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Comparative destaining properties of protease enzyme extracted from jasmine (*Jasminum sambac*), banana (*Musa paradisiaca*) and ginger (*Zingiber officinale*)

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Abstract*:* Enzymes act as biocatalyst which has several advantages over chemical catalysts due to their eco-friendly nature that make them a favorable choice for almost all industrial applications. The present study aims to assess the destaining properties of protease enzymes purified from three different plant sources *i.e.*, jasmine flower (*Jasminum sambac*), ginger rhizome (*Zingiber officinale*) and banana leaf (*Musa paradisiaca*). Various parameters were determined like plant protein content, protease activity, specific activity, optimal temperature, pH and kinetics of protease enzyme. The result indicated that the jasmine flower showed the maximum protein content and protease activity followed by ginger rhizome and banana leaf. The optimal temperature for the activity of protease enzyme was observed between 70°C and 100°C in ginger rhizome while around 40°C in jasmine flower and banana leaf. The highest protease enzyme activity was observed at pH 7 in jasmine flower and ginger rhizome while at pH 6 in banana leaves. The kinetics indicated that maximum V_{max} (3.27 µM/sec) was observed in jasmine flower followed by ginger rhizome and banana leaf which values were 2.405µM/sec and 1.628 µM/sec respectively. The maximum K_m value of banana leaf was 0.0091 mM/l followed by jasmine (0.003 mM/l) and ginger rhizome (0.00531 mM/l). The specific activity of partially purified extract of ginger rhizome and jasmine flower was highest (at 60% salt saturation) while it was found 90% in banana leaf. Based on their efficient protease activity, crude enzymes partially purified from different sources were competent in removing the different stains such as roli, coffee, turmeric, tea, and ink from the stained cloth. Hence, these plants can be a potent candidate for enzyme sources as an additive in the large-scale detergent industry.

Keywords: **Protein, protease enzyme, kinetics, destaining, stain, plant source.**

1. INTRODUCTION

The demand for the enzyme is steadily increasing due to their biocatalytic and eco-friendly nature. The detergent industry is among the top consumers of enzymes. Proteases, lipases and amylases are used as additives in detergent formulations. Lipases are used in the detergent industry to decompose fatty materials and dominate the enzyme market which accounts for 70% of enzyme sales, along with proteases. Proteases or proteolytic enzymes function to break down the protein into free amino acids or small peptides by breaking the peptide bonds present between amino acids (Alnahdi, 2012). They help to degrade the long chain of protein into short chains of protein. Proteases are the class of hydrolases a large group of enzymes and playout a critical part as for their applications in both physiological and commercial fields. Today, generally utilized industrial enzymes are proteases, carbohydrates (sugar)- hydrolyzing proteins, and ester cleavage fat hydrolyzing enzymes (Mahajan et al., 2016). The first enzyme to be produced in large quantities was alkaline proteases and for the production of enzyme animal, plant and microbial sources are considered to be vital. Proteases have significant applications in various industries such as detergent and food industry and this group of enzymes is considered one of the most important for industrial purposes(Merheb et al., 2007). There are many plant proteases, which belong to several unrelated families. Protease performs various biological functions such as meiosis, embryogenesis, cuticle deposition, gametophyte survival, stomata development, and defense mechanisms(Van der, 2008). Based on the ability to hydrolyze native collagen the activity of enzyme extracted from the ginger rhizome is better than as compared to other plant cysteine proteases and different varieties of ginger rhizome could have different proteolytic activities(Kim et al., 2007). Ascorbic acid help in maintaining the stability of freshly extracted ginger rhizome protease from oxidation(Adulyatham & Owusu-Apenten, 2005).

On a commercial scale, proteases find many applications in major areas of food processing, detergent industries, leather, paper, textiles, brewing, etc (Poldermans, 1990). The use of protease enzyme in detergent depends on its compatibility with the detergent(Kumar et al., 2003). An enzyme that can be used in detergent must be active and stable in the alkaline solutions and should be thermally stable to be operative in a varied temperature range during washing(Bhosale et al., 1995). In the present paper, proteases extracted from plants i.e. Jasmine flower *(Jasminum sambac),* ginger rhizome *(Zingiber officinale)* and banana leaf *(Musa paradisiaca)* were proven to be stable over a wide range of parameters such as temperature and pH. Although, different proteases perform the same reaction but have a different catalytic mechanisms. Since these sources are easily available, a large number of proteases can be extracted at a cheaper cost(Subathra et al., 2012; Agrahari & Sharma, 2014). Hence, the current study can serve as a potential source of proteases, which further have great importance in the detergent industry.

2. MATERIALS AND METHODS

2.1. Plant Source

A fresh sample of Jasmine flower (*Jasminum sambac),* banana leaf (*Musa paradisiaca)* and ginger rhizome (*Zingiber officinale)* was collected around the area of Amity University Uttar Pradesh, Noida, India. The samples were washed gently with sterile distilled water.

2.2. Crude extract preparation

An equal amount of each sample was crushed using pestle and mortar. Phosphate buffer prepared of 0.1M concentration and pH 6 was maintained. Banana leaf extract was prepared by adding ice-cold acetone along with extraction buffer to remove pigments and lipids. The extract was subjected to centrifugation around 10,0000 rpm for 10 minutes at 4 ^{o}C.

2.3. Determination of protein concentration

The concertation of protein in the enzyme extract was determined using Folin's Ciocalteu reagent as per the standard procedure of Lowry et al., 1951. Standard protein, crystalline BSA (Bovine Serum Albumin) was used for standard curve preparation. The different concentrations of BSA were reacted with Folin's reagent. The absorbance was computed at 750 nm with the help of a UV Spectrophotometer of the blue color solution that was developed (Eppendorf Biospectrometer basic, SW- Version: 4.3.1.0).

2.4. Protease assay (casein assay)

The activity of extracted protease was determined using 1% (w/v) casein as substrate followed by the method of Cupp-Enyard, 2008. Absorbance readings were taken at 660 nm with the help of a UV spectrophotometer. To prepare a standard curve tyrosine was used. The enzyme activity is expressed as µg of tyrosine released per ml per min under standard conditions(Hagihara et al. 1958).

2.5. Effect of temperature on the activity of the enzyme

To determine the optimal temperature for the activity of protease, the reaction mixture was incubated at different temperatures such as 4^{o}C, 40^{o}C, 70^{o}C and 100^{o}C. Enzyme activity was recorded and determined by a standard curve.

2.6. Effect of pH on the activity of the enzyme

The enzymatic activity was computed at varied values of pH such as 5.27, 6, 7 and 9. The pH of reaction mixtures was adjusted using different buffers such as acetate buffer of pH 5.27, phosphate buffer of pH 6 and 7 and tris HCl buffer of pH 9. The activity of the reaction mixture was measured and determined by using a standard curve after it was kept for 30 minutes at 40^{o}C.

2.7. Effect of concentration of substrate on the activity of the enzyme

To estimate the effect of different concentrations of a substrate on the enzymatic activity of protease, casein (1% (w/v) was used as the substrate and was taken in the volume of 0.5, 1, 1.5, 2 and 2.5 ml. At 40^{o}C for 30 minutes the reaction mixture was incubated. The rate of tyrosine produced was measured with the help of the standard curve and the kinetic constants V_{max} and K_m of the product tyrosine released were estimated by double reciprocal plots(Ramachandran & Arutselvi, 2013; Lineweaver & Burk, 1934).

2.8. Ammonium sulfate precipitation method

For the partial purification of crude extract, ammonium sulfate was added to the culture supernatant to obtain 30%, 60% and 90% saturation, stirred for 30 minutes and was allowed to settle overnight at 4℃. The precipitate of the mixture was collected by centrifuging it at 8000 rpm for 20 minutes at 4℃. The pellet that was obtained was mixed with 0.1M phosphate buffer of pH 6. Protease assay and protein concentration were determined for each saturation and their specific activity was calculated.

2.9. Destaining property of the crude enzyme

To study the effectiveness of protease in stain removal and as an additive in detergent. 1% (w/v) detergent solution (Ghari detergent) was prepared. White muslin cloth was cut into pieces of
5 cm x 5 cm. The cloth pieces were kept in the Petri plates. 0.5 ml of each strain was put on the cloth pieces and the stained cloth pieces were dried in a hot air oven at 40℃ for 20 min(Alnahdi, 2012). The following sets were prepared:

1. 10 ml distilled water in Petri plate + cloth with stain (control).
2. 10 ml distilled water in Petri plate + cloth with stain + 1ml detergent (Ghari).
3. 10 ml distilled water in petri plate + cloth with stain + 1 ml detergent (Ghari) +1 ml of crude extract.
4. 10 ml distilled water in Petri plate + cloth with stain + 1 ml of crude extract.

For each set, the activity of the crude enzyme on stained cloth pieces at regular intervals of 5 minutes up to 20 minutes was visually examined.

2.10. Statistical analysis

The data obtained were evaluated using the Graph Pad Prism (version 8) software. Unpaired 't-test was used as comparative analysis. The probability value was found less than 0.05 ($P<0.0001$) and was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Protein content and protease activity

The total protein content was assessed in the crude extract as well as at 60% salt saturation in jasmine flower, gingers rhizome and banana leaf. Results indicated that the total protein content in the crude extract was highest in jasmine

flower (720 mg/ml) followed by ginger rhizome (448 mg/ml) and banana leaf (440 mg/ml) vice versa similar trend was also observed at 60% salt saturation in which the total protein content was highest in jasmine flower (483 mg/ml) followed by ginger rhizome (275 mg/ml) and banana (160 mg/ml) leaf (Table 3.1). The total protease enzyme activity was observed in all these three materials. Results indicated that the highest enzyme activity at 60% salt saturation was observed in jasmine flower (209 U/ml) followed by ginger rhizome (62 U/ml) and banana leaf (32.8 U/ml) while in enzyme activity in the crude extract was observed 240 U/ml, 120.4 U/ml and 32.8 U/ml in jasmine flower, ginger rhizome and banana leaf respectively (Table 3.1). The leaf is the main part of the plant where protein is synthesized and it was stored in different plant parts like rhizome, root, flower, fruit and seed. According to Chinnadurai et al. (2018), the older leaf has a higher content of protein than the younger leaf. Thus in the present finding jasmine flower showed the highest protein content and protease activity than the ginger rhizome and banana leaf.

Table3.1. Partial purification of protease from the crude extract of jasmine flower, ginger rhizome and banana leaf in which higher specific activity was observed

Plant source	Extract	Total protein contents (mg/ml extract)	Total enzyme activity (U/ml)	Specific activity (U/mg)	Purification folds	Yield (%)
Jasmine flower	Crude enzyme	720	240	0.330	0	100
	60% salt saturation	483	209	0.432	1.30	87.3
Ginger rhizome	Crude enzyme	448	120.4	0.268	0	100
	60% salt saturation	275	62	0.516	1.92	51.4
Banana leaf	Crude enzyme	440	76.4	0.173	0	100
	60% salt saturation	160	32.8	0.205	1.18	42.9

3.2. Effect of temperature and pH on the activity of enzyme

The protease enzyme activity of partially purified extract from the ginger rhizome was found to be stable at a temperature ranging from 40°C to70°C and showed maximum activity at 70°C. A similar finding was also observed by Thompson et al.(1973) while the other two samples were found to be stable at a temperature ranging from 40°C to 70°C. After 70°C the proteolytic capability of all the three sources was declined (Fig. 3.1a). The crude extract of jasmine flower and ginger rhizome showed its maximum activity at pH 7 while banana leaf showed its maximum activity at pH 6 (Fig. 3.1b). Thus, jasmine flower and ginger rhizome are neutral proteases while the banana leaf is an acidic protease. This result is in agreement with that of the previous finding by a different worker in the case of ginger rhizome using phosphate buffer at pH 7(Kim et al. 2007; Nafi et al. 2013).

3.3. Effect of concentrations of a substrate on the activity of enzyme

The effect of different concentrations of a substrate on the protease activity showed that the crude extract of jasmine flower showed higher V_{max} having a value of 3.27 µM/sec, followed by ginger rhizome and banana leaf having V_{max} values as 2.405 µM/sec and 1.628 µM/sec respectively. Banana leaf showed a higher K_m value having a value of 0.0091 mM/l, followed by Jasmine and Ginger's rhizome having values Km as 0.003 mM/l and 0.00531 mM/l respectively.

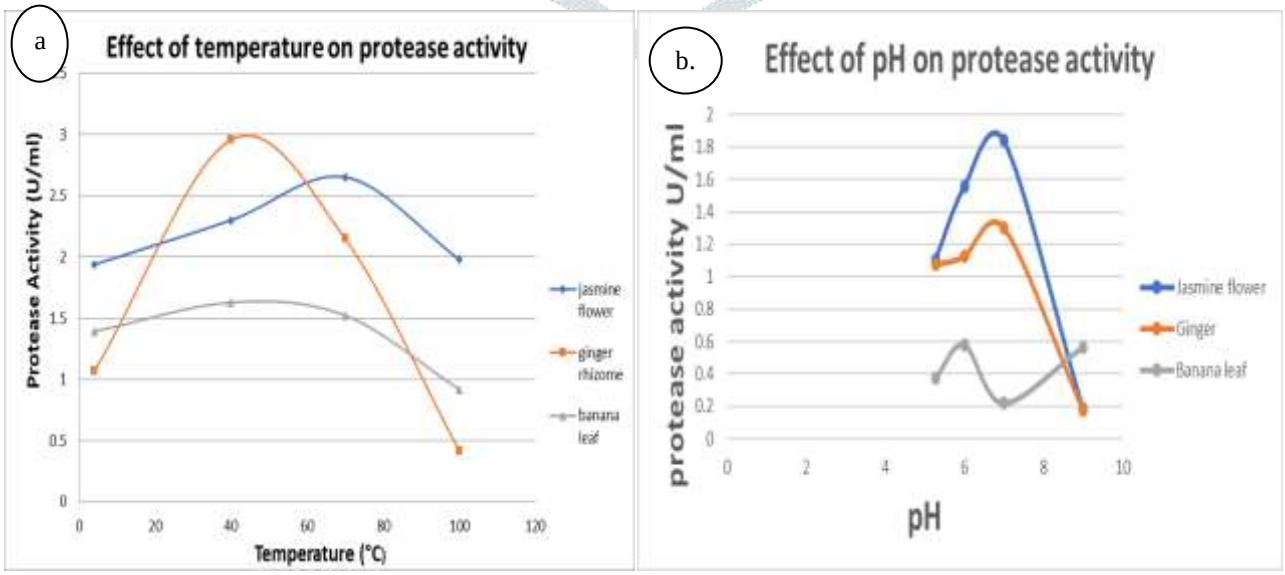

Figure 3.1. Effect of temperature (a) and pH (b) on the protease activity of the crude extract partially purified from jasmine flower, ginger rhizome and banana leaf.

3.4. Destaining efficiency of the crude enzyme

Destaining efficiency of protease enzyme partially purified from the jasmine flower, ginger rhizome and banana leaf was studied without detergent (E1, E2 and E3 respectively) and with detergent (D+E1; D+E2; D+E3 respectively) for the destaining of roli, coffee, turmeric, tea and ink from the cotton cloth. Results indicated that jasmine flower protease(E1) was showed medium destaining for coffee, turmeric and tea stains while the low for roli and very low for ink stain but when enzyme used with detergent (D+E1) which enhanced the destaining efficiency as very high for coffee, high for turmeric and tea while the low for ink(Table 3.2). Protease from the ginger rhizome (E2) showed very low destaing for coffee and ink and low for roli and tea while medium destaining in turmeric. Protease from a ginger rhizome with detergent (D+E2) showed very high and high destaining for coffee and turmeric respectively while medium for tea and ink but low for roli. Protease from banana leaf (E3) was showed high destaining for turmeric, medium for ink, low for coffee and tea and very low for roli. Protease from the banana leaf with detergent (D+E3) showed very high destaining for turmeric and high for ink, medium for coffee and tea while the low for roli (Table 3.2). Thus, the destaining property was greater in the crude enzyme of jasmine flower and ginger rhizome due to these plant sources containing higher protease activity than the crude extract of banana leaf.

Table 3.2. Destaining efficiency (out of 5) of the crude extract of jasmine flower, ginger rhizome and banana leaf.

Stains	Destaining efficiency					
	E1	D+E1	E2	D+E2	E3	D+E3
Roli	++	+++	++	++	+	++
Coffe	+++	+++++	+	+++++	++	+++
Turmeric	+++	++++	+++	++++	++++	+++++
Tea	+++	++++	++	+++	++	+++
Ink	+	++	+	+++	+++	++++

E1: Jasmine flower; E2: Ginger rhizome; E3: Banana leaf; D: Detergent (Ghari)

Destaining efficiency notation: +(very low), ++(low), +++(medium), ++++(high), +++++(very high)

4. CONCLUSION

Although, there are many promising protease sources are in the market for enzyme production, but still, research community preferably isolates and study new resources because there could be alternative for commercial use. In the present investigation, plant sources such as Jasmine flower *(Jasminum sambac)*, ginger rhizome *(Zingiber officinale)* and banana leaf *(Musa paradisiaca)* were partially purified and biochemically analyzed. As, ginger is well known for its several antibacterial and medicinal properties; jasmine has application in relieving abdominal pain, liver diseases and helps in the treatment of cancer. Moreover, banana leaves are utilized for their healing properties and also the antioxidants in them play a vital in fighting against cancer. The protease activity of the jasmine flower and banana leaf has not been reported before. Among the three-plant source, the crude enzyme of the jasmine flower showed higher protease activity and showed better stain removal of the coffee stain. Hence, the jasmine flower is considered as the viable source of protease because it is thermostable, in-expensive and easily available.

5. REFERENCES

[1] Adulyatham, P., Owusu-Apenten R. 2005. Stabilization and partial purification of a protease from ginger rhizome (*Zingiber offinale* Roscoe). Journal of Food Science., 70(3), 231-234.

[2] Agrahari, S. and Sharma, N. 2014. Extraction and characterization of protease from senesced leaves of Papaya (*Carica papaya*) and It's application. International Journal of Genetic Engineering and Biotechnology. 5, 29-34.

[3] Alnahdi, H.S. 2012. Isolation and screening of extracellular proteases produced by new isolated Bacillus sp., Journal of Applied Pharmaceutical Science. 2(9), 71.

[4] Annamalai, N., Rajeswari M.V., Balasubramanian, T. 2014. Extraction, purification and application of thermostable and halostable alkaline protease from Bacillus alveayuensis CAS 5 using marine wastes. Food and Bioproducts Processing. 92(4), 335-342.

[5] Bhosale, S.H., Rao M.B., Deshpande V.V., Srinivasan M.C. 1995.Thermostability of high-activity alkaline protease from Conidioboluscoronatus (NCL 86.8. 20). Enzyme and Microbial Technology. 17(2), 136-139

[6] Cupp-Enyard, C. 2008. Sigma's Non-specific Protease Activity Assay-Casein as a Substrate. Journal of Visualized Experiments, 19.1-2. doi:10.3791/899

[7] Hagihara, B., Matsubara, H., Nakai M., Okunuki K. 1958. Crystalline bacterial proteinase., The Journal of Biochemistry. 45(3), 185-194.

[8] Kim, M., Hamilton S.E., Guddat L.W., Overall C.M. 2007. Plant collagenase: unique collagenolytic activity of cysteine proteases from ginger. Biochimica et BiophysicaActa (BBA)-General Subjects. 1770(12), 1627-1635.

[9] Kim, M. Hamilton, S.E., Guddat, L.W. 2007. Overall, C.M. Plant collagenase: Unique collagenolytic activity of cysteine proteases from ginger. Bba-Gen. Subjects. 1770, 1627–1635.

[10] Kumar, D., Bhalla, T.C. 2003. Bacillus sp. APR-4 protease in protein recovery from waste bones., National Academy Science Letters. 26(11-12), 332-335.

[11] Lineweaver, H. and Burk, D. 1934. The determination of enzyme dissociation constants., Journal of the American Chemical Society. 56(3), 658-666.

[12] Lowry, O.H., Rosebrough, N. J., Farr, A. L., Randall, R. J. 1951. Protein measurement with the Folin phenol reagent.,Journal of Biological Chemistry. 193(1), 265-275.

[13] Mahajan, R., Chaudhari, G., Chopadaa, M. 2016. Report on Biotechnological applications of proteolytic enzymes from lattices of euphorbian plants.,Journal of Applied Biotechnology Reports. 2(4), 333-337.

[14] Merheb, C.W., Cabral, H., Gomes, E., Da-Silva, R. 2007. Partial characterization of protease from a thermophilic fungus, Thermoascusaurantiacus, and its hydrolytic activity on bovine casein., Food Chemistry. 104(1), 127-131.

[15] Nafi, A., Foo, H.L., Jamilah, B., Ghazali, H..M. 2013. Properties of proteolytic enzyme from ginger (Zingiber *officinale* Roscoe). International Food Research Journal. 20, 2133–2138.
[16] Poldermans, B. 1990..Proteolytic enzymes.,Enzymes in Industry. 108-118.
[17] Ramachandran, N., Arutselvi, R. 2013. Partial purification and characterization of protease enzyme from Nomuraearileyi. International Journal of Pharmaceutical Sciences and Research. 4(9), 3460
[18] Subathra, K., Jeevitha, G.C., Deepa, R. 2012. Aqueous two-phase extraction of protease from neem leaves [*Azadirachta indica*]. International Journal of Chemical Sciences and Applications. 3(3), 346-351.
[19] Thompson, E. H., Wolf, I.D., Allen C.E. 1973. Ginger rhizome- A new source of proteolytic enzyme.,Journal of Food Science. 38(4), 652-655.
[20] Van der, Hoorn R. A. 2008. Plant proteases: from phenotypes to molecular mechanisms. Annual Review Plant Biology ,59, 191-223.