Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Review Article

Evaluation of Antiproliferative and Antifungal Properties of a Serine Protease Inhibitor in Some Cucurbitaceae Members: A Review

Sreenu Barla[1], Dowluru Svgk Kaladhar[2,*] Narasinga Rao V[3]

Received on: 08-01-2016
Accepted on: 16-01-2016
Published on: 15-02-2016

Corresponding Author:

*** DSVGK Kaladhar,**
Dept. of Microbiology and Bioinformatics,
Bilaspur University,
Bilaspur-495001, Chhattisgarh.
India.
(M): 9885827025

*Email Id- dkaladhar@gmail.com

ABSTRACT

Plants produce complecx set of biological components that acts as active principles. A complete study of protein components provides an understanding about the complex network and the changes of functions within a system. The plant system contains both useful and harmful sources that can act as medicinal sources. The plant proteins are using from the ancient times but the principle understanding about these sources are arised from the modern times. The plant seed and testa in some Cucurbitaceae members as the medicinal sources in the form of protease inhibitors has been provided in the present study. The plant proteins isolated from seed coat is having good antiproliferative and antifungal properties. As the seeds containing testa is using as the edible sources, there is multiple interlinks in the control of various metabolic diseases like cancer. The protease inhibitors are also having good antifungal properties.

Key-words: Diabetes, Medicinal plants, Chhattisgarh, Disorder, Blood glucose level.

Cite this article as:
Sreenu Barla , Dowluru Svgk Kaladhar, Narasinga Rao V, Isolation, Evaluation of Antiproliferative and Antifungal Properties of a Serine Protease Inhibitor in Some Cucurbitaceae Members: A Review, Asian Journal of Pharmaceutical Technology & Innovation, 04 (16); 2016, 44-60. www.asianpharmtech.com*

1, 3 Department of Biochemistry, GIS, GITAM University, Visakhapatnam-530045, India
2 Deputy Registrar and HOD (Microbiology and Bioinformatics), Bilaspur University, Bilaspur 495001, India

INTRODUCTION

Plants contain pure and active principles that have rich source of medicament's associated in the treatment of human ailments [1, 2]. In the current research, medicinal plants involve healthcare approach for drug design and discovery by intricate approaches through combining techniques like Phytochemistry, Botany, Biology and Molecular Technology [3]. The ancient medicinal systems like ayurvedic, siddha, unani and folk medicine, with an integration of traditional, scientific and modern systems of medicine provides insights in the treatment of diseases with contemporary scientific practice [4].

Most of the prominent scientists and experts have compiled comprehensive survey of research in various disciplines of science [5]. The analysis and challenges in the broader information community systems from the past performs high quality science research [6]. Advances in Genetics, Molecular Biology, Biotechnology, Phytochemistry and Bio-engineering involve new manufacturing concepts from cellular systems for the production of medicinal compounds that can lead to a new manufacturing paradigm [7].

Plants produce complex sets of proteins/peptides including ribosome-inactivating proteins (RIP), protease inhibitors, lectins, antiproliferation and antifungal peptides, etc., that play a key role in defense systems, An increased levels of protease inhibitors like Trypsin, Protease K and Chymotrypsin are directly associated with the plants and provides resistance to the pathogenic microbes within the system [8]. Hence there is a need for screening of protease inhibitors from Cucurbitaceae members seed coat (or testa) that can potently been inhibiting the growth of microbes//tumors which causes pathogenic activities.

MEDICINAL PLANTS

Government of India was focused on Indian medicinal plants that requires stimulus of war and consequence scarcity of drugs. The drugs are extracted from plants grown in the country and provide more sustainable study in the subject of drug production [9]. One of the prerequisites in the primary health care is the availability of medicines in the use of sustainable drugs from plants that have common source of medicaments. Plants from traditional preparations with pure bioactive principles have regulatory mechanisms in therapy. Traditional plant medicines are used throughout the world that clearly depicts the importance of health and economy. Characterization of biological samples provides meaningful, interpretations through metabolic analysis [10].

MEDICINAL FOOD PLANTS FROM CUCURBITACEAE MEMBERS

Plants have been revered throughout history with medicinal properties that has natural forms of components using in the modern medicine [11]. The edible plants may also show medicinal properties and provides good health to living species. The edible plants including seeds provide healthy alternatives as foods and pharmaceuticals for the human community [12]. The edible seeds are providing good health to birds from the past. The geographical isolation of medicinal herbs and vegetable used by locals as food medicine permits the existence of a rich folk phytotherapy as health resources [13]. Humans are using plant resources to accomplish various necessities in developing countries. These plant sources derive as a substantial part of the components from herbal and wild plants to provide healty society with good revenue [13].

The protease inhibitor therapies are characterized by the ability to block activation of harmful enzymes [14]. Some protease inhibitors have good kinetic profiles that may be administered with food [15]. An administration of the plant protease inhibitors in single or combination with other drugs provides important concerns in pharmacokinetic against microbial species in patients. The knowledge regarding plants that serve both as food and medicine is vital in the present decades. Most of the edible members from Cucurbitaceae family have medicinal and nutritious values in all regions of the world.

Cucurbitaceae members have an important vegetable property that has good shelf line with good source of water soluble vitamins like carotene [16]. Pumpkin is popularly used in various traditional systems of medicine in treatment of several aliments, used as anti-diabetic, antibacterial, antitumor, antihypertensive, anti-hypercholesterolemia, immune-modulation, anti-inflammation, anti-parasitia, antalgic and anti-helmenthic activities. The plants provided an attention on various investigations towards metabolic diseases. The plant materials are being investigating by several scientists to know the effect of pharmacological activities with medicinal properties to elucidate mechanisms that reduce disease risk [17].

Table 1 has shown that seeds of Cucurbitaceae members have importance in food and medicine based on previous studies.

Table 1: Seeds of Cucurbitaceae members as food and medicine

Nutritious Plants	Medicinal properties
Actinostemma tenerum[18]	Snake bite
Benincasa hispida[18]	Diabetes, dropsy, demulcent, vermicide, gonorrhea, diseases related to liver, leucorrhea and fever.
Bryonia alba[19]	Diabetes mellitus treatment
***Citrullus colocynthis*[19]**	Diabetes mellitus treatment
***Citrullus lanatus*[18]**	Hypoglycaemic activity, an expectorant
***Cucumis melo*[18]**	Hematoma, hepatitis, appendicitis and pulmonary abscess
***Cucumis sativus*[20]**	Diabetes mellitus treatment
***Cucurbita ficifolia*[19]**	Diabetes mellitus treatment
***Cucurbitamaxima*[21]**	Stomach pain, anti-inflammatory and antipyretic, treatment of worms
***Cyclantherapedata* Schrab[18]**	Heart disease, hyperglicemia associated with type 2 diabetes mellitus, tumors, anti-viral activity
***Luffa aegyptiaca*[19]**	Diabetes mellitus treatment
***Momordica charantia*[19]**	Diabetes mellitus treatment
Raphanussativus[22]	Action against syphilis
***Richosanthes*[23] *dioica* Roxb**	Antihyperglycemic effect
***Telfairia occidentalis*[24]**	high-protein breads, blood tonic
***Trichosanthes dioica* Roxb[25]**	Antihyperglycemic effect

The three plants that are mostly used as food and medicine are *Citrullus lanatus, Cucurbita maxima* and *Cucumis sativus.*

***Citrullus lanatus* L.**

Citrullus lanatus (Thub.) commonly known as watermelon (Telugu vernacular name: Puchhakaya) belongs to family Cucurbitaceae. The plant is cultivated in northern and western parts of India. The fruits are available during summer season that contains rich water source [11]. The crops of Cucurbits contain significant sources of bio-components like proteins, lipids, carbohydrates and, secondary metabolites that play a key role on human health [26].

The plant is cultivated for the massive fruits at times throughout the year. The fruit is almost putrid, febrifuge and can protect from several diseases. Fruit of *C. lanatus* is used as diuretic, antioxidant, cooling, demulcent, pectoral and tonic.

C. lanatus is used in treatment of various diseases like diabetes, diarrhea, dysuria, jaundice, beri-beri and rheumatis [27]. Seeds contain biochemical compounds that show medicinal properties in the treatment for kidney

stones, demulcent, good vermifuge and hypotensive action that can paralyze tapeworms and roundworms, diuretic, pectoral urethral problems and tonic [28].

C. lanatus is a prostrate herb with robust tendrils, bi-fid and villous branchlets. Leaves are soft, pubescent, deeply tri-fid, with lobes ovate, oblong or obtuse, round and acute. Calyx is tubular with five lobes. The flower contains five petals with yellow and villous structures. There are three stamens in the flower. Fruits are globular or oblong, striped, green and sweet red with green or yellow flesh. Seeds are white, black or reddish with flat and smooth surface.

Figure 1 was shown the *Citrullus lanatus* fruits, testa and testa powder that was used as edible sources and contain protein parts. The fruit, seed and testa has been shown in Figures 1 (A), (B) and (C) respectively. Figure 1 (D) was shows the testa powder of *Citrullus lanatus*. The testa powder collected from *Citrullus lanatus* is thick brown color and soft.

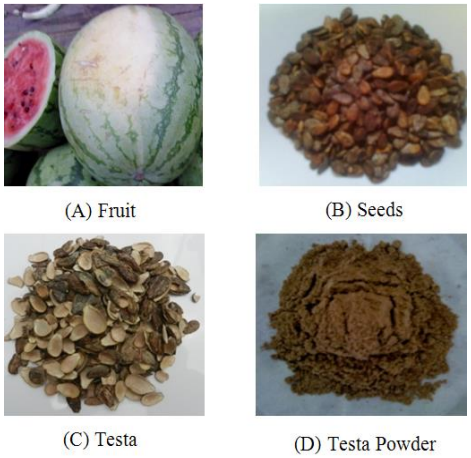

Figure 1: *Citrullus lanatus* L.

Figure 2 shows the Trasverse section of the seed of *Citrullus lanatus.* The seed contains cotyledon, embryo, hilum and testa. The testa part is selected for the present experimentation.

Figure 2: *Citrullus lanatus* L. testa

C*ucurbita maxima* L.

The common name of *Cucurbita maxima* is pumpkin, winter squash fruit, Squash gourd, Melon pumpkin, Red gourd or Red pumpkin (Telugu vernacular name: Gummadikaya, Tiyyagummadi or Erragummadi). The plant shows interpretations with components of other species that can be analyzed through metabolic analysis [29]. A winter squash fruit is an important vegetable fruit that has good shelf line with good source of water, soluble vitamins and carotene [16].

Pumpkin is popularly used in various traditional systems of medicine in treatment of several aliments. The fruit is used as antidiabetic, antibacterial, antitumor, anti-hypertensive, anti-hypercholesterolemia, immunomodulation, anti-inflammation, antiparasitia, antalgic and antihelmenthic. The edible fruits and seeds of the plant provided an attention on various investigations in biology. The plant materials are being investigating by various scientists to know the effect of pharmacological activities along with medicinal properties to elucidate mechanisms that reduce disease risk [17].

The plants of *C. maxima* are long running climbers with many tendrils. Leaves are not rigid, moderately upright, orbicular, shallow serrate with soft spines. Corolla has refluxed lobes. Fruits are flaucous or bluish. The plant is cultivated for its fruits as vegetable throughout the year.

C. maxima fruit, seeds, testa and testa powder (**Figure 3**) are used as dysuria alcoholism boils, burns, inflammations. Seeds are toxic to expel intestinal worms, diuretic and taeniacide (killing tapeworms).

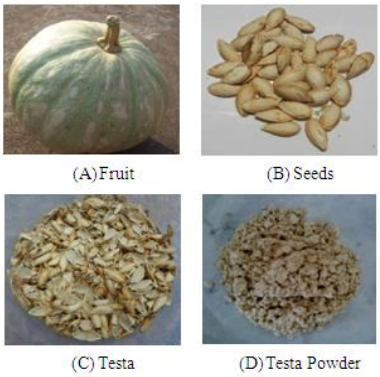

Figure 3: C*ucurbita maxima* L.

***Cucumis sativus* L.**

Cucumis sativus L., commonly known as cucumber (Telugu vernacular name: Dosakaya) is a yellow Indian cucumber using in curries that has sweet taste and neutral flavor [30]. *C. sativus* is used widely in the preparation of curries, mostly during the summer season, in the southern parts of India and Sri Lanka. It is used as diuretic, an antipyretic agent and in treatment for skin diseases [18]. The *C. sativus* fruit is very useful in terms of human health like purification of blood, removal of constipation, good for digestion and giving energy to the living system [31].

The medicinal plant, *C. sativus* is a prostrate or climbing herbs. Leaves are broad and cordate or ovate, hispid, palmately five angled and denticulate. Flowers are yellow and in axillary clusters. Calyx is with five lobes and linear. Corolla show five lobes and is tubular. There are three stamens and filaments much reduced. Fruits are oblong, green/ yellow with longitudinal stripes (**Figure 4**). The plant is cultivated widely with flowers and fruits during December-February.

The fruits and seeds are used for anaemia, hyperacidity, dysuria, haemorrhage, urinary disorders, *Oliguria* or hypouresis, retention of urine disorders, retention of urine and urinary calculi, leucorrhoea, diabetes and skin irritation in children.

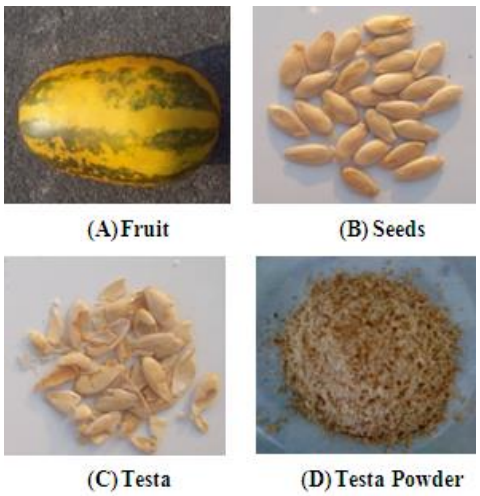

Figure 4: *Cucumis sativus* L.

PROTEINS

Proteins are the biomolecules that show interactive and diverse functions within the cell [32]. The protein components within the cell are interlinked with other protein components and Perform biological functions for beneficial, harmful or neutral effects [33]. The protein molecules are produced from the DNA that are arranged as a string of amino acid and perform a particular function [34]. Sequence-to-structure-to-function is an important mechanism that can predict the defensive mechanism of the plant against microbes. Plant proteins are the important biological sources that will interact with diseased proteins within or foreign cells [35]. The cellular and molecular studies provide significant information regarding aging and disease [36].

The most accurate and efficient method in determining the protein concentration is by acid hydrolysis and amino acid analysis. The other methods in estimation of proteins are sensitive to the amino acid composition in a protein. The procedure of protein estimation by Lowry method [37] is suitable and accurate. As the sensitivity is moderately constant from protein to protein, Lowry protein estimation method is widely used that are completely acceptable from the past to present decades. In Lowry method, peptide bond reacts with copper sulphate in an alkaline medium gives a blue colored complex. Tyrosine and tryptophan residue of proteins causes' reduction of the phosphotungstate and phosphomolybdate components (in 1:3 ratios) of Folin-ciocalteau reagent gives a bluish product. The final color obtained in Lowry's method is a result of biuret reaction of protein with copper ions in the alkali reduction of the phosphomolybdic phosphotangstic reagent (Fc reagent) by tyrosine and tryptophan residues present in the treated protein. A simple, objective, rapid, and more generally applicable of the protein assay for estimation of proteins is by Lowry method [37].

PLANT PROTEINS AND PROTEOME

The genome and proteome of plants provides the foundation for complete comparison of conserved processes by establishing rapid systematic ways to identify genes that perform specific gene functions [38]. The excretion in regulation of biological processes in the plant species requires specific interaction with numerous proteins. A regulator protein interaction form the network mediated cellular responses that determine the operation of the signal transduction systems and complex regulatory circuits [39].

The functional screening of plant proteins provides a valuable association with components in the intracellular protein-trafficking system [40]. Proteins are essential nutrient that plays a key role in our bodies function. The proteins are also composite in many stages during the growth and development of plant's life. Most of the proteins are coupled with several diseases that can be found out through protein interaction networks. Excessive proteins are leads to cancers like colon, breast, prostate and pancreas. The interactions are also related with other metabolic diseases including kidney disease and osteoporosis. Proteins are molecules that assist in the formation of complex structures like enzymes, hormones, antibodies and new tissues. Proteins also replace new cells instead of accumulation and non-functional components like old cells. Proteins are positively an essential nutrient that plays several key roles in regulatory processes and networks within and outside the living system.

The seeds from plants contain small hydrophilic proteins which are structurally related showing similar or different functions. The proteins may also play a role during imbibitions by controlling water uptake. The propagation, dispersal and development of plant seed are harvested by civilization with healthy plant tissues. The concentration of proteins varies from 10% in cereal seed to 40% in legumes and oilseeds by dry weight. The mature seed proteins are the major dietary protein source contain either structural or metabolic roles (Figure 5)

PIs are universal occurrence of molecules mostly isolated from the plant kingdom. Plant PIs (PPIs) are the small proteins or peptides that occur in seeds, tubers and aerial parts of plants. Protease inhibitors shows important biological functions like control of blood clotting, digestion of proteins, microbes, apoptosis, diseases and signaling-receptors interaction in the multicellular systems. PPIs are endogenous regulators of proteases act as plant defense agents that shows network of interactions and causing blockage of insect and microbial proteinases [41]. PPIs found in plants, which are effective against phytophagous insects and microorganisms.

Most of the proteins show native conformation with hydrophobic amino acids present in interior parts of the protein molecule [42]. Protein kinases are the targets for the treatment of many diseases like inflammation and cancer. The structural insights provides targeting of the residual molecule at ATP sites that are showing less conserved active sites into targeting on catalytic domains[43].

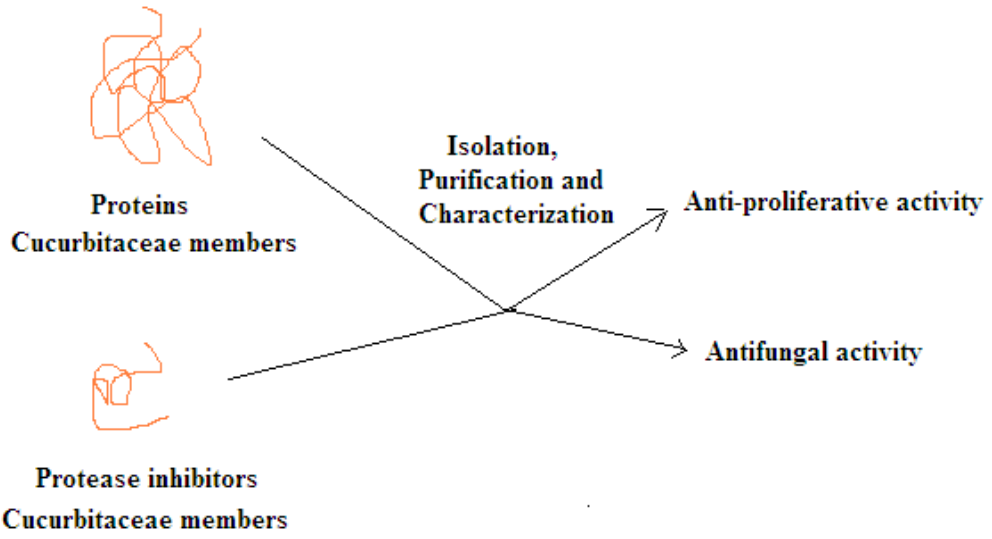

Figure 5: *Cucumis sativus* L. as Anti-proliferative and Antifungal agents

Plants have been co-evolving from thousands of years with defense mechanisms the show protection against most herbivores; defense involves the production of protein inhibitors [PIs]. The PIs are proteins that are found in various parts of the plant provide defense against insects and microbes show the ability to adapt species that overcomes the effect of plant PIs [44]. 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. The fundamental experiments from previous decades provided the most advances in the cellular processes that can shape the major drug targets of the 21st century [45].

A druggable target is a protein, peptide or nucleic acid that show activity and perform modulation by a drug .The experimental screening methods show advantages because the drug targets that are identified in the cellular modeled systems and the engineered are selected that 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 [46].

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 powerful weapons in the society [47].

The "proteome" approach can be used for identification of the unknown proteins within the multicellular systems. The proteomics data can be useful in protein localization and gene expression in an organism that provides evidence for chemo taxonomic evolution [48]. Most of the research scientists gain access of proteomic data in understanding of protein level heterogenisity applied in Top-down proteomics approaches. The post genomic bioengineering and biomedicine from plant requires better insights at protein level. These systemic approaches are significant to determine molecular changes and reasons for the development of multigenic diseased phenotypes. The systems biology provides development of infract proteins which specifies are both orthologous and paralogous proteins with peptide base proteomics [49].

PROTEASE INHIBITORS

A drug target is a protein, peptide or nucleic acid that show activity and perform modulation by a drug. The targets are to be addressed with experimental screening methods [50] show advantages for future treatments. The advances of isolation of drug targets provide closer relationships that are identified in the cellular modeled systems. The drug targets should be engineered or selected that will reflect to the disease model [51].

Academic and industrial researchers are investigating on protease inhibitors that could possibly be used to treat cancer [52]. Antimicrobial peptides present in plants, insects, amphibians and mammals are playing vital roles in the immune responses producing innate host-defense mechanisms. The peptides possess effective antibiotic activities against pathogenic microbes like fungi, bacteria and viruses [53, 54]. Hence an investigation on isolation

and mode of action of plant antimicrobial peptides are important that act against rapid emergence of pathogens which are resistant to antibiotics.

Proteases are indispensable to the maintenance and survival of plant and animal systems [55]. The proteolytic events catalyzed in many of cellular events like inflammation, hormone processing, blood clotting, and apoptosis pathways by the enzymes that serve as mediators of signal initiation, processing, and termination. The information's of enzymes are vital to the living cells and organisms in the host that may be potentially damage the system when present in higher concentrations or overexpressed. The activities are strictly regulated and controlled by the enzymes that are needed within the cell.

Proteases are the protein cutting machines involves in central to diverse physiological networks throughout bio-systems. Proteases are classified into six broad groups. They are Serine proteases, Metallo-proteases, Cysteine proteases, Threonine proteases, Aspartate proteases and Glutamic acid proteases. Among the classified proteases, the most common proteases in the regulation of cellular processes are Serine proteases.

Seeds are a vital component of the world's diet [56]. The seed epithelium has a vital role in the enzymatic breakdown of reserve substances stored in the endosperm tissues than in the aleurone layer [57].Proteinases are the hydrolytic enzymes that perform a wide variety of biological functions like break down or modify proteins or peptides, control of humoral and cellular immune responses, prostate cancer and microbial diseases [58]. A glycoside hydrolase enzyme, α-galactosidases in protein storage vacuoles of developing seeds [59] is important in germination of cereal seeds is the result of an action of hydrolytic enzymes [60]**.** Hydrolytic enzymes do not decrease until fairly severe desiccation has taken place [61] in the cellular system. In the hydrolysis of canola seeds, the crude hydrolytic enzymes were found more efficient when compared with purified enzymes [62]. An enzyme named Hydrolase cleaves N α-acylated amino acids by catalyzing the hydrolysis of chemical bond from small peptides.

Serine Proteases

Serine proteases are also called as serine endopeptidases. Serine is an aminoacid that provides nucleophilic amino acid at the active site. Serine proteases are the proteases present both in prokaryotes like *E.coli* and eukaryotes like Cucurbitaceae members. Trypsin and subtilisin are the two broad categories of serine proteases based on their structure. Serine proteases are responsible for coordination of physiological functions such as immune reaction, digestion and clotting. Trypsin, Chymotrypsin, and Proteinase K are the serine proteases selected for experimentation in the present study as these proteins are highly applicable in protease inhibition studies.

Trypsin

Trypsin (Molecular weight: 23.3 KD and Enzyme Commission Number: 3.4.21.4) is a serine protease and an enzyme that was found in the digestive system. Trypsinogen is an inactive form of zymogen that converts into trypsin (active form) by proteolytic cleavage in present of enteropeptidase within the pancreas. Trypsin cleaves peptide chains specifically at the carboxyl side of the amino acids such as arginine (R) or lysine (L) [63].

Chymotrypsin

Chymotrypsin (Molecular weight**:** 25.6 kDa and Enzyme Commission Number 3.4.21.1) is a digestive enzyme produced as a component of pancreatic juice. It performs breakdown of proteins and polypeptides within the system. Chymotrypsin cleaves peptide chains mostly at the carboxyl side of the amide bonds likes tryptophan (W), tyrosine (Y) and phenylalanine (F).

Proteinase K

Proteinase K (Molecular weight 28.9 kDa and Enzyme Commission Number: 3.4.21.64) can hydrolyze insoluble keratins. Keratinous substance is Protease resistant proteins that can digest Keratinous substance, hence, the name "Proteinase K". Proteinase K cleaves peptide bond adjacent to the carboxyl side of the aromatic and aliphatic amino acids. It is frequently used for its broad specificity.

The inhibition of protease is a protein-protein interaction process that shows directive control mechanism within cell [64]. *Helicoverpa armigera* contains protease inhibitors that were experimented using X-ray film contact print technique [65]. *Simmondsia chinensis* cotyledonary extracts were inhibited due to enzymatic activity by trypsin,

chymotrypsin, and pepsin. Trypsin inhibitor activity was decreased with the germination of seed. Germination can enhance the degradation of both Kunitz soya bean trypsin inhibitor and Bowman–Birk soya bean trypsin inhibitor [66].

Simmondsia chinensis seeds were found to contain a heat-labile Trypsin inhibitor substance(s) [67, 68]. The extracellular proteinases are produced by many phytopathogenic fungi that shows proteinases components and plays active role in the development of human diseases [69]. Plants inturn produce some inhibitory proteins that suppress the activities of extracellular proteinases produced by phytopathogens [70].

An isolation of chitin-binding antimicrobial peptide was conducted previously from *Ginkgo biloba* seeds [71, 72]. Proteinase inhibitors extracts from various sources, like *Ginkgo biloba* seeds they were found to contain some inhibitory activities against the Cys proteinase papain and the Asp proteinase pepsin [73]. The Chymotrypsin inhibitor activity shows less significant than Trypsin inhibitor activity in seed varieties of chickpea [74]. Vacuolar processing enzyme is a Cys proteinase responsible for the maturation of vacuolar proteins [75]. Studies on different cultivars of dry beans (*Phaseolus vulgaris* L.) with phytomolecules like phytic acid, tannins, trypsin, chymotrypsin and α-amylase inhibitory activities were previously been investigated [76].

ZYMOGRAPHY

Zymography is advanced electrophoresis technique that is used to identify the proteolytic activity of enzymes under non-reducing conditions. The proteolytic enzymes can be separated in polyacrylamide gels. The technique is extensively used in qualitative evaluation of the proteases present in cell cultures, cellular systems and tumors [77]. Distinct varieties of specific electrophoretic zymograms were previously observed for acid phosphatase, alcohol dehydrogenase, amylase and tetrazoliumoxidase [78]. The evolutionary relationships between accessions in collection of PIs can be determined based on allozyme seed protein variability and cross ability relations [79]. There are very less number of applications that have been made in zymogram technique to plant taxonomic problems [80]. The plant extracts and dermatological formulations comprising of one or more plant extracts provide inhibition of one or more extracellular proteases from the group of Matrix MetalloProteases (MMPs) in MAPkinase pathway [81].

Zymography is useful for the analysis of proteinase composition present in complex systems [82, 83]. Zymography is important to assess the enzymatic activity of proteins either *in situ* or by separating them with electrophoresis [84, 85] **.** The enzyme converts the substrate into a product which is detected by different staining methods [86].

There are various types of zymographic methods. They are substrate zymography, Gelatin zymography, Casein zymography, Collagen zymography, Heparin-Enhanced Substrate zymography, reverse zymography and *in situ* zymography. Zymography is considered as a standard technique to examine the activity of enzymes involved in the cellular processes[87]. Gelatine zymography is an efficient and sensitive method compared to other methods to detect both pro- and active form of proteins [88]. Reverse zymography is an efficient technique used to characterize proteins using degradomics [89]. In gel zymography, the visualization of bands are formed based on molecular weights are characterized as enzymes.

PROTEASE INHIBITION BY X-RAY FILM METHOD

Sensitive and simple method for visualization of electrophoresis separated protease inhibitors can detect as anti-aging biological constituents [90]. The analyses in recognition of PIs are conducted using X-ray film or X-ray gel film by utilizing the gelatin on the surface of an unprocessed substrate [91, 92].

PROTEOMIC TECHNIQUES

Sodium DodesylSulphate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) is general simple and reliable technique for protein separation [93]. Matrix-Assisted Laser Desorption and Ionisation-Time of Flight (or MALDI-TOF) in connection with Mass Spectroscopy (MS) is the soft ionization technique and the powerful tool used for serving small proteins and peptides [48, 94].

PROTEOME TECHNIQUES IN PI STUDIES

The amino acid sequences for number of proteins contain conserved tracts with domain functionality. The conserved sites have been deduced from several species in the past years [95] for constructing phylogeny.

Advanced technological proteomics cover experimentations such as SDS-PAGE, mass spectrophotometry, bioinformatics etc. These experimentations are relevant in plants proteomics that addresses plant biology with relevant to other organisms.

The widest spread technique for peripheral membrane proteins and soybean seed coats are compared with standards that are known based on SDS-PAGE. The plant extracts were exhibited positive for Trypsin Inhibitor (TI) activity based on the studies. The research on isolation and characterization of the low molecular weight inhibitor(s) are needed in the present decades [96]. Salts can be removed and digested by site specific proteases like trypsin and the small fractions of mono isotopic peptide ion masses that will be determines using MALDI-TOF MS, The experimental peptides searched against proteins was predicted from genomics data [97]. Proteomic analysis by modern methods has been investigated in various parts of plants like rice root [98], Arabidopsis seed germination [99], grape berry ripening [100], tomato seeds [101], etc.

SDS-PAGE is very simple, general, accurate and reliable technique used for protein separation in the mass that ranges from 1kDa to 30 kDa [93].

Table 2.2 shows the previous reports conducted for protein separation by SDS PAGE in Cucurbitaceae members.

Table 2.2: Protein separation by SDS-PAGE technique from Cucurbitaceae members taken from previous literature

Plant name	Partof plant
***Citrulus lanatus* [79]**	Leaf discs
***Momordica charantia* [102]**	Seed
Cucurbita maxima [103]	Seed
Citrullus vulgaris [103]	Seed
Cucurbita ficifolia [104]	Phloem
Citrullus colocynthis [105]	Seed
Trichosanthes [106]	Seed
Cucumis melo [107]	Placenta part of the fruit
Lagenaria siceraria [108]	Seed

TESTA PROTEINS

Seed coat also called the testa develops from the tissue called the integument that is originally surrounding the ovule. *Citrullus lanatus* is distinguished by a four-layered seed coat with a distinct inner layer[79] (**Navot and Zamir, 1987**). The testa protein from seed was extracted, purified and analyzed using modern techniques[109] **(Keller et al., 1989).**

PROTEASE INHIBITORS AS ANTIPROLIFERATIVE AGENTS

A Bowman-Birk trypsin inhibitor of 8 kDa was reported by Vincent and NG in 2008 from black soybeans with antiproliferative activity. A trypsin inhibitor was isolated on DEAE-cellulose shown anti-proliferation in hepatoma (Hep G2) and breast (MCF-7) cancer cells shown IC_{50} values as 35 and 140 μM respectively [110].

Human diet contains rich seed components that control cellular processes. Consumption of fruits and vegetables were associated with the reduced risk of several chronic diseases like cardiovascular disease. Fruits and vegetables contains phenolics as major bioactive compounds that improves health conditions [111]. Flavonoids from Citrus plant were shown antiproliferative activities that act against several tumor and normal human cell lines [112]. Coccinin and barley seed peptide proteins were shown antifungal with antiproliferative activities [108]. Plant tissues from Cucurbitaceae family members were shown anti-gonadal, antiproliferative, immunomodulatory, antifungal, antitumor and anti-HIV activities [113].

Purification and characterization of Serine protease inhibitors like anapin-like ribosome-inactivating peptide and charantin were evaluated from bitter gourd (*Momordica charantia*) seeds [114]. Lycopene from guava (*Psidium guajava*), phytocompounds from watermelon (*Citrullus lanatus*) and Zingiberaceae plants shown antioxidant activity [115]. The offensive nature of both tumour and endothelial cells are mediated by the proteolytic activities of MMPs [116].

PROTEASE INHIBITORS AS ANTIFUNGAL AGENTS

Fusarium oxysporum and *Mycosphaerella arachidicola* contains trypsin inhibitor showing antifungal activity. Trypsin inhibitor was reported from seeds of *Clausena lansium* (Lour) with a molecular mass of 54 kDa that exerted antifungal (*Physalospora piricola*) and antiproliferative (human leukemia HL60 and hepatoma Hep G2) activities [117]. Several Trypsin inhibitors has been reported in various plants but not reported in testa of *C.lanatus*.

Specific chitinases accumulate in seeds can also be induced in developing and germinating seeds in response to microbial attack, including antifungal properties [118]. Chitinase is often produced in higher plants as a general defence response after acerbic or pathogenic attack. Since germinating seeds are exposed to soil pathogens.

Medicinal plants are widely utilized for various purposes as antibacterial, antifungal and antioxidants according to their components [119]. The albumins are second novel class of antifungal proteins that are strongly antagonized by cations [120]. All plant lectins present in plants plays an important function in plant defensive mechanisms. Lectins occurs at lower concentrations may be involved in recognition processes of specific mechanism within the plant system.

Molecular, cellular, physiological, biochemical and evolutionary experimentations from previous studies indicates that lectins play an important role in plant defense mechanisms [121]. Cysteine proteinase inhibitor gene present in transgenic tobacco plants can induce resistance against two plant potyviruses like potato virus Y (PVY) and tobacco etch virus (TEV) [122].

Cucurbits and cereals trees contain Serine proteases (EC 3.4.21) share a number of physiological and biochemical properties provides an understanding of numerous sub-cellular mechanisms in Bottom-up or Top-down systems approaches [123]. A common observation on many species of seeds becomes infected by fungi within and on the inner layers of the seed coat and tissues. The existence of resistance systems within embryos and other tissues inside the seed coat provides protection. When a cotton seed is infected by a fungus, the hyphae penetrate through the chalaza, but do not immediately invade the embryo.

The embryo is surrounded by thin tissue called the nucellus, which apparently is derived from crushed endosperm cells. The tissue appears to act as a barrier to fungi when the embryo is infected. Infection occurs primarily within the half closest to the chalaza which does not contain the embryonic axis [124].

Table 2.3 shows the reports done for protease inhibitors from the seed of Cucurbitaceae members. The reports suggest that seeds contain PIs. In the present work, the seed coats are important that produces PIs for protection of seed is to be isolated that show health benefits in humans.

Table 2.3: Members from seeds of Cucurbitaceae as protein inhibitor

Plant name	Protein inhibitor
Bryonia diotica[125]	Trypsin inhibitor
Cucurbita ficifolia[125]	Trypsin inhibitor
Citrullus vulgaris[125]	Trypsin inhibitor
Cucurbita maxima[126]	Trypsin inhibitor
Cucurbita pepo[126, 125]	Trypsin inhibitor
Costelytra zealandica[127]	Serine proteinases
Cyclanthera pedata[126]	Serine proteinase inhibitors
Ecballium elaterium[128,129]	Trypsin iso-inhibitors, Trypsin inhibitor
Linum usitatissimum[126]	Trypsin inhibitor
Momordica charantia[130]	Ribosome-inactivating proteins
Momordica cochinchinensis[131]	Chymotrypsin-specific potato type I inhibitor

Sechium edule[132]	Trypsin inhibitor
Sicyos australis[133]	Proteinase inhibitor

IN SILICO ANALYSIS

In silico analysis supports genes encoding proteins related to various protease inhibitors [134, 135]. Inhibitory peptides from food proteins facilitate the interactions in large scale networks which particularly release bioactive peptides [136]. The *in silico* analysis might provide further tools to re-construct extinct plants from archaeological remains useful for conservation genetics [137].

The gels obtained from *in vitro* studies provide further scope through *in silico* method-based complete catalogue that exists between different protein patterns [138]. Protein identification and analysis is usually based on *in silico* techniques that match the peptide sequences paves the way in metabolomics, transcripotomics and other large Omics techniques into system biology [139-142]. There are tremendous opportunities for molecular biologists, biochemists and bioinformaticians to define protein nature showing transducer genetic information and energy transfer that obtains from plants. These proteomic methods are very much important for the study of different components in the plants that states plant function [143].

CONCLUSION

As there is exploiting association of protein information through sequencing and *in silico* analysis along with protein interactions at the system level there is a need to conduct and assess the nature of a peptide present from testa of *Citrullus lanatus.*

Conflict of Interests

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

Acknowledgment

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

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