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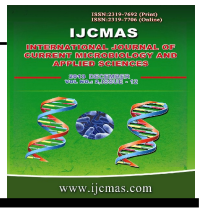

Original Research Article

Detection of protease from latex producing plant by X-ray film by DOT-BLOT method

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A B S T R A C T

Many plants contain latex that exuded when leaves are damaged & number of protein & enzymes have been found in it. The latex of some plant families such as *Asclepiadaceae, Apocynaceae, Caericaceae, Euphorbiaceae, Moraceae, Meliaceae, Sopodilla* contains endopeptidase. In presence study fourteen various latex producing plants were identify for the presence of proteolytic activity by dot-blot X ray film method. The *Euphorbia synudenium, Caloteopisprocera, Thevetia Peruviana, Ficusreligiosa, Caricapapaya, Azarirachta indica, Ficusbengalensis, Manikarazopota, caloteopisgigantea,* degrade the gelatin on the x-ray film & the clear zone is formed at the site of application on x-ray film which indicates the presence of protease in the sample. The *Jatrophacurcus, Plumeriarubera, Euphorbia triucalli, Ficusracemosa, Ricinuscommanis* show no zone of clearances at the site of sample on x-ray film which shows absence of protease. The protein estimation of a various latex containing plants were done by lowery's method. The *Euphorbia synudenium ,Caloteopisprocera, Thevetiaperuviana, Ficusreligiosa ,Caricapapaya ,Azarirachtaindica, Ficusbengalensis, Manikarazopota, caloteopisgigantea, Jatrophacurcus, Plumeriarubera, Euphorbia triucalli Ficusracemosa, Ricinuscommanis*in show protein concentration in between the rang of 45µg-390µg/ 0.1ml respectively. The proteolytic activities of enzymes preparation isolated from latex containing plants were estimated by using casein as substrate.In the present paper we described a simple and inexpensive procedure to detect protease of latex containing plants by the X- ray film dot-blot method.

Keywords

Plant latex; protease assay; X-ray film; protein estimation.

Introduction

Latex is widely distributed in plant more than 12000-35000 species have been reported to contain it. Many proteases from plant latex have been isolated and their properties extensively investigated, e.g., ficin from *Ficuscarica*, euphorbains from Euphorbia spp., papain and related proteases from *Carica papaya*(Arnon R. Papain, 1970, Liener IE, Friedensen B. Ficin, 1970, Pal G, Sinha NK, 1980) and

calotropain from *Calotropis gigantean(*Abraham KJ, Joshi PN,1979). Proteases have also been purified and characterized from oat, wheat flag, maize, *Phaseolus vulgaris*, *Onopordum turcicum*, *Spinacia oleracea* and *Petroselinum crispum* leaves (Jiang WB, Lers A, Lomaniec E, Aharoni N, 1999). Proteases are important enzymes of plant metabolism and are instrumental in regulating senescence (Lauriere C. 1983). They are responsible for the degradation of proteins. Proteolytic enzymes are used extensively inindustrial and medical applications (WardO P. 1985**).** As latex often contains toxic compounds against herbivorous insects (e.g. cardenolide in milk weed and alkaloid in poppy) (Dussourd 1993; Farrel et.al., 1991; Harborne, 1993),and as large amount of fluid intensely exudes immediately after an insect attack at the point of damage in spite of the relatively small total amount of latex suggested to exist in whole plant (Dussourd & Denno, 1991; Farrel et.al. 1991),some biologists hypothesized that latex provides plants with an ideal defense mechanism against insect herbivores (Dussourd ,1993,Dussourd & Denno, 1991; Farrel et.al., 1991; Harborne, 1993; Dussourd & Eisner,1987).However, neither apparent toxicity nor toxins have been reported from the majority of latex producing plants. For e.g., no apparent toxins have been reported from the latex of papaya, Ficus species, dandelion, mulberry, or the rubber tree, although these plants are well known latex producing plants. In such cases, the defensive role of latex has usually been attributed partly to its sticky nature, which would enable the plants capture and immobilize the mouth parts of insects (Dussourd, 1993, Dussourd& Denno, 1991; Farrel et.al., 1991). However, the absence of apparent toxicity from such plants appears to be inconsistent with and even undermining the widely accepted defense hypothesis. Meanwhile, latex is known to be a rich source of enzyme such as proteases (Arima et al.2000; Arribere et.al., 1998; Cohen et al., 1986; Kimmel &Smith et al., 1954; Kramer& Whitaker, 1964; Sgarbieri et al.,1964), chitinase (Azarkan, 1997; O'Riordain et al., 2002) etc. In particular, cysteine proteases are found in the latex of several plants, such as papaya and fig, in great abundance (Arribere et.al., 1998; Cohen et al., 1986; Kimmel&Smith et al., 1954; Kramer& Whitaker, 1964; Sgarbieri et al., 1964), although their physiological roles remain unknown.The enzymes that cleave peptide bonds of a protein are referred to as proteolytic enzymes or proteases. In the present paper, we described a simple and inexpensive procedure to detect protease of latex on the X- ray film by dot-blot method.Gelatine, a denatured form of collagen is a substrate commonly used to detect proteolytic activity [D.E.kliener 1994]. [A.L.cheung et.al.1991] have demonstrated the use of gelatin coating present on X-ray film as a substrate for detecting aggregate proteolytic activity in a dot-blot assay. With the help of X-ray film assay variety of proteolytic enzymes including serine proteinases, Metalloproteinase,thiolproteinases, and acid proteinases have been demonstrated [A.L Cheung., 1991].The present study was conducted to detect the protease from fourteen various latex producing plants by dot-blot X -ray film method.

Materials and Methods

Collection of latex from the sample

The plants were obtained from the rural area around the Vaijapur village. The latex was collected in a sterile container by

breaking of the leaves while the other parts of the plant were obtained by up-rooting the plant.

Protease activity of latex by the dot-blots method

10µl of latex sample, spotted on to strip of X-ray film .The protease present in latex degrade the gelatin on the X-ray film and the clear of zone is formed at the site of sample applied on X-ray film [Vinod Borde et.al.2012].[Fig.1].

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. [Table: 1].

Protease activity was assayed by a modified method of [Tsuchicla et.al.1986] by using casein as substrate.100µl 0f enzymes solution was added to 900 µl of substrate solution [2% casein in 10mM. Tris-Cl buffer pH 8.0] the mixture was incubated at 50°C for 20 min. Reaction was terminated by the addition of an equal volume of 10% chilled Trichloro acetic acid (TCA) then the reaction mixture was allowed to stand in ice for 15 min to precipitate the insoluble protein.The supernatant was separated by centrifugation at 10,000 rpm for 10 min at 4^0c,the acid soluble product in the supernatant was neutralized with 5ml of 0.5M Na_2CO_3 solution .The colour developed after adding 0.5 ml of 3 fold diluted Folin ciocalteau reagent was measured at 660 nm.All assay were done in triplicate .One protease unit is defined as the amount of enzymes that release 1µ mol of tyrosine per ml per minute under the above assay condition. The specific activity is expressed in unit of enzymes activity per milligram of protein. [Table: 2].

Results and Discussion

The fourteen samples collected were analyzed for protease activity. *Euphorbia synudenium, Caloteopisprocera Thevetia Peruviana, Ficusreligiosa, Caricapapaya, Azarirachta indica, Ficusbengalensis, Manikarazopota, caloteopisgigantea* degrade the gelatin on the X-ray film & a clear of zone is formed at the site of sample on X-ray film. The *Jatrophacurcus, Plumeriarubera, Euphorbia triucalli, Ficusracemosa, Ricinus commanis* show no zone of clearance at the site of sample on X-ray film. Total protein concentration of *Euphorbia synudenium, Caloteopisprocera, Ficus religiosa, Ficusracemosa, Jatrophacurcus, Thevetia peruviana, Plumeria rubera, Euphorbia triucalli, Carica papaya, Azarirachta indica ,Ficusbengalensis, Manikarazopota, caloteopisgigantea, Ricinus commanis* were found in range of 45µg-390 µg/0.1ml respectively. The specific activity of crude enzymes preparation isolated from latex containing plants were estimated by using casein as substrate and is in rang of 0.61827 to 9.444 units. *Euphorbia synudenium* show highest specific activity 9.444 unit/mg, *Jatrophacurcus* 6.75 unit/mg, *Manikarazopota* 6.51786 unit/mg, *Ricinus commanis* 5.9375 unit/mg, *Euphorbia triucalli* 3.20513 unit/mg, *Ficus religiosa* 2.1794 unit/mg, *Thevetiaperuviana*

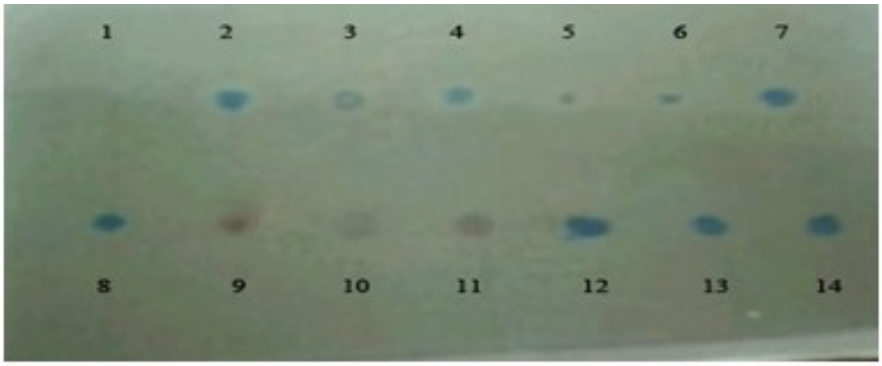

Fig.1: Dot-blot assay on x-ray film

Jatrophacurcus, Plumeriarubera, Euphorbia triucalli, Ficusracemosa, Ricinuscommanis show no zone of clearances is formed at the site of sample on x-ray film, in fig.1,5,9,10,11.The *Euphorbiasynudenium, Caloteopisprocera, Thevetia Peruviana, Ficusreligiosa, Caricapapaya, Azarirachtaindica, Ficusbengalensis, Manikarazopota, caloteopisgigantea*, degrade the gelatin on the X-ray film & show the clear zone at the site of sample on X-ray film, in fig.2,3,4,6,7,8,12,13,14.

Table.1 Total protein concentration in latex
Determination of protease activity**:**

S.No	Sample name	Concentration in of protein µg/0.1ml
1	*Euphorbia synudenium*	112.5
2	*AzadirachtaIndica*	390
3	*Ricinuscommanis*	367.5
4	*Carica papaya*	290
5	*Manikarazapota*	195
6	*Jatrophacurcas*	100
7	*Thevetia peruviana*	120
8	*Calotropis gigantea*	70
9	*Ficusreligiosa*	70
10	*Ficusbengalensis*	140
11	*Ficusracemosa*	87.5
12	*Calotropisprocera*	45
13	*Plumeriarubera*	85
14	*Euphorbia triucalli*	110

Table.2 Total proteolytic activity of latex containing plant

S.No	SAMPLE NAME	TOTAL VOLUME (ml)	TOTAL ACTIVITY (unit)	TOTAL PROTEIN (mg)	SPECIFIC ACTIVITY (unit/mg)
1	*Euphorbia synudenium*	7.5	796.875	84.375	9.444
2	*Azadirachta indica*	7.5	140.623	82.5	1.70458
3	*Ricinus commanis*	7.5	534.375	90	5.9375
4	*Carica papaya*	7.5	257.8125	275.625	0.93537
5	*Manikarazapota*	7.5	684.375	105	6.51786
6	*Jatropha curcas*	7.5	506.25	75	6.75
7	*Thevetia peruviana*	7.5	576.5625	337.5	1.70833
8	*Calotropis gigantea*	7.5	403.125	652.025	0.61827
9	*Ficus religiosa*	7.5	637.5	292.5	2.1794
10	*Ficus bengalensis*	7.5	421.875	525	0.80357
11	*Ficus racemosa*	7.5	304.6875	217.5	1.40036
12	*Calotropis procera*	7.5	520.3125	637.05	0.81675
13	*Plumeria rubera*	7.5	543.75	525	1.03571
14	*Euphorbia triucalli*	7.5	468.75	146.25	3.20513

1.70833 unit/mg, *Azarirachta indica* 1.70458 unit/mg, *Ficus racemosa* 1.40036 unit/mg, *Plumeria rubera* 1.03571 unit/mg, *Carica papaya* 0.93537 unit/mg, *Caloteopis procera* 0.81675 unit/mg, *Ficus bengalensis* 0.80357 unit/mg, respectively, were as *Caloteopis gigantea* show the lowest specific activity 0.61827 unit/mg .

From the above result it was concluded that, all the selected plant containing latex from varies family show the proteolytic activity as common biological activity. India has a large tribal population, which is regularly using plant latex for the treatment of various diseases. Though so many utilities plant latex are known but their overall ethnobotanical use is still unknown that might be more helpful for development novel antibiotics from plant latex. However, before its clinical medicinal and industrial uses its phytochemical analysis is highly needful. Most of these properties are need to be explored. No doubt, plant latex is an industrially important raw material that can be made easily available for production of valued products such as much cheaper antibiotics for common microbial infections. In addition, there is a possibility to generate many more commercialized products by using plant latex especially fires, glues, adhesives, paints, flourings, films ,contraceptives, finger stalls, teats and immunodiagnostic materials. More specifically, use of latex and its products are environmentally much safer and these are easily recyclable or biodegradable in nature.Today proteases have become an integral part of the food and feed industry, and plant latex could be a potential source of novel proteases with unique substrate specificities and biochemical properties. And hence the present study was conducted to detect the protease from fourteen various latex producing plants by dot-blot X -ray film method.

References

Abraham KJ, Joshi PN, 1979. Studies on Proteinases from *Calotropis gigantea* Latex. I. Purification and some properties of two proteinases containing carbohydrate. Biochim Biophys Acta; 568: 111-119.

A.L.cheung, P.ying 1991. A method to detect protease activity using unprocessed x-ray film, anal .Biochem; 193, 2013.

Arima K, Uchikoba T.Yonezawa H.,2000. Cucumisin like protease from the latex of *Euphorbia supine*. Phytochem. 53:639-644.

Arribere, M.C., Cortadi, A.A., Gattuso, M.A., Bettiol, M.P., Priolo, N.S. and Caffini,N.O. 1998. Comparis on of Asclepiadaceae latex proteases and characterization of *Morrenia brachystephana* Griseb. cysteine peptidases. Phytochem. Anal. 9, 267–273

Arnon R. Papain,1970. Methods Enzymol; 19: 226- 244.

Azarkan M., Amrani A., Nijs M., 1997. Carica papaya latex is a rich source of a class II chitinase. Phytochem. 46,1319-1325.

C.D. Dayanand January 2013. Evaluation of comparative total proteolytic activity in plant lattices. Int. J. Life Sci. Bt Pharm. Res. Vol.2: 47-55.

Cohen L.W.,Coghian V.M.,1986.Cloning and sequencing of papain encoding complementary DNA. Gene, 48; 219-228.

Dangles JL, Jones JDG: 2001. Plant pathogen & integrated defense response to infection, Nature, 411; 826-833.

D.E. kliener 1994.Quantitative zymography, detection of pictogram quantities of gelatinases. Anal.biochem.218, 325-329.

Dussourd, D. E., 1993. Foraging with finesse: caterpillar adaptations for circumventing plant defense. In Caterpillars: Ecological and Evolutionary Constraints on Foraging (ed. N. E. Stamp and T. M. Casey), pp.92 -131. London: Chapman & Hall

Dussourd, D.E., and Denno R.F. 1991. Deactivation of plant defense: correspondence between insect behavior & secretory canals architecture. Ecology, 72; 1383-1396.

David E. Dussourd and Robert F. Denno. 1994. Host Range of Generalist Caterpillars: Trenching Permits Feeding on Plants with Secretory Canals. Ecology 75:69–78

Kotaro Konno, and Chikara Hirayama 2004.Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. Plant J. 37 :(3):370-8.

Lauriere, C., 1983.Enzymes and leaf senescence. Physiol Veg; 21: 1159-1177.

Liener, I.E., Friedensen B. Ficin,1970. Methods Enzymol; 19: 261-273.

Lowry O.H,Rosebrough.1951. Protein measurement with the Folin phenol reagent. J.Biol.Chem193:265.

PhanuphongChaiwut and SaroteNitsawang. 2006. A Comparative Study on Properties and Proteolytic Components of Papaya Peel and Latex Proteases.Chiang Mai J. Sci. 2007; 34(1): 109-118.

Farrell B.D., Dussourd D.E. 1991. Escalation of plant defense: do latex and resin canals spur plant diversification. The American Naturalist 138,881-900.

Harborne, J.B., 1993. Introduction to Ecological Biochemistry, 4th edn. London: Academic Press, pp. 186–210.

Jiang, W.B., Lers A, Lomaniec E and Aharoni N, 1999. Senescence related serine protease in parsley. Phytochem.; 50: 377-382.

Kimmel, J.R., and Smith E.L.1954. Crystalline papain .Part I, preparation, specificity and activation. J. Biol. Chem. 207: 515-531

Kramer, D.E. and Whitaker, J.R. 1964. Ficusenzymes II: Properties of the proteolytic enzymes from the latex of Ficus carica variety Kadota. J. Biol. Chem. 239, 2178–2183

O'Riordain, G., Radauer C.2002. Cloning and molecular characterization of the Heveabrasiliensis allergen Hev B11,a class I chitinase. Clin Exp. Allergy. 32(3):455-62.

Pal, G., Sinha NK,1980. Isolation, crystallization, and properties of *calotropins*DI and DII from *Calotropis gigantea*. Arch. Biochem.Biophys; 202: 321-329.

Sgabieri V.C.,Gupte S.M.1964. *Ficus* enzyme. Part 1.Separation of the proteolytic enzyme of *Ficus carica* and *Ficus glabrata* lattices. J Biol Chem. 239, 2170-2177.

Tsuchida, O., and Yamagota, Y. 1986. An alkaline proteinase of an alkalophilic *Bacillus* sp. Current microbial.14:7-12.

Vinod Borde, Vandana Hivrale, Manvendra Kachole. 2012. Detection &purification of mucunapruriens seed protease inhibitors, 49B 10178-10181. Bio/Technolgy ; Elixir Bio Tech. 49B 49B:10178-10181

Ward, O P., 1985. Proteolytic Enzymes. In: Comprehensive Biotechnology, Vol. 3, Moo-Young M, editors., Oxford: Pergamon Press; pp. 789–818.