

Various Biochemical Parameters of Protease Isolated From *Adhatoda Vasica*: A Medicinally Important Plant

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Abstract

Adhatoda vasica, also known as malabar nut, is part of the Acanthaceae plant family which grows on wastelands and in a variety of habitats and soil. The leaves, flowers, fruits and roots are extensively used for treating cold, cough, chronic bronchitis and asthma. It has also been used by proponents of alternative therapies or identified in materials of traditional or folk medicine. In the present study, leaves of *Adhatoda vasica* have been explored for the presence of proteases, an important group of enzymes that helps in degradation of proteins by hydrolysis of peptide bond. Proteases have immense importance in treatment of various diseases like sepsis, digestive disorders, inflammation, cystic fibrosis, retinal disorders, psoriasis, cardiovascular, and others. This research work was undertaken to study the various biochemical aspects of protease enzyme isolated from *Adhatoda vasica*. The specific activity of the crude enzyme was found to be 3.38 Units/mg. Further, studies included pH optima, temperature optima, time course along with pH and thermal stability. The enzyme was found to show maximum activity at pH 5.0 and temperature values ranging between 20-30°C in 40 minutes of reaction time. The thermal and pH stability values of the enzyme were 30°C and 4.0-5.0, respectively. The results obtained suggest that *Adhatoda vasica*, a medicinally important plant can serve as potential source of proteases, which further have great importance in therapeutics.

Keywords: *Adhatoda vasica*, protease, biochemical parameters.

1. Introduction

Adhatoda vasica is an immensely important plant drug in Ayurvedic and Unani

medicine. Its leaves have been used widely in Ayurvedic Medicine primarily treating the respiratory disorders. The plant is found all over the plains of India and in lower Himalayan ranges. *Adhatoda vasica* (Vasaka) is used in various chest affections and is well known as an expectorant in the indigenous system of medicine (Godghate, 2013). The roots, leaves and flowers of *Adhatoda vasica* are widely used in indigenous medicine to treat cold, cough, bronchitis and asthma (Rajani et al., 2008). This plant has medicinal uses, mainly antispasmodic, fever reducer, anti-inflammatory, anti bleeding, bronchodilator, antidibetic, antihelminthic, disinfectant, anti-jaundice, antiseptic, oxitoxic and expectorant and has many other medicinal applications (Chakraborty, 2001; Singh, 2011) Because of its varied uses in treatment of various chronic diseases (Chandra et al., 2011), the plant looked promising candidate and therefore used as protease source. Protease obtained from this plant may be a potential candidate for targeting many diseases. Proteases are important enzymes of plant metabolism and are instrumental in regulating senescence (Maurya et al., 2001). In view of unrestricted availability, the plant sources would be a possible alternative of microbial and animal proteases. Proteases are becoming a potential target for developing therapeutic agent against life threatening diseases (Channalia et al., 2011; Craik2011).The vast variety of proteases have attracted worldwide attention in the attempt to exploit their biotechnological applications (Fox et al., 1991). In the present study, various biochemical aspects of protease enzyme isolated from *Adhatoda vasica* leaves have been studied.

2. Materials And Methods

Adhatoda vasica was obtained from Amity University farmhouse. All chemicals were of reagent grade and obtained from standard commercial firms.

2.1 Extraction of Protease enzyme: The pre-weighed and washed leaves of *Adhatoda vasica* were crushed in distilled water and centrifuged at 10,000 rpm for 7 minutes at 4⁰C. The filtrate was treated as crude extract.

2.2 Protein determination: Protein determination was estimated spectrophotometrically by Lowry method (1951) using Bovine serum albumin was used as a standard.

2.3 Protease Assay: Protease activity was assayed using Folin-Ciocalteu reagent. The 5ml reaction mixture contained casein (1%), enzyme, 0.05 M sodium acetate buffer (pH 5.0) incubated for 30 min at 30⁰C. After that 0.5 M NaOH and folins reagent was added. 1International Unit of enzyme is defined as 1µg of tyrosine released per minute per ml under standard assay conditions. The activity was reported as mean of three determinations.

Determination of specific activity: Specific activity was determined by using the following relationship:

Specific activity= Total enzyme units /Total protein (mg)

2.4 Biochemical characterization of Protease: The crude Protease enzyme was characterized as follows:-

2.4.1 Time course: The reaction mixture containing enzyme and substrate was incubated at 30°C for time period ranging between 10-90 minutes and the product released estimated by Folin's method.

2.4.2 pH and temperature optima: Suitable buffers (0.05 M) of various pH values ranging from 3.0 to 11.0 were used to study the effect of pH on the enzyme activity. The optimum temperature for the enzyme activity was determined by incubating the reaction mixture in 0.05 M buffer (appropriate pH) from temperature ranging from 10-90°C.

2.4.3 pH and temperature stabilities: After 2 h pre-incubation in appropriate buffers corresponding to pH 3.0-11.0 at room temperature, activities were measured using standard assay conditions. For determining the thermal stability, aliquots of enzyme samples were incubated at temperatures 10-90°C for 2 h and activities determined.

3. Results and Discussion

The major objective of the work reported here was to screen various medicinally important plants for high specific activity protease enzymes. Out of the four plants studied, the leaf extract of *Adhatoda vasica* was found to contain protease with maximum specific activity (3.38 Units/mg) as shown in Table 1, and was further characterized. Figure 1 illustrates the time course for the protease-catalysed reaction showing 40 minutes to be the optimum time period, above which a slight decline was observed (Figure. 1). The enzyme was found to show maximum activity at pH 5.0 and temperature values ranging between 20-30°C as shown in fig. 2 and fig. 3, respectively. Figure 4 depicts the thermal stability curve of the isolated protease showing activity retention up to 30°C. The pH stability range of the enzyme was 4.0-5.0, as shown in fig. 5. It can be inferred from the results that leaves of *Adhatoda Vasica* is a potential source for proteases which further have immense importance in treatment of various diseases like sepsis, digestive disorders, inflammation, cystic fibrosis, retinal disorders, psoriasis, cardiovascular and others. Thus, it may be concluded that *Adhatoda vasica*, a medicinally important plant can serve as potential source of proteases, which further have great importance in therapeutics.

Table 1: Specific activity of protease obtained from various plant sources.

S. No.	Plant Source	Specific Activity (Units/mg)
I	<i>Adathoda vasica</i>	3.38
II	<i>Acorus calamus</i>	2.95
III	<i>Rosmarinus officinalis</i>	2.65
IV	<i>Apium graveolens</i>	1.81

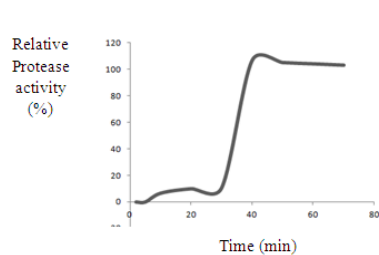


Figure 1. Time course of protease catalysed reaction.

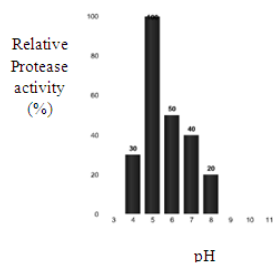


Figure 2. pH optima of protease enzyme isolated from *Adhatoda vasica* leaves.

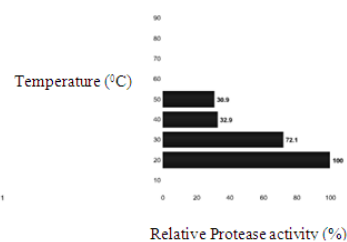


Figure 3. Temperature optima for protease enzyme isolated from *Adhatoda vasica* leaves.

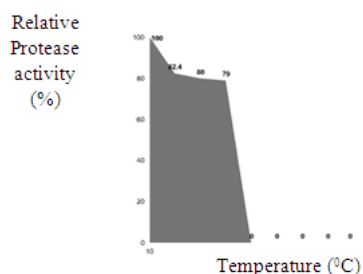


Figure 4. Thermal stability curve of protease enzyme isolated from *Adhatoda vasica* leaves.

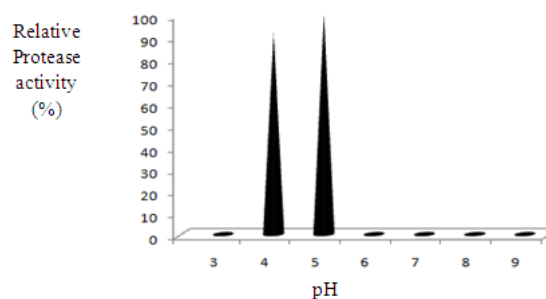


Figure 5. pH stability profile of protease isolated from *Adhatoda vasica* leaves.

4. Conclusion

From the present study it has been observed that *Adhatoda vasica*, a medicinally important plant used in various Ayurvedic and unani medicines for treatment of various diseases is, is also a potential source of proteolytic enzymes; which further have immense therapeutic value. Therefore it may be concluded that *Adhatoda vasica* may be used for curing many more diseases related to protein degradation directly or indirectly. Though a lot of work needs to be done in this direction.

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