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AMINO ACIDS, ENZYME ACTIVITY AND EFFECT OF CHEMICAL AGENTS, METALLIC SALTS ON THE STABILITY OF α-AMYLASE AND PROTEASE FROM *ALOE BARBADENSIS* MILLER

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Abstract

The investigation on Aloe vera was carried out and the results revealed that out of 22 amino acids 14 were found in dried and fresh skin, gel of Aloe vera leaf in which 6 were essential and the rest 8 were non-essential. The results showed that a maximum content of amino acid in total dried leaf i.e. glutamic acid was 660 mg/100 g. Enzyme activity was maximum in total dried leaf rather than fresh total leaf, skin and gel parts of leaf. The enzyme protease, amylase and urease activity were 1.93, 2.92 and 0.13 U/gm, respectively in total dried leaf of Aloe vera. The activity of urease was not detected in fresh gel. The absence of urease activity indicates that no toxicity was present there. In dried Aloe vera powder, the presence of 0.5M calcium chloride ($CaCl_2$) increased 39.85% activity in both the α-amylase and protease enzymes. The activities of two enzymes were completely destroyed in the presence of 8 M urea and 30% acetic acid. Metallic salt (Mg^{2+}) increased the enzymes activity while Zn^{2+}, Cu^{2+} and Fe^{2+} reduced the activity of both enzymes remarkably.

Introduction

Aloe vera (*Aloe barbadensis* Miller) belongs to Liliaceae is a succulent plant, typically growing in tropics or sub tropics and may tend to grow 80 - 100 cm long (Kulveer *et al*. 2011). It was indigenous to the Mediterranean regions and Africa but also distributed in the different parts of India. It has a large history of being used for thousands of years. Aloe vera is cultivated almost everywhere in the world specially for medicinal purposes (Mukherjee *et al*. 2006). The diversified products which are made from mucilaginous tissue i.e. Aloe vera gel are also used in cosmetics and conventional pharmaceutical field. It is the most widely used plant species both commercially and therapeutically. The solid plant parts possess different amino acids, enzymes, vitamins, minerals and phenolic compounds (Burn 2003). Various amino acids and enzyme activity of fresh and dried plant parts of Aloe vera were observed. Amino acids are biologically important molecules (Lehninger 1996). Of the 22 amino acids, 8 - 9 are known as essential amino acids. Amino acids are used for a variety of applications in drug, cosmetic and food industry (Rajeswari 2012). Available literature demonstrated that no study on amino acids and enzyme activity were done on individual parts of Aloe vera plants. Thus, the present efforts have been made to determine the amino acids and enzyme activity of fresh and dried Aloe vera plant parts and also the effect of chemical agents, metallic salts on the stability of α-amylase and protease of Aloe vera powder.

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Materials and Methods

The Aloe vera plants selected for this study was collected from the local market of Dhaka city. The samples used were dried powder, dry total (skin + gel), fresh (skin + gel), only gel (fresh) and only skin (fresh). The protein content of different plant parts was determined according to Micro-Kjeldahl method (AOAC 2000).The samples were ground in a mortar with cold 0.1M phosphate buffer of respective pH, for amylase (6.7) and for protease citrate buffer (5.5) and finally crushed into paste using a homogenizer. The temperature was maintained at 4ºC by putting ice in the outer chamber of the homogenizer. The suspension was then filtered through a few layers of cheese cloth (trade ford) in the cold room. The filtrate was collected and clarified further by centrifugation in a refrigerated centrifuge at 10,000 rpm for 20 min at 4°C and used as crude enzyme extract.

The protease activity was assayed following the modified method using haemoglobin as substrate (Mahadevan and Sridhar 1982) and was measured by estimating the amount of leucine released from haemoglobin. The amount of leucine released was calculated from the standard curve prepared with leucine. One unit of protease activity was defined as the amount required for liberating 1 mg of leucine in 30 min at 37°C. After 30 min of incubaton, the reaction was stopped by adding 3 ml of 5% trichloro acetic acid (TCA). The sample was then centrifuged and filtered and prepared for counting. From the supernatant, 0.5 ml was taken in a test tube and 0.5 ml distilled water and 1 ml ninhydrin solution were added. The sample was heated for 20 minutes in a boiling water bath at a temperature of 100°C. Thereafter, the sample was cooled and 5 ml of diluents (n-propanol : distilled water, 1 : 1) was added and the optical density (OD) was measured at 570 nm with a GBC Cintra 6 spectrophotometer.

Amylase activity was assayed using 1% starch solution (1g in 100 ml of 0.1M phosphate buffer, pH 6.7 Jayaraman (1981). The activity was measured by estimating the release of maltose. The amount of maltose released was calculated from the standard curve prepared with maltose. One unit of amylase activity was defined as the amount required for liberating 1 mg of maltose in 15 min at 37°C.

The urease activity was assayed by the method of Jayaraman (1981) where urea was used as substrate. It was estimated by the release of ammonium calculated from the standard curve. One unit of urease activity was defined as the amount of required for liberating 1µg of ammonium in 15 minute at 55°C.

Chemicals and metallic salts such as calcium chloride, urea and acetic acid at various concentrations were added to 5.0 ml of both amylase and protease enzymes extract solutions from dry, total (skin + gel) and incubated for 10 minutes at 20°C. The mixtures were again incubated with the substrate for 15 min at 37°C and the enzyme activities were assayed.

The amino acid detected by amino acid analysis system instruction manual (Anon.1993).

Results and Discussions

The results on protein and amino acids of fresh and dry Aloe vera leaves are shown in Table 1, which revealed that among amino acids content, aspartic acid was maximum (17.80 mg/100 g) followed by threonine (9.50 mg/100 g), lysine (8.50 mg/100 g) and glycine (8.40 mg/100 g) in fresh gel but the content of histidine was minimum (1.8 mg/100g). In fresh skin, the glycine content was maximum (157.10 mg/100 g) followed by alanine, aspartic acid etc. Furthermore, in *Aloe vera* fresh total leaves (skin + gel), glutamic acid was recorded (90.60 mg/100 g) followed by alanine (70.00 mg/100 g), lysine (39.93 mg/100 g) and glycine (38.96 mg/100 g).

In dried total Aloe vera leaves (skin + gel), showed better amount of all essential amino acid contents than fresh conditions. The highest amount of amino acid content was found as glutamic

acid followed by aspartic acid, glycine etc. Thus, it was observed that the contents or amount of different amino acids were different. No specific amino acid content was found as maximum in fresh and dried *Aloe vera* leaf parts.

Table 1. Protein and amino acids of *Aloe vera* leaf.

Protein (%) and amino acids (mg/100 g)	Results of *Aloe vera*			
	Dry, total (skin + gel)	Fresh, total (skin + gel)	Skin (Fresh)	Gel (Fresh)
Protein	10.50	0.67	1.08	0.13
Aspartic acid	560.00	27.70	78.20	17.80
Threonine	280.00	16.60	52.40	9.50
Serine	480.00	18.20	36.00	5.60
Glutamic acid	660.00	90.60	48.10	6.80
Glycine	540.00	38.96	157.10	8.40
Alanine	140.00	70.00	85.70	4.67
Valine	240.00	15.97	38.90	3.40
Methionine	330.00	15.10	20.70	2.92
Isoleucine	260.00	23.40	32.80	7.68
Leucine	200.00	11.80	17.60	2.54
Tyrosine	390.00	16.90	26.90	3.56
Histidine	280.00	8.82	15.40	1.86
Lysine	450.00	39.93	56.28	8.50
Arginine	320.00	32.70	40.38	6.79

The data on enzyme activity of *Aloe vera* fresh and dried leaf are summarized in Table 2. Among three enzyme activities, the most interesting feature was observed in case of urease. In fresh gel of Aloe vera leaf, the urease activity was not detected. But in fresh skin, gel, total leaf (skin + gel) and dried total leaf (skin + gel), the urease enzyme activity was obtained as trace amount. The α-amylase was found to be maximum (2.92 U/g) in dried leaf and minimum in gel, skin of fresh and dried Aloe vera leaves. Maximum (1.93 U/g) protease activity was seen in dried leaves and minimum in fresh and dried skin and gel of leaf parts. No reports were available on protease activity of Aloe vera fresh and dried leaf.

Table 2. Enzyme activity of *Aloe vera* leaf.

Enzyme activity (U/gm)	Results of *Aloe vera*			
	Dry, total (skin + gel)	Fresh, total (skin + gel)	Skin (Fresh)	Gel (Fresh)
Protease	1.93 ± 0.015	0.60 ± 0.014	0.92 ± 0.015	0.43 ± 0.015
áα - amaylase	2.92 ± 0.015	1.99 ± 0.01	1.20 ± 0.0015	0.39 ± 0.014
Urease	0.13 ± 0.001	0.022 ± 0.002	0.021 ± 0.0005	Not Detected

Data are represented as mean ± standard deviation of triplicate measurements.

The effect of calcium, urea and acetic acid on the activities of α-amylase and protease are presented in Table 3. The activity was gradually increased with the increase of calcium concentration and maximum (39.85%) increase was observed due to 0.5 M calcium chloride ($CaCl_2$). As shown in the Table 3, the activity of α-amylase and protease decreased gradually with increasing concentration of urea and acetic acid while the activity of the enzymes was destroyed completely in the presence of 8 M urea and 30% acetic acid.

The effect of various metallic salts on the activity of α-amylase and protease are shown in Table 4. The results revealed that the presence of Mg^{2+} increased the activity of α-amylase and protease. On the contrary, the other metallic salts such as Zn^{2+}, Cu^{2+}, Fe^{2+} reduced the activity of both the enzymes remarkably.

Table 3. Effect of calcium, urea and acetic acid on the activities of α-amylase and protease of *Aloe vera* powder.

Concentration of $CaCl_2$ (M)		0.00	0.005	0.01	0.05	0.10	0.50
Relative activities (%)	α-amylase	100.00	110.36	117.28	126.40	132.68	139.85
	Protease	100.00	108.62	115.36	120.26	124.62	133.52
Conc. of urea (M)		0.00	1.00	2.00	4.00	6.00	8.00
Relative activities (%)	α-amylase	100.00	92.18	78.40	45.12	22.46	0.00
	Protease	100.00	90.44	72.26	41.52	15.48	0.00
Conc. of acetic acid (%)		0.00	2.50	5.00	10.00	20.00	30.00
Relative activities (%	α-amylase	100.00	87.28	76.32	45.88	21.46	0.00
	Protease	100.00	85.68	70.24	44.28	19.16	0.00

Table 4. Effect of various metallic salts on the activities of α-amylase and protease of *Aloe vera* powder.

Test salts	Concentrations (M)	Relative activities	
		α-amylase	Protease
None	-	100.00	100.00
$MgCl_2$	0.001	103.58	104.32
	0.002	107.14	109.48
$ZnCl_2$	0.001	83.26	86.92
	0.002	72.42	77.64
$CuCl_2$	0.001	85.72	73.25
	0.002	74.94	62.58
$NaCl_2$	0.001	100.00	99.28
	0.002	99.26	98.56
KCl_2	0.001	100.00	100.00
	0.002	100.00	100.00
$FeCl_2$	0.001	68.14	63.38
	0.002	57.46	51.64

The amino acids are building block of protein (Lehninger 1996). Human requires 22 amino acids and the body can synthesize all of them except for eight essential amino acids which our body gets from different food/drinks (Agary 2005, Barcroft and Alasdair 1999). The Aloe vera fresh and dried total leaf, skin, gel can serve as the maximum source of major essential amino

acids. Every essential amino acid is available in Aloe vera and they include isoleucine, leucine, lysine, methionine, threonine and valine. Some of the other non-essential amino acids are also found in Aloe vera (Barcroft and Alasdair 1999). In this way, the amino acid contents of fresh and dried Aloe vera leaves, skin etc. can influence human brain function. In case of α-amylase activity, the results were partially in accordance with the findings of Ahmed *et al*. (2013). No reports are available on protease activity of Aloe vera fresh and dried leaf parts. The activity of α-amylase and protease of dry Aloe vera powder (skin + gel) were totally destroyed by 8.0 M urea and 30% acetic acid which are in agreement with the results of Rahman *et al.* (2001).The presence of metallic salt (Fe^{2+}) reduce the activities of both enzymes. On the other hand, Mg^{2+} increased the enzyme activities about 3 - 9%. Other metallic salts such as Zn^{2+}, Cu^{2+} ,K^{+} and Na^{+} showed very little inhibitory effect on the activities of α-amylase and protease, which are partially in accordance with the results of Akand *et al.* (2003).

Aloe vera is undoubtedly a natural unique medicinal herb. Overall, the observations of the present studies have given a brief information regarding the amino acid composition, enzyme activity of fresh and dried Aloe vera leaf parts. However, this is a preliminary study and a further intensive scientific studies on the enzyme activity of this valuable medicinal plant is required to promote its utilization large scale.

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