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Screening of Plant Extracts to Identify Extracts Containing Inhibitors Against Larval Gut Proteases of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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Abstract **– Plant protease inhibitors (PPIs) are plant defensive proteins and often get induced in response to injury or attack by insects or pathogens and are being explored as pest control agents. In the present study plant extracts were screened to identify extracts containing protease inhibitors against the gut proteases of *S. litura*. Protease inhibition was done using azocasein as substrate at pH 9.0. Out of the 50 plant extracts screened, 13 plant extracts showed greater than 40% inhibition. The highest inhibition (88.60±0.50%) was given by *Ardisia solanaceae*. Six of the plant extracts contain inhibitors which are proteinaceous in nature and are of potential use in pest control.**

Keywords **– Plant Protease Inhibitors, *Spodoptera litura*, Gut Proteases, *Ardisia solanaceae.***

I. INTRODUCTION

Several insect pests invade many agricultural crops resulting in loss of yield [1, 2]. Plants have evolved certain defensive proteins which confer resistance against the attack of phytophagous insects, nematodes and bacterial fungal and viral infections. The plant defensive proteins mainly fall under four categories, inhibitors of proteolytic enzymes, lectins, ribosome-inactivating proteins and glycohydrolases [3]. Protease inhibitors (PIs) inhibits the proteolytic enzymes. The plant protease inhibitors (PPIs) are generally small proteins, which regulate significant physiological processes, and are also induced upon attack by insects or pathogens [4, 5, 6]. Protease inhibitors are grouped primarily as serine, cysteine, aspartic or metallo protease inhibitors [7, 8] based on the class of protease they inhibit. Protease inhibitors retard the larval growth and development by inhibiting the larval gut proteinase activity which results in impairment in the absorption of amino acids [9]. Most of the insect pests fall under order lepidoptera which have alkaline midgut fluid with pH 9-11. Trypsin and chymotrypsin are the major serine proteinases in the midgut fluid of the lepidopteran [10, 11]. Serine protease inhibitors have anti-nutritional effects against several lepidopteran insect pests [12, 13]. Plant protease inhibitors exhibit different binding affinity towards different proteinases in the insect midgut. It is essential to screen and identify potent PPIs against gut proteases of insect pests for pest management. In the present study we screened 50 plant extracts to identify extracts that inhibit the larval gut proteases of *Spodoptera litura.*

II. MATERIALS AND METHODS

Azocaesin was from Sigma Aldrich (USA) and Proteinase K from Qiagen. Other reagents used in this study were of analytical grade.

Collection of Plants and Plant Extract Preparation

Plant materials for the study were collected from Palakkad district and Malappuram district of Kerala, India. Seeds / other plant parts were washed in clean water, wiped thoroughly to remove water and were soaked in bic-

-arbonate buffer, pH 9.0 (1 ml/g of tissue) overnight and homogenized. The homogenates were centrifuged at 10,000g at 4°C for 10 minutes. The supernatant containing soluble proteins recovered and stored at -20°C until use.

Rearing of Spodoptera litura Larvae

The pupae of *S. litura* were purchased from NBAIR Live Insect Repository (National Accession No: NBAII-MP-NOC-02), Bangalore, India. The emerged adult moths were kept in glass beakers covered with muslin cloth and fed with diluted honey (30%). The moths were allowed to mate and lay eggs. Larvae hatch out within4-5 days and were reared in glass beakers with tender leaves of *Ricinus communis* initially and later transferred to plastic troughs with somewhat mature leaves as they grew in size. The culture was maintained at 25±2°C, relative humidity (RH, 75 ± 3%).The total larval period was found to range from 19-21 days and consisted of 6 larval instars.

Preparation of larval gut extract of Spodoptera litura

Actively feeding fifth-instar larvae of *S. litura* were chilled on ice and killed by decapitation to collect the midgut and stored it at -20°C until use. The gut was homogenized in sodium bicarbonate buffer, pH 9.0 (1ml/g of tissue).The homogenates were centrifuged at 10,000 g for 10 minutes at 4°C. The soluble protein recovered from the supernatant was stored as aliquots at -20°C until use.

Protease Assay

Crude extracts were assayed for total protease activity by estimating hydrolytic activity towards the synthetic substrate azocasein. The total proteinase activity was assessed by incubating 5µl of crude gut extract with azocasein (11.48µg/µl) as substrate in 100 mM bicarbonate buffer, pH 9.0 at 37°C for 30 minutes in a total volume of 20.2µl. After incubation, the reaction was stopped by adding 80 µl of 5% Trichloro acetic acid (TCA). Then it was centrifuged at 10,000g for 10 minutes and 50 µl of the supernatant from each tube was mixed with 150 µl of 50mM NaOH. The dye released was measured at 440nm in a Microplate reader. All assays were done in triplicate.

The Protease Inhibition Assay

Protease inhibition assay was done by pre-incubating 5µl of gut extract with 10µl of plant extract (inhibitor) for 10 minutes and then proceeded with protease assay as described earlier.

Determination of Protein Concentration

Protein concentration of the plant extracts were determined by Lowry's method [14] using bovine serum albumin as standard.

Heat Inactivation of the Inhibitor

The heat stability of the inhibitor was checked by incubating 100µl the plant extract in boiling water bath for 5 minutes and after cooling, the protease inhibition assay was done with using the larval gut extract of fifth instar larvae of *Spodoptera litura*.

Proteinase K Treatment

The proteinaceous nature of the inhibitor was tested by measuring the percentage inhibition of the extract after Proteinase K treatment of the extract. For this 90µl of plant extract was incubated with 2.3µg of Proteinase K at 56°C overnight followed by heat inactivation of the Proteinase K enzyme by heating the mixture at 96°C for 5 minutes. Appropriate controls were also maintained.

Calculation of percentage inhibition

For calculating the percentage inhibition the absorbance of the control was taken as 100% enzyme activity. Absorbance of the control (azocasein alone) was subtracted from the absorbance of plant extract (inhibitor) alone and the value thus obtained represents the protease activity present in the plant extract. This value was subtracted from the absorbance of the test (in presence of the inhibitor) to get the actual absorbance in the absence of any protease activity from the plant extract. The absorbance thus obtained is converted into percentage enzyme activity taking the absorbance of control as 100% activity. This value was subtracted from 100 to get the percentage inhibition.

Statistical Analysis

Statistical analysis was done using R program.

III. RESULTS AND DISCUSSION

Table 1. List of Plants Screened for the Protease Inhibition against the Gut Proteases of *Spodoptera litura* 5th Instar Larvae.

Sl. No	Plant species	Plant part used	Inhibition % (Mean ± SD)
1	*Ardisia solanaceae*	Seed	88.60 ± 0.50
2	*Eleusine coracana*	Seed	76.50 ± 0.61
3	*Accacia concina*	Seed	73.84 ± 0.57
4	*Coccinia grandis*	Seed	69.91 ± 1.06
5	*Murraya koenigii*	Seed	70.80 ± 0.73
6	*Abelmoschus manihot*	Seed	69.53 ± 0.60
7	*Mimusops elengi*	Leaf	57.95 ± 1.23
8	*Piper nigrum*	Seed	60.42 ± 1.24
9	*Mucuna pruriens*	Seed	60.00 ± 0.84
10	*Celosia cristata*	Seed	48.50 ± 4.82
11	*Amaranthus dubius*	Seed	48.88 ± 0.311
12	*Prunus cerasus*	Seed	47.46 ± 1.14
13	*Linum usilatissimum*	Seed	46.35 ± 1.54
14	*Nyctanthes arbor-tristis*	Seed	47.23 ± 1.68
15	*Ipomea tricolor*	Seed	37.34 ± 4.70
16	*Canavalia maritime*	Seed	31.71 ± 1.42
17	*Lawsonia inermis*	Seed	31.08 ± 1.07
18	*Anogeissus latifolia*	Leaf	30.78 ± 1.55

19	*Malviscus arboreus*	Flower	30.71 ± 0.35
20	*Citrullus lanatus*	Seed	29.81 ± 0.92
21	*Tribulus terestrius*	Seed	29.21 ± 0.30
22	*Mangifera indica*	Seed	28.60 ± 0.32
23	*Cardeospermum halicacabum*	Seed	28.08 ± 0.09
24	*Lantana camera*	Flower	27.90 ± 0.25
25	*Oscimum sanctum*	Leaf	26.24 ± 0.13
26	*Heliconia caribaea*	Leaf	25.47 ± 0.26
27	*Fittonia verschaffeltii*	Leaf	23.25 ± 1.13
28	*Immpatiens flaccid*	Seed	22.87 ± 0.53
29	*Biophytum sensitivum*	Leaf	22.17 ± 0.22
30	*Swietenia mahagoni*	Seed	19.56 ± 0.12
31	*Callistemon lanceolatus*	Leaf	19.27 ± 0.27
32	*Musca sp.*	Seed	19.22 ± 0.18
33	*Curcuma longa*	Seed	18.91 ± 0.56
34	*Passiflora pomifera*	Seed	18.40 ± 0.16
35	*Spathiphyllum cupido*	Leaf	18.03 ± 0.05
36	*Artemisia Vulgaris*	leaf	17.09 ± 0.38
37	*Bougainvillea glabra*	Leaf	17.01 ± 0.14
38	*Clitoria ternatea*	Seed	15.62 ± 0.33
39	*Mussaenda erytrophylla*	Leaf	15.14 ± 0.31
40	*Bacopa monnieri*	Leaf	13.70 ± 0.46
41	*Monsteradeliciosa minima*	Leaf	13.46 ± 0.36
42	*Trichosanthus dioica*	Seed	12.50 ± 0.26
43	*Cynodon dactylon*	Leaf	12.26 ± 0.27
44	*Punica granatum*	Seed	10.68 ± 0.58
45	*Clerodendrum infortunatum*	Leaf	10.14 ± 0.23
46	*Musca sp.*	Leaf	7.63 ± 0.64
47	*Nerium oleander*	Leaf	6.16 ± 0.13
48	*Zanthoxylum rhesta*	Seed	4.83 ± 0.50
49	*Rosa indica*	Leaf	2.89 ± 0.27
50	*Jasminum auriculatum*	Leaf	2.31±0.21

Table 2. Proteinase K Treatment.

Sl. No	Plant species	Control	Test (Mean ± SD)
1	*Ardisia solanaceae*	88.60 ± 0.50	17.63 ± 1.22
2	*Accacia concina*	73.84 ± 0.57	13.60 ± 1.40
3	*Abelmoschus manihot*	69.53 ± 0.60	11.13 ± 2.48
4	*Mimusops elengi*	59.18 ± 1.23	15.44 ± 1.47
5	*Piper nigrum*	57.95 ± 1.23	19.05 ± 1.70
6	*Amaranthus dubius*	48.88 ± 0.311	18.63 ± 1.92
7	*Prunus cerasus*	47.46 ± 1.14	40.36 ± 2.47

Plant protease inhibitors are extensively exploited class of plant defensive molecules for developing insect resistant plant [15]. Lepidopteran insect pests depend mainly on serine proteinases, particularly trypsin and chymotrypsin- like enzymes, for the digestion of food proteins [16]. Developing pest- resistant crops with potent PPIs constitutes a strategy for increasing plant defense against lepidopteran pests. Non host PPIs are found to be potentially more effective plant defense agents [17] than the host PPIs, which due to continuous exposure to the pest increase the chance of resistance development in the pest [18, 19, 20]. Thus identification of potent PPIs is essential for pest management. In the present study we screened 50 plant extracts (Table 1) to check their inhibitory activity against the larval gut proteases of 5th instar larvae of *Spodoptera litura.* Out of the 50 plant extracts tested, 13 plants (*Ardisia solanaceae, Eleusine coracana, Accacia concina, Abelmoschus manihot, Mimusops elengi, Piper nigrum, Mucuna pruriens*, *Celosia cristata, Phyllanthus amarus, Amaranthus dubius , Prunus cerasus, Linum usilatissimum* and *Nyctanthes arbor-tristi)* inhibited the gut protease activity of *Spodoptera litura* larvae greater than 40%. To the best of our knowledge there is no report of protease inhibitors from *Ardisia solanaceae, Accacia concina, Abelmoschus manihot, Mimusops eleng, Piper nigrum, Amaranthus dubius*, *Prunus cerasus*.

The highest inhibition is given by the seed extract of *Ardisia solanaceae* (88.60 ± 0.50 %). It has been reported to have antioxidant, thrombolytic and cytotoxic activities. But the active compounds responsible for these activities are yet to be discovered [21]. The seed extract of *Accacia concina* inhibited the larval gut protease activity to an extent of 73.84 ± 0.57%. The pods of this plant are used traditionally for many skin diseases, cough, as a laxative and for dandruff treatment. The larval gut protease activity of *S. litura* was inhibited by the seed extracts from *Abelmoschus manihot* up to 69.53±0.60%. The seeds of *A.manihot* is used to treat pain, inflammation, urinary infection and chronic bronchitis .Seed extract from *Mimusops elengi* was found to inhibit the larvla gut protease activity of *S. litura* to an extent of 57.95 ± 1.23%. Bark of this plant is used to treat gum and teeth diseases [22], the flowers cures liver disorders, headache and smoked flower is good for treating asthma [23] and the seeds cure nasal congestion and headache [24]. *Piper nigrum* seed extracts inhibited the gut protease activity of *S. litura* larvae to an extent of 57.95 ± 1.23%. It is traditionally used as a medicine for cough and cold. Piperine the bioactive compound in pepper is reported to have immune-modulatory, anti-carcinogenic, anti-inflammatory and hepatoprotective effect [25]. The seed extracts of *Amaranthus dubius* was found to inhibit the larval gut protease of *S. litura* up to 48.88 ± 0.311%. The leaves of this plant is widely used as food. The larval gut extract of *S. litura* was inhibited by the seed extract of *Prunus cerasus* to an extent

of 47.46 ± 1.14%. The seeds of this plant are known its immune modulatory, antioxidant, anti-diabetic and anti-inflammatory activities and also it is reported to enhance sleep [26].

Presence of protease inhibitors were reported from the plant extracts of *Eleusine coracana* [27], *Coccinia grandis* [28], *Mucuna pruriens* [29], *Celosia cristata* and *Linum usilatissimum* [30, 31]. Though the protease inhibitors were reported from these plants, there are no reports of protease inhibition against insect gut enzymes from these plants.

The inhibitors present in *Ardisia* solanaceae, *Accacia concina*, *Abelmoschus manihot*, *Mimusops elengi*, *Piper nigrum* and *Amaranthus dubius* are proteinaceous in nature as there is a significant reduction in inhibition on proteinase K treatment (Table 2). In the case of *Prunus cerasus* the inhibition percentage dropped down only from 47.46±1.14 to 40.36±2.47 on proteinase K treatment which indicates the non-protein nature of the inhibitor or a protein protease inhibitor insensitive to proteinase K digestion. Thus from the above findings it is clear that the protease inhibitors from the plant extracts of *Ardisia* solanaceae, *Accacia concina*, *Abelmoschus manihot*, *Mimusops elengi*, *Piper nigrum* and *Amaranthus dubius* are proteins and the major inhibitor from *Prunus cerasus* is of non-protein in nature. Majority of the protease inhibitors reported from plants are proteinaceous in nature including many well characterized protease inhibitors such as Soybean trypsin inhibitor (SBTi) and Winged bean trypsin inhibitor (WBTi) [32]**.** Further isolating and characterizing these plant protease inhibitors will be useful to formulate better pest control strategies including expressing the gene coding for the protease inhibition in the host plants. The first successful transfer of protease inhibitor gene of plant origin was that from cowpea, encoding a double-headed trypsin inhibitor (CpTI) into tobacco plants) followed by the successful trials of this transgenic crop against *Manduca sexta* [33]. Furthermore the transgenic rice with CpTI genes conferred enhanced resistance level against rice stem borers [34].

IV. Conclusions

Out of the 50 plants screened to identify plant protease inhibitors against the gut proteases of *S. litura*, 13 plant extracts showed greater than 40% inhibition. The highest inhibition was given by *Ardisia solanaceae* (88.60 ± 0.50). Out of which 6 plant extracts were found to contain proteinaceous protein inhibitor. Further isolation and characterization of these plant protease inhibitors will be useful for formulating better pest control strategies.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Pawar C.S. (1998). Wasps- predators of Heliothis on pigeon pea. *Int. Pigeon pea Newsletter* 2: 65- 66.
[2] Rajapakse C.N.K. and Walter G.H. (2007). Polyphagy and primary host plants: oviposition preference versus larval performance in the lepidopteran pest Helicoverpa armigera. *Arthropod–Plant Int*. 1: 17–26.

[3] Carlini, R, and Maria Fa. 2002. "Plant Toxic Proteins with Insecticidal Properties. A Review on their Potentialities as Bio-insecticides." *Toxicon* 40 1515–1539.
[4] Xavier-Filho J. (1992). The Biological Roles of Serine and Cysteine Proteinase Inhibitors in Plants. *Rev. Bras. Fisiol. Veg* 4: 1-6.
[5] Schaller A. and Ryan C.A. (1995). System in-a polypeptide defense signal in plants. *Bio Essays* 18: 27–33.
[6] Shewry P.R. (2003). Tuber storage proteins. *Ann. Bot* 91: 755- 769.
[7] Richardson M. (1991). Seed storage proteins: the enzyme inhibitors. *Methods in Plant Biochemistry* 5: 259–305.
[8] Birk Y. (1994). Protein proteinase inhibitors in legume seeds-overview. *Arch. Latinoam. Nutricion*. 44: 26S–30S.
[9] Zhu-Salzman Keyan and Zeng Rensen. (2015). Insect response to plant defensive protease inhibitors. Annu Rev Entamol 60: 13.1-13.20.
[10] Milne R., Kaplan H. (1993). Purification and characterization of a trypsin like digestive enzyme from spruce budworm (*Choristoneura fumiferana*) responsible for the activation of δ-endotoxin from *Bacillus thuringiensis*. *Insect Biochem. Mol. Biol.* 23: 663–673.
[11] Srinivasan A., Giri A.P., Gupta V.S. (2006). Structural and functional diversities in lepidopteran serine proteases. *Cell. Mol. Biol. Lett.* 11: 132–154.
[12] Shulke R.H., Murdock L.L. (1983). Lipoxygenase trypsin inhibitor and lectin from soybeans: Effects on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae). *Environmental Entomology* 12: 787-791.
[13] Broadway R.M. and Duffey S.S. (1986). "Plant protease inhibitors: mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. *Journal of Insect Physiology* 32: 827-833.
[14] Lowry O H, Rosebrough N J, Farr A L, Randall R J. (1951). Protein measurement with Folin phenol reagent. *J. Bio. Chem* 193: 265-275.
[15] Jouanin L, Bonade-Bottino M, Girard C, Morrot G and Giband M. (1998). Transgenic plants for insect resistance. *Plant Sci.* 131: 1–11.
[16] Bown DP, Wilkinson HS, Gatehouse JA. (1997). Differentially regulated inhibitor sensitive and insensitive protease genes from the phytophagous pest, *Helicoverpa armigera*, are members of complex multigene families. *Insect Biochem. Mol. Biol* 27: 625-638.
[17] Harsulkar M. Abhay, Ashok P. Giri, Aparna G. Patankar, Vidya S. Gupta, Mohini N. Sainani, Prabhakar K. Ranjekar and Vasanti V. Deshpande. (1999). Successive Use of Non-Host Plant Proteinase Inhibitors Required for Effective Inhibition of *Helicoverpa armiger*a Gut Proteinases and Larval Growth. *Plant Physiology* 121: 497–506.
[18] Broadway R.M. (1995). Are insects resistant to plant proteinase inhibitors? *Journal of Insect Physiology* 41 107-116.
[19] Jongsma M.A., Bakker PL Peters J, Bosch D and Stiekema WJ. (1995). Adaptation of *Spodoptera exigua* larvae to plant proteinase inhibitors by induction of gut proteinase activity insensitive to inhibition. *Planta* 195: 29-35.9.
[20] Jongsma M A and Bolter C. 1997. The adaptation of insects to plant protease inhibitor. *J. Insect Physiol* 43: 885-895.
[21] Mohammed Nurul Amin, Shimul Banik, Md Ibrahim, Md. Mizanur Rahman Moghal, Mohammed Salim Majumder, Rokiya Siddika, Khorshed Alam, Rahat Maruf Jitu K M and Shamima Nasrin Anonna. (2015). A study on Ardisia solanace for evaluation of phytochemical and pharmacological properties. *Int J Pharmacognosy and Phytochemistry Res.* 7(1): 8-15.
[22] Basavaraj C K and Purmina A. (2010). Diuretic activity of extracts of *Mimusops elengi* Linn. Bark. *Int J Green Pharm.* 90-92.
[23] Manjeshwar S B, Ramakrishna J P, Harshith P B, Princy L P and Rekha B. (2011). Chemistry and medicinal properties of the Bakul (*Mimusops elengi* Linn.): A review. *Food Res Int.* 44(7): 1823-1829.
[24] Bharat G and Parabia M H. (2010). Pharmacognostic evaluation of bark and seeds of *Mimusops elengi* L. *Int J Pharm Pharmac. Sci.* 2(4): 110-113.
[25] Darshan S and Doreswamy R. (2004). Patented anti-inflammatory plant drug development from traditional medicine. *Phytother Res* 18: 343-357.
[26] Ahmad Imtyaz, Shamsi Shariq, and Zaman Roohi. (2016). A review on sour cherry (*Prunus cerasus*): A high value unani medicinal fruit. *Int. Jou of Green Phar* 11(1): 1-6.
[27] Shivaraj, B and Pattabhiraman T N. (1981). Natural Plant Enzyme Inhibitors. *Biochemistry Journal* 193: 29–36.
[28] Pramanik, Asmita, Dibyendu Paik, Pijush Kanti Pramanik and Tapati Chakraborti. (2019). "Biomedicine and Pharmacotherapy of serine protease inhibitors rich *Coccinia grandis* (L). Voigt Leaf extract includes protective immune response in Murine visceral leishmaniasis." *Biomed and Pharm.* 111: 224-235.
[29] Borde, Vinod, Vandana Hivrale, and Manvendra Kachole. (2012). Bio Technology Detection and Purification of *Mucuna Pruriens* Seed Protease Inhibitors . Elixir Bio Tech 49B: 10178–81.
[30] Rosu ana. Eremia Michaela-Carmen, Spiridon Maria, Guidea Silvana, Lupescu Irina and Juracoane Stefana (Stefan's Juracoane). (2010). In Search of Plant Sources for Serine Protease Inhibitors : I . Detection of Serine Protease Inhibitors in Callus Cultures Induced from Somatic Explants of Flax (*Linum Usitatissimum* L .). Romanian Biotechnological Letters 15(5): 5668–74.
[31] Lorenc Kubis I, Kowalska J, Pochron B, Zuzlo A, Wilusz T. (2001). Isolation and amino acid sequence of a serine proteinase inhibitor from common flax (*Linum usitatissimum*) seeds. Chem. Bio Chem. 2 (1): 45 -51.
[32] Hiroshi Shibata, Saburo Hara Tokuji Ikenaka, Jiro ABE. (1986). Purification and Characterization of Proteinase Inhibitors from Winged Bean (Psophocarpus tetragonolobus (L.) Seeds. J. Biochem 99 (4): 1147-1155.
[33] Ussuf K, Laxmi N, and Mitra R. (2001). Proteinase inhibitors: Plant-derived genes of insecticidal protein for developing insect-resistant transgenic plants. Current Science 80(7): 847-853.
[34] Xu D P, Xue Q Z, McElroy D, Mawal Y, Hilder V A and Wu R.(1996). Constitutive expression of a cowpea trypsin-inhibitor gene, CpTI, in transgenic rice plants confers resistance of two major rice insect pests. Mol. Breeding 2: 167È73.

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