WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 12, 1696-1711. Research Article ISSN 2277– 7105

A STUDY ON PLANT LATEX, A RICH SOURCE OF PROTEASES AND CUTTING EDGE FOR DISEASE INVASION

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Article Received on
20 Oct 2015,

Revised on 10 Nov 2015,
Accepted on 30 Nov 2015

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ABSTRACT

India is one of the twelve mega biodiversity centers having more than 45,000 plant species. A vast ethnobotanical lore exists in India from ancient time which can be of real use in the formulation of effective therapeutics. Plant based medications had served from the earliest period of the human civilization as the most important therapeutic weapon available to man to fight various human and animal diseases. The world is now looking towards India for new drugs to manage various challenging diseases due to its rich biodiversity of medicinal plants and abundance of traditional knowledge such as Siddha, Ayurveda and Unani. Many plants exudate latex when injured. The latex of some plant families such as Asteraceae, Caricaceae, Moraceae, Asclepiadaceae, Apocynaceae and Euphorbiaceae contains number of proteins and enzymes. The proteolytic activities of plant lattices was estimated by using casein as substrate. Cysteine proteases (EC 3.4.2.2), with a cysteine residue in their active center form the well distributed class. They are usually identified based on the effect of their active site inhibitors like iodoacetate, iodoacetamide, chloroacetate, para chloromercuribenzoate, EDTA and pepstatin. Activation of the enzymes was done by thiol compounds. A novel plant protease has been identified from *Vallaris solanacea* and screened for its caseinolytic activity. Its protein concentration was found to be 5mg/ml. Thus this information emphasizes that further research on *Vallaris* can be explored for new drugs.

KEYWORDS: Plant Latex, Proteases, Vallaris Solanacea and Proteins.

INTRODUCTION

For about a century, the enzymes that play the central role in protein degradation by hydrolyzing peptide bonds were known as "proteases" or "peptide hydrolase". Proteases have been identified and studied from the latex of several plant families such as Asteraceae, Caricaceae, Moraceae, Asclepiadaceae, Apocynaceae and Euphorbiaceae. Proteases have a first place in the world market of enzymes, estimated at ~US$3 billion.[1]

Proteolysis is an irreversible process of polypeptide cleavage with important physiological roles in a number of cellular processes. These enzymes can also hydrolyze peptides when a pteroyl moiety or acyl groups replaces the peptidyl group. In the course of discussion of the mechanism of proteolysis, two subdivisions of proteases was embodied in the alternative names endopeptidases for those acting in the interior of polypeptide chains and exopeptidases for those acting at the termini. These names are, of course, analogous to those used for hydrolases acting on other polymers such as polysaccharides and polynucleotides.

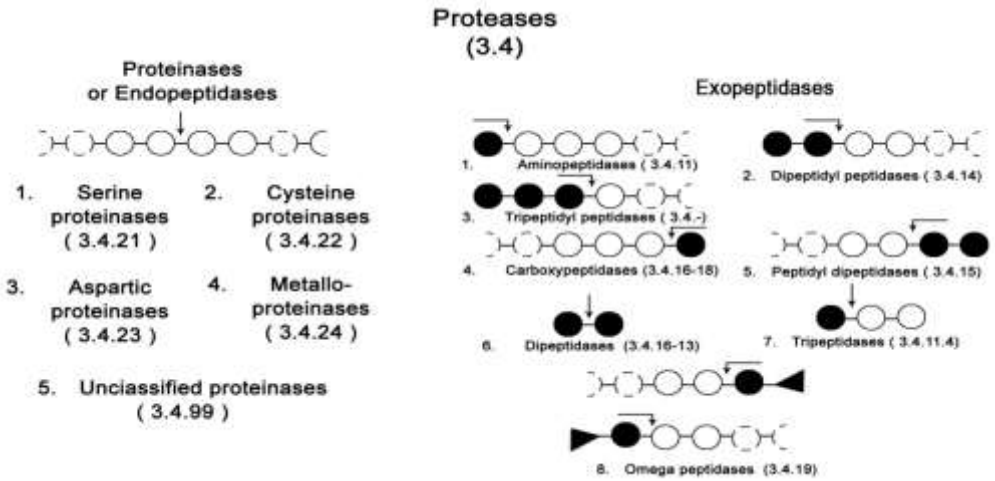

Figure 1. Classification of Proteases.

(The numbers given in parentheses indicate the divisions in which the enzymes have been placed in the enzyme nomenclature given by International Union of Biochemistry and Molecular Biology).

Proteases or endopeptidases

Proteases differ from all the other enzymes in that their substrate specificities are invariably difficult to define and certainly do not represent an acceptable basis on which one can name and classify the enzymes. A solution to this problem was put forward by Hartley (1960), who

showed that the proteases acted through four distinct catalytic mechanisms and hence regarded as serine, thiol, acid or metal proteases. Now that much more is known about the chemistry of the catalytic sites, three of the four names have been amended, as serine, cysteine, aspartic and metallo-proteases, but the concept of distinguishing these groups of enzymes remains completely valid.

Evolution of the endopeptidases

It is one of the valuable aspects of the system of classifying proteases by catalytic mechanism that the groups thus created do not cut across evolutionary relationships in the way that classification by specificity would do. Nevertheless, the four major groups of proteases (serine, thiol, acid or metal) are not all monophyletic. Evolutionary homology of the proteases is demonstrated by similarities in amino acid sequence and molecular structure as revealed by x-ray crystallography. Similarities of amino acid sequence are susceptible to statistical analysis and the valuable term "super family" is used to describe the largest group of proteins that show statistical evidence of evolutionary homology with each other. The modern aspartic proteases seem to have arisen from a single primitive enzyme, whereas the serine proteases are derived from two stocks, the cysteine proteases probably from atleast four and the evolution of metallo- proteases is still unclear. The aspartic proteases appeared recently.

Distribution of proteases

Proteases are distributed widely in different parts of the biological sources. In occurrence of proteases, plant kingdom occupies the highest rank (43.85%) followed by bacteria (18.09%), fungi (15.08%), animals (11.15%), algae (7.42%) and viruses (4.41%). Isolation of proteases from biological sources contributes 27 to 67% either animal, microbial or plant origin. Cysteine protease occurs abundantly in plants (34.92%). Microbes secrete serine and aspartic proteases in large quantities of 13.21% and 8.81% respectively. In animals the commonly found proteases are cysteine, serine and aspartic. In plants, proteases are present virtually in every part i.e. stem, fruit, flower, seed, root, leaf, gum and latex. Plant latex is the richest source of protease.

Phytochemistry of latex

Latex is an inducible defense system, mobilized and transported to the site of damage immediately after the damage. It is a protein rich multi-component milky sap secreted by the specialized plant cells called laticifers. Plant latex is a good source of secondary metabolites

such as alkaloids, tannins, saponins, phenolic components with potent antibacterial, antiviral, antifungal bioactivities. These bioactive constituents of latex also exhibits potent pharmacological activities like anti-tumors, anti-angiogenic, anti-diabetic, antiproliferative, anti-arthritis, anti-inflammatory, antioxidant, antiasthmatic, anti-fertility, analgesic, immunomodulation, wound healing, cytotoxicity, vasodilatory activities.

Applications of proteases

All proteolytic enzymes have characteristic properties with regard to temperature, pH, ion requirement, specificity, activity and stability. These biochemical parameters determine the application of protease in industry and other fields.

Detergent Industry

Development and improvement of household and industrial detergents are greatly contributed by proteases. The enzymes used in detergent industry are proteases, lipases, amylases, cellulases etc. Among these, protease plays a major application as detergent additive due to their ability to hydrolyze and remove proteinaceous stains such as blood, gravy, egg and milk at high pH conditions.

Leather Industry

In leather industry, processing of leather involves three main steps known as soaking, dehairing and bating. The conventional methods of leather processing which involves the usage of chemical reagents (sodium sulfide, sodium hydroxide, hydrogen sulfide, etc. release a large amount of hazardous pollutants into the surrounding environment. However application of proteases in place of chemical treatments has been identified as an environmental friendly alternative for leather processing. Keratinolytic activity of novel proteases has the potential to replace sodium sulfide in the dehairing process.

Food and Feed Industry

Proteases are used widely in the preparation of protein hydrolysates to be used as additives to food and feed to improve their nutritional value. These are also used in brewing, cheese elaboration and bread manufacturing. Proteases also play a role in protein storage, mobilization, senescence, programmed cell death, hormone signaling and defense and are regulated by various types of environmental stresses. These have a role as meat tenderizers and as plant milk clotting enzymes for novel dairy products. Chymosin is preferred in cheese making industry due to its high specificity for casein. Measuring hydrolytic activity on

synthetic substrates is a simple way to know the cleavage specificity of these enzymes, which provide important information for biotechnological applications, as with the production of bioactive peptides from food proteins.

Silk Degumming

Threads of raw silk must be degummed to remove sericin protein that covers the silk fiber. Traditionally, degumming is performed in an alkaline solution containing soap. This is a harsh treatment because the fiber itself is attacked. Use of alkaline proteases to remove the sericin without attacking the fiber is a better method.

Photographic Industry

The photographic films contain 1.5-2.0% silver by weight in their gelatin layers. The conventional method used for silver recovery is the burning of films which causes the problem of environmental pollution and in addition, the polyester based film cannot be recycled by this method. Thus, the use of enzymatic hydrolysis of gelatin layers not only extracts silver from the films but also polyester based films can be recycled.

Pharmaceutical Industry

Proteases are also used in pharmaceutical industry in developing therapeutic agent. Proteases from the plant extracts have been used as traditional medicine in treating cancer as antitumorals, for digestive disorders and for immune modulation problems. Several plant latex proteases are known to interfere in homeostasis as procoagulant suggesting its unique substrate preference over other proteases. Some peptides are hidden and inactive in the original peptides, but when liberated they can have diverse biomedical applications, as antihypertensive or antioxidant agents. Plant latex is a natural source of pharmaceuticals and pesticides.

Cysteine proteases

Cysteine proteases (CPs), also known as thiol proteases, are widely distributed among living organisms. Cysteine proteases are represented by 70 families.[2] Most plant-derived proteases have been classified as cysteine proteases and rarely as aspartic proteases.[3] The best-known CPs are caspase-like proteins, vacuolar-processing enzymes, papain-like peptides and cathepsin-type proteases.[4] CPs have great potential in the food, biotechnology and pharmaceutical industries owing to their property of being active over a wide range of temperature and pH.

Inhibition of cysteine proteases

The sensitivity of papain, ficin and bromelain to inactivation by thiol-blocking reagents and their activation by reagents expected to regenerate thiol groups from disulfides, led to classify them as "thiol proteases". The cysteine proteases are inhibited by thiol blocking agents such as iodoacetate, chloroacetate and mercuribenzoate. These reagents are reactive with low molecular weight thiol compounds too, so that they are not easy to use under the usual conditions of assay of the cysteine proteases i.e., in the presence of thiol activators. Cysteine proteases (EC 3.4.2.2), endopeptidyl hydrolases with a cysteine residue in their active center are usually identified basing on the effect of their active site inhibitors (iodoacetate, iodoacetamide and E64) and activation of the enzymes by thiol compounds. They are susceptible to sulfhydryl agents such as PCMB but are unaffected by DFP and metal-chelating agents.

Overview on the industrial applications of cysteine proteases

Cysteine proteases are anti-edematous[5], anti-thrombic and anti-inflammatory.[6,7] They are also used as antitumour agents[8] and reduce inflammation by altering migration and activation of lymphocytes.[9,10,11,12] The three potential roles of cysteine proteases in plant defense include invader perception, downstream signaling pathway activation and defense response.[13] Salas *et al*, (2008) reviewed the pharmacological activity of plant cysteine proteases, emphasizing their role in mammalian wound healing, immunomodulation and neoplastic alterations.

Appreciating the varied applications of these proteases the present study aimed to screen different plant lattices for the proteolytic activity.[14] From these plant proteases cysteine proteases have been identified using thiol blocking agents.

MATERIALS AND METHODS

A number of plant lattices belonging to different plant families have been screened for proteolytic activites from the gardens in and around Visakhapatnam, A.P, India.

Latex Collection

Latex of the plant was collected fresh from the cuttings of leaf stalks with capillary tubes into glass containers kept in ice. The latex was a white thick fluid with pungent odour. The pH of the fluid was about 6.3 as measured with glass electrode.

Enzyme Isolation

The freshly collected latex was diluted with 5 volumes of ice cold distilled water and centrifuged at 10000 rpm for 20 minutes in high speed refrigerated centrifuge and the clear, colourless supernatant was collected and stored at 0ºC. The pellet containing the white insoluble gums was discarded. All the experiments on the crude preparation of solanain were carried out using freshly collected latex.

Screening and selection of plant lattices for proteases

Protease activity in the latex samples[15] were measured according to the Kunitz method.[16]

Protease Activity

The assay system prepared has a total volume of 2.0 ml containing 50mM Glycine-NaOH buffer (pH 9.0), 10mM β-mercaptoethanol, 2mM EDTA, 10 mg casein and 3-5µg of enzyme protein. 3ml of TCA arrested the reaction and to 0.9 ml of TCA soluble peptide filtrate, 0.1 ml of iodate (5-25mM), 5.0 ml of alkaline copper reagent was added immediately without any prior incubation followed by 0.5 ml of Folin Ciocalteau reagent. The colour developed after 30 min was measured by visible spectrophotometer at 670 nm. Zero controls were run by the addition of TCA prior to the addition of casein.[17] A tyrosine standard curve drawn for tyrosine (0.5µ moles/ml) by Lowry's method was used for calculating the activities.

Protein determination

Protein content of the latex was determined by the Lowry method[18] using crystalline bovine serum albumin as the standard.

A. Enzyme unit

One unit of protease activity is defined as the amount of enzyme yielding an increase in colour with TCA soluble peptides, equivalent to 1µ mole of tyrosine per minute with Lowry's method under the assay conditions.

B. Specific activity

Specific activity of the proteolytic enzyme is expressed as the number of units per milligram of protein.

A tyrosine standard curve drawn for tyrosine (0 to 0.5µ moles) by Lowry's method was used for calculating the activities.

Assay of enzyme using haemoglobin as substrate

The assay of protease using haemoglobin as substrate was also carried out.

Urea denaturation of haemoglobin

2.5 g of haemoglobin and 36.0 g of urea were suspended in 100 ml of distilled water. This was stirred for 1 hour with a magnetic stirrer and then dialysed for 24 hours with three changes of distilled water at 4ºC. The dialysed solution was centrifuged to remove the insoluble material and the supernatant solution was used as haemoglobin substrate.

Enzyme Assay

To 0.5 ml of appropriate buffer, 0.3 ml of activated and unactivated enzyme (3-5µg) was added and incubated for 5 minutes at 37°C. The reaction was then started by the addition of 1.2 ml of 2.5% denatured haemoglobin solution. After incubation at 37°C exactly for 15 minutes the reaction was terminated by the addition of 3.0 ml of 5% TCA. Zero controls were run by adding TCA prior to the addition of haemoglobin solution. The reaction mixture was centrifuged after 30 minutes keeping at room temperature. The supernatant was analyzed for the TCA soluble peptides by Lowry's method as described before. The readings were corrected for zero time controls.

Identification of cysteine protease

To determine the class and specificity of the protease, enzyme extracts were incubated for 30minutes to 1h at 37ºC with the inhibitors and then added to 1% Casein. The inhibitors used were10mM PCMB (ρ-chloro mercurybenzoate) and10mM PMSF (phenyl methyl sulphonyl fluoride). Pepstatin is the inhibitor for aspartate proteases and EDTA for metalloproteases. These inhibitors help in identifying the class of proteases.

RESULTS

Proteases are distributed widely in different biological sources namely plants, animals and microbial sources but the results confirm plants as a rich source of proteases.

In plants, proteases are present virtually in every part i.e. stem, fruit, flower, seed, root, leaf, gum and latex. The results showed that plant latex is the richest source of protease.

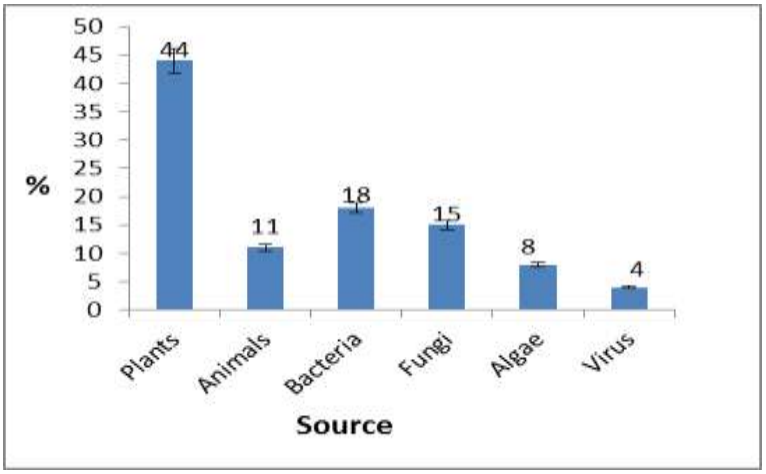

Figure 2: Distribution of Proteases.

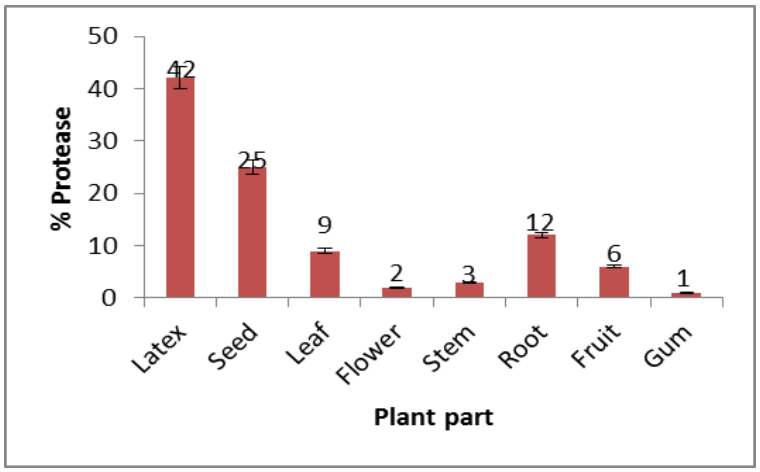

Figure 3: Distribution of Proteases in Plant Parts.

Screening and selection of plant lattices for proteases

About 70 plant lattices when screened for proteolytic activites, of which 61 plants showed proteolytic activity confirming several plant families such as Asteraceae, Caricaceae, Moraceae, Asclepiadaceae, Apocynaceae and Euphorbiaceae as the protease bearing plant families. (Table 1).

Table1. Screening of different plant lattices for protease activity (+ positive _ Negative).

S.No	Plant	Proteolytic Activity	S.No	Plant	Proteolytic Activity
1	*Artocarpus altilis*	+	36	*Ficus longifolia*	+
2	*Artocarpus hetrophyllus*	+	37	*Asclepias speciosa*	+
3	*Carrica candamarcensis*	+	38	*Calotropisgigantea*	+
4	*Croton celtidifolius*	+	39	*Asclepias syriaca*	+
5	*Euphorbia tirucalli*	+	40	*Calotropis procera*	+
6	*Euphorbia nivulia*	+	41	*Araujia hortorum*	+
7	*Euphorbia antiquorum*	+	42	*Asclepiascurassavica*	+

8	*Euphorbia hirta*	+	43	*Morreniabrachystephana*	+
9	*Euphorbia heliscopia*	+	44	*Asclepias fruticosa*	+
10	*Euphorbia dendroides*	+	45	*Funastrum clausum*	+
11	*Ficus lyrata*	+	46	*Tabernamontana gradiflora*	+
12	*Brassica napus*	_	47	*Pergularia extensa*	+
13	*Himatanthus drasticus*	+	48	*Ervatamiacoronaria*	+
14	*Hancornia speciosa*	+	49	*Philibertia gilliesii*	+
15	*Helianthus annuus*	_	50	*Morus indica*	+
16	*Vallaris solanacea*	+	51	*Euphorbia milii*	+
17	*Pedilanthus tithymaloides*	+	52	*Pedilanthus tithymaloids*	+
18	*Synadenium grantii*	+	53	*Jatropha curcas*	+
19	*Pentaclethra macrophylla*	_	54	*Euphorbiaulcherrema*	+
20	*Linum usitatissimum*	_	55	*Taraxacumofficinale*	+
21	*Euphorbia pulcherrima*	+	56	*Crinum asiaticum*	+
22	*Centaurea calcitrapa*	+	57	*Euphorbia neerifolia*	+
23	*Cocos nucifera*	_	58	*Crystostegia grandiflora*	+
24	*Mirabilis jalapa*	+	59	*Plumeria alba*	+
25	*Euphorbia supina*	+	60	*Nerium indicum*	+
26	*Taraxacum officinale*	+	61	*Nerium oleander*	+
27	*Carica papaya*	+	62	*Ficus religiosa*	+
28	*Nigella sativa*	_	63	*Ananas comosus*	+
29	*Jarilla chocola*	+	64	*Ananas sativus*	+
30	*Zea mays*	-	65	*Pileus mexicanus*	+
31	*Avena fatua*	_	66	*Ficus carica*	+
32	*Hordeum vulgare*	_	67	*Ficus benghalensis*	+
33	*Cryptolepis buchananii*	+	68	*Ficus elastic*	+
34	*Ficus glabrata*	+	69	*Helianthus annuus*	-
35	*Ficus racemosa*	+	70	*Cocos nucifer*	_

Identification of cysteine proteases

To determine the class and specificity of the isolated proteases, enzyme extracts were incubated for 30minutes to 1h at 37ºC with the inhibitors (PCMB and PMSF) and then added to 1% Casein.10mM PCMB (ρ-chloro mercurybenzoate) was used to identify cysteine proteases and10mM PMSF (phenyl methyl sulphonyl fluoride) was used to identify serine proteases and the results were presented in Table 2.

Table 2: Identification of cysteine proteases from different plant families.

S.No	PLANT	FAMILY	TYPE OF PROTEASE ISOLATED
1	*Artocarpus altilis*	Moraceae	Cysteine protease
2	*Artocarpus hetrophyllus*	Moraceae	Serine protease
3	*Carrica candamarcensis*	*Caricaceae*	Cysteine protease
4	*Croton celtidifolius*	Euphorbiaceae	Cysteine protease
5	*Euphorbia tirucalli*	Euphorbiaceae	Serine protease &cysteine protease

6	*Euphorbia nivulia*	Euphorbiaceae	Cysteine protease
7	*Euphorbia antiquorum*	Euphorbiaceae	Cysteine protease
8	*Euphorbia hirta*	Euphorbiaceae	Serine protease
9	*Euphorbia heliscopia*	Euphorbiaceae	Serine protease
10	*Euphorbia dendroides*	Euphorbiaceae	Serine protease
11	*Ficus lyrata*	Moraceae	Cysteine protease
12	*Ficus religiosa*	Moraceae	Serine protease
13	*Himatanthus drasticus*	Apocynaceae	Cysteine protease
14	*Hancornia speciosa*	Apocynaceae	Cysteine protease
15	*Hevea brasiliensis*	Euphorbiaceae	Cysteine protease & serine protease
16	*Vallaris solanacea*	Apocynaceae	Cysteine protease
17	*Pedilanthus tithymaloides*	Euphorbiaceae	Cysteine protease
18	*Synadenium grantii*	Euphorbiaceae	Serine protease
19	*Ananas comosus*	Bromeliaceae	Cysteine protease
20	*Ananas sativus*	Bromeliaceae	Cysteine protease
21	*Euphorbia pulcherrima*	Euphorbiaceae	Serine protease
22	*Centaurea calcitrapa*	Asteraceae	Aspartate protease
23	*Cynara cardunculus*	Asteraceae	Aspartate protease
24	*Mirabilis jalapa*	Nyctaginaceae	Aspartate protease
25	*Euphorbia supina*	Euphorbiaceae	Serine protease
26	*Taraxacum officinale*	Asteraceae	Serine protease
27	*Carica papaya*	Caricaceae	Cysteineproteases
28	*Pileus mexicanus*	Caricaceae	Cysteineproteases
29	*Jarilla chocola*	Caricaceae	Cysteineproteases
30	*Ficus carica*	Moraceae	Cysteineproteases
31	*Ficus benghalensis*	Moraceae	Cysteineproteases& serine protease
32	*Ficus elastic*	Moraceae	Cysteineproteases& serine protease
33	*Cryptolepis buchananii*	Asclepiadaceae	Serine protease
34	*Ficus glabrata*	Moraceae	Cysteineproteases
35	*Ficus racemosa*	Moraceae	Cysteineproteases
36	*Ficus longifolia*	Moraceae	Cysteineproteases
37	*Asclepias speciosa*	Asclepiadaceae	Cysteineproteases
38	*Calotropisgigantea*	Asclepiadaceae	Cysteineproteases
39	*Asclepias syriaca*	Asclepiadaceae	Cysteineproteases
40	*Calotropis procera*	Asclepiadaceae	Cysteineproteases
41	*Araujia hortorum*	Asclepiadaceae	Cysteineproteases
42	*Asclepiascurassavica*	Asclepiadaceae	Cysteineproteases
43	*Morreniabrachystephana*	Asclepiadaceae	Cysteineproteases
44	*Asclepias fruticosa*	Asclepiadaceae	Cysteineproteases
45	*Funastrum clausum*	Asclepiadaceae	Cysteineproteases
46	*Tabernamontanagradiflora*	Apocynaceae	Cysteineproteases
47	*Pergularia extensa*	Apocynaceae	Cysteineproteases
48	*Ervatamiacoronaria*	Apocynaceae	Cysteineproteases
49	*Philibertia gilliesii*	Apocynaceae	Cysteineproteases
50	*Morus indica*	Urticaceae	Cysteineproteases& serine protease
51	*Euphorbia milii*	Euphorbiaceae	Cysteineproteases& serine protease
52	*Pedilanthus*	Euphorbiaceae	Cysteineproteases

	tithymaloids		
53	*Jatropha curcas*	Euphorbiaceae	Cysteineproteases
54	*Euphorbia pulcherrema*	Euphorbiaceae	Cysteineproteases
55	*Taraxacumofficinale*	Asteraceae	Cysteineproteases
56	*Crinum asiaticum*	Amaryllideae	Cysteineproteases
57	*Euphorbia neerifolia*	Euphorbiaceae	Serine protease
58	*Crystostegia grandiflora*	Apocyanaceae	Cysteine protease
59	*Plumeria alba*	Apocynaceae	Cysteine protease
60	*Nerium indicum*	Apocyanaceae	Cysteine protease
61	*Nerium oleander*	Apocyanaceae	Cysteine protease

Though all the four classes of proteases occur in plants most of them are cysteine proteases. The results clearly showed that plant derived proteases are mostly cysteine proteases.

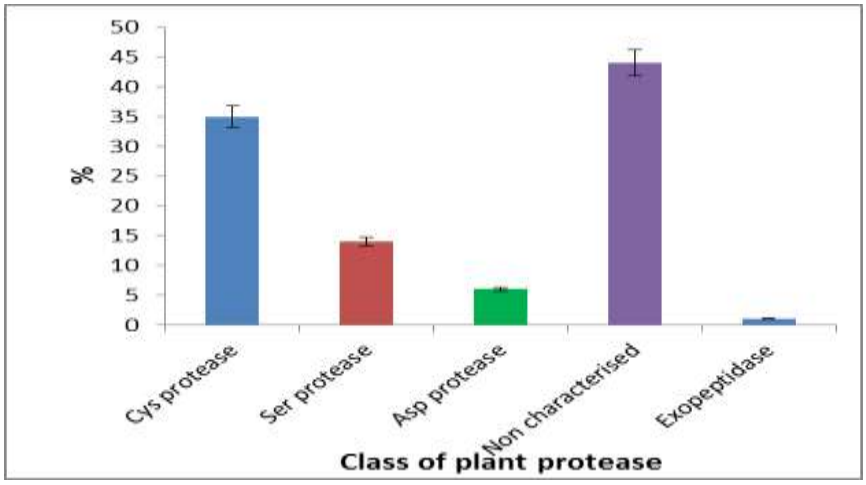

Figure 4: Plant Protease.

DISCUSSION

Plant latex is the richest source of protease and several plant families such as Asteraceae, Caricaceae, Moraceae, Asclepiadaceae, Apocynaceae and Euphorbiaceae as the latex protease bearing plant families. Plant latex is a natural source of pharmaceuticals and pesticides.[19] Among the 61 latexprotease positive plants tested 11 were serine protease positive (Moraceae, Euphorbiaceae, Asclepiadaceae, Asteraceae) three were aspartate protease positive (Asteraceae, Nyctaginaceae), six were positive to both cysteine protease and serine protease (Euphorbiaceae, Urticaceae, Moraceae). The rest were cysteine protease positive, which shows that the cysteine proteases form the well distributed class. Particularly cysteine proteases are active over wide range of pH and temperature.[20] Among the screened plants, lattices of Ficus *longifolia* and *Vallaris solanacea* were not studied and characterized previously thus they were selected for further studies. Further, due to the advantages like their

easy availability and high protein concentration (5mg/ml), *Vallaris solanacea* was finally selected for further purification, characterization and disease invasion studies.

The latex of *Vallaris solanacea* has high proteolytic activity, the activity being comparable to those of other known latex proteases such as papain, ficin, bromelain, asclepain and calotropain etc. The specific activity of the crude latex towards casein at pH 9.0 is 87.5 Usually, proteolytic enzymes lose their activity during storage due to autolysis in addition to denaturation. To prevent autolysis, thiol proteases may be stored in reversibly inhibited state. For example, papain was prepared as its mercury derivative. The mercury derivative was more stable and lost little activity during storage for long periods of time. In place of mercury, PCMB also can be used to cause reversible inhibition of the enzyme. Similar inhibitory effects of heavy metal ions were previously described for b-NAHAs from jack bean meal[21], germinating fenugreek seeds[22], maize seedlings[23], rice seeds and mungbean seedlings.[24]

CONCLUSION

Thus in the search of novel plant proteases, a new plant protease has been identified and screened effectively for its proteolytic activity. Using thiol blocking reagent PCMB (ρ-chloro mercurybenzoate), this protease was confirmed as cysteine protease. Among the 61 plants screened *Vallaris solanacea* was finally selected considering its high protein concentration (5mg/ml), ease of availability and stability. *Vallaris solanacea* (Roth) Kuntze (Family: Apocyanacea) locally named as Agarmoni, bread flower is a tall climbing shrub. It is distributed throughout India and cultivated as an ornamental plant for its white fragrant flowers traditionally used against ring worms and skin infections. The plant extract contains reducing sugars, tannins, saponins, gums, steroids, alkaloids and glycosides and was found medicinally important with its cytotoxic, antioxidant, antinociceptive, antimicrobial, analgesic, anti-inflammatory and antidiarrhoel activities. As an accumulators of pollutants it has an an important role in phytoremediation. Barks and roots of some Apocynaceae species have anticancer and antimalarial properties The vinca alkaloids of Apocynaceae are antimitotic agents and primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers, including leukemias, lymphomas, advanced testicular cancer, breast and lung cancer and Kaposi's sarcoma. It was shown that a glucoside mixture from the plant leaves possess powerful digitalis-like activity in addition to general musculotropic activity on smooth muscle. It also caused emesis and its glycosides also

possess cardiotonic activity. The seeds of *V solanacea* are very rich in cardiac glycosides. A new cardenolide glycoside, vallarisoside showed potent TRAIL-resistance-overcoming activity in human gastric adenocarcinoma (AGS) cells and cell-growth-inhibitory activity against HeLa and SW480 cells. It was demonstrated that latex of *V solanaceae* completely suppres the citrinin, which is a lemon yellow toxin produced by *Penicillium citrinumto* which cause enlarged kidney in rats with degeneration and dilation of lower nephrons. This information emphasizes that herbal world can be explored for new drugs. Thus this information emphasizes that further research on *Vallaris* can be explored for new drugs and this work also stresses on the necessity for the isolation and characterization of valuble proteases in the latex of *Vallaris solanacea.*

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