



Crinumin, a novel substitute of chymotrypsin may control pancreatic insufficiency and inflammation: Purification and characterization

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Abstract

Crinumin, a glycosylated serine protease with chymotrypsin like catalytic specificity was purified from a medicinally important plant *Crinum asiaticum* of family Amaryllidaceae. Ethno-botanical information and Pharmacological studies confirming about the presence of active bio- molecules in the Crinum played crucial role in injury, inflamed joint, local pain and arthritis. Biomedical research suggests chymotrypsin was not only used as digestive aid but also helpful in the above disease. These findings support towards the crinumin may be the active bio-molecule. Crinumin shows activity over a wide range of pH (4.5-11.5 and optimum at 8.5), temperature (75 °C and optimum at 70 °C) and is also functional against chaotrophs, organic solvents, and detergents even after prolonged exposure. The molecular mass (67.7kDa), extinction coefficient (17.7) and isoelectric point (6.9) were also estimated. The denatured natural substrates, such as casein, azocasein, azoalbumin and haemoglobin were hydrolysed by crinumin with very high specific activity. The enzyme also showed amidolytic activity against synthetic substrates, N-succinyl-Phe-p-nitroanilide and -leucine-p-nitroanilide. The apparent Km, Vmax and Kcat values obtained from the Lineweaver-Burk plot were 5 × 10⁴ M, 0.316 M/min and 0.73 with N-succinyl-L-phenylalanine-p-nitroanilide as substrate. The proteolytic activity of the enzyme is inhibited by PMSF but not by SBTI make it more special and useful than other serine proteases being used in the food industry. Easy and economic purification with high yield (33%), stability and activity in adverse conditions with chymotrypsin like functioning make it better among known chymotrypsin enzymes from different sources.

Introduction

Chymotrypsin, a type of serine protease is a digestive enzyme preferentially hydrolyzes peptide amide bonds where the carboxyl side of amide bond is a tyrosine, tryptophan or phenylalanine (1).

Chymotrypsin is most often used for proper digestion of proteins and also crucial for making the small intestine free from parasites. As an anti-inflammatory agent, the chymotrypsin is used to treat inflammation and reduce swelling (i.e., soft tissue injuries, acute traumatic injuries, sprains, contusions, hematomas, infections, edema of the eyelids and genitalia, muscle cramps, and sports injuries) (2).

Crinum asiaticum, a medicinal plant is also used to treat inflamed joints and sprains (3, 4, 5), for the treatment of hemorrhoids, contusions, fractures, luxations (4), and earache (3). Its extract is anti-inflammatory (5).

Similar role of the plant and chymotrypsin in many aspects combindly indicating the presence of a serine protease with chymotrypsin like specificity, as an active constituent of the plant extract.

Aim

Identification, purification and characterization of the protease constituents of the plant, if any.

Why interesting

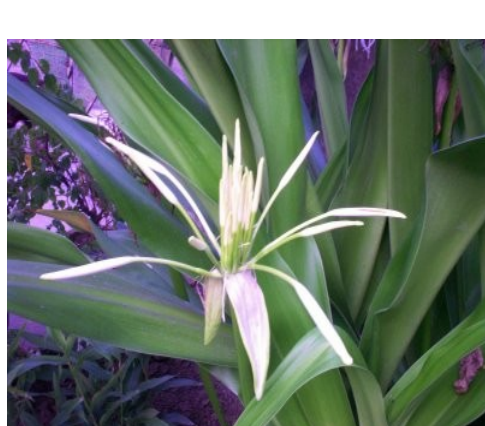
Although the serine protease with Chymotrypsin like specificity is widely distributed in nature, but the enzymes from plant sources are better suited to understand its basic mechanism in biomedical science due to broad substrate specificity, high stability in extreme conditions, good solubility, and activity over a wide range of pH and temperature .

Efforts

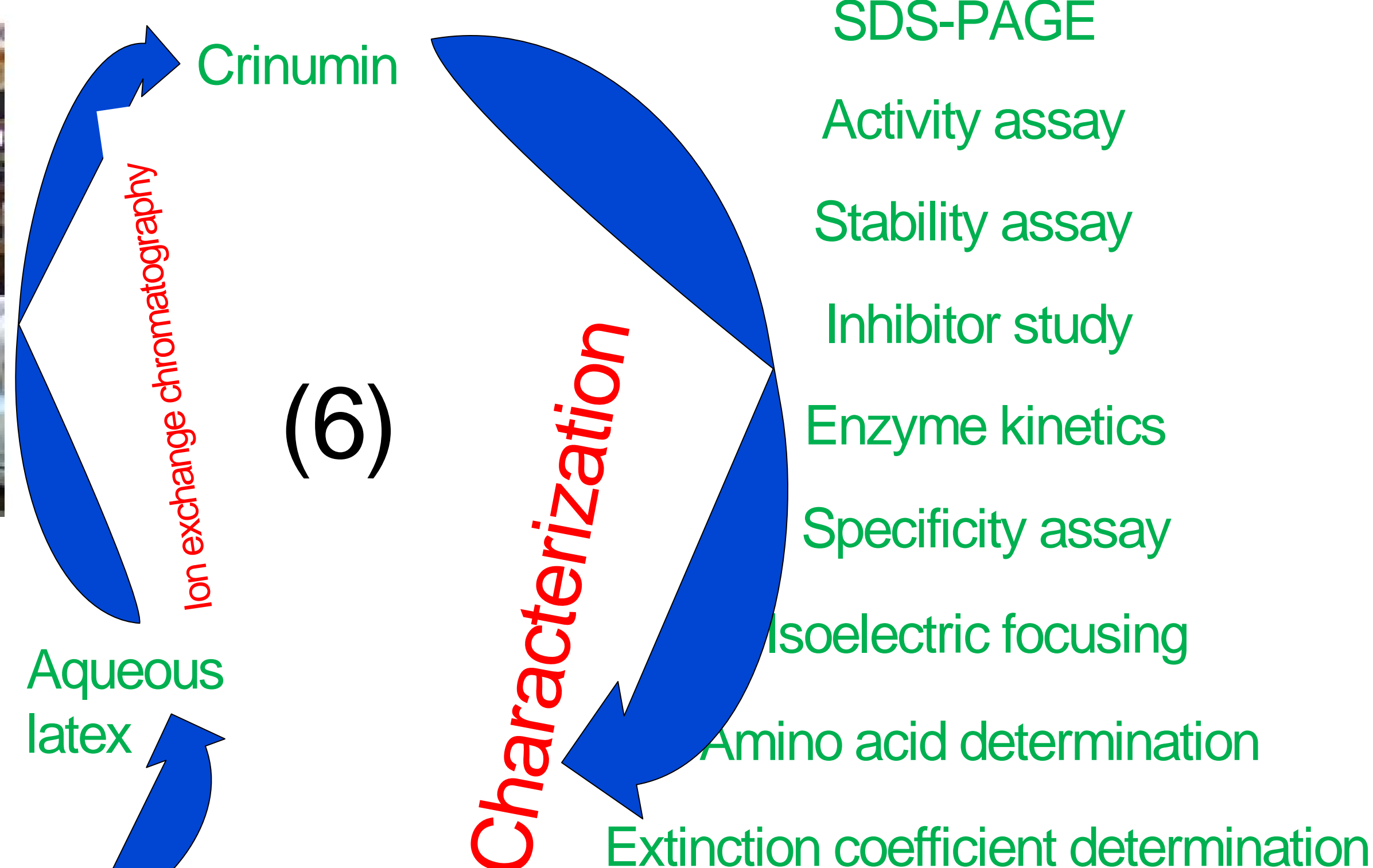
The efforts were focused on identifying and purifying the protein constituents of plant and while screening different parts of the plant, the aqueous latex was found to possess a considerable amount of proteolytic activity.

Here, we report the purification and physicochemical characterization of crinumin, a serine protease from the aqueous latex of *C. asiaticum*

Methods



Crinum asiaticum



Results

Step	Volume (ml)	Total protein (mg)	Total activity (Units)	Specific activity (Units/mg)	Fold purification	Recovery(%)
Crude	352.00	66.83	2746.78	41.10	1.0	100.00
CM-Sepharose	56.7	22.27	1780.00	79.93	1.94	33.32

Table 1. Purification of crinumin from the *Crinum asiaticum*

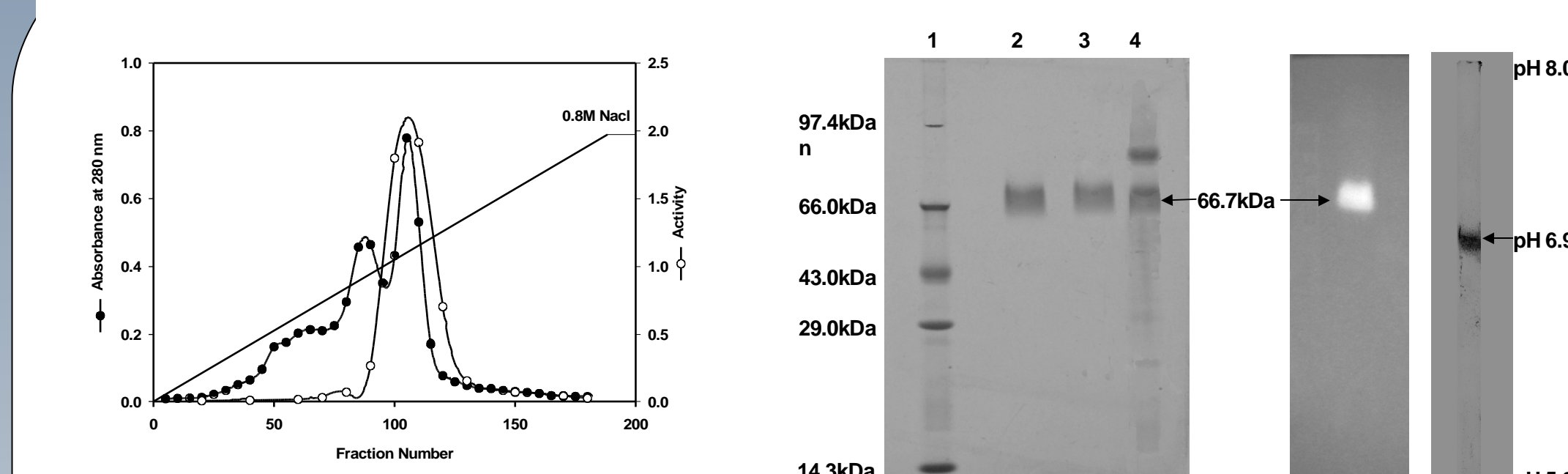


Figure 1. The bound proteins were eluted and fractions were assayed for activity () and for protein content (●). Figure 2. (a) SDS-PAGE of proteins includes molecular weight marker (lane 1), inactive (incubated with PMSF) protease (lane 2), active pure protease (lane 3), and crude latex (lane 4). (b) Zymogram pure protease (c) Isoelectric focusing of crinumin

Enzyme	Mol. Mass (kDa)	1% 280nm	pl	Glyco sylation (%)	Tyr	Tr p	Free cys	Tota l cys
Crinumin	67	17.7	6.9	13	27	14	1	15
Milrin	51.4	29	7.2	7-8	14	23	2	14
Cryptolepain	50.5	26.4	6.0	6-7	41	15	0	08
Carnein	80.2	37.1	5.6	No	76	35	3	07
Wrightin	57.9	36.4	6.0	8	75	20	1	09
Ara12	76.1	N	N	N	N	N	N	N
Euphorbains Ic	70	N	8.0	yes	27	13		20
Protease	67.0	N	N	N	N	N	N	N
Protease	50	N	N	N	N	N	N	N
Cucumisins	67	N	N	N	N	N	N	N

Table 2. Physicochemical properties of crinumin in comparison with other plant serine proteases

Synthetic Substrate	Activity
N-succinyl-Phe-p-nitroanilide	+
-Leucine nitroanilide	+
N- -Benzoyl-dl-Arginine-p-nitroanilide	-
Ala-Ala-Ala-p-nitroanilide	-
P-Nitrophenyl acetate	-
L- -Glutamyl-p-nitroanilide	-
L-Ala-p-nitroanilide	-

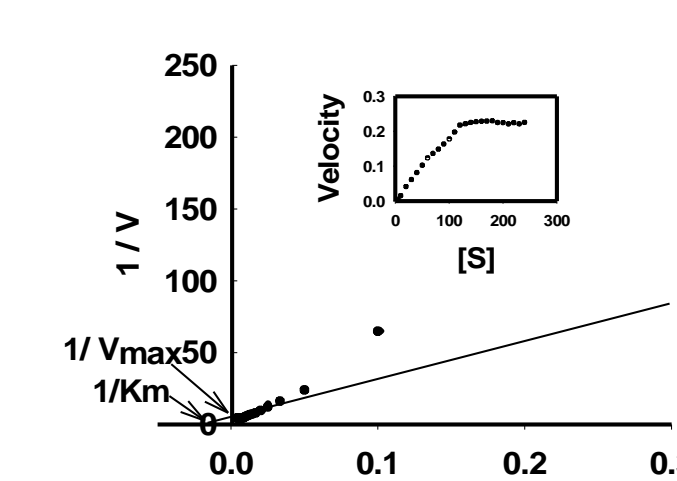


Figure 3. Effect of substrate concentration on reaction velocity (inset). The apparent Km, Vmax and Kcat values obtained from the Lineweaver-Burk plot (1/(S) vs 1/(V)) were 5 × 10⁴ μM, 0.316 μM min⁻¹ and 0.73 respectively with N-succinyl-L-phenylalanine P-nitroanilide as a substrate.

Table 4. Activity of crinumin with different synthetic substrates

Conditions	Concen tration ^a	%Residua l activity
Protease Inhibitor	IAA	1mm 97
	Mercuric chloride	1mm 99
	EDTA	1mM 99
	o- phenanthroline	1mM 95
	PMSF	1mM 5
	SBTI	1mM 95
Chaotrophs	GuHCl	3.0M 98
	Urea	6.0M 99
Organic solvents	Methanol	50% 98
	Ethanol	40% 92
	Isopropanol	90% 87
	Butanol	40% 87
	Acetonitrile	70% 95
	DMSO	50% 97
	Dioxane	90% 98
	Dete rgen	SDS 0.05% 88
	Triton X-100 2% 98	
	Exaline 0.3% 89	

Table 3. Activity of crinumin under various Conditions

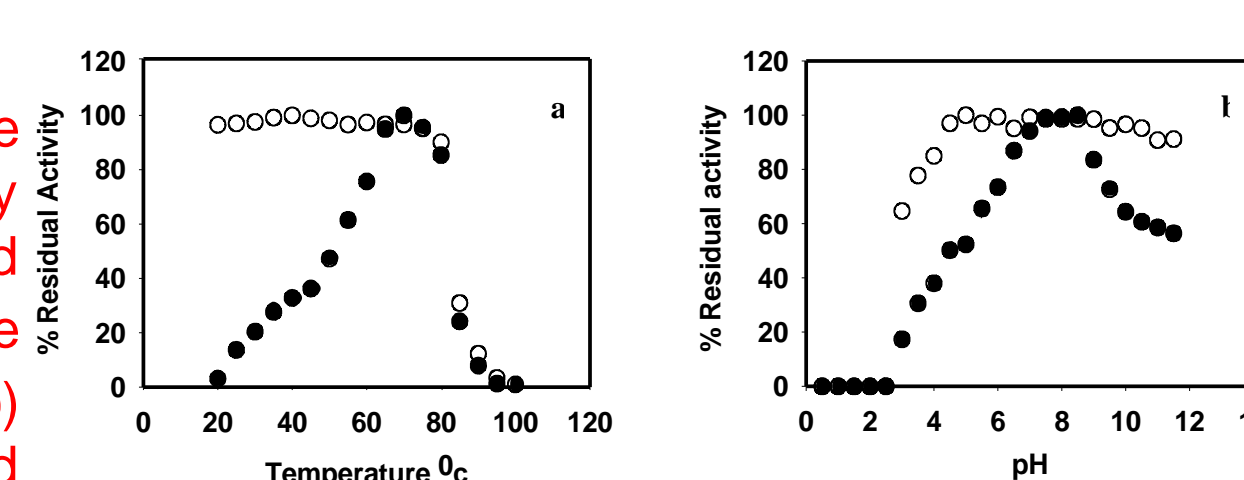


Figure 4. Effects of temperature (a) and pH (b) on stability () and optimum (●).

Conclusion

Crinum asiaticum, a medicinal plant contain crinumin, a serine protease with chymotrypsin like specificity.

Its purification is easy and economic with high yield.

Crinumin is stable as well active in adverse conditions.

Enzyme is functional even with inhibitor having proteinatious nature.

Bibliography

- Appel W. "Chymotrypsin: molecular and catalytic properties". *Clin. Biochem.* (1986)19 (6): 317-22.
- Sims, J. (2001). Encyclopedia of Alternative Medicine. "Chymotrypsin" Page 1 http://findarticles.com/p/articles/mi_q2603/is_0002/ai_2603000281
- International Cyber Business Services, Inc., Herb Information (*Crinum asiaticum*), (1998-2000) http://www.holisticonline.com/Herbal-Med/_Herbs/h_crinum-asiaticum.htm.
- Nguyen Van Dan & Doan Thi Nhu, Medicinal Plants in Vietnam ,World Health Organisation (1989) ISBN 92.9061.101.4.
- Awatef M. Samud, M. Zaini Asmawi, Jagdish N. Sharma and Ahmad Paupi M. Yusuf, Anti-inflammatory activity of *Crinum asiaticum* plant and its effect on bradykinin-induced contractions on isolated uterus, *Immunopharmacol.* (1999), 43(2-3): 311-316.
- Singh, K.A., R.K., Rao,G.R.K., Jagannatham, M.V. Crinumin, a chymotrypsin like but glycosylated serine protease. Purification and Physicochemical characterization, *Food Chemistry.* (2010),119:1352-1358.



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