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Evaluation of Proteolytic Activity of Some Euphorbian Garden Plants

Raghunath T. Mahajan[1*], Javed V. Khan[1], Tanveer A. Khan[2]

[1]Department of Biotechnology, Post Graduate College of Science, Technology and Research, Jalgaon, Maharashtra, India

[2]Department of Botany, H. J. Thim College of Arts and Science, Jalgaon, Maharashtra, India

[*]**Address for Correspondence:** Dr. Raghunath T. Mahajan, Head, Department of Biotechnology, PGCSTR College, Jalgaon, (MS), India

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ABSTRACT- Most of the Euphorbian plants secrete fluid which contain a proteolytic enzyme for defensive role against insects, pests and hence eco physiological inheritance to sustain vegetation eventually in adverse environmental conditions. Evaluation has been carried out on twenty five Euphorbian garden plants for their proteolytic activities using casein as a substrate. Out of these, *Euphorbia nerifolia, Euphorbia milli, Euphorbia tirucalli, Euphorbia lactea, Synadenium granti, Jatropha curcas, Euphorbia nivulia, Euphorbia antiquoram, Pedilanthus tithymaloides, Euphorbia viguieri, E. heterophylla* and *E. leucocephala* are the good enzyme source. Moderate activity found in *Jatropha integerrima, Jatropha multifida, Jatropha podagrica, Euphorbia pulcherrima,* and *Dalechampia scandens*. While different tissues of *Acalypha hispida, Acalypha wilkesiana, Breynia nivosa, Cicca acida, Codiaeum variegatum, Drypetes roxburghii* are devoid of proteolytic activity. This paper describes in detail about name of plants, habitat and presence of proteolytic enzyme in them. Results show that the out of twenty five plants 50% plant tissue synthesize protease in appreciable amount, while 10% are not able to produce it. However 40% plants demonstrate only detectable amount of protease. A comparative account of proteolytic activity reveals some promising plants good source of enzyme. Some plants produce combination of cysteine and serine proteases. A single plant i.e. *Euphorbia nerifolia* latex contains cysteine, serine, metallo-protease and aspartic proteases. In turn, these proteases may be used in various industrial uses in general and cheese production in a particular.

Key-words- Garden Euphorbian plants, Cysteine and serine protease, *E. leucocephala, Euphorbia viguieri*

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INTRODUCTION

Botanical plants are considered as god's gift to human being in the form of natural medicine. Euphorbiaceae comprises more than 2000 species. Some of Euphorbian plants are cultivated as ornamental/garden plants in national and international gardens. Due to rich cultural heritage and relatively rich flora, a wealth of knowledge on traditional and folk medicine has been accumulated in India [1]. An exhaustive and a comprehensive review on proteolytic enzyme of biological sources appeared in literature which includes study on properties of various proteases with mechanism of action of proteolysis of protein [2].

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The used parts of Euphorbian plant species include latex, roots, seeds, stem bark, wood, leaves and whole plant [3-5]. The plants in the family Euphorbiaceae are known for chemical diversity of secondary metabolites and have various curative properties against different ailments [6]. Most of member of this family synthesis proteases in different tissues for defensive purpose [7-9]. A good source is latex and juices. Lynn described occurrence, properties of different proteases of Euphorbiaceae family [7]. An excellent article is appeared in literature stating importance of a chemotaxonomic marker of Euphorbia species pertaining presence of proteolytic activity in the latex of Euphorbian genera [9]. This aspect is confirmed recently in next year in the form of review of Euphorbiaceae family and its medicinal features [10]. Further such study is extended for the production of plant proteases *in vivo* and *in vitro* [11]. A scientific article on research into Euphorbia latex and various ingredients is published [12]. Very recently, article entitled a study on plant latex, a rich source of proteases and cutting edge for disease invasion is appeared in literature mentioning that, out of the 35 latex

bearing plants, 16 plants possess proteolytic activity belongs to family of Euphorbiaceae [13]. Very very recently, a very good article entails medicinal importance and biochemistry of latex of certain Euphorbian taxa [8]. In our laboratory, we mentioned 13 industrial bio-applications of proteases of some Euphorbian wild and weed plants [14]. In this communication, we report here studies on proteases in garden Euphorbian plants aiming to search a vegetable rennin source for production of cheese and some allied industrial applications.

MATERIALS AND METHODS

Plants are procured from campus of Moolji Jaitha College, Jalgaon and plant nurseries of Jalgaon city, Maharashtra, India. Dr. Tanveer taxonomist, identified plants for study. Different parts of plant such as leaf, stem, root, flower, and latex of the garden Euphorbian plants was collected from during June 2014 to December 2015. The cuttings of leaf stalks with capillary tubes into glass container and was kept in ice. The latex was a white thick fluid with pungent odour brought to laboratory and kept in refrigeration till use. Experiments were conducted at department of Biotechnology, PGCSTR, Jalgaon, India. A photo plate of some promising Euphorbian garden plants is given in Fig. 1.

Fig. 1: Photo plates of 10 Euphorbian garden plants

Enzyme Isolation

The freshly collected latex was diluted with 5 volumes of ice cold phosphate buffer pH 7.4 and centrifuged at 10,000 rpm for 20 minutes in high speed refrigerated centrifuge and supernatant was collected and stored at 4^0C. The pellet containing the white insoluble gum was discarded. All the experiments on the crude preparation were carried out using freshly collected latex and preserved in refrigerator at 4^0C. From other parts 10% homogenate was prepared in phosphate buffer at pH 7.4 and centrifuged and supernatant was used as a source of enzyme.

Screening and Selection of Garden Euphorbian Plant Proteases

Protease Activity

Proteolytic activity of different plant tissues was determined by the colorimetric assay using 1% casein as a substrate as described by [15]. The protease activity was expressed as amount of enzyme required to produce peptide equivalent to µg of tyrosine/min/mg protein at 37^0C and protein content was determined according to Lowry's method [16] using Bovine serum albumin as the standard protein.

Enzyme Unit

One unit of protease activity is defined as the amount of enzyme to release 1 µg of tyrosine per minute at 37^0 C. A tyrosine standard curve was calibrated (10 to 100 µg/ml) using Folin Phenol reagent. Specific activity of the proteolytic enzyme is expressed as the number of units per milligram of protein.

RESULTS AND DISCUSSION

Proteases are distributed widely in different biological sources namely plants, animals and microbial sources. In Euphorbian plants protease are present in virtually every part i.e. stem, fruit, flower, leaf, root, gum and latex. We have communicated presence of proteolytic activity in various parts of plant indicated, plant latex is the richest source of protease [14,17]. Table 1 summarizes habitat of some Euphorbian plants. They are grouped into three category i. Wild, ii. Weed, and iii. Garden. The distribution of wild, weed and garden is 47%, 22%, and 31% respectively. The order of occurrence of protease in garden Euphorbian plant is serine< cysteine< serine and cysteine < metallo protease<aspartic protease (Fig. 2). This finding is good in agreement and supports our findings as results reported by number of authors [9,10, 18,19] pertaining to the occurrence and distribution of proteolytic enzymes.

Table 1: List of Some Euphorbian Plants

Wild (A)	Weed (B)	Garden (C)
Acalypha ciliate	*Acalypha malabarica*	*Acalypha hispida*
Acalypha indica	*Chrozophora prostrate*	*Acalypha wilkesiana*
Baliospermum raziana	*Chrozophora rottleri*	*Breynia nivosa*
Bridelia airy-shawii	*Euphorbia hirta*	*Cicca acida*
Cleidion spiciflorum	*Euphorbia indica*	*Codiaeum variegatum*
Croton bonplandianum	*Euphorbia notoptera*	*Drypetes roxburghii*
Emblica officinalis	*Euphorbia prostrate*	*Euphorbia milii*
Euphorbia clarkeana	*Euphorbia prunifolia*	*Euphorbia pulcherrima*
Euphorbia cristata	*Euphorbia thymifolia*	*Euphorbia tirucalli*
Euphorbia fusiformis	*Phyllanthus airy-shawii*	*Jatropha integerrima*
Euphorbia nerifolia	*Phyllanthus amarus*	*Jatropha multifida*
Euphorbia nivulia	*P. maderaspatensis*	*Jatropha podagrica*
Euphorbia pycnostegia		*Pedilanthus tithymaloides*
Homonoia riparia		*Synadenium granti*
Jatropha curcas		*Dalechampia scandens*
Jatropha gossypifolia		*Euphorbia viguieri*
Kirganelia reticulate		*Jatropha podogrica*
Mallotus philippensis		*Euphorbia nerifolia*
Manihot esculenta		*Euphorbia nivulia*
Micrococca mercurialis		*Euphorbia* species 1
Phyllanthus urinaria		*Euphorbia* species 2
Ricinus communis		
Securinega leucopyrus		
Securinega virosa		
Tragia plukenetii		

The life form of above plants ranging from small herbs, herbs, shrubs, small tree and tree. Some of them are seasonal and perennial.

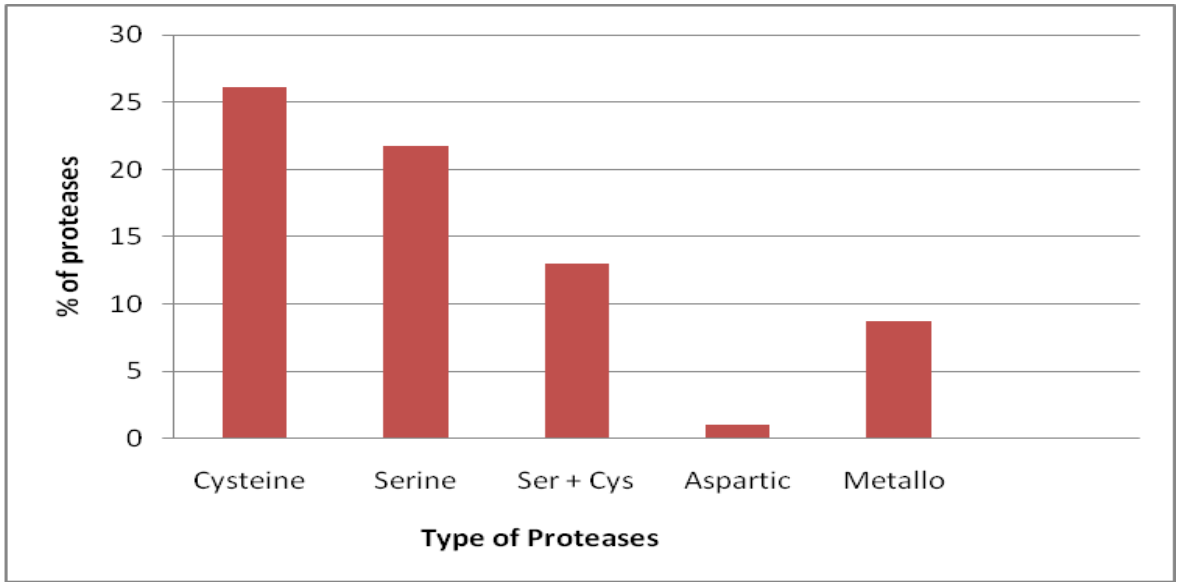

Fig. 2 Occurrence of protease of Euphorbian garden plants

Table 2: Studies on Proteases from Garden Euphorbian Plants

Plant Name	Type of Protease
Acalypha hispida	NR
Acalypha wilkesiana	NR
Breynia nivosa	NR
Cicca acida	NR
Codiaeum variegatum	NR
Drypetes roxburghii	NR
Euphorbia milii	Serine & Cysteine
Euphorbia pulcherrima	Serine & Cysteine
Euphorbia tirucalli	Serine
Jatropha integerrima	NR
Jatropha multifida	NR
Jatropha podagrica	NR
Pedilanthus tithymaloides	Cysteine
Synadenium granti	Serine
Dalechampia scandens	NR
Euphorbia lactea	Serine
Euphorbia antiqourum	Cysteine
Euphorbia heterophyll	Cysteine
Euphorbia nerifolia	Serine,cysteine, metallo, aspartic
Euphorbia tirucalli	Serine, cysteine
Euphorbia nivulia	Cysteine
Euphorbia prunifolia	Serine
Jatropha curcas	Cysteine
E. leucocephala	Serine & Cysteine
E. viguieri	Serine

NR- *Not reported*

Table 2 indicates 8.6% of plant tissue are able to synthesis both Cysteine and serine proteases, whereas 47.8% of them produce either Cysteine or serine proteases. Whereas 43.4% plant tissues are free of any detectable enzyme.

Table 3: Evaluation of Protease Activity of Some Garden Euphorbian Plants

Plant Name	Proteolytic Activity
Acalypha hispida	-
Acalypha wilkesiana	+
Breynia nivosa	+
Cicca acida	-

Codiaeum variegatum	+
Drypetes roxburghii	-
Euphorbia milii	+++
Euphorbia pulcherrima	++
Euphorbia tirucalli	+++
Jatropha integerrima	+
Jatropha multifida	+
Jatropha podagrica	-
Pedilanthus tithymaloides	+++
Synadenium granti	+++
Dalechampia scandens	-
Euphorbia lactea	-
Euphorbia antiqourum	-
Euphorbia heterophyll	-
Euphorbia nerifolia	+++
Euphorbia tirucalli	+++
Euphorbia nivulia	+++
Euphorbia prunifolia	+++
Jatropha podogrica	+
Euphorbia species 1	++
Euphorbia species 2	++

+ : Less activity , ++: Moderate activity, +++ : Highest activity, - : No activity

Out of the 25 garden plants 32% show highest proteolytic activity and 40% plants have no proteolytic activity, while contribution of moderate and less activity plant is same i. e. 12% (Table 3). Our observations are good in agreement with comparative total proteolytic activity in plant lattices [14,17-19]. In contrast to this presence of serine in each laticiferrous plant is reported [9]. Surprisingly, while collection of plants we noted occurrence of some weed garden plants such as *E. hirta, E. indica, Phyllanthus amarus* and *E. heterophylla*. Among them *E. hirta* and *E. heterophylla* are good source of enzyme [17]. A seen from Table 4, some very common plants though appeared in literature as reported by earlier investigators for their proteolytic activity; we have taken them for validation of our experiments and comparison.

Table 4: Caesinolytic Activity of Some Promising Garden Euphorbian Plants

Name of Plant	**Proteolytic activity (U/gram tissue)**
Euphorbia milii	17.76 ±5.24
Euphorbia tirucalli	26.56±2.78
Euphorbia lactea	22.56±3.25
Euphorbia nivulia	15.87±5.35
Synadenium granti	20.48±1.85
Euphorbia nerifolia	30.15±2.05
Euphorbia viguieri	10.58±4.85
Jatropha curcas	8.48±3.15
Pedilanthus tithymaloides	48.89±4.68
E. leucocephala	16.18±4.67

±: SD of 6 observations

In our previous communication, we have reported various aspects of our study which has been carried out in our laboratory based on economic importance of Euphorbian plants. Here we focused ethanomedicinal importance of laticiferrous plants used by tribal people of North Maharashtra, India to treat various diseases [1,3,6]. Also we extended our study on phytochemical investigation of some laticiferous plants belonging to khandesh region of Maharashtra, India. [6] Latex is a rich in secondary metabolites like sterols, glycosides, alkaloids, and enzymes

specifically proteases, amino oxidases, esterases and lipases. A report on proteolytic enzyme of some laticiferrous plants belonging to khandesh region of Maharashtra, India which include plants from other families like Moraceae, Asclepidaceae and Apocynaceae and Caricaceae is published [3]. Economical importances of forty ethnobotanical Euphorbian plants of North Maharashtra region include their applications in various diseases along with some industrial uses [4,5]. In this article, emphasis is given for the most promising Euphorbian garden plants to evaluate potential of them. The morphological features are shown in Fig. 1.

Richest source of proteolytic enzyme is latex, followed by seed, leaf, stem, root, fruit, and flower [14,17]. Out of above twenty five Euphorbian garden plants, *Euphorbia nerifolia* occupy the first rank as it possesses combination of four proteases namely serine, cysteine, metalloprotease and aspartic proteases, four plants namely *Euphorbia milli*, *Euphorbia tirucalli*, *E. leucocephala*, *Euphorbia pulcherrima* do have serine and Cysteine proteases. A single protease is observed in rest of the plant. It is interesting to note, no threonine protease is recorded in any Euphorbian plant. Presence of proteases of latex along with secondary metabolites like diterpene, and alkaloids exhibit defensive properties against the pest. Additionally latex possesses the medicinal as well as agriculture applications [9,10,14,17]. We would like to put here worthiness of Euphorbian plants for their medical importance and enzymes of these plants as biomarkers. Such statements are hold true for earlier findings of different investigators [9,10,12].

CONCLUSIONS

In a nutshell, on evaluating proteolytic activity of 25 Euphorbian garden plants, 10 plants are found promising activity, out of them 2 plants namely *E. leucocephala*, *E. viguieri* are not yet explore for such finding. Three plants namely *E. pulcherrima*, *E. species* 1 and *E. species* 2 have moderate activity, followed by six plants exhibit less activity, whereas remaining plants are devoid of any activity**.** The presence of proteolytic activity of the latex of *E. viguieri* and *E. leucocephala* motivated to us to analyze biochemical characterization of enzymes with their possible bioapplications of commercial use.

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