2.4. Techniques for Preliminary Analysis of Protease Inhibitors**

Various techniques have been conducted for identification, isolation and activity for protease inhibitors.

**2.4.1. Protease Inhibition Assay**

An Activity of protease inhibitor against serine proteases (Trypsin/ Chymotrypsin/ Proteinase K) was assayed based on the protocol provided by Kunitz [15]. The reaction mixture was prepared as 1 ml of protease (Trypsin, Chymotrypsin and Proteinase K respectively). The enzyme sample was preincubated with 1 ml of protease inhibitor at 37°C for 15 minutes. About 2ml of 1% Hammerstein casein was added to the reaction mixture and again incubated at 37°C for 30 minutes. About 2.5ml of 0.44 M trichloroacetic acid (TCA) solution was added to the reaction mixture for stopping the reaction. Above reaction mixture was centrifuged at 10,000rpm for 15 minutes and the protein was precipitated. The clear supernatant absorbance was measured at 280nm in UV-Visible spectrophotometer against blanks. The TCA soluble amide fractions of casein shaped by the action of proteinase within the presence and absence of inhibitor were quantified by analysis with amino acid as normal. One unit of enzyme activity was determined in the present experimentation.

The Inhibitory activity is calculated by subtracting amounts of tyrosine released without an inhibitor with the amount of tyrosine released with inhibitor and dividing by the amount of tyrosine released without inhibitor. The protease Inhibitory activity (%) was calculated by multiplying inhibitory activity with 100.

Specific activity = Inhibitory activity (%)/ Protein (mg/ml)

**2.4.2. Detection of protease inhibitors by Dot - Blot method**

The dot-blot method is used to detect protease inhibitors based on procedure mentioned below.

**2.4.2.1. Enzymes preparation**

In the present studies, the enzymes (serine proteases) selected are Trypsin, Chymotrypsin and Proteinase K.

**2.4.2.1.1. Trypsin**

Prepare 0.1 M tris HCl buffer (pH 7.8). Add 0.1mg of Trypsin in 1ml buffer (i.e. 0.1mg Trypsin/ml)

**2.4.2.1.2. Chymotrypsin**

Prepare 100 ml of 0.1M Glycine-NaOH buffer (pH 10). To the buffer, about 0.5mg of Chymotrypsin is added in 1ml of buffer (i.e. 0.5 mg chymotrypsin/ml)

**2.4.2.1.3. Proteinase K**

Prepare storage buffer by adding 50ml of glycerol with 1ml of 1MTris HCl and 0.29g of $CaCl_2$. Makeup the solution to100ml with double distilled water. Finally 10ml of storage buffer is taken and add 100mg of Proteinase K to get 1mg Proteinase K/ml enzyme solution.

**2.4.2.2. X-Ray Film Method for Analysis of Crude and Buffer Extracts from Cucurbitaceae Members as Protease Inhibitors**

About 10µl of protease inhibitor (6 mg/ml) was mixed with 10µl protease (0.5mg/ml) and spotted on X-ray film. The solutions were made with 50% serial dilutions to get 10, 5, 2.5, 1.25 and 0.625 concentrations. Mixing of 10µl of testing serine

protease with 10µl of 0.lM phosphate buffer (pH 7.0) as the control and marked onto the X-ray film Incubate the X-ray film for 10 minutes at 37°C. The film is washed under tap water. The protease cannot degrade the gelatin on the X-ray film due to the presence of protease inhibitory activity in the extracts of Cucurbitaceae members. Protease was hydrolyzing the gelatin clear spot was visualized due to the absence of protease inhibitor.

## 3. RESULTS AND DISCUSSION

Different proteins from plants are governed by amino acid composition determines the nutritional and functional properties within and outside the species. Proteins, carbohydrates, lipids, vitamins, minerals, and non-nutrients play a key role of the plant during seed development and germination [16]. A major problem in plant food processing industry represents the concerned and usage of reasonable quantities of waste materials like seeds or seed coats, peels and their by-products. The plant sources may be demanding with natural ingredients with safe and health-promoting aspects that show the potential effects on human health [17,18].

Proteins are the vital components that are present in living species. *Citrullus lanatus* testa contains the biological components that play a vital role in the regulation of plant growth and defense. Naturally occurring primary and secondary metabolites are of plants, animals, microbes and mineral origin. The plant kingdom has shown various phytocompounds and proteins that are useful in the treatment of various diseases showing important source of world's pharmaceuticals. Most of the medicinal plants are used as food and spices meant for medicinal purposes [19].

The experimentation was shown that the standard BSA solution has shown ODs values as 0 (Blank), 0.18, 0.35, 0.53, 0.70 and 0.90 at working standards 0, 0.2, 0.4, 0.6, 0.8 and 1ml of protein solutions. The results were plotted on the graph (**Figure 1**). The graph shows a straight line. By using standard graph, the protein concentrations of testa crude and buffered extracts from Cucurbitaceae members has been assayed.

The protein samples of the extracts are measured as per OD values and cut on the standard graph. *Citrullus lanatus*, *Cucurbita maxima* and *Cucumis sativus* testa crude extract was shown as 6.0±0.07, 4.8±0.04 and 5.8 ±0.04 mg/ml of protein respectively. In the selected species, *Citrullus lanatus* has shown good protein content in the testa crude extract. The protein content of crude extracts of Cucurbitaceae members were shown in **Table 1 and Figure 2**.

*Citrullus lanatus* testa crude extract was shown as 6.0±0.07 mg/ml of protein. The protein component in 30%, 50%, 70% and 90% of isolated extracts from *C. lanatus* was shown 2.9±0.04, 3.0±0.04, 3.8±0.04, and 4.1±0.06 mg/ml respectively. The high protein content was shown in 90% isolated extracts from *C. lanatus* compared with other isolated extracts.

*Cucurbita maxima* testa crude extract was shown 4.8±0.04 mg/ml of protein. The protein component in 30%, 50%, 70% and 90% of isolated extracts was shown 1.5±0.07, 2.9±0.04, 3.2±0.04 and 3.9±0.07 mg/ml respectively. The high protein content was shown in 90% of isolated extracts from *C. maxima* compared to isolated extract.

*Cucumis sativus* testa crude extract was shown 5.8±0.04 mg/ml of protein. The protein component in 30%, 50%, 70% and 90% of isolated extracts was shown 2.3±0.04, 3.2±0.04, 3.5±0.08 and 3.7±0.04mg/ml respectively. The high protein content has been shown in 90% of isolating compared to all extract. When compared with standards, 30% and 50% isolated crude extracts have shown low concentrations of proteins in *Citrullus lanatus*, *Cucurbita maxima* and *Cucumis sativus* testa (**Figure 2**).

**Figure 3 and 4** shows the Inhibitory activity and specific activity from testa of some Cucurbitaceae members with Trypsin. Based on the OD values, inhibitory activity and specific activity values have been calculated. Cucurbitaceae members were shown the activities of Trypsin on crude extract testa. Trypsin inhibitory activity is more in *Citrullus lanatus* (65%) followed by *Cucumis sativus* (40%) and *Cucurbita maxima* (58%). Trypsin specific activity is more in *Citrullus lanatus* (10.8) followed by *Cucurbita maxima* (8.3) and *Cucumis sativus* (10). Chymotrypsin activity on the crude extract testa of Cucurbitaceae members was conducted. Chymotrypsin inhibitory activity is more in *Cucumis sativus* (53%) followed by *Citrullus lanatus* (47%) and *Cucurbita maxima (*46%). Chymotrypsin specific activity is more in *Cucurbita maxima* (9.3) followed by *Citrullus lanatus* (9.5) and *Cucumis sativus* (8.1).

Proteinase K activity on crude extract testa of Cucurbitaceae members was conducted. Proteinase K inhibitory activity is more in *Citrullus lanatus* (15%) followed by *Cucurbita maxima* (5%) and *Cucumis sativus* (13%). Proteinase K specific activity is more in *Citrullus lanatus* (2.5) followed by *Cucurbita maxima* (1.04) and *Cucumis sativus* (2.24).

**Figure 5** shows the protease inhibitor activity for Cucurbitaceae members on Proteases using the X-ray film method. In the present experiment, trypsin was shown inhibition with isolated testa extract of *C. lanatus*. Chymotrypsin has shown inhibition with isolated extract of *C. lanatus*. Proteinase K has not

shown any inhibitory activity with isolated extract of *C. lanatus*. Hence the isolated testa crude extract of *C. lanatus* has shown the protease inhibitor activity. Trypsin has not shown inhibition at 10 dilutions. Chymotrypsin has shown inhibition in all the dilutions and the activity decreasing on further dilutions. Proteinase K has not shown any inhibitory activity. Trypsin has shown inhibition at 10 dilutions. Chymotrypsin has shown inhibition in all the dilutions and the activity decreasing on further dilutions. Proteinase K has not shown any inhibitory activity. The protease Inhibition activity for Cucurbitaceae members on 90% buffer extracts using the X-ray film method shown good results.

The protease inhibition activity of *Citrullus lanatus* testa buffer extracts was shown in **Figure 6**. Chymotrypsin inhibition in four dilutions of all buffered extracts was shown inhibition but control was not shown any inhibition. Inhibitory activity was not shown with Proteinase K.

The protease inhibition activity of *Cucumis sativus* testa buffer extracts was shown in **Figure 7**. Trypsin inhibition at all the dilutions used in the experimentation was not shown for *Cucumis sativus* testa buffer extracts. Chymotrypsin was shown inhibition in all the dilutions of all buffered extracts and control has not shown any inhibition. *Cucumis sativus* testa buffer extracts were not shown any Proteinase K inhibitory activity.

The protease inhibition activity of *Cucurbita maxiama* testa buffer extracts was shown in **Figure 8**. *Cucurbita maxiama* testa buffer extracts were not shown any Trypsin inhibition at the dilutions used in the experiment. *Cucurbita maxiama* testa buffer extracts were shown Chymotrypsin inhibition activity in all the dilutions of all buffered extracts but control has not shown any inhibition. *Cucurbita maxiama* testa buffer extracts was not shown any Proteinase K inhibitory activity.

An antimicrobial protein (Ace-AMP1) of about 10 kDa has been isolated by Cammue et al., 1995 from onion (*Allium cepa* L.) seeds [20]. Two antimicrobial peptides named Mj-AMP1 and Mj-AMP2 was isolated previously from the seeds of *Mirabilis jalapa* L. A plant seed possess potent, broad-spectrum antimicrobial peptides for a wide range of microbes [21]. The defense system in plants is naturally developed in the testa as protease inhibitors that will protect from pathogenic microbes at the early stages of seed germination.

Biochemical analysis of proteins relies on accurate quantization of protein concentration [22]. The most suitable method for the quantification of protein was the Lowry method [23]. In Lowry method, different proteins give different responses [24].

Most of the seed protein constitutes approximately 70% of the total seed protein at maturity and 30%-40% of the seed weight during germination [25]. Watermelon seeds have a high protein content of approximately 35% [26]**.** Pumpkin seeds have rich protein contain from approximately 39.25% [27, 28].

Several technologies have been developed in biomolecules during the past 80 years provided good scope in biological sciences. The role of enzymes in biochemical reactions in bio-systems has potential applications in food and medicinal industries. Proteolytic enzymes have many physiological functions that are ranging from protein digestion to specific regulated processes like activation of zymogens within and outside the system [29]. The zymogram method is a technique developed by Hunter and Markert in 1957 was found many research applications. Cucurbitaceae members were shown good serine protease inhibition activities in testa of *C. lanatus* [30]. The technique of gel electrophoresis of enzymes with specific staining methods provides good activity in the gel that show molecular understanding of enzymes and proteins.

**Figure 9** shows the comparative activity of the substrate in the absence and presence of the inhibitor with enzyme.

Protease inhibitors have an irreversible suppressive effect on the carcinogenic process that shows oncogene expression and inhibit carcinogen induced protease activity. Protease inhibitors stop the process of changing the normal cells to the malignant state even at the advanced stages of carcinogenesis [31]. Detection of protease inhibitors by using a new, sensitive and simple method is X ray film (dot blot) method. By this method found that pigeon-pea seed extract was contained nine trypsin and seven chymotrypsin inhibitors [12].

Proteolytic enzymes acting on protein substrates like casein within the cell. By the action of caseinolysis, the amount of proteolytic inhibitory activity was theoretically estimated [31]. The method has been applied to an evaluation of the chymotrypsin inhibitor activity of crude soybean extracts [32]. The inhibitory activity of PIs used to determine the $EC_{50}$ using a nonlinear regression routine [33] procedure following equation.

**Inhibition (%) = I max [inhibitor] n /inhibitor**

General serine protease inhibitors, heavy metal cations ($Zn^{2+}$ and $Hg^{2+}$), and the heavy metal chelator 1, 10-phenanthroline partially or totally inhibited the proteolytic activity [34]. Buffer maintains a neutral pH and some of the metals may show inhibition [35].

The protease Inhibition activity for Cucurbitaceae members on 30%, 50%, 70% and 90% buffer extracts

using the X-ray film method being studied. *Citrullus lanatus* testa buffer extracts have shown Trypsin inhibition at 90% dilution used in the experiment. *Citrullus lanatus* testa crude 10% extract was not shown trypsin inhibition based on previous results [35]. Chymotrypsin inhibition has shown in all the dilutions of all buffered extracts but control has not shown any inhibition. All extracts of *Citrullus lanatus* testa buffer extracts have not shown any Proteinase K inhibitory activity. *Cucumis sativus* testa buffer extracts have not shown any Trypsin inhibition at all the dilutions used in the experiment. Chymotrypsin inhibition has shown in all the dilutions of all buffered extracts but control has not shown any inhibition. *Cucumis sativus* testa buffer extracts has not shown any Proteinase K inhibitory activity. *Cucurbita maxima* testa buffer extracts have not shown any Trypsin inhibition at all the dilutions used in the experiment. Chymotrypsin inhibition is shown at 5 dilution but not in all the dilutions of buffered extracts but control has not shown any inhibition. *Cucurbita maxiama* testa buffer extracts have not shown any Proteinase K inhibitory activity.

In contrast, most of the recently isolated serine proteases from plants have isoelectric points in the range of 4.0–7.0 [36]. Hence the present work provided the quantification of proteins and serine protease inhibitory activity from aqueous extracts in some Cucurbitaceae members. Cucurbitaceae members contain proteases that can inhibit serine proteases.

## 4. CONCLUSION

Further analysis on protease inhibition and antimicrobial studies are needed to know the activities of peptides in Cucurbitaceae members. The present work has shown that *Cucumis sativus*, *Cucurbita maxima* and *Citrullu lanatus* testa extracts shows good protease inhibitor activity. In the present experiment *Citrullus lanatus* testa was shown good inhibition activity with trypsin compared to other two Cucurbitaceae members.

## 5. CONFLICT OF INTERESTS

The author declares that there is no conflict of interests regarding the publication of this paper.

## 6. ACKNOWLEDGMENT

The authors would like to thank the management and staff of GITAM University, India and Bilaspur University, India for their kind support in bringing out the above literature and providing lab facilities.

**Table 1**
**Protein concentration from *Citrullus lanatus, Cucurbita maxima* and *Cucumis sativus* testa of crude and isolated extracts**

| Crude/Diluted extracts | Protein concentration (from graph) in mg/ml Mean ± SE(n = 3) | | |
|---|---|---|---|
| | ***Citrullus lanatus*** | ***Cucurbita maxima*** | ***Cucumis sativus*** |
| Crude | 6.0±0.07 | 4.8±0.04 | 5.8±0.04 |
| 30% | 2.9±0.04 | 1.5±0.07 | 2.3±0.04 |
| 50% | 3.0±0.04 | 2.9±0.04 | 3.2±0.04 |
| 70% | 3.8±0.04 | 3.2±0.04 | 3.5±0.08 |
| 90% | 4.1±0.06 | 3.9±0.07 | 3.7±0.04 |

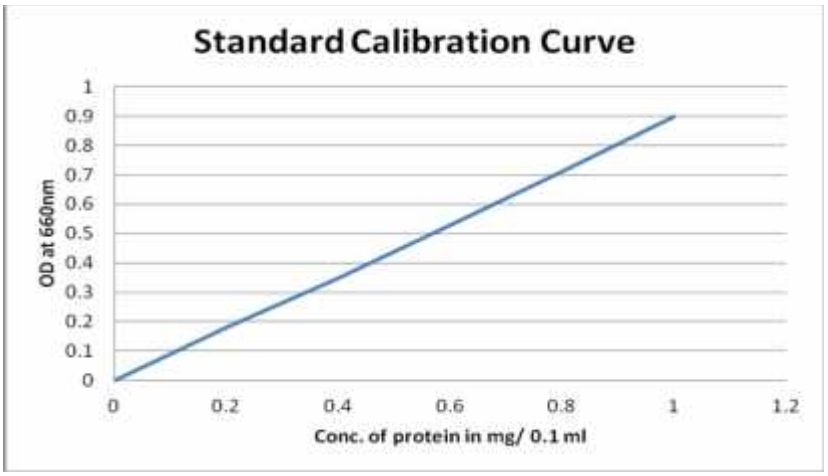



**Figure 1**
**Standard plot for BSA protein**

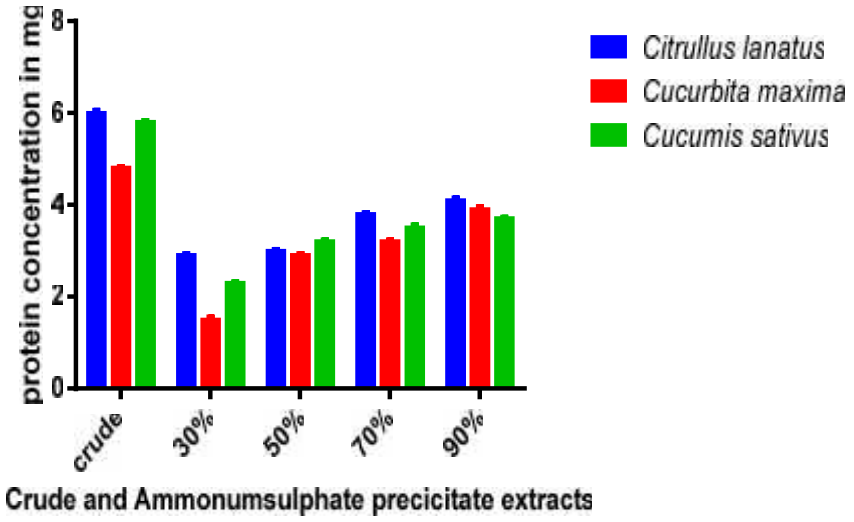



**Figure 2**
**Protein concentration (in mg/ml) of testa extracts in some Cucurbitaceae members**

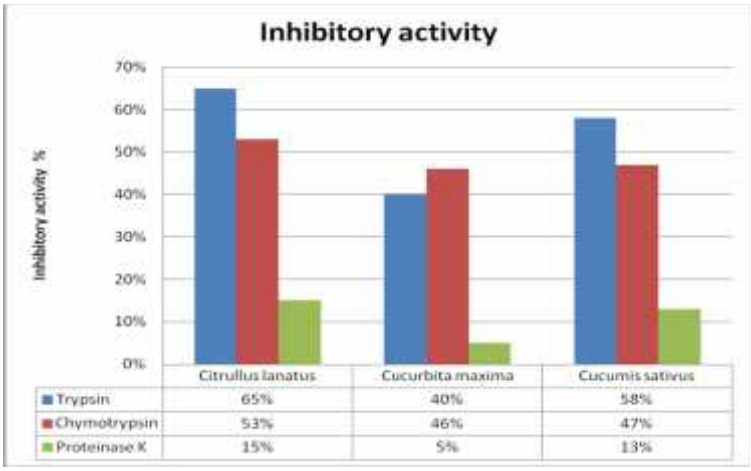



**Figure 3**
**Inhibitory activity from testa of some Cucurbitaceae members**

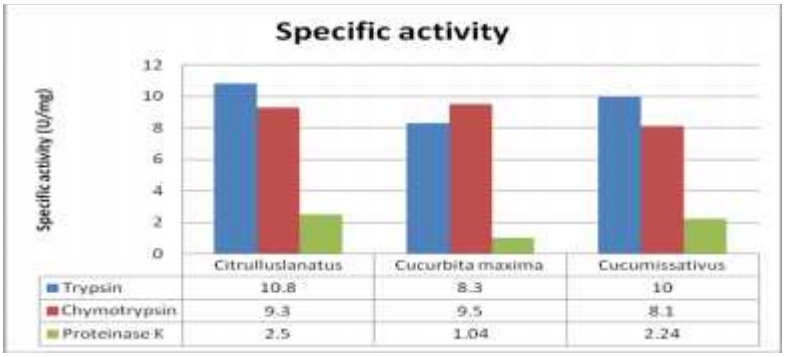
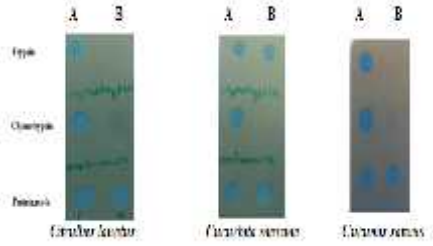
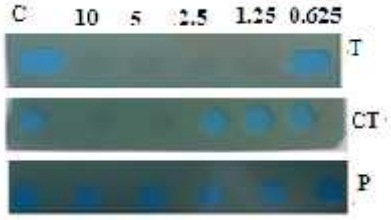



**Figure 4**
**Specific activity from testa of some Cucurbitaceae members**



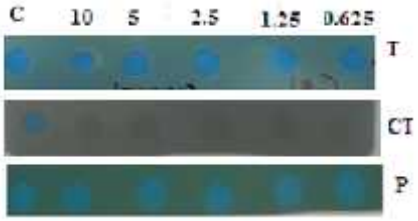

***Note: A – Control; B- Crude extract***
**Figure 5**
**Protease inhibitor activity from testa crude extracts of Cucurbitaceae members on Proteases using X-ray film method**




**Note: C-Control; T-Trypsin; CT-Chymotrypsin; P-Proteinase K**

**Figure 6**

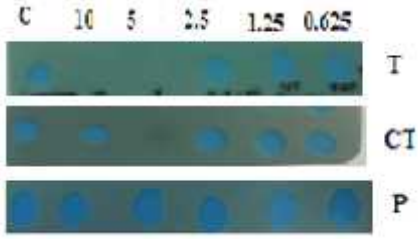
**Protease Inhibition activity of *Citrullus lanatus* of isolated buffered samples by X-Ray film method**




**Note: C-Control; T-Trypsin; CT-Chymotrypsin; P-Proteinase K**

**Figure 7**

**Protease Inhibition activity of *Cucurbita maxiama* of isolated buffered samples by X-Ray film method**




**Note: C-Control; T-Trypsin; CT-Chymotrypsin; P-Proteinase K**

**Figure 8**

**Protease Inhibition activity of *Cucumus sativus* of isolated buffered samples by X-Ray film method**

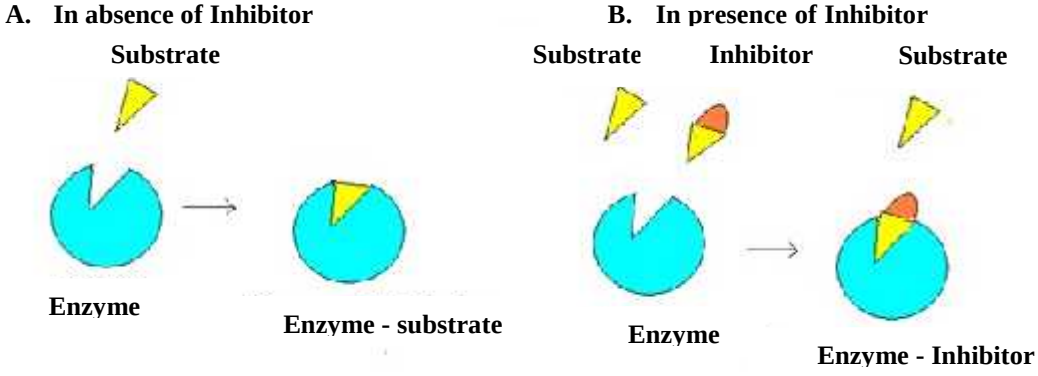



**Figure 9**

**Activity of substrate in absence and presence of inhibitor**

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