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Research Paper

Protease Inhibition Studies and Metallic Responses of Cucurbita maxima and Citrullus lanatus Seed Coat Extracts

Biochemistry

KEYWORDS : Cucurbita maxima, Citrullus lanatus, seed coat extracts, Protease inhibition, metals

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ABSTRACT

The impact of medical specialty activities with medicative properties to elucidate mechanisms will scale back unwellness risk. This experimentation has been conducted to seek out the proteolytic enzyme restrictive impact of Cucurbita maxima and genus Citrullus lanatus seed coat extracts. The restrictive action of enzyme and Protease-K has not shown any restrictive impact. Chymotrypsin inhibition has been determined at 10µl of crude extract. Pumpkin seed coat crude extract has shown enzyme (at 10µl) and Chymotrypsin (10 to 0.625µl) inhibition. All the tested concentrations has not shown any Protease-k inhibition. The isolated Cucurbita maxima and genus Citrullus lanatus seed coat extracts has not shown any inhibition with Trypsin and Protease-k however shown inhibition with Chymotypsin at 30%, 50% and 90% concentrations. FeSO4, Na2EDTA and (CH3COO)2Pb has shown Trypsin inhibition. All the metals except FeSO4, Na2EDTA and (CH3COO)2Pb has not shown any Trypsin inhibition. Typsin inhibition has not been seen once genus Citrullus lanatus seed coat extract (10µl) has been else before adding metal. Thus this shows the competitive binding of Citrullus lanatus seed coat extract. All the metals has not shown any Chymotrypsin inhibition. Addition of genus Citrullus lanatus seed coat extract has shown competitive inhibition in Phosphate buffer, MgSO4, HgCl2 and Na2EDTA. Protease-k has shown inhibition with FeSO4 and not shown any inhibition with different metal compounds. Competitive inhibition of genus Citrullus lanatus seed coat extract has been found in CuSO4 and HgCl2. Competitive binding of the FeSO4 with protease-k has been determined. Typsin inhibition has been seen once Cucurbita maxima seed coat extract (10µl) has been else before adding HgCl2. Thus this shows the binding of extract. All the metals haven't shown any trypsin inhibition. Addition of Cucurbita maxima seed coat extract has shown competitive inhibition with all the metals in chymotrypsin. Protease-k has not shown inhibition with all metal compounds of Cucurbita maxima seed coat.

1. Introduction

Plants contain pure active principles of substances that have source of medicaments [1], associated in treatment of various human ailments [2]. Medicinal plants involve a healthcare approach in current research in drug design and discovery by combining with techniques like Botany, Phytochemistry, Biology and Molecular Technology [3]. Various systems of medicines like ayurvedic, unani, siddha, folk medicine with integration of traditional and modern systems of medicine provides insides in treatment of diseases with contemporary scientific practice [4].

Characterization of biological samples provides meaning full, interpretations through metabolic analysis [5]. A winter squash fruit (Cucurbita maxima duchesna variety; Telugu vernacular- gummadikaya; common name- pumpkin) is an important vegetable fruit has good shelf line with good source of water soluble vitamins carotene [6].

Pumpkin is popularly used in various traditional systems of medicine in treatment of several aliments, used as anti-diabetic, antibacterial, antitumor, antihypertensive, antihyper-cholesterolemia, immune-modulation, anti-inflamation, antiparasitia, antalgic, antihelmenthic, provides an attention on various investigations on this plant. The plant materials are being investigating by various scientists took know the effect of pharmalogical activities with medicinal properties to elucidate mechanisms that reduce disease risk [7].

Citrullus lanatus (Thub.) commonly known as water million belongs to family cucurbitaciae. The plant is cultivated in northern and western parts of India, fruits are available during summer season[8]. Citrullus lanatus (or Citrullus vulgaris; Telugu vernacular - puchhakaya; common name - watermelon) is used in treatment of various diseases like diabetes diarrhea, dysuria, jaundice, beri-beri, rheumatism [9], seeds contains a biochemical compound that show medicinal properties in the treatment for kidney stones, demulcent, diuretic, pectoral urethral problems and tonic [10].

t2.1. Plants for the study

Plants that are locally available and belonging to the family cucurbitaceae were used as the source material to screen for protease inhibitory activity. Cucurbita maxima and Citrullus lanatus seed coat extracts were used for the study.

2.2. Extraction and recovery of protease inhibitor

Plant materials for the study were washed thoroughly in distilled water and air-dried. A buffer extract was prepared in a 500 ml conical flask by homogenizing 10 g of plant materials in 60 ml of 0.1M phosphate buffer with pH 7.0 in an electrical blender. The homogenate was further mixed thoroughly by incubating the contents at room temperature in a rotary shaker for 30 minutes at 150 rpm. The slurry was then filtered through cheesecloth and the filtrate was centrifuged at 10,000 rpm for 15 minutes at 4°C for removing any cell debris that remains in the preparation. The clear supernatant obtained represented the crude extract and was assayed for protease inhibitor activity and protein content in the present experimentation.

2.3. Ammonium sulphate precipitation

The fractionation using ammonium sulphate precipitation has the advantage of intermediate removal of unwanted proteins and simultaneously the protein of interest could be concentrated. Ammonium sulphate (SRL, India) required to precipitate the protease inhibitor was optimized by adding varying concentrations (30%, 50%, 70% and 90%) to the crude extract.

1) To precipitate the protein, ammonium sulphate was slowly added initially at 30% (w/v) saturation to the crude extract while keeping in ice with gentle stirring.
2) After complete dissolution of ammonium sulphate, the solution was kept at 4"C for overnight precipitation.
3) Protein precipitated was collected by centrifugation at 10,000 rpm for 15 minutes at 4"C.
4) To the supernatant, required ammonium sulphate for next level of saturation was added and the procedure mentioned above was repeated. The precipitation was continued up to 90% (w/v) of ammonium sulphate saturation.

2.4. Dialysis

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The precipitate obtained after ammonium sulphate precipitation was further dialyzed against 0.1M phosphate buffer (pH 7.0), in order to remove the ammonium sulphate from the precipitate. Dialysis tube (Sigma-Aldrich) was treated to remove the humectants and protectants like glycerin and sulfur compounds present in it, and to make the pores of the tube more clear. The treated tube retain most of the proteins of molecular weight 12 kDa or greater. The method followed for the treatment of the dialysis tube.

a) Wash the tube in running water for 3-4 hrs.
b) Rinse in 0.3% (w/v) solution of sodium sulfide, at 80°C for 1 minute.
c) Wash with hot water (60°C) for 2 minutes.
d) Acidified with 0.2% (v/v) sulphuric acid.
e) Rinse with hot water (60°C).

2.5. Analysis of protease inhibitor by Dot - Blot method
The purified fraction collected from ion exchange chromatography is analyzed for its protease inhibitory activity.

1. 10µl of protease inhibitor was mixed10µl with protease (0.5mg/ml) and spotted on to a strip of X-ray film.
2. 10µl of protease was mixed with10µl phosphate buffer 0.lM (pH 7.0) as the control and spotted on to the X-ray film.
3. Incubated the X-ray film at 37°C for 10 minutes.
4. Washed the film under tap water till the zone of gelatin hydrolysis by protease was visualized.
5. Where the inhibitor is present, the protease does not degrade the gelatin on the x-ray film. If the inhibitor is absent, a clear zone is formed at the site of sample application on the X-ray film.

2.6. Effect of various metal ions on protease inhibitor activity
Effect of various metal ions on activity of protease inhibitor was evaluated by incubating the protease inhibitor along with different concentrations of various metals ions in the inhibitor solution for 30 minutes. The metals studied included sodium chloride, calcium chloride, magnesium sulphate, cupric sulphate, sodium molybdate, zinc sulphate, ferric chloride, manganese chloride, nickel chloride, mercury chloride, barium chloride, cadmium sulphate, and aluminum sulphate which contributes the metal ions like KI, Na+, Ca2+, Pb2+, Mg2+, Zn2+, Cu2+, Fe3+, Mn2+, Hg2+. Ba2+, Al2+, Cr2+Cd2+, and Mo6+, each at 5 mM final concentrations respectively.

3. Results & Discussion
Protein inhibitory activity from plant and animal components provide a significant role in medicinal usage in control of various diseases. The present work has been conducted to find the protease inhibitory effect of Cucurbita maxima and Citrullus lanatus seed coat extracts.

Figure 1 has provided the protease inhibitory activity of crude samples. The inhibitory action of Trypsin and Protease-K has not shown any inhibitory effect. Chymotrypsin inhibition has been observed at 10µl of crude extract. Pumpkin seed coat crude extract has shown trypsin (at 10µl) and Chymotrypsin (10 to 0.625 10µl) inhibition. All the tested concentrations have not shown any Protease-k inhibition.

Figure 1. Protease inhibition activity of crude samples

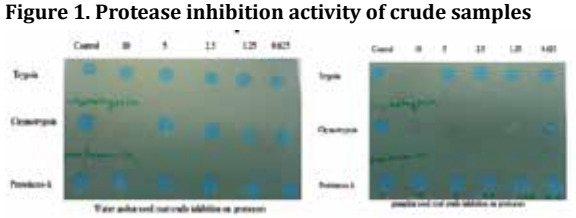


Figure 2 has shown the inhibition of Trypsin, Chymotypsin and Protease-k activity of Cucurbita maxima and Citrullus lanatus seed coat extracts. The isolated samples has not shown any inhibition with Trypsin and Protease-k but shown inhibiton with Chymotypsin at 30%, 50% and 90% concentrations,

Figure 2. Protein inhibition of isolated samples

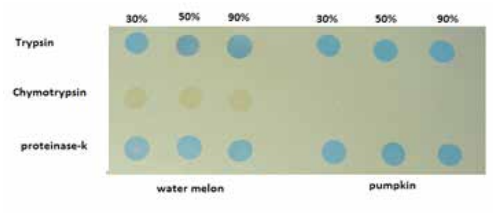


Figure 3 has shown the metal inhibition activity of Citrullus lanatus seed coat extracts. FeSO4, Na2EDTA and (CH3COO)2Pb has shown Trypsin inhibition. All the other metals has not shown any Trypsin inhibition. Typsin inhibition has not been seen when Citrullus lanatus seed coat extract (10µl) has been added before adding metal. Hence this shows the binding of extract. All the metals have not shown any Chymotrypsin inhibition. Addition of Citrullus lanatus seed coat extract has shown competitive inhibition in Phosphate buffer, MgSO4, HgCl2 and Na2EDTA. Protease-k has shown inhibition with FeSO4 and not shown any inhibition with other metal compounds. Competitive inhibition of Citrullus lanatus seed coat extract has been found in CuSO4 and HgCl2. Competitive binding of the FeSO4 with protease-k has been observed.

Figure 3. Metal inhibition with Water melon

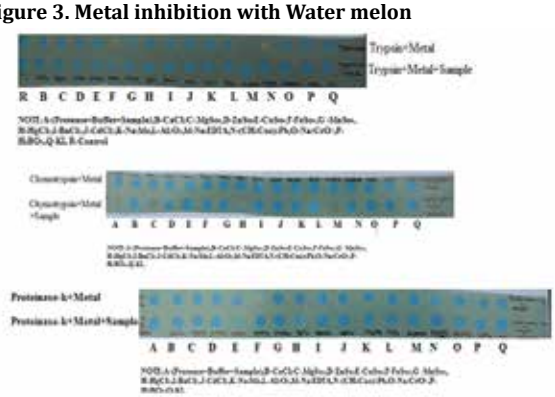
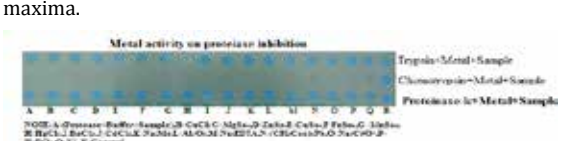


Figure 4 has shown the metal inhibition activity of Cucurbita maxima seed coat extracts. Typsin inhibition has been seen when Cucurbita maxima seed coat extract (10µl) has been added before adding HgCl2. Hence this shows the binding of extract. All the metals have not shown any trypsin inhibition. Addition of Cucurbita maxima seed coat extract has shown competitive inhibition with all the metals in chymotrypsin. Protease-k has not shown inhibition with all metal compounds of Cucurbita maxima.



Proteins are the biomolecules that show particular functions within the cell. The protein components are interlinked with other protein components. Perform biological functions for beneficial or no harmful or neutral effects. These molecules are produced from the DNA, arranged as amino acid strings. Plant proteins are the important protein biological source that interacts with disease proteins within or foreign cells. The cellular and molecular studies provide significant information regarding aging and disease.

Most of the proteins show motive conformation with hydrophobic amino acids that are typically located in the interior of the protein molecule [11]. Protein kinases are the targets for the

treatment of many diseases like inflammation and cancer the structural in sights provides targeting the residual of the molecule at ATP sites are less conserved active sites into targeting non catalytic domains [12]. Plants have been co-evolving the thousands of years with defense mechanisms the show protection against most herbivores, defense involves the production of protein inhibitors [PIs]. These inhibitors are proteins that are found in various parts of the plant provide defense against insects show the ability to adapt species that overcomes the effect of plant PIs [13]. Active protein kinase inhibitor shows the approval of drugs for clinical use those regulators protein phosporelation in most aspects of cell life, that can treat the cause are consequence of disease. These experiments provides the most advances in the cellular processes that can shape the major drug targets of the 21st century [14]

A druggable target is a protein, peptide or nucleic acid that show activity and perform modulation by a drug .For the targets to be addressed with experimental screening methods show advantages because the targets that are identified in the cellular modeled systems and the engineered or selected reflect to the disease model provide closer relationships .A target has been successfully validated and might be necessary show therapeutic use provide an indication that has a role in common mechanism potential that allows the broadening of therapeutic activity [15].

An understanding on signal transduction and the protein phosphorelation in the cell show the key regulatory components in signal transduction pathways in raveling the reasons of protein inhibitors that can serve as powerful therapeutic power full weapons in the society who are battling with human diseases [16]

Sodium dodesyl sulphate poly accrylamide gel electrophoresis (SDS-PAGE) is general and very simple, reliable technique for protein separation in the mass range 1-30KDa [17]. MALDI-TOF MS is the soft ionization technique and the power full tool used for serving small proteins and peptides [18]. This "proteome" approach can be used for identification of previously unknown proteins that can be usefulled in experimenting regulation of gene expression and protein localization in an organism provides evidence for chemo taxonomic evolution [19]

Most of the research groups gain as access to MS instrument that removes the barriers in detection and understanding of protein level heterogenisity, one of the promising technique of Top-down proteomics . The post genomic bioengineering and biomedicine from plant requires better insights into biology at the protein level that are critical to determine molecular indicators and the causes of multigenic diseased phenotypes. This technology provides the development of infract proteins which are both orthologous and Paralogous proteins with peptide base proteomics [20].

4. Conclusion

The present work has shown that, Cucurbita maxima and Citrullus lanatus seed coat extracts shows good protease inhibitory activity. Metals can also shown competitive role in either inhibiton or binding with enzyme/sample at active site and provide inhibition/non-inhibition of Trypsin, Chymotypsin or Protease-k.

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