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In vitro assessment of Trypsin Inhibitory Activity in Seed Extracts of Medicinal Legume *Mucuna pruriens*

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Abstract:
Disturbances in the tight regulation of trypsin activity was identified as the cause of certain fatal human diseases. Trypsin inhibitory proteins are recognized as a potential treatment strategy against such diseases. Seeds of legumes have been recognized as potential natural source of novel trypsin inhibitory proteins. The present study was designed to assess the activity of trypsin inhibitory proteins present in seed extract of M. pruriens local breed. Trypsin inhibitory activity of a concentration series of the extract was assessed by method explained by modified Kunitz (1947). Crude protein extract was subjected to fractionation using ammonium sulphate precipitation. The total protein content of the extract was estimated by modified Bradford assay (1976). The highest trypsin inhibitory activity (83.60 ±3.60%) was exerted by 20% concentration of the extract. Total protein content of crude protein extract was 2.11 ±0.05 mg/ml. The maximum specific trypsin inhibitory activity was observed in 20% crude protein extract. The specific trypsin inhibitory activity of M. pruriens seeds indicated a dose-dependent (r = 0.94) activity. Maximum percentage trypsin inhibitory activity (81.20 ± 1.21%) was shown by the protein fraction precipitated by the 60% ammonium sulphate saturation. The present study revealed that the seed of local breed of M. pruriens demonstrate a substantial trypsin inhibitory activity.

Keywords: *Mucuna pruriens*, Proteases, Proteins, Trypsin Inhibitors

1. Introduction

Serine proteases are a group of enzymes that catalyze the breakdown of proteins within organisms and are therefore involved in the regulation of a wide range of functions including protein digestion in food, inflammation, blood clotting mechanism and immunity. Trypsin is one of the widely distributed serine proteases throughout nature which is mediated in many vital physiological functions in organisms. Although it is essential for survival, it was reported that disturbances in the tight regulation of trypsin activity lead to fatal human diseases such as cardiovascular diseases, inflammatory diseases, cancers, neurodegenerative disorders, etc. [1].

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Hence, trypsin inhibitory proteins have been gained attention as a potential treatment strategy against such diseases. Seeds of legumes store high protein content and thereby they have been recognized as a potential natural source of novel trypsin inhibitory proteins. The genus *Mucuna* which belongs to Family Fabaceae is classified under the subfamily Papilionaceae. *Mucuna pruriens* is a pulse that is native to tropical Asia and also a popular medicinal plant from ancient times, which has been used to treat male infertility and certain nervous disorders [2]. It is widely known as velvet beans and in Sinhala, it is known as wanduru mae.

Currently, *M. pruriens* is one of the most popular green crops in the majority of tropical countries. However, in Sri Lankan context *M. pruriens* is an under-utilized wild legume that was ignored by cultivators during past decades, due to the low yield of crops. However, the studies done on seeds revealed that they contain a comparatively higher protein concentration with excellent digestibility. Therefore, *M. pruriens* is recognized as an invaluable source of dietary proteins.

The Field Crops Research and Development Institute of Sri Lanka (FCRDISL) has been made efforts to produce novel breeds to obtain high yield and successfully released a novel local breed of *M. pruriens* recently for the purpose of cultivation. Scientific investigations on protease inhibitory proteins present in this breed have not been carried out yet. Therefore the present study was designed to assess the activity of trypsin inhibitory proteins present in seed extract of *M. pruriens* local breed.

2. Methodology

Fresh mature seeds of the local breed of *M. pruriens* were collected from FCRDISL. A concentration gradient (20, 10, 5, 2.5 and 1.25 % w/v) of the crude extract of the seed sample was prepared using distilled water.

Trypsin inhibitory activity of each concentration of the extract was assessed by the method explained by Kunitz (1947) with slight modifications. The crude extract was incubated with trypsin solution at 37^0C for 15 minutes. Thereafter, 1% Hammerstein casein substrate was incubated with the mixture at 37^0C for 60 minutes. Following the addition of 5% trichloroacetic acid, the reaction mixture was centrifuged. The absorbance of the supernatant was measured at 280 nm. Percentage trypsin inhibitory activity (TIA) of each sample was calculated as below, [3].

$$\textit{Percentage Trypsin activity} = \frac{\textit{(Absorption of test- Absorption of control)}\ X100}{\textit{Absorption of test}}$$

The crude extract (20%) was subjected to fractionation using ammonium sulphate salting out procedure. First, 30% ammonium sulphate saturation was gradually added to the crude extract and precipitated proteins were obtained by centrifuging. The remaining supernatant was treated with 60% and 90% saturated ammonium sulphate respectively. The precipitates were dialyzed against 0.01 M solution of phosphate buffer (pH 7.0) using a dialysis tube (8 kDa).

The total protein content of 20% crude extract was estimated by modified Bradford assay (1976) [4]. Then specific trypsin inhibitory activity of each concentration was calculated using the following formula,

$$\textit{Specific trypsin inhibitory activity} = \frac{\textit{Trypsin inhibitory activity}}{\textit{Total protein content}} \times 100$$

3. Results And Discussion

Trypsin inhibitory activity of crude extract

The average percentage trypsin inhibitory activity of crude extracts of seed sample is presented in table 01. Among five different concentrations, the highest trypsin inhibitory activity (83.60 ±3.60%) was exerted by 20% crude extract.

Table 01: The average percentage trypsin inhibitory activity of crude extracts of seed samples of *M. pruriens*

Concentration of the crude extract (%)	The average percentage trypsin inhibitory activity (%)
20	83.60 ±3.60
10	70.94 ±0.28
5	55.40 ±1.77
2.5	41.38 ±1.96
1.25	28.72 ±1.55

A study done by Machuka (2000) observed the percentage trypsin inhibitory activity within the range of 23-83 % in eleven varieties of *M. pruriens* collected from Nigeria. A similar kind of study done in India reported that the percentage trypsin inhibitory in the seed of *M. pruriens* was 48.01±0.23% [5]. The results of the present study showed that the local breed exerts comparatively higher trypsin inhibitory activity.

Total protein content and specific trypsin inhibitory activity

The total protein content of the crude extract was 2.11 ±0.05 mg/ml. Previous studies observed that seeds of *M. pruriens* growing in different countries also contain comparatively higher protein amounts (Machuka, 2000).

The specific trypsin inhibitory activity of each concentration is presented in Table 02. The maximum specific trypsin inhibitory activity was observed in 20% crude extract. The specific trypsin inhibitory activity of *M. pruriens* seeds indicated a dose-dependent ($r = 0.94$) activity.

Table 02: The specific trypsin inhibitory activity of crude extracts of seed samples of M. pruriens

Concentration of the crude extract (%)	The specific percentage trypsin inhibitory activity (%)
20	39.60±0.75
10	33.60±0.96
5	26.25±0.57
2.5	19.61±0.48
1.25	13.61±0.25

Fractionation with ammonium sulphate precipitation

The proteins contained in crude extract (20%) was precipitated using ammonium sulphate salting-out method in order to remove the unnecessary secondary products. With increasing ammonium ions concentration within the crude extract, the solubility of globular proteins gradually decreased resulting in precipitation of the proteins. The maximum solubility of each protein reach in the presence of a specific ammonium ions concentration. This theory was applied to perform the initial fractionation of the proteins in the crude extract. Thereby, ammonium sulphate precipitation was applied for partial purification of proteins in the seed sample.

The ammonium sulphate saturation of 30 %, 60 % and 90 % was used to fractionate the proteins in the seed extract. Among the resulting fractions, the maximum percentage trypsin inhibitory activity (81.20 ± 1.21%) was shown by the proteins precipitated by the 60 % ammonium sulphate saturation. The percentage trypsin inhibitory activity of the fractions obtained by 30 % and 90 % ammonium sulphate saturations were 79.81 ± 2.31% and 68.41 ± 3.23% respectively. Hence, protein fraction precipitated by 60 % ammonium sulphate saturation is selected for future experiments for purification of trypsin inhibitory active proteins from the seed sample.

The results of dialysis using a semipermeable dialysis tube with 8kDa pore size, revealed that the trypsin inhibitory proteins present in the tested seed sample are larger than 8kDa. The observations of the experiments on partial purification and dialysis will be beneficial for purification studies in future.

Natural protease inhibitory proteins have been gaining attention due to their specific activity against proteases leaving other proteins unaffected, unlike chemically synthesised protease inhibitors. Therefore, protease inhibitors available in nature are recognized as promising candidates for protease targeted therapy against certain diseases. The remarkable trypsin inhibitory activity demonstrated by the test extract suggests that the seeds of the local breed of *M. pruriens* as a potential source of therapeutically active trypsin inhibitory proteins.

5. Conclusion

The results of the present study revealed that the seed of the local breed of *M. pruriens* demonstrates a considerable trypsin inhibitory activity, reflecting its therapeutic significance. Cell based or *in vitro* model experiments are suggested to confirm the

therapeutic efficiency of the seed extract. In order to discover effective trypsin inhibitory proteins, further studies on protein purification are recommended.

References

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