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Identification and extraction of papain enzyme from papaya leaf in adigrat towen, northern Ethiopia

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Abstract
Papain is a proteolytic enzyme currently in papaya that breaks down proteins and widely it has a dozens of food processing applications. Generally, the objective of this study was to extract papain enzyme by different extraction technique mainly using grinding method and ultrasonication. Experimental study was conducted from February 2017- May 2017. The collection of fresh papaya leaf was taken from locally grown papaya from Adigrat in region of Tigray by using cutting and then it was taken in molecular laboratory of Biotechnology department. By using the reagent for purification of the extracted papain was done by using ammonium sulfate and sodium chloride precipitation. The concentration of enzyme was then determined by Bradford method. The enzyme for pyrolytic activity was determined according to the procedure of Arnon with some addition of modifying his manual. The concentration of extract papain was from 0.055 - 0.003 mg/ml. The maximum mean value was shown by the grinded sample and the minimum mean concentration was 0.003 obtained from the sonicated sample within temperature of 50^0C for 25min. The maximum mean absorbance for enzyme activity was shown by sonicated sample at 1h by 60^0c and the minimum mean absorbance was from the sonicated sample within temperature of 50^0C for 20min. Finally, papaya leaves contain papain enzyme, and this needs further the widest application of prosecuting research.

Keywords: *Carica papaya***,** Papain, Proteolytic Enzymes

1. Introduction
Enzymes are biological catalyst that increase the rate of reactions by decreasing the reactions activation energy, the absence of undergoing any well ordedred change in their structures at the end of a reaction [15]. When the enzyme is neat the reaction occurs at much higher rate and the enzyme itself is not consumed in the activity, and also the first named in the late nineteenth century by [17] who partially purified the product from the sap of papaya [3]. When named, it was simply recognized as a proteolytically active constituent in the latex of tropical papaya fruit [17]. In the 1980s, the geometry of the active site was reviewed and the three-dimensional structure was determined to a 1.65 Angstrom resolution [16]. The precursors and inhibitors of papain were studied into the 1990s [16]. In this finding concluded that papaya leaves contain, papain enzyme.

This plant is lead to well-known by its weak and usually un branched 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 [2]. According to [1] Papain is prolytic enzyme from cystein protease family.

The carica papaya belongs to family caricaceaus commonly known as papaya in English, papita in Hindi and erandakarkati, in Sanskrit [11] and [6]. It is native to tropical America [13]. And was introduce to India in 16th century. It is an enzyme present in a papaya and is used for breaking down meat fibers. Papain is another type of 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, papain also has a number food processing application.

In the twenty first century is the era try to advance technique which is Biotechnologically it has subsequently spread the sec of commercially valuable complicated biochemical processes. This enzyme core branches are enzyme technology that makes different industrial procedures convenient, economical and simple.

This enzyme the product of technology has wide applications in chemical and food industries. Unripe papayas are the principal source of papain enzyme [4]. Pproteolytic activity carries and

belongs to cysteine proteinase family. Active papain enzymes can be isolated and purified fromthe latex of green papaya fruits [14]. The proteolytic activity of papain has been well described in the literature, including the degradation of elastin and proteoglycans [7]. According to Dr John and Whitman Ray Papain possesses a very powerful digestive action superior to pepsin and pancreatin. Recently Kinoshita in 2003 reported that Papain a major chemical compound extracted from latex of papaya used in several industries for various industrial and pharmaceutical products.

Papaya is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and foliate and fiber. Moreover, 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. 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. In this finding 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 60ºC 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

2. Materials and Methods

2.1 Study area

The study area was found in the Adigrat region of Tigray which is located in the North East part of Ethiopia mainly in the Tigray regional stat. It is located about 904km from Addis Ababa, capital city of Ethiopia. Geographically Adigrat is located It is located in the Misraqawi Zone at longitude and latitude 14°16′N 39°27′E, with an elevation of 2,457 metres above sea level [5]

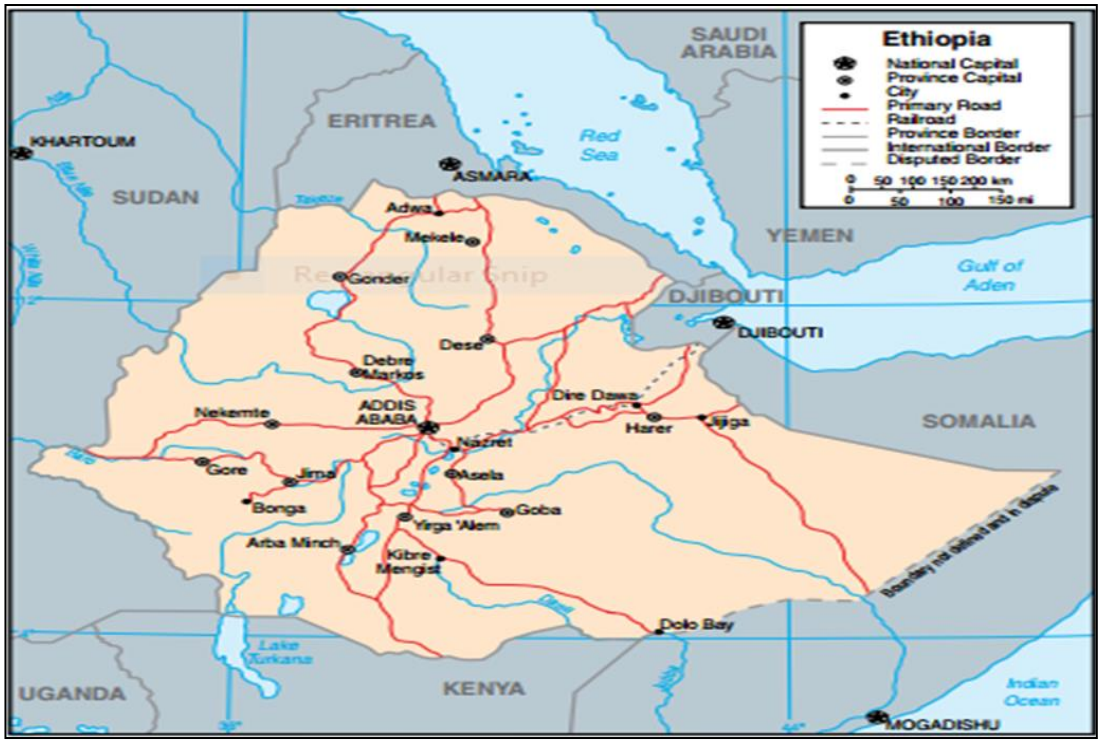

Fig 1: Ethiopia

2.2 Study design and period

This study design was experimental and it was conducted from to isolate papain enzyme February 2017- May 2017. In the area of Northern part of Ethiopia from papaya leaves using ultrasonication and grinding methods.

2.3 Collection of sample

The papaya leaf C. papaya grown locally in northern part of Ethiopia was used as a starting leaf material. The fresh leaf was taken from locally grown papaya from around Adigrat University by using knife and then it was taken in molecular laboratory department of Biotechnology.

2.4 Extraction enzymes from papaya leaf

2.4.1 Extraction by grinding assisted

The fresh papaya leaves were cut and wash with distilled water. It would be dried in the laboratory room by using atmospheric air for eight days. The leaves were ground using a grinder. Accurately weight 5g of grinded powder papaya was dissolve in accurately measured 20ml distilled water, the water was added in ratio 1:5 and Water papaya mixture was filtered by using filter paper [8] and [15].

2.4.2 Extraction by ultrasound-assisted

The sample was harvested cue in to 3cm by 3cm of length and width by using stainless scissor. Samples in triplicate were pretreated with**,** ultrasonication time (25, 30 and 60 minutes) and extraction temperature (50, 60 and 70°C).

2.4.3 Two-step salt precipitation to used purification of papain from fresh leaf

It was used ultrasound and grinding pretreated crude enzyme

was mixed with 40mM cysteine 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 4^0c [15]. The mixture was filtered and p^H of the filtrate was adjusted 9.0 using 6M NaOH. The insoluble material was removed by centrifugation at 9000xg for 30min at 4°c. The supernatant was precipitated with $(NH_4)_2SO_4$ at 45% saturation. The salt –enriched solution was incubated at 4^0c for 30min. The precipitation was collected by centrifugation as above, and dissolved using 20mM cysteine. The solution was kept at 4^0c 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°C [12].

2.4.4 Identification of papain
Three drops of papain extract were added to 10ml of 20% powdered skim milk pH 5.5 and it was incubated at 37°C [18].

2.4.5 Determination of protein content
The amount of protein in the samples during purification was determined by Bradford method [9].

2.4.6 Protease activity determination
Prolytic activity of the enzyme was determined according to the procedure of Arnon with addition of some 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 37^0c for 5min before starting the reaction by adding 3ml of 50% (v/v) trichloro acetic acid (TCA) and then cooled for 1h. The mixture of reaction 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 adition of TCA [12].

3. Result and Discussion
This enzyme was extracted from where the total samples which were coagulated when incubated at 37°C as shown in Fig 1. The concentration of extract papain was from 0.055 - 0.003 mg/ml. Where, 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 this significant difference [15]. The mean value was shown by the grinded sample and the minimum mean concentration was 0.003 obtained from the sonicated sample within temperature of 5^0C for 25min. But the efficiency 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 the cellular compartments. This minimum mean concentration obtained from the sonicated sample there are different concentrations which have significantly similar concentrations as shown in the Table-1. 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.

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 papin enzyme was about 60°C.

Therefore, in the extraction of papain enzyme from papaya leaves, water is applied as the extraction media. This is because water can maintain the structural stability of papain besides it is also very good extractive properties for polar substances compare to the organic solvent

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)	
25min	50	0.003±01^a
	60	0.02± 0.002^b
	70	0.028±0.012^b
30min	50	0.015±0.0012^{ab}
	60	0.016±0.0012^{ab}
	70	0.033±0.002^b
1h	50	0.032±0.0012^b
	60	0.004±01^a
	70	0.034±01^b
Total		0.026±0.0034

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

The revealed result from this treatment has been in proteolytic assays were made using casein as substrate as shown in the Table-1. Absorbance of the supernatant was measured at 275 nm and mean absorbance of extract papain was from 0.057-0.013. The maximum mean value was shown sonicated sample at 60min by 60^0c and the minimum mean absorbance was recorded in the grinded sample. However, in the activity of those enzyme compare to the past time it's some advance in the causes of slightly modification of the protocol for identification of papain enzymes.

Table 2: Phytochemical evaluation of papaya leaf

Test	Amount of enzyme
Proteins	+++
Steroids	+
Glycosides	+
Amino acids	++

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

Table 3: The activity of extracted papain from papaya leaves

Pretreatment		Mean Absorbance at 275nm± Standard deviation
Grind		0.013± 0.0023 ^{a}
Sonicated		
Time	Temp(^{0}c)	
25min	50	0.015± 0.0012^a
	60	0.0291±0.037 ^{ab}
	70	0.024±0.0021 ^{ab}
30min	50	0.019±0.002 ^{a}
	60	0.022±0.001^{ab}
	70	0.021±0.00 1^a
60min	50	0.043±0.004 ^{bc}
	60	0.057±0.004 ^{bc}
	70	0.024±0.004 ^{ab}

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

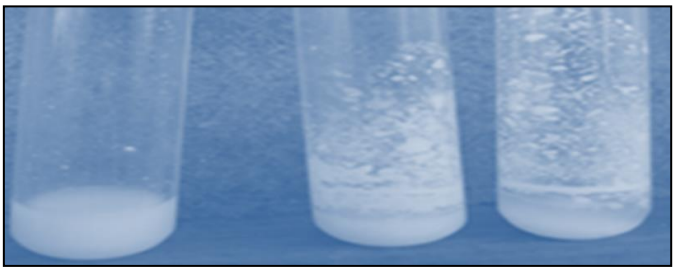

Fig 2: extracted of papain enzyme from papaya tree.

The amount of extraction of papain enzyme from the leaf by using grinding 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 [12]. While this study for higher 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. But slightly difference their used sonicated time at 25 min that may be the difference of their source of the weather condition. The amount this papain enzyme was steadily increased as temperature and time of sonication [15]. Strongly agreement the current study

4. Conclusion

The present day of this study, we have found that the using different application of this enzyme can be applicable by papain enzyme for different purpose. So, concluded that it need further investigating is essential and also it helps supporting economic aspect of under developing country especially Ethiopia.

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