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Partial Purification and Kinetic Studies of Thermostable Protease Isolated from Ocimum Basilicum: A Medicinally Important Culinary Herb

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Abstract—*Ocimum basilicum (Basil) is a commonly used household culinary herb. It has been therapeutically used as an antioxidant, antiproliferative, antiulcer, antibacterial and anticancer agent. It has extensive applications in various industries such as food, detergent, leather, pharmaceutical industries etc. In the present study, dried sample of O. basilicum has been explored for the presence of protease enzyme. Proteases are a group of enzymes that catalyze hydrolytic reactions resulting in breakdown of peptide bonds and have industrial as well as therapeutic applications. The specific activity of the crude enzyme was found to be 25.4U/mg. The protease enzyme isolated from O. basilicum was partially purified by ammonium sulphate fractionation into three parts viz; 0-30%, 30-60% and 60-90% according to saturation level, out of which 0-30% fraction showed highest specific activity (71.2 U/mg) with 2.8-fold of purification level. This enzyme fraction on further biochemical characterization showed maximum enzyme activity at pH 5.0 and temperature 30^0C in 30 minutes of reaction time. The pH stability value for the enzyme was observed to be 4.0-5.0 whereas it retained its activity upto 50^0C of thermal treatment upto 24 h. The values of kinetic parameters; Michaelis-Menten constant (K_m) and Maximum Velocity (V_{max}) were found to be 0.28 mg/ml and 20 units/min/ml, respectively. Out of various metal ions tested; $Fe^{3+}>Na^{+}>Mn^{2+}>K^{+}>Co^{2+}$ were found to inhibit the protease activity in mentioned order. The results obtained suggest that O. basilicum is a potential source of protease and can be explored for various industrial and therapeutic purposes.*

Keywords: *Ocimum basilicum*, protease, industrial importance

1. INTRODUCTION

Ocimum basilicum (sweet basil) has recently attracted great attention because of its uses in ancient system for the treatment of various diseases [1]. It is an annual herb belonging to the Lamiaccae family, commonly found in India, Asia and Africa [2]. It is used as a component in cosmetic products viz. perfumes, shampoos, lotions, oils and soaps [3, 4]. It is considered good for digestion, stomach cramps, vomiting, skin infection, snake bites and acne. Its application in the treatment of various diseases, suggest, that the plant may also be a promising source of protease [5]. Proteases are class of hydrolytic enzyme which has various physiological functions in plants, animals and microbes [6, 7]. These constitute a large and complex group of enzymes, which differ in properties such as substrate specificity, active site, catalytic mechanism; pH, temperature optima and stability profile [8].They are the only class of enzymes which occupy an important position because of their physiological and commercial applications [9]. Their importance can be elucidated by the fact that they represent about 60% of all the commercially used enzymes [10]. They play multiple roles in plants e.g. in growth, senescence, apoptosis, accumulation and mobilization of storage proteins etc. [11, 12]. They are also required in signaling pathways and are critical mediators of homeostasis, thrombosis, biotic and abiotic stress [13]. Because of various reasons, plant sources of proteases are preferred over animal and microbial sources. In the present study various biochemical properties of the protease enzyme isolated from *O. basilicum* have been studied.

2. MATERIALS AND METHODS

O. basilicum was obtained from certified shops. All chemicals were of reagent grade and obtained from standard commercial firms.

2.1 Extraction of Protease enzyme

The pre-weighed sample of *O. basilicum* was crushed in distilled water and centrifuged at 10,000 rpm for 10 minutes at 4^0C. This filtrate was treated as crude extract.

2.2 Protein determination
Amount of protein was determined spectrophotometrically by Lowry method (1951) using Bovine serum albumin as a standard [14].

2.3 Protease Assay
Protease activity was assayed using Folin-Ciocalteau reagent. The 5ml reaction mixture contained casein (1%), enzyme, 0.05 M sodium acetate buffer (pH 5.0) incubated for 30 min at 30^0C. After that 0.5 M NaOH and Folin's reagent was added. One International 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. Specific activity was determined by using the following relationship:

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

2.4 Partial purification of protease isolated from *O. basilicum* by Ammonium Sulphate Fractionation:
O. basilicum extract was fractionated into 3 parts corresponding to 0-30%. 30-60% and 0-90% based on saturation level of ammonium sulphate. Specific activity of the fractions was calculated. The fraction with highest specific activity was used for further characterization

2.5 Biochemical characterization of Protease
The crude Protease enzyme was characterized according to the following parameters:

2.5.1 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.

2.5.2 pH and temperature optima
Suitable buffers (0.05 M) of varying pH range, 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 10-90^0C.

2.5.3 pH and temperature stabilities
After 2 h of 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 10-90^0C for 2 h and activities determined.

2.5.4 Effect of varying substrate concentration
The varying substrate concentration in the range of 0.02 mM and 0.5 mM was used to study the effect on enzyme activity. The Michaelis-Menten constant (Km) and maximum velocity were calculated by using Lineweaver Burk plot (1/v vs. 1/s).

2.5.5 Effect of various metal ions
The effect of various metal ions like Cu^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, Zn^{2+}, Na^+, K^+ in their respective salts $CuSO_4$, $CoCl_2$, $MnCl_2$, $FeCl_3$, $ZnSO_4$, NaCl, KCl were studied at 0.25 mM concentration.

3. RESULTS AND DISCUSSION

The main objective of the work reported here, was to screen various herbs for protease enzyme with high specific activity. From the five plants that were studied, the extract of *O. basilicum* showed the presence of protease with maximum specific activity (25.4 Units/mg) as shown in table 1 and hence was further characterized. The protease enzyme isolated from *O. basilicum* was partially purified by ammonium sulphate fractionation into three parts viz; 0-30%, 30-60% and 60-90% according to saturation level, out of which 0-30% fraction showed highest specific activity (71.2 U/mg) with 2.8-fold of purification level (fig.1). The time course for the protease-catalyzed reaction showing 30 minutes to be the optimum time period is illustrated in fig.2. In contrary to Vidyalakshmi and Selvi, 2013[15], protease enzyme isolated from *O. basilicum* showed maximum activity at pH and temperature conditions corresponding to 5.0 and 30^0C (as shown in fig. 3 and 4) respectively. The enzyme was found to be stable in pH range of 4.0- 5.0 (fig. 5) and retained its activity upto 50^oC as shown in fig 6. The values of kinetic parameters; Michaelis-Menten constant (K_m) and Maximum Velocity (V_{max}) were found to be 0.28 mg/ml and 20 units/min/ml (fig. 7), respectively. Out of various metal ions tested; $Fe^{3+}>Na^+>Mn^{2+}>K^+>Co^{2+}$ were found to inhibit the protease activity in mentioned order (fig.8). From the above mentioned results, it may be concluded that *O. basilicum*, a culinary and medicinally important herb can serve as a potential source of protease, which can be used for various other applications.

Table 1: Specific Activity of different types of herbs.

Herbs	Specific Activity(U/mg)
Ocimum basilicum(Basil)	25.4
Oreganum vulgare (Oregano)	18.6
Mentha piperita (Mint)	15.2
Aloe barbadensia (Aloe Vera)	10.0
Corriander sativum (Corriander)	8.83

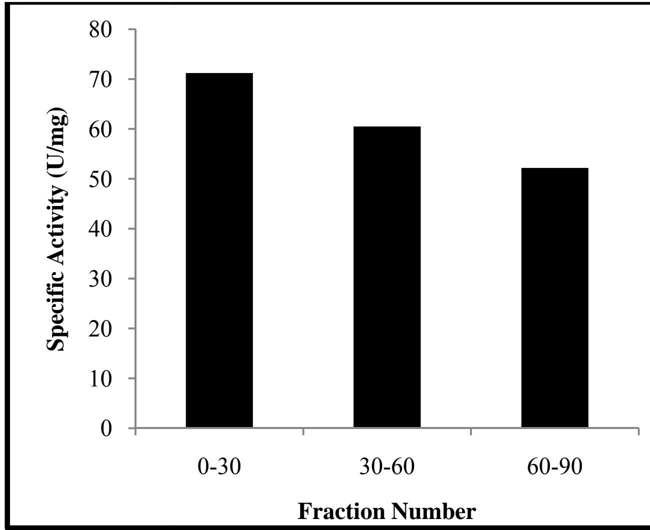

Fig. 1: Specific activity of protease fractions obtained after ammonium sulphate fractionation.

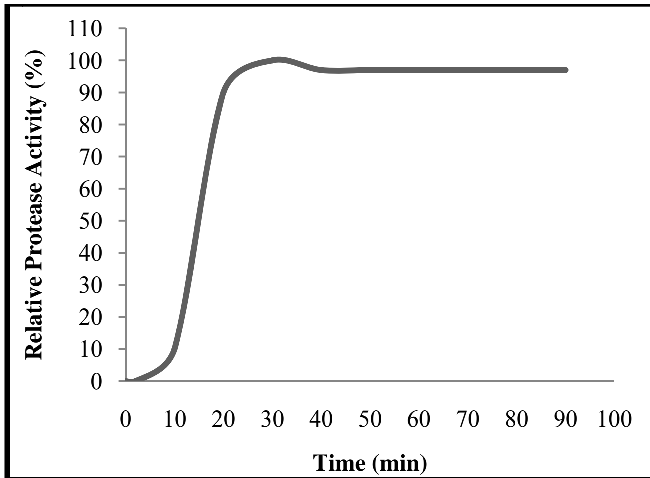

Fig. 2: Time course of protease catalyzed reaction isolated from *O. basilicum*..

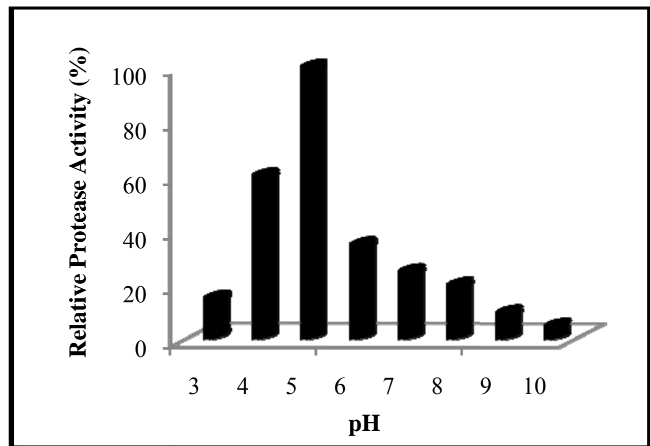

Fig. 3: pH optima of protease enzyme isolated from *O. basilicum* incubating the reaction mixture at pH ranging

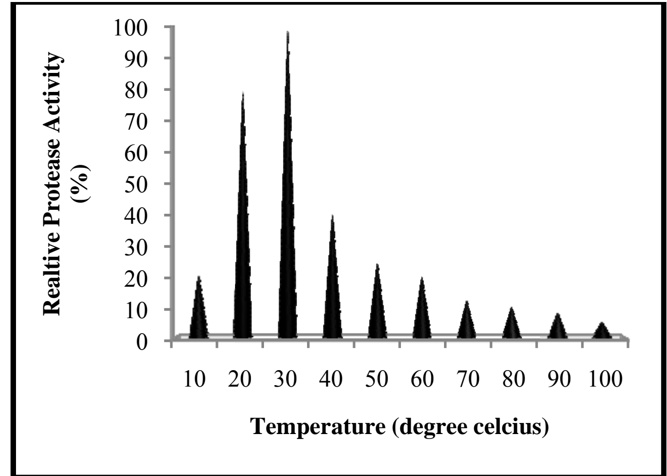

Fig. 4: Temperature optima of protease isolated from *O. basilicum* by incubating the reaction mixture at different temperatures.

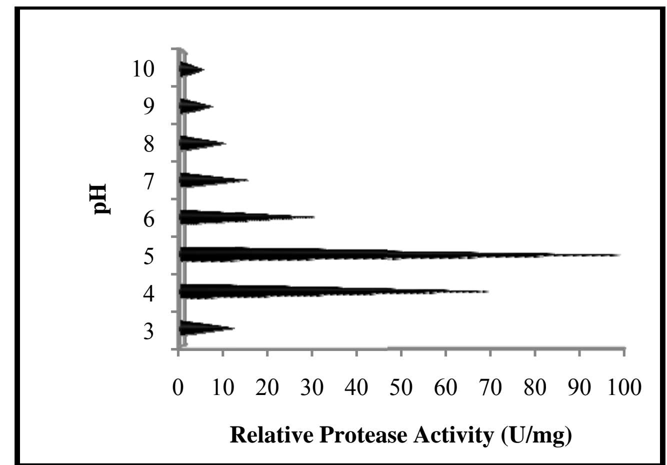

Fig. 5: pH stability of protease enzyme isolated from *O. basilicum* (after 2hr of incubation).

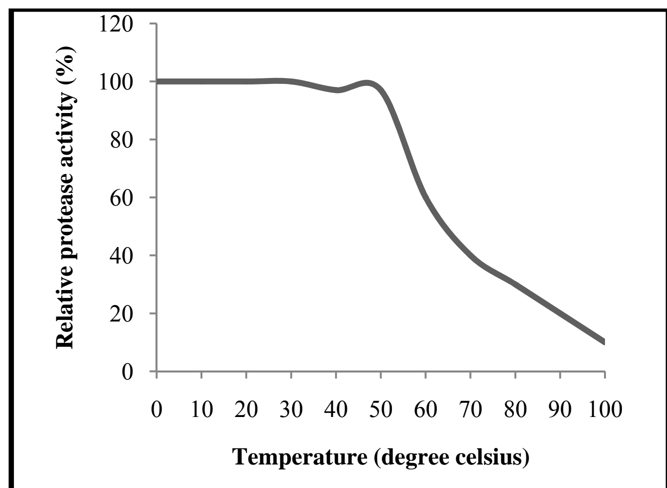

Fig. 6: Thermal stability of protease enzyme isolated from *O. basilicum* (after 2 hr of pre- incubation).

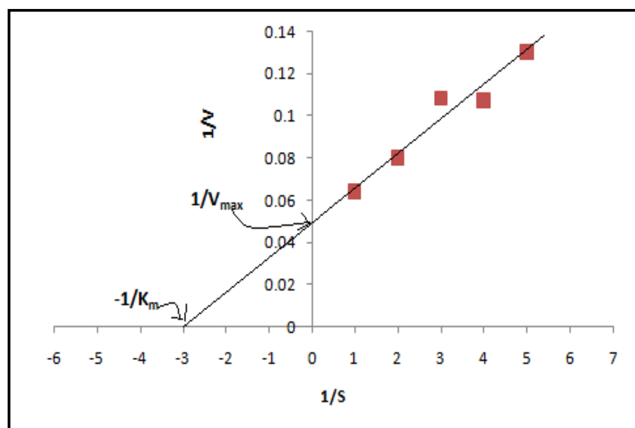

Fig. 7: Lineweaver plot showing K_m and V_{max} values of protease isolated from *O. basilicum*.

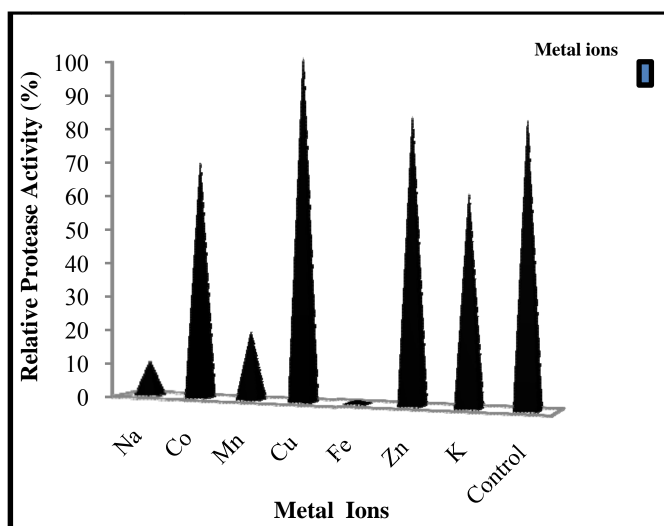

Fig. 8: Effect of metal ions on protease isolated from O. basilicum.

4. CONCLUSION

It has been observed from present study that *O. basilicum* which has attracted huge attention in ancient systems (Ayurvedic and Unani) of Indian medicine for its use in the treatment of various ailments, is also a potential source of protease enzyme which further has various therapeutic and industrial applications. Hence it can be concluded that *O. basilicum* may be used as a potential source of protease enzyme which can be further be used as a cure for diseases associated with protein degradations directly or indirectly. But, more work needs to be done in this direction..

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