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# Protease Inhibitor Activity of Selected *Calatropis* Species

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**Abstract:** *Protease inhibitors are widely distributed in all plant groups. There are several reports on application of protease inhibitors in medicines as well as in agriculture fields; The present study aimed to screen the plants for its protease inhibitor activities (PIs) by using the dot blot assay technique. The selected plants were screened for protease (Trypsin) inhibitor activities viz. Calotropisprocera (Aiton) W. T. Aiton and Calotropisgigantea (L.) W. T. Aiton. The plant samples (leaf, stems flower)shows potent protease inhibitor activity For screening of PI's activity. Trypsin and inhibitors (Plant sample) are loaded on gelatin coated X-ray films with respective concentration [3:1], [1:1], and [1:3] Appearance of The faint spot (gelatin) indicates total inhibition of enzymes, while dark blue spot (gelatin hydrolysis) indicates no inhibition. In present experiment C. procera (Aiton) W. T. Aiton leaf extract and C. gigantea (L.) W. T. Aiton leaf as well as stem extract shows the Protease Inhibitor activity.*

**Keywords:** *Calatropis*; Dot Blot assay; Protease inhibitor activity

## I. INTRODUCTION

Plant it is autotrophic organism which contains Proteins, lipid, carbohydrate, vitamin, and minerals also. Presence of protease inhibitor activity are recorded in plants(H. Azzouz*et al*. 2005) animals and microorganisms(C. Shee& A. K. Sharma 2007)and algae (R. A. Bagwan *et al.* 2021).Protease inhibitors are well known as one of the prime candidates to have numerous applications in biotechnology and medicine and Agricultural sector.

The one best counter action to control of *H. armigera*infestation in *Cicerarietinum*(L.) fields is the use of chemical insecticide and pesticides. But due to the high costs of insecticides, pesticides, their risk andhazardous for the balance of environment and human health, proteinase inhibitor becomes a defense alternative by creating an insect-resistant plant(Nair *et al.*2013).

## II. MATERIALS AND METHODS

### 2.1 Materials and Chemicals

Gelatin coated X-ray films were purchased from Agfa (CP-BU New). Electrophoresis systems and Molecularweight markers were obtained from Genei (Bangalore) India. Trypsin and Bovine serum albumin (BSA) wereobtained from HiMedia. Solvent viz. hexane and acetone from Qualigens, India.

The plant sampleswere collected from the Government Institute of Science Aurangabad and were authenticated by using standard literature Flora of Maharashtra Naik(1998)

**Plant Material**

| Sr. No. | Botanical Name | Family |
|---|---|---|
| 1 | *Calotropisprocera* (Aiton)W.T. Aiton | Apocynaceae |
| 2 | *Calotropisgigantea*(L.) W.T.Aiton. | Apocynaceae |

**Table 1:** List of Plant materials used for screening

### 2.2 Removal of fats and pigments from sample

1 gram of the powdered sample were de-pigmented by repeatedly washing with ice chilled acetone (1:6 w/v) forfour to five times and further defatted with n-hexane for two times and it was allow to air dry.The air dried material wasused for extraction of PIs.

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### 2.3 Extraction of PIs

Extraction of protein from defatted and de-pigmented plants samples was performed in 0.1M phosphate buffer (pH7.0) containing 1% Poly Venyl Pyruvate. 0.1 gram of each sample was dissolved in 10 ml extraction buffer at room temperature(28°C ±2°C) on shaker for 12 hours. Later the mixtures were centrifuged at 8000 rpm for 20 min at 40 C. Theclear supernatant pipette out and this crude extract was used for Screening of protease inhibitor activity, quantification total protein contents.

### 2.4 Screening of PIs

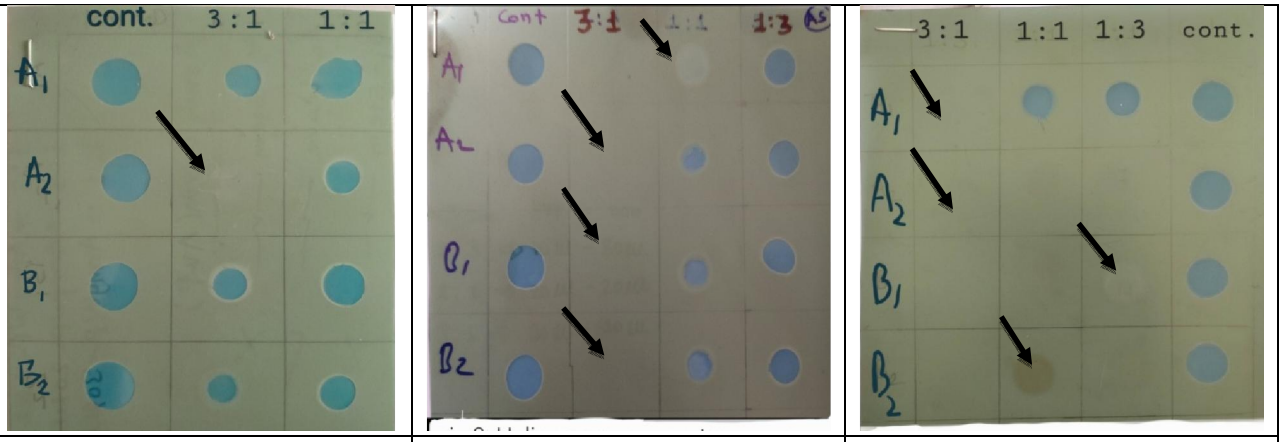
The Screening of Protease inhibitor activity was performed as follow:10ml of Trypsin was prepared in 100mM Tris-HCL (pH 7.8) buffer with final concentration 0.1 mg/ml. Three different concentrations of the enzyme and crude extracts cocktail was prepared in ratio 3:1, 1:1, and 1:3 (v/v).The total volumes of the cocktails were adjusted to 30µl with Tris-HCL buffer out of this 20µl each samples werespotted on undeveloped X ray film with spots of trypsin with buffer as a control. The reaction mixture were incubated on X-ray film for 20 min at 37°C and then the X ray film was washed under running tap water. Change in the color of X ray film at the point of spot of mixture was visually observed to note the activity of PIs.

## III. RESULTS

| **Sample code** | **AWP** | | | **HWP** | | | **Trypsin** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Cont.** | **1:3** | **1:1** | **3:1** | **1:3** | **1:1** | **3:1** | **1:3** | **1:1** | **3:1** |
| **A1***(C.procera* leave) | - | - | + | - | + | + | - | - | + |
| **A2***(C.procera stem)* | - | - | - | - | - | + | + | + | + |
| **B1***(C.gigantea* leave) | - | - | - | - | - | + | + | + | + |
| **B2***(C.gigantea stem)* | - | - | - | - | - | + | + | + | + |

**AWP: Army warm protease, HWP: Helicoverpa warm protease.**

**Table 2:** Screening of PIs from extracts by spot test




| **AWP** | **HWP** | **Trypsin** |
|---|---|---|

**Photo no.** Detection of protease inhibitor activity of AWP, HWP and trypsin by the spot test. In lane 1 only Protease loaded ascontrol while three different concentrations of Protease and inhibitors are loaded in lane withrespective concentration [3:1], [1:1], and [1:3] for the screening of PIs. The faint spot (gelatin) indicates totalinhibition of enzymes, while dark blue spot (gelatin hydrolysis) indicates no inhibition.

## IV. CONCLUSION

The present study deals with primary screening of protease inhibitor activity in the commonly growing Calotropis species. Interestingly to record that Trypsin Inhibitor activity is found to be positive in all four samples (leave & stem) studied except the 1:3 & 1:1 concentration for C. procera leave. While in HWP minimum inhibition was recorded in

C.procera leave only. Similarly minimum activity was also recorded in AWP at very low concentration of protease. This study will help in developing insect resistant varieties in near future with the help of biotechnological tools.

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