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RESEARCH ARTICLE

A PRELIMINARY STUDY OF THE PROTEASES FROM MOMORDICA CHARANTIA (BITTER GOURD) AND VICIA FABA (BROAD BEANS) SEEDS

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ABSTRACT

A preliminary analysis of the proteases from the seeds of *Momordica charantia* and *Vicia faba* has been performed. The study has revealed that *Momordica charantia* and *Vicia faba* seed extracts show protease activities of 3.9024 and 6.708 U/mg, respectively, and specific activities of 0.1904 and 0.2178. This study showed that protease activity is higher in *Vicia faba* extract. The protease from *Momordica charantia* seeds showed maximum activity at a temperature of 40□ and the protease from *Vicia faba* seeds showed maximum activity at a temperature of 60□ when the activities were studied at 20□, 40□, 60□ and 80□.

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INTRODUCTION

Proteases catalyze the break down of proteins into amino acids. Proteolytic enzymes are multifunctional enzymes that have many physiological functions in plants and animals. Commercially they are extremely important as more than 60% of the total enzyme market is made up of proteases; they are isolated from plants, animals, bacteria and fungi. For many years, studies on proteases focused on their original roles as blunt aggressors associated with protein demolition. However, the realization that, beyond these nonspecific degradative functions, proteases act as sharp scissors and catalyze highly specific reactions of proteolytic processing, producing new protein products, inaugurated a new era in protease research (Neurath and Walsh, 1976). The current success of research in this group of ancient enzymes derives mainly from the large collection of findings demonstrating their relevance in the control of multiple biological processes in all living organisms (López-Otín and Overall, 2002). Thus, proteases regulate the fate, localization, and activity of many proteins, modulate protein-protein interactions, create new bioactive molecules, contribute to the processing of cellular information, and generate, transduce, and amplify molecular signals. As a direct result of these multiple actions, proteases influence DNA replication and transcription, cell proliferation and differentiation, tissue morphogenesis and remodeling, heat shock and unfolded protein responses, angiogenesis, neurogenesis, ovulation, fertilization, wound repair, stem cell mobilization, hemostasis, blood coagulation, inflammation, immunity, autophagy, senescence, necrosis, and apoptosis (Gupta *et al*., 2002). Proteases also play key roles in plants and contribute to the processing, maturation, or destruction of specific sets of proteins in response to developmental cues or to variations in environmental conditions (Garcia-Lorenzo et al., 2006). Proteolytic enzymes from the plant sources have received special attention because of their broad substrate specificity as well active in wide range of pH, temperature, and in presence of organic compounds as well as other additives. Search for valuable proteases with distinct specificity is always a continuous challenge for varied industrial and medical applications (Devaraj, 2008).

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MATERIALS AND METHODS

Collection of Samples and Preparation of Crude Seed Extracts

Momordica charantia (bitter gourd) fruits and *Vicia faba* (broad beans) bean pods were obtained fresh from the local market and the seeds were collected from them. 50 gm of *Momordica charantia* and *Vicia faba* seeds were washed thoroughly, air dried and homogenized in 100 ml of Tris HCl buffer (pH 7.5).

Screening the *Momordica charantia* and *Vicia faba* Seed Extracts for Protease Activity by Casein Agar Plate Assay

Casein agar plate assay was performed according to the method of Cheeseman (Cheeseman, 1963). Casein agar solution was prepared by dissolving 500 mg of casein in a 1% agar solution. The solution was then poured into glass petriplates and allowed to solidify. After solidification, a single well was punched in the centre of the gel and the extract was loaded into the well taking care to prevent overflow of the extract from the well. The petriplates were then incubated at room temperature for 16 h after which the diameter of the zones of clearance formed due to protease activity were measured.

Estimation of Total Protein Concentration in the Crude Extracts

Total protein was estimated by the Lowry's method (Lowry *et al*., 1951). To 1 ml properly diluted protein sample (10-60 fig/ml), 5 ml alkaline solution.(working solution) was added, mixed well and incubated at 55°C for 10 min. To the above mixture 0.5 ml Folin Ciocalteau reagent was added, mixed well and incubated at 55°C for 30 min. The absorbance of the colour was measured at 680 nm in a spectrophotometer. The amount of protein present in the sample was calculated from a standard curve prepared using varying concentrations of bovine serum albumin (BSA). The concentration of the standard BSA solution was 100µg/ml.

Determination of Protease Activity and Specific Activity of the Crude Extracts

The protease activity was determined by the modified procedure of Tsuchida (Tsuchida *et al*., 1986). Using 2% casein in 0.2 M carbonate buffer (pH 10) as substrate. Casein solution (0.5 ml) was incubated with 0.5 ml enzyme extract at 55°C for 10 min. After 10 min, the reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid. The mixture was centrifuged and to the supernatant was added 5 ml of 0.44 M sodium carbonate and 1ml of two-fold diluted Folin Ciocalteau reagent. After 30 min, the colour developed was read at 660 nm against a reagent blank prepared in the same manner. Tyrosine served as the reference standard. One unit of enzyme activity was defined as the number of µmols of tyrosine formed per min per mg of seed sample (U/mg). Specific activity of the enzyme in the seed extracts was also determined.

Determination of the Activity of Protease in the Crude Extracts at Varying Temperatures

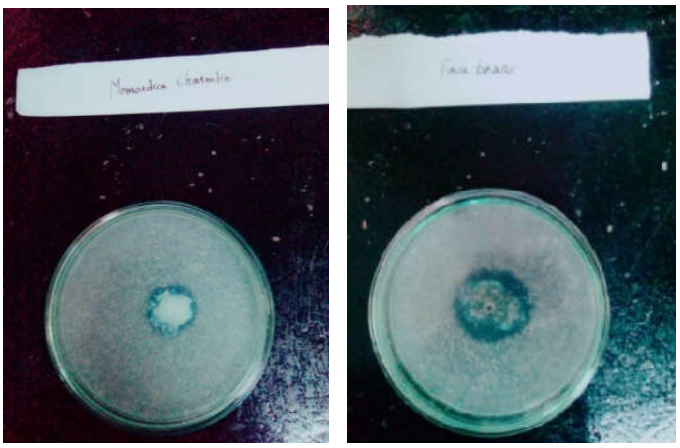
The activity of proteases in the crude extracts was determined by the modified procedure of Tsuchida *et al*. (1986). Casein solution (0.5 ml) with an equal volume of suitably diluted enzyme solution was incubated at varying temperatures of 20□, 40□, 60□ and 80□. After 10 min, the reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid.

The mixture was centrifuged and to the supernatant was added 5 ml of 0.44 M sodium carbonate and 1ml of two-fold diluted Folin Ciocalteau reagent. After 30 min, the colour developed was read at 660 nm against a reagent blank prepared in the same manner. Tyrosine served as the reference standard.

RESULTS

Screening the *Momordica charantia* and *Vicia faba* Seed Extracts for Protease Activity by Casein Agar Plate Assay

Zones of clearance were observed with both the extracts on the casein agar plates as shown in Fig. 1. The diameters of the zones of clearance for the *Momordica charantia* and *Vicia faba* seed extracts after16 h of incubation were 2.5 and 4.5 cm.



(A) **(B)**

Fig. 1 Casein agar plates showing the zones of clearance due to protease activity in (a) *Momordica charantia* and (b) *Vicia faba* extracts

Estimation of Total Protein Concentration in the Crude Extracts

The *Vicia faba* extract showed higher protein concentration of 30.8 mg/ml than that of *Momordica charantia* extract of 20.5 mg/ml (Fig. 2).

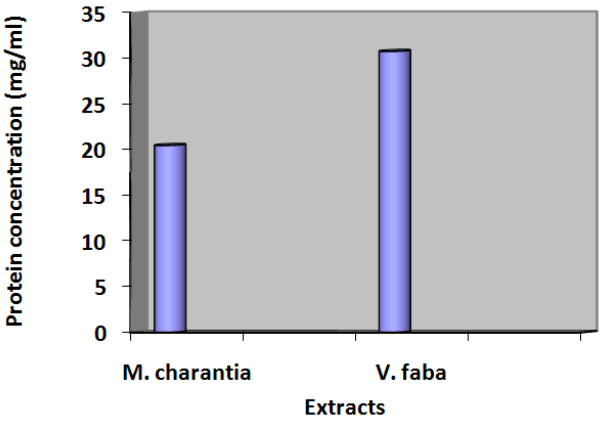


Fig. 2. Protein concentration in *Momordica charantia* and *Vicia faba crude* extracts

Determination of the Protease Activity and Specific Activity in the Seed Extracts

The protease activities in the *Momordica charantia* and *Vicia faba* extracts were found to 3.9024 and 6.708 U/mg, respectively. The specific activities of proteases in the *Momordica charantia* and *Vicia faba* extracts are 0.1904 and 0.2178, respectively. The *Vicia faba* extract shows a higher protease activity (Fig. 3) and specific activity (Fig. 4) than *Momordica charantia* extract.

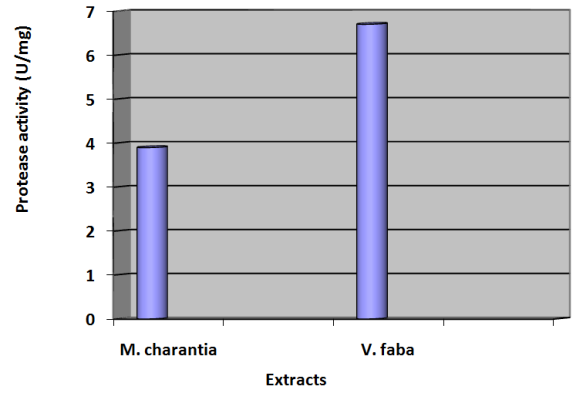


Fig. 3. Protease activities in *Momordica charantia* and *Vicia faba* seed extracts

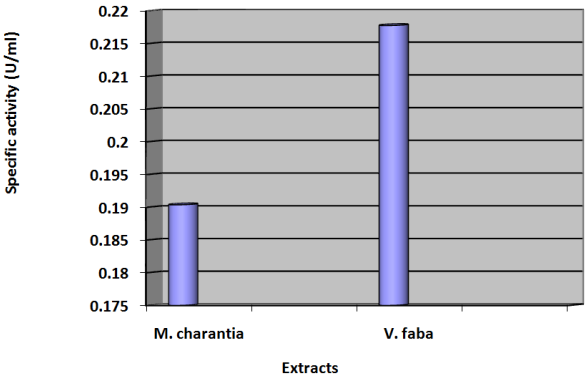


Fig. 4. Specific activity of proteases in *Momordica charantia* and *Vicia faba* seed extracts

Determination of the Activity of Protease in the Crude Extracts at Varying Temperatures

The protease activities in the crude extracts at different temperatures are shown in Table 1. The *Momordica charantia* protease showed maximum activity at 40□ while the *Vicia faba* protease showed maximum activity at 60□ (Fig. 5).

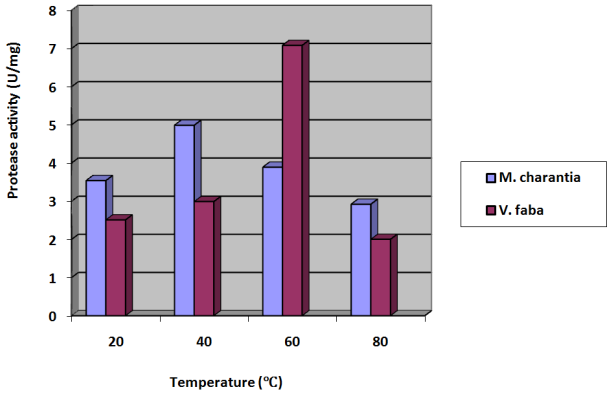


Fig. 5. Protease activities (U/mg) in the crude extracts at different temperatures

Table. 1 Protease activities (U/mg) in the crude extracts at different temperatures

Samples	Temperatures (□)			
	20	40	60	80
Momordica charantia	3.5407	4.9901	3.892	2.9210
Vicia faba	2.5110	2.9902	7.0822	2.0020

DISCUSSION

The *Vicia faba* extract has greater protease activity compared to *Momordica charantia* as observed by the casein agar plate assay. This result is consistent with the measurement of protease activity carried out in this study, wherein the *Vicia faba* extract has shown greater protease activity and specific activity compared to *Momordica charantia.* This may probably be due to the presence of greater amounts of protease inhibitors in *Momordica charantia.* Species of the Fabaceae family, part of the Leguminosae, store large amounts of proteins in their seeds as a consequence of an extensive protein metabolism, which is more expressive in comparison with plants from other families. In order to regulate the protein metabolism, plants express high levels of proteolytic enzymes and of their inhibitors (Silva-López, 2009 – Gomes *et al*., 2011). Generally, temperature increases the rate of a reaction as long as the structure of the enzyme is maintained in its native state. *Vicia faba* protease seems to be more stable at temperatures greater than 40□ in contrast to *Momordica charantia* protease whose activity decreases at temperatures higher than 40□. This corresponds with the reports in scientific literature that most proteases show optimum activity in the range of 40 to 60□.

Conclusion

This study is a preliminary analysis of the proteases from two plant species, i.e., *Momordica charantia* and *Vicia faba.* The study has revealed that *Vicia faba* and *Momordica charantia* extracts show protease activity. Microbial sources of proteases are widely used for avariety of industrial and medical purposes. The production of microbial enzymes involves costly and time consuming culturing and purification steps. Use of alternative proteases from plant sources such as *Momordica charantia* and *Vicia faba* would lead to the availability of more cost effective and less toxic proteases for industrial and medical purposes. Further investigations of these proteases could be useful in the use of these proteases in many industrial and medical fields.

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