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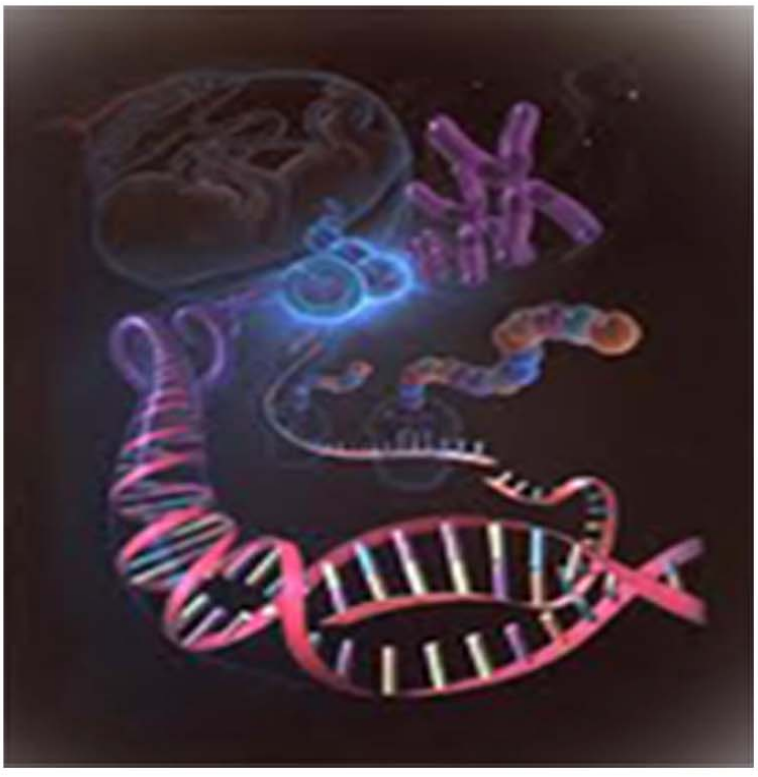

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Research Paper

EVALUATION OF COMPARATIVE TOTAL PROTEOLYTIC ACTIVITY IN PLANT LATTICES

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Proteases catalyze the hydrolysis of a peptide bond of a protein; they are widely distributed in nature. They perform various biological functions in plants and particularly in humans they participate in zymogen activation, fibrinolysis, complement activation, blood coagulation and digestion process. **Objectives:** The present study focused on evaluation of *in vitro* total proteolytic activity from various plant lattices belongs to families such as apocynaeceae, asclepiadaceae, caricaceae, euphorbiaceae, moraceae at different pH conditions and compared with latex of *Carica papaya* as standard protease activity. **Methods:** The total protease activity in enzyme extract assayed in terms of caseinolytic activity using one percent casein substrate at 37 °C and pH 7.6 (0.2 M buffer). The soluble casein fragments released during proteolysis were measured by Lowry reaction. Protein content in enzyme extract was determined by using Folin Ciocalteau method. **Results:** From the present study, total proteolytic activity reported in terms of specific activity (activity/mg protein) in different pH condition and were found that majority of plants shows Protease activity in alkaline pH in comparison with water. **Conclusion:** The conclusion of the study states that lattices of the above selected plant families contains protease activity as common biological activity.

Keywords: Plant protease, Latex, Specific activity, Endopeptidase

INTRODUCTION

The enzymes that cleave peptide bonds of a protein are referred to as proteolytic enzymes or proteases. Several proteinases (endopeptidase) are well known in broad sense of catalyzing the same reaction. However, these enzymes differ widely in their specificity towards certain peptide bonds as well as in pH-optima and many other reaction parameters.

Proteases are widely distributed in nature, present in animals and man (Akihiko Moriyama and kenjitakahashi, 1980; Neurath, 1986; and Donohue *et al*., 1998) microorganisms (Endo, 1962; and Wu *et al*., 2006) and in plant lattices (Robbins and Lamson, 1934; Wurtz and Bouchut, 1879; and Lynn and Clevette-Radford, 1983). The characteristic property of each protease through irreversible cleavage is crucial for functional

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activation of proteins that are involved in blood coagulation, fibrinolysis, complement activation and protein digestion in biological system. Therefore, they have become the focus of wide range of industrial and medical application.

Even though there is a vast information on plant protease, majority of these proteases have been subjected for isolation to homogeneity and characterized. The survey of literature revealed that the availability of seldom information on protease activity from plant lattices. They are Papain (*Carica papaya Lynn*), Euphorbain (*Euphorbia lathyris Willd*), Curcain (*Jatropa curcas Lynn*), Calatropin (*Calatropis gigantean Robert brown*) and Ervamatin (*Ervatomia coronaria Jacq*.) etc.

However, considerable research works were carried out for proteolytic activity in individual latex under specified conditions, the present study taken up laticeferous perennial shrubs belongs to different families were selected and screened for detection of protease activity. However, the comparative evaluation of total proteolytic activity in the plant lattices is the prevailing research gap and this necessitates the study. The protease activity is justified with well-known papaya latex protease. This holds up the justification of authenticity procedure adopted in the present research. The aim of the present study is to isolate proteolytic active principle on solubilization in to various buffers at different pH conditions from regional higher plants grown around Kolar, Karnataka for comparative protease activity in lattices.

MATERIALS AND METHODS

Plant Material

Latex has been used as a term for the fluid substance in plants. The fluid present in vessels or cells make the laticeferous system formed from rows of cells laid down in the meristem of the stem or root (Mahlberg, 1993). It serves as defense against herbivorous insects (Agrawal and Konno, 2009). In the year 1887, Joseph, James noticed the development of the seedling latex cells that differentiate as the plant grows, and these latex cells grow into a branching system extending throughout the plant. Dried latex from the opium poppy is opium, the source of many useful opiates and other alkaloids of high value.

The latex collected from the following plants on breaking the twigs or from petiole in to dry tube were processed and stored at 4 ^{o}C. The plants bearing latex are *Carica papaya Lynn*, *Calatropis procera Robert brown*, *Euphorbia pulcherrima wild*, *Ficus bengalensis Lynn*, *Synadenium grantii Hook 'f', Artocapus hetrophyllus Lynn*, *Euphorbia antiquoram Lynn*, *Morus alba Lynn*, *Ficus religiosa Lynn*, *Euphorbia heterophylla Lynn*. All the chemicals used in the study are analytical grade from Sigma chemical company St Louis [USA]. Papain and casein from Hi Media Bombay.

Methods

Protein Estimation: Protein concentration in the enzyme extract was determined using Folin Ciocalteu reagent as per the procedure of Lowry *et al*. (1951), Crystalline Bovine Serum Albumin used as standard protein for preparation of standard curve. The different aliquots of protein standard allowed reacting with Folin phenol reagent. The absorption of the blue color developed was measured at 540 nm using spectrophotometer.

Protease Assay: The protease assay was done according to the method of Kunitz (1947), which was adapted in a research work described by Sumathi and Pattabiraman (1975). The enzyme extract of processed latex was incubated with 1.0

mL of 1% casein substrate prepared using 0.2 M phosphate buffer at pH 7.6 for 20 min at 37 °C. The reaction was arrested by addition of 3.0 mL of 5% Trichloro acetic acid (TCA). The TCA soluble fragments and total protein of the enzyme extract estimated by the procedure of Lowry *et al.* (1951).

Isolation of Protease: Clarification of crude latex performed on suitable dilution of latex in different buffer system with varying pH conditions. The buffers used were 0.2 M glycine-HCl, 0.2 M Sodium acetate, 0.2 M phosphate buffer, Tris-HCl buffer and 0.1 M sodium hydroxide. These were subjected for freezing and thawing to remove scum, gum until it produce clear latex sera separated by centrifugation at 10,000 g for 15 min at 4 ^{0}C the same was used for protease assay in the study.

RESULTS AND DISCUSSION

The results obtained from the study presented in tabular format. Accordingly, Table 1 shows the total proteolytic activity of lattices solubilized in water and Table 2 indicates total proteolytic activity at pH 7.6 when Lattices dissolved in 0.2 M phosphate buffer as shown in Figures 1 and 2. The appropriate dilution criteria of the latex either water or buffer arrived by analysis of the specific activity. The degree of dilution selected in a way to measure of protease activity in a linear range. The degree of dilution indicates the amount of the protein content present in the latex responsible for the protease activity.

An innovative aspect of the current study helps to determine the protease activity in the lattices of plant that could have possible positive effect on local economics in relation to garden composting, leather industries, food technology, breweries, or modification of functional and nutritional properties of food proteins through controlled enzymic hydrolysis, etc.

In the present study by comparison of the results from the above Tables 1 and 2 evinced the protease activity in water and phosphate buffer system. As per this data, water was found appropriate medium for solubilization of the

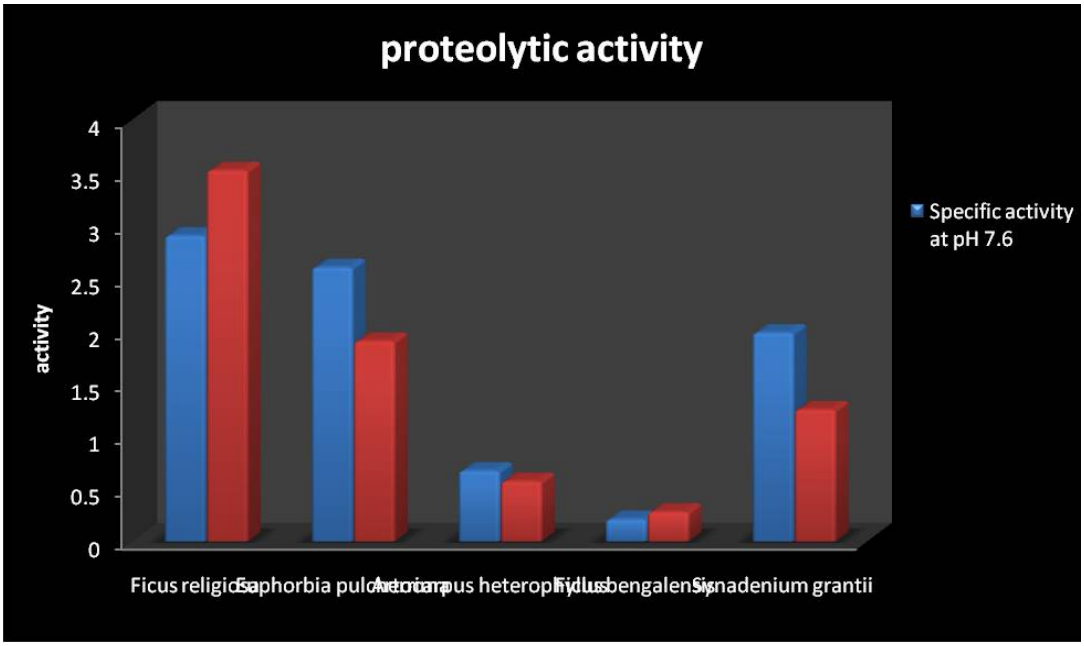
Table 1: Total Proteolytic Activity of Lattices Solubilized in Water

Name of the Plant	Total Units	Total Proteins	Specific Activity (activity/mg protein)
Carica papaya	1,750	128	13.6
Calatropis procera	600	31.25	19.0
Euphorbia heterophylla	440	87.50	5.0
Ficus religiosa	380	136	3.52
Morus alba	108	53	2.03
Euphorbia antiquorum	165	16	4.10
Euphorbia pulcherrima	95	50	1.90
Artocarpus heterophyllus	75	131.2	0.57
Ficus bengalensis	28	100	0.28
Synadenium grantii	30	23.75	1.25

Table 2: Total Proteolytic Activity at pH 7.6 in Latex Bearing Plants

Name of the Plant	Total Units	Total Proteins (mg protein)	Specific Activity (activity/mg protein)
Carica papaya	2,650	140	19.0
Calatropis procera	760	49	16.0
Euphorbia heterophylla	400	100	4.0
Ficus religiosa	380	136	2.9
Morus alba	83	45	1.84
Euphorbia antiquorum	330	29	11.5
Euphorbia pulcherrima	120	48	2.6
Artocarpus heterophyllus	92	137	0.67
Ficus bengalensis	21.6	100	0.21
Synadenium grantii	77.0	39.0	1.98

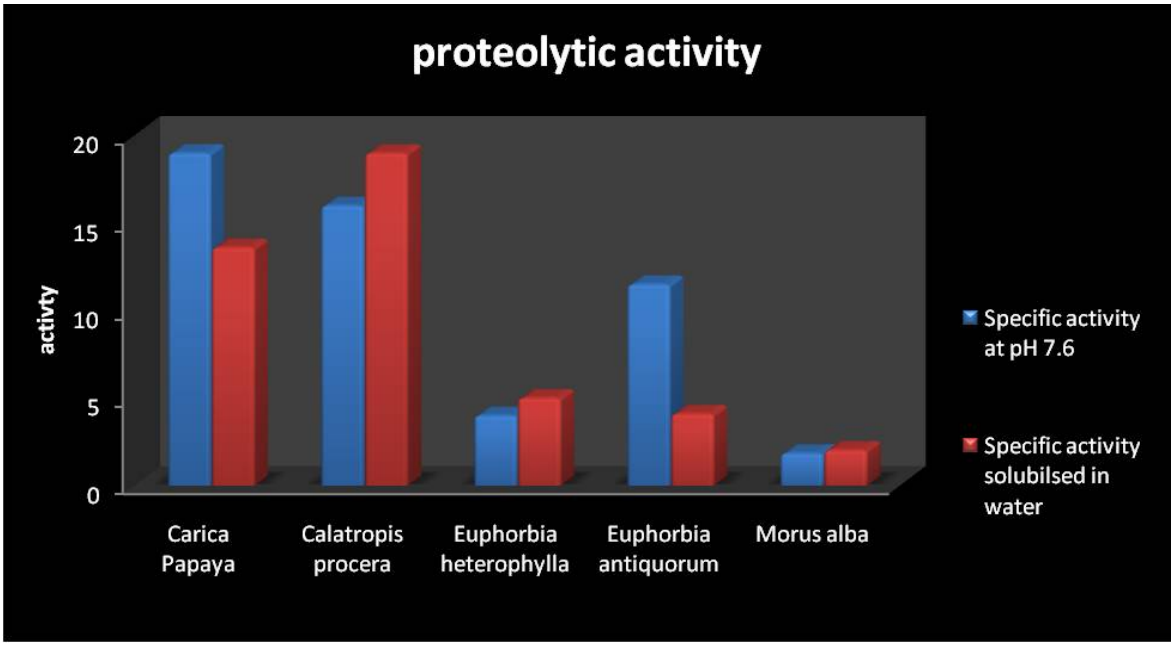
Figure 1: Proteolytic Activity at Various pH (Group I)



protein responsible for the protease action for few plants such as *Calatropis procera*, *Euphorbia heterophylla*, and *Ficus religiosa*. However, it is not good environment for few plant lattices from *Carica papaya, Euphorbia antiquoram*.

Buffer acts as suitable medium for *Euphorbia antiquoram* and *Carica papaya* where they exhibit increased activities nearly more than 75% and 50%, respectively. In order to measure the total protease activity for the generalized screening

Figure 2: Proteolytic Activity at Various pH (Group II)



procedure, phosphate buffer system found ideal for extraction of protease active principle rather than water due to proportionate enhanced activity from all the lattices.

Table 3 results presents the consolidated data of total protease activity of plant lattices selected in the present study when measured in different buffer system at varying pH conditions of pH 2.0-

Table 3: Total Proteolytic Activity in Latex Bearing Plants at Different pH Conditions

Name of the Plant and Dilution	Dilution	pH					
		2.0	5.0	6.0	8.0	9.0	12.0
Carica Papaya	(1:500)	4.90	7.30	21.3	18.0	18.0	13.0
Calatropis procera	(1:200)	8.0	13.0	24.8	21.3	8.60	5.6
Euphorbia heterophylla	(1:400)	0.16	3.43	3.40	8.40	8.60	Nil
Ficus religiosa	(1:200)	1.13	2.17	2.0	3.60	4.26	2.93
Morus alba	(1:100)	1.35	1.38	2.05	23.88	1.86	Nil
Euphorbia antiquorum	(1:100)	13.4	4.10	8.0	17.6	7.04	Nil
Euphorbia pulcherrima	(1:100)	0.25	0.93	2.20	2.60	2.90	Nil
Artocarpus heterophyllus	(1:100)	0.67	0.66	0.62	0.66	0.55	Nil
Ficus bengalensis	(1:100)	0.23	0.16	0.21	0.16	0.16	Nil
Synadenium grantii	(1:100)	0.60	0.60	1.30	1.98	2.30	Nil

Figure 3: Proteolytic Activity at Various pH (Group I)

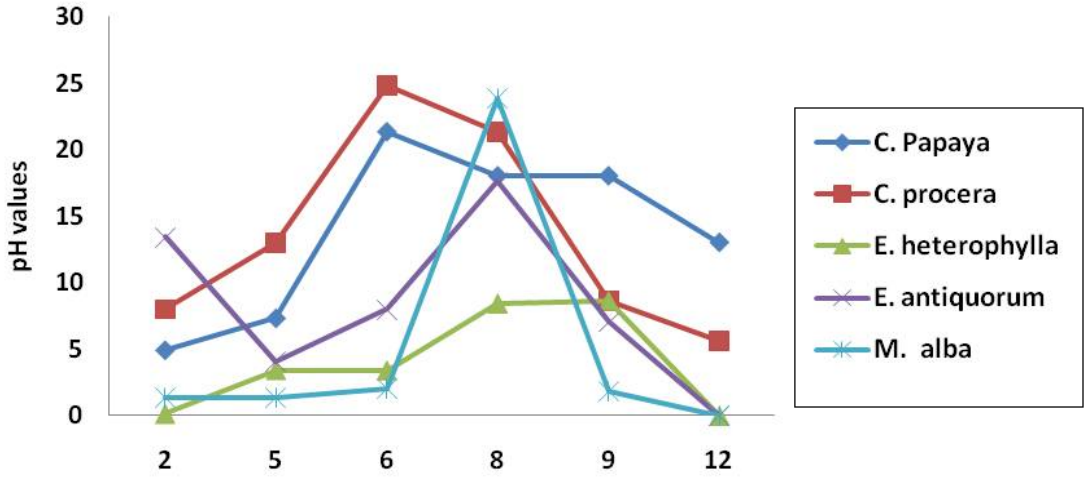
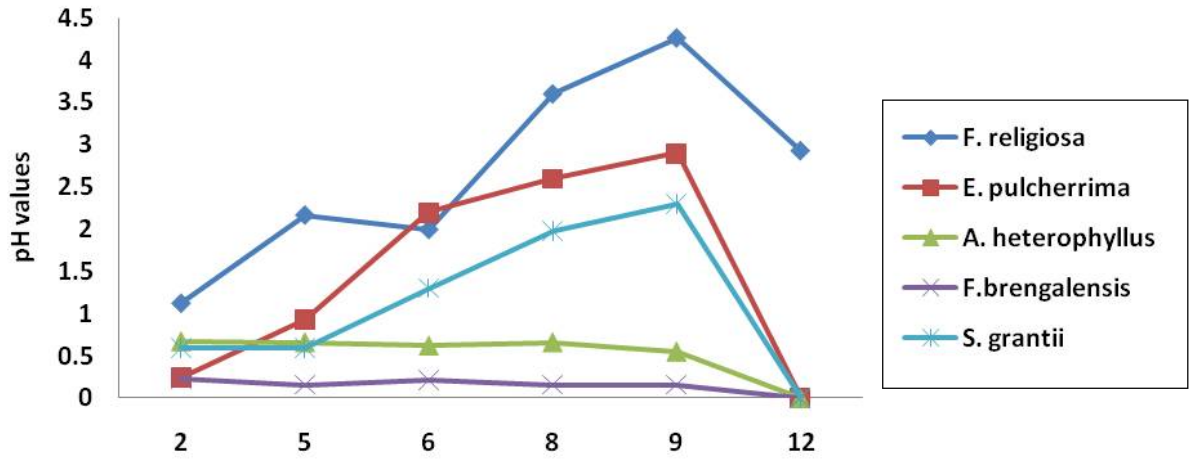


Figure 4: Proteolytic Activity at Various pH (Group II)



12.0. In exemption to *Artocarpus heterophylla* and *Ficus bengalensis* all the remaining lattices exhibited pH dependent activity and also twenty percent of the plants in the study were found to be inactive at extreme pH 12.0 due to enzyme instability as shown in Figures 3 and 4. In contrary to other plants in our study Ficus *bengalensis*, *Euphorbia pulcherrima* showing considerable amount of activity in acidic pH.

Under the assay conditions, *Artocarpus heterophyllus* and *Euphorbia tirukalli* did not show any significant measurable amount of protease activity. These results generate an idea that the substrate casein used in the study might not be ideal or presence of any protease inhibitor in the crude latex. However, the remaining lattices contains moderate amount of protease action thus this study helps to 'Know' the plant latex that

was not received due attention for protease activity measurement helps to carry out the study to find out the utility in the applied science.

In the world market of enzymes, the bulk of protease obtained from microorganisms has antremendous role in biotechnology, food industries and other applied fields. However, some plant cysteine proteinases namely papain, bromelain, and ficin are still preferred in economic processes. Even though there are only limited number of plant proteases that have been isolated and characterized. Thus, the similar type of study helps to explore the protease content in various latex bearing plants.

The proteins are modified by hydrolysis that leads to chemical, physical, biological, and immunological properties. These changes cause improvement of the nutritional characteristics, retarding deterioration, modification of different functional properties such as solubility, foaming, coagulation and emulsifying capacities, prevent undesired interactions, changes in flavors, odors and removal of toxic or inhibitory factors.

The enzymatic hydrolysis is strongly preferred over chemical methods because it yields hydrolysates containing well-defined peptide and amino acid mixtures (aminosol) and avoids the destruction of L-amino acids and the formation of toxic substances like lysino-alanine (Lahl and Brown, 1994; and Mahmoud, 1994).

Peptides that show antihypertensive activity are the most commonly isolated from enzymatic hydrolysates of various food materials (Haileselassie *et al*., 1999; and Miguel *et al*., 2007). From these digests Peptides with other biological activities, such as opioid agonistic and antagonistic, antioxidants, anticancerous, and immuno modulatory actions have also been identified.

The above mentioned nutraceutical products are suitable for use in the food and pharmaceutical industries or both in the form of a hydrolysate or bioactive peptides (Wang *et al*., 2005). In the fore going discussion, the protease activity measured in the lattices is similar to few latex proteases studied by several workers. They are papain of *Carica papaya*, ficin of *Ficus carica*, calatropin from *Calatropis gigantia*, euphorbain from *Euphorbia lathyris* and Ervamatin from *Erratomia coronaria* (Pal and Sinha, 1980; Lynn and Clevette-Radford, 1983; Sundd M *et al*., 1998; and Sarote Nitsawang *et al*., 2006;).

According to Lynn Clevette-Radford (1988), several species of *Euphorbiaceae* family known for protease content and their physical and chemical properties (Lynn and Clevette-Radford, 1983). This commonest observation of latex protease enzyme also adds a feature to taxonomical study. The present study shows the chemical property of all crude latex when released from the plant has pH 6.0 but the magnitude of protease activity was found high in alkaline pH due to more solubilization of protein and stability. Freezing and thawing helps to obtain the clear aqueous extract.

Due to various factors contributing towards enzyme activity in the crude latex state, it is desirable to measure the protease total activity. However, further isolation and purification necessary for characterization such as determination of three dimensional structures, kinetic properties, to explore biological activity. This screening study leads to an avenue to select the plant latex for further study to explore the economic utility.

CONCLUSION

The present study showed that all the selected

plant lattices from various families contain proteolytic activity and found significant in alkaline range on suitable buffer extraction.

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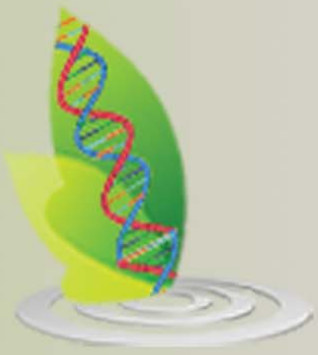

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