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Research Article

EXTRACTION AND PURIFICATION OF PAPAIN ENZYME FROM PAPAYA LEAF AND THE PHYTOCHEMICAL COMPONENTS OF THE LEAF

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ABSTRACT

Papain is a proteolytic enzyme present in papaya that breaks down proteins and has a number of food processing applications. The objective of this study was to extract papain enzyme by using grinding and ultrasonication extraction techniques and determination of the phytochemical components of the leaf. The fresh papaya leaf was collected from locally grown papaya plant. Purification of the extracted papain was done by using ammonium sulfate and sodium chloride precipitation. The concentration of the enzyme was then determined. Papain enzyme was identified by using casein protein. The prolytic activity of the enzyme was also determined according to the procedure of Bradford method with slight modification. The concentration of extract papain was from 0.054 - 0.002 mg/ml. The maximum mean value was shown by the grinded sample and the minimum mean concentration was 0.002 mg/ml obtained from the sonicated sample within a temperature of 50^{o}C for 20min. The maximum mean absorbance for enzyme activity was shown by sonicated sample at 1h by 60^{o}C and the minimum mean absorbance was from the sonicated sample within temperature of 50^{o}C for 20min. Inrelative comparison proteins and alkaloids are the most abundant component of papaya leaf. Papaya leaves contain papain enzyme, and the different methods of isolation, concentration and application of the enzyme needs further study.

Keywords: *Carica papaya*, concentration of papain, Proteolytic enzymes.

INTRODUCTION

Enzymes are biological catalyst that increase the rate of otherwise slow reactions by decreasing the reactions activation energy, without undergoing any neat change in there structures at the end of a reaction

(Trived *et al*., 2013). They are mostly protein in nature. When the enzyme is present the reaction occurs at much higher rate and the enzyme itself is not consumed in the activity, and it was first named in the late nineteenth century by Wurtz and Bouchut (1945) who partially purified the product from the sap of papaya (Mendrd and Storer, 1998). When named, it was simply recognized as a proteolytically active constituent in the latex of tropical papaya fruit (Wurtz and Bouchut, 1945). In the 1980s, the geometry of the active site was reviewed and the three-dimensional structure was determined to a 1.65 Angstrom resolution. The precursors and inhibitors of papain were studied into the 1990s (Vernet, 1991).

The *Carica papaya* belongs to family *Caricaceaus* commonly known as papaya in English, papita in Hindi and erandakarkati, in Sanskrit (Anonymous, 1994; Chaudhari, 1996 and Evans, 2011).It is native to tropical America (Milnd and Gurditta, 2011). And it was introduce to India in 16th century. The plant is recognized by its weak and usually unbranched soft stem yielding copious white latex and crowded by terminal cluster of large and long stalked leaves, is rapidly growing and can grow up to 20m tall (Banerjee,1986). *Carica papaya contains* biologically active compounds. The two most common important compounds are papain and chymopapain (Parel and Gurditta, 2011). According to Allan *et al*., 1983; Bodansky and Rose, 1982, papain is prolytic enzyme from cystein protease family which is present in a papaya. Papain is a prolytic enzyme unlike pepsin and trypsin, which are synthesized in the human body, and it is typically found in papaya plants roots including papaya and pineapple.

Twenty first century is the era of biotechnology has spread its wings towards commercially valuable complicated biochemical processes. One of its major branches is enzyme technology that makes different industrial procedures convenient, economical and simple. Papain is one of the product of this technology has diverse applications in chemical and food industries. Unripe papayas are the principal source of papain enzyme (Broklehurst, 1981). It carries proteolytic activity and belongs to cysteine proteinase family. Active papain enzymes can be isolated and purified from the latex of green papaya fruits (Nakason and Paul, 1998). Papain possesses a very powerful digestive action superior to pepsin and pancreatin (Johanson, 1972).Papain is a single chained polypeptide with three disulfide brigs and sulfhydryl group that are highly essential for the activity of the enzyme. Papain is expressed as an inactive precursor prepropapain (Biswajit *et al.,* 20013*)*.

Papaya is a power house of nutrients and is available throughout the year. It is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and foliate and fiber. In addition to all this, it contains a digestive enzyme papain that effectively treats causes of trauma, allergies and sports injuries. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer and the

enzyme used to treat arthritis (Parel and Gurditta, 2011). Some studies indicated that the enzyme helps in the prevention of diabetic heart disease (Chaudhari, 1996).The fruit is an excellent source of beta carotene that prevents damage caused by free radicals that may cause some forms of cancer. It is reported that it helps in the prevention of diabetic heart disease. Papaya lowers high cholesterol levels as it is a good source of fiber. It has a number of food processing applications especially n considering fiber digestion of meat (Chaudhari, 1996).

During the last few decades there are advances in technology and awareness regarding the extraction and isolation of plant based enzymes. There has been a tremendous increase in the demand of these enzymes, due to this there is a need for standardization to extract and purify enzymes from the different part of the plant. Although modern techniques are being widely used for standardization process, the pharmacognostic and physical approach is still reliable for identification of the raw material. To this effect, the objective of the study was to isolate papain enzyme from papaya leaf and knowing of the extraction methodology, concentration of the enzyme and creating awareness about the significance of the papaya which contains different enzymes and nutrients. The finding of this study could serve as baseline method for further research on related topics.

MATERIALS AND METHODS

Study area

The study was conducted in the town of Gondar which is located in the North Western part of Ethiopia, within the Amhara Regional State. It is located about 738km from Addis Ababa, capital city of Ethiopia. Geographically Gondar is located12^{o} 35' 07''North latitude and 37^{o} 26' 08' East longitudes and altitude range from2000–2200m above sea level (David, 2011).

Study design and period

The design of this study was experimental and it was conducted from January 2014 to June 2014to isolate papain enzyme from papaya leaves using ultra sonication and grinding methods.

Sample collection

The papaya leaf *C. papaya* grown locally in northern part of Gondar University in Tewodros campus was used as a starting material. The fresh leaf was taken by using knife and then it was taken in Molecular Biology laboratory of Biotechnology department.

Phytochemical Screening

For phytochemical screening 5 grams dried powdered material was taken in a flask containing 100 ml of Hydro-Alcohol, and kept on shaker at 100rpm for 24 hrs. After 24 hours the extract was filtered and evaporated to dryness and were used for the analysis of different phyto-constituents viz. alkaloids, tannins, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins (Khandelwal, 2008 and Roseline, 2011).

Enzymes extraction from papaya leaf

Grinding assisted extraction

The papaya fresh leaves were cut and washed with distilled water. Then it was dried in the laboratory room by using atmospheric air for seven days. 1The leaves were ground using a grinder. Accurately 5g of grinded papaya leaf powder was dissolved in accurately measured 20ml

distilled water, the water was added in ratio 1:5 and Water papaya mixture was filtered by using filter paper (Mahmood *et al*., 2005).

Ultrasound-assisted extraction

The harvested sample was cut in to 3cm by 3cm of length and width by using stainless scissor**.** Samples in triplicate were pretreated with**,** ultrasonication time (20, 30 and 60 minutes) and extraction temperature (50, 60 and 70^0C).

Purification of papain from fresh leaf by two-step salt precipitation

Ultrasound and grinding pretreated crude enzyme was mixed with 40mM cystein at a ratio of 3:1(w/v) and the suspension was adjusted to pH 5.6 using 6M HCL and then stirred for 15 min at 4^oC. The mixture was filtered and pH of the filtrate was adjusted at 9.0 using 6M NaOH. The insoluble material was removed by centrifugation at 9000xg for 30min at 4^oC.The supernatant was precipitated with $(NH_4)_2SO_4$ at 45% saturation. The salt–enriched solution was incubated at 4^o C for 30min. The precipitation was collected by centrifugation as above, and dissolved using 20mM Cystein. The solution was kept at 4^oC before adding sodium chloride (10% w/v). The mixture was slowly stirred for 30min before separating the papain by centrifugation. The enzyme was dissolved in water and stored at 4^oC (Sarote *et al*., 2006).

Identification of papain

Three drops of papain extract was added to 10ml of 20% powdered skim milk pH 5.5 and it was incubated at 37^oC (Trivedi *et al*., 2013).

Determination of papain content

The protein content in the samples during purification was determined by Bradford method (Bradford, 1976).

Protease activity determination

The proteolytic activity of the enzyme was determined according to the procedure of Arnon with slight modification. The reaction mixture contained 200µl of 50mM casein, 20mM EDTA (disodium salt), pH 8.0, 700µL 50 Mm Tris-HCl buffers, and pH 8.0 and1000µl enzyme solutions. The mixture was incubated at 37^o C for 5min before starting the reaction by adding 3ml of 50% (v/v) trichloroacetic acid (TCA) and then cooled for 1h. The reaction mixture was centrifuged, and absorbance of the supernatant was measured at 275nm.The reading was corrected for a blank in which the enzyme was added after addition of TCA (Sarote *et al*., 2006).

Phytochemical Screening: For phytochemical screening 5 grams dried powdered material was taken in a flask containing 100 ml of Hydro-Alcohol, and kept on shaker at 100rpm for 24 hrs. After 24 hours the extract was filtered and evaporated to dryness and were used for the analysis of different phyto-constituents viz. alkaloids, tannins, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins (Khandelwal, 2008 and Roseline, 2011).

RESULT

Papain enzyme was extracted from the whole samples which were coagulated when incubated at 37^0c as shown in Figure 1. The concentration of extract papain was from 0.054 - 0.002 mg/ml. The maximum mean value was shown by the grinded sample and the minimum mean concentration was 0.002 obtained from the

sonicated sample within temperature of 5^{o}Cfor 20min. Even if this minimum mean concentration obtained from the sonicated sample there are different concentrations which have significantly similar concentrations as shown in the Table-1.

Table1. The concentration of extracted papain from papaya leaves.

Pretreatment		Mean Concentration (mg/ml) ±Standard deviation
Grinded		0.054± 0.008^{ab}
Sonicated Time	Temp(^{0}c)	
20min	50	0.002±0.00^{a}
	60	0.021± 0.001^{b}
	70	0.027±0.011^{b}
30min	50	0.015±0.001^{ab}
	60	0.016±0.001^{ab}
	70	0.033±0.001^{b}
1h	50	0.032±0.001^{b}
	60	0.004±0.00^{a}
	70	0.034±0.00^{b}
Total		0.025±0.003

Key; Values within the same column followed by different superscripts are significantly different at (P≤ 0.05).

Proteolytic assays were made using casein as substrate as shown in the Table-2. Absorbance of the supernatant was measured at 275 nm and mean absorbance of extract papain was from 0.056- 0.012. The maximum mean value was shown sonicated sample at 60min by 60^{o}C and the minimum mean absorbance was recorded in the grinded sample.

Papaya leaf contains protein and alkaloids in appreciable amount. The leaf also contains amino acids in a moderate amount. Carbohydrates, steroids and glycosides are also present in a detectable label.

Table 2. The activity of extracted papain from papaya leaves.

Pretreatment		Mean Absorbance at 275nm± Standard deviation
Grind		0.012± 0.002 [a]
Sonicated		
Time	Temp(^{0}c)	
20min	50	0.014± 0.001[a]
	60	0.029±0.036[ab]
	70	0.023±0.002[ab]
30min	50	0.019±0.002 [a]
	60	0.022±0.001[ab]
	70	0.021±0.001[a]
60min	50	0.042±0.003[bc]
	60	0.056±0.003[bc]
	70	0.023±0.003[ab]

Key; Values within the same column followed by different superscripts are significantly different at ($P \leq 0.05$).

Table 3. Phytochemical evaluation of papaya leaf

Test	Amount
Proteins	+++
Alkaloids	+++
Carbohydrates	+
Steroids	+
Glycosides	+
Amino acids	++

Key, + Present, ++ Moderate amount, +++ Appreciable amount

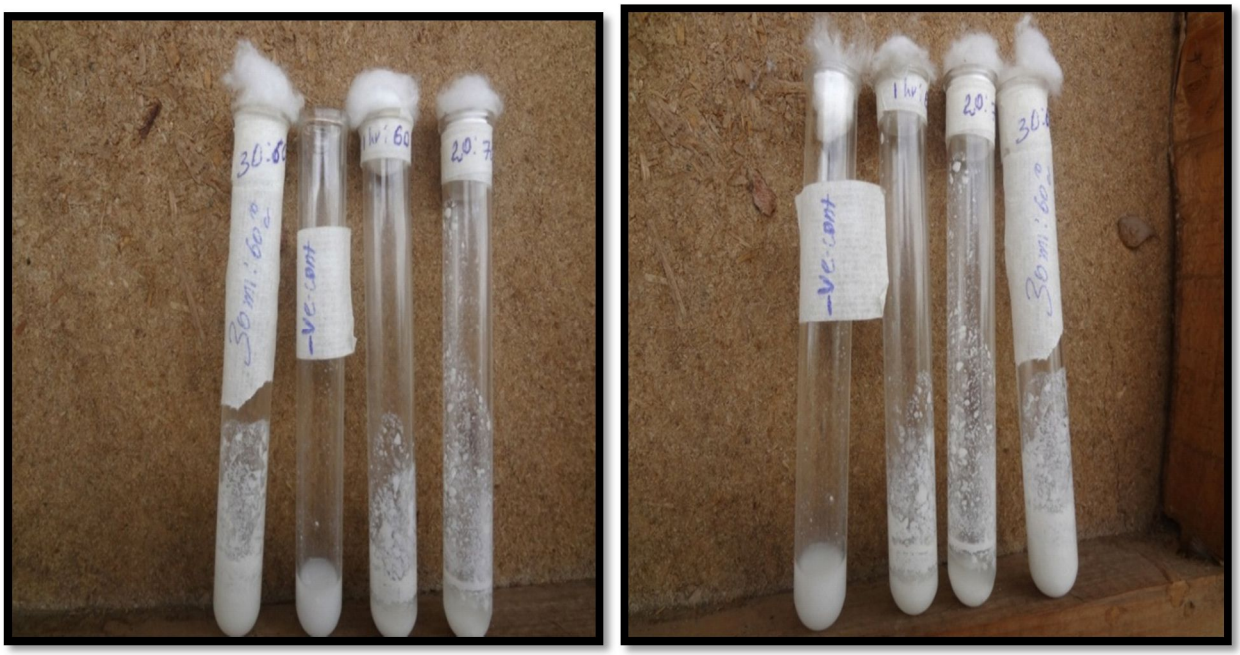

Figure 1. Identification of papain enzyme extracted from papaya leaf.

DISCUSSION

The amount of papain enzyme isolated from grinded leaves of papaya was higher than the sonicated papaya leaves and this might be grinding of the leaf used to avoid the outer parts of the leaf that enclosed the cytoplasm. Highly pure papain was obtained in a much shorter processing time than the long processing time (Sarote *et al*., 2006).This is in agreement with the current study, the efficiency of the papain enzyme was more in the sonicated leaf sample and this indicated that relatively pure enzyme was isolated from the sonicated samples; papain enzyme isolated from the grinded leaf was more crud and this might the contamination of papain enzyme with the grinded particles and the cellular compartments.

The concentration of papain enzyme was steadily increased as temperature and time of sonication. This indicated that temperature facilitated the disruption of the unwanted cellular parts and also time of sonication was critical pretreatment condition for the isolation of this enzyme (Sarote *et al*., 2006).

The activity of enzymes is affected by different conditions like temperature, pH as well as time. Different enzymes require different temperature range for their activities. And the optimum temperature for the isolated papain enzyme was about 60^{o}C. The latex of the papaya contains higher concentration of enzyme than the leaf as compared to our result from which they done on the latex parts (Sarote *et al*., 2006). Extraction of papain enzyme from the leaf is simpler than extraction of papain enzyme from the latex part, as stated by Sarote *et al*., 2006 the amount of contamination is minimized in the later one.

Papaya leaf contains protein and alkaloids in appreciable amount; amino acids in moderately amount; carbohydrates steroids and glycosides as a detectable label. Deepa and Meenakshi, 2015 reported that alkaloids, tannins, phenolics and flavonoids exist in papaya leaf as an appreciable amount; carbohydrates, glycosides, saponins, proteins, amino acids, steroids and terpenoids as a detectable amount in the leaf of male and female papaya leaf.

CONCLUSION

In this finding it is concluded that papaya leaves contain, papain enzyme. Also from the result, it can be deduced that higher concentration of papain was extracted from grinding assisted extraction as compared to sonicated one but more active papain enzyme was extracted from sonication pretreatment at 60^{o}C for 60min. Grinding pretreatment for the isolation of papain from papaya leaves is more effective method than the sonication pretreatment method but more active papain enzyme is obtained from the effectiveness of the enzyme was more effective in extraction of ultrasonication. Papaya leaf contains protein, alkaloids, amino acids, carbohydrates, steroids and glycosides.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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