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Comparative Analysis of Papain Enzyme Activity in Different Parts of *Carica papaya*

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ABSTRACT

Carica papaya, a neutraceutical plant known for its wide range of pharmacological activities, contains various enzymes, particularly papain, and vitamins, making it a valuable plant in the neutraceutical industry. This study aimed to investigate the enzyme activity of papain derived from unripe Carica papaya root, latex, and leaves, and determine the most effective extraction process among boiling, grinding, and latex extraction. Standard methods were employed to assess enzyme activity. The results revealed that the boiling method for leaf extraction exhibited the highest enzyme activity at a concentration of 0.1, specifically for the effect of cysteine. The stability of protease activity was observed to be highest at pH 8 (0.14 ± 0.03 nm) and pH 10, while the optimal temperature for enzyme activity was 55°C (0.11 ± 0.02 nm). At 60°C, the activity reading decreased to 0.09 ± 0.02 nm. For root enzyme activities, the optimal temperature was found to be 65°C with an activity reading of 0.10 ± 0.00 nm. Moreover, the phosphate buffer at pH 2 resulted in the optimal pH value of 0.13 ± 0.01 nm, while at pH 7, the enzyme activity reading was 0.13 ± 0.00 nm. Comparatively, the enzyme activities obtained from the latex exhibited the lowest values across different temperatures. Among the leaves, those treated with Citrate buffer at pH 3 demonstrated the highest enzyme activity of 0.16 ± 0.01 nm, whereas at pH 6, the activity reading was 0.15 ± 0.00 nm. Surprisingly, the extraction of latex showed the least value in terms of proteolytic assay of papain activity, indicating that it possessed the greatest enzymatic activity. In conclusion, this study demonstrates the diverse enzymatic activity of papain extracted from different parts of Carica papaya. It highlights the effectiveness of the boiling method for leaf extraction and provides insights into the optimal pH and temperature conditions for enzyme activity.

Keywords: Papain, *Carica papaya*, Enzyme activity, Extraction, Protease

INTRODUCTION

Pawpaw, scientifically known as Carica papaya, is a small deciduous tree native to the United States and parts of Canada. It is characterized by its large, simple leaves and distinct fruits. Pawpaw is not only a delicious tropical fruit but also possesses various medicinal properties. The entire plant, including the fruit, roots, bark, peel, seeds, and pulp, is known to have medicinal benefits [1]. The leaves of the pawpaw tree are clustered symmetrically and have a distinct appearance. The fruits of the tree develop after flowering and mature to yellow or brown. Pawpaw fruits are rich in nutrients, including vitamins A, B, and C, as well as proteolytic enzymes like papain and chymopapain, which

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have antiviral, antifungal, and antibacterial properties [2, 3]. The nutritional content of pawpaw includes vitamins A, B, C, and E, as well as minerals such as magnesium and potassium. These nutrients improve cardiovascular health, protect against heart diseases, strokes, and prevent the oxidation of cholesterol[4]. Pawpaw also contains phytochemicals, non-nutritive plant chemicals that have protective and disease-preventive properties. These phytochemicals contribute to the plant's ability to protect it and may also offer benefits to humans[5, 6]. The medicinal uses of pawpaw are categorized based on different parts of the plant. The leaves of pawpaw have numerous benefits, including treating dengue fever, inhibiting cancer cell growth, and possessing anti-malarial and anti-plasmodial activity [7]. The peel of pawpaw is used in cosmetics and home remedies, such as sunscreen and soothing salve, fighting dandruff, and acting as a muscle relaxant. The roots of pawpaw are used to ease urinary troubles and remedy various digestive disorders [8-9]. Pawpaw has been associated with various health benefits, including preventing colon cancer, reducing inflammation, promoting lung health, and preventing prostate cancer. However, it is important to note that some individuals may be allergic to pawpaw, experiencing side effects such as skin rash, nausea, vomiting, or diarrhea [10-12]. Pawpaw is a versatile fruit with a wide range of medicinal properties. Its nutritional content, phytochemicals, and various plant parts offer numerous health benefits. However, it is essential to be aware of potential allergies and side effects associated with pawpaw consumption. Further research is needed to explore the full potential of pawpaw in the field of medicine and health. The objectives of this study are to compare the extraction efficiency of papain from Carica papaya root, leaves, and latex; to evaluate the enzymatic activity of papain derived from the different sources (root, leaves, and latex). As well, to compare the enzymatic activity levels of papain from each source. In addition we also aimed to investigate the pH and temperature optima for the enzyme activity of papain derived from each source. Lastly to compare the stability and shelf life of papain obtained from Carica papaya root, leaves, and latex.

MATERIALS AND METHODS

Fresh Carica papaya Leaves and Root were procured from Papaya trees in the vicinity of Babcock University Ilishan-Remo and identified by Professor Edward B. Esan, a plant scientist in the Department of Biosciences and Biotechnology, Babcock University.

Methods of Collection and Preparation of Extracts

Fresh leaves and roots were collected. The leaves were thoroughly washed with tap water and then rinsed with sterile distilled water. Two different methods were used for extraction: a. in one treatment, 5g of the fresh leaves and root were weighed and boiled in distilled water for 15 minutes with constant stirring. The extract was then filtered [13]. b. In another treatment, 5g of the fresh leaves and root were weighed and pulverized using a sterile laboratory mortar and pestle. The resulting thick paste was suspended in 100ml of sterilized water and filtered to obtain the extract [14]. Both extracts were stored in airtight glass containers sealed with foil and protected from sunlight until further use.

Extraction of Papain from *Carica papaya* Latex

The unripe but almost mature papaya fruit was selected, and the skin was cut, allowing the latex to flow from the cuts. The fruit was tapped during a period of high humidity, preferably in the morning. The papaya fruit

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was cut from top to bottom with a razor blade, ensuring the cut was not more than 2mm deep and meeting at the base of the fruit. The fruit was held over a collection dish, and the liquid latex was allowed to drip onto the dish for a minimum of 6 minutes. On subsequent tapings, the cuts were spaced between earlier ones. The collected liquid papain was transferred to a vacuum oven. The oven was set to 38°C, and the liquid papain was dried for 5 hours. The resulting dried papain had a crumbly appearance [15]. The dried papain was gently scraped from the collection dish with a stainless steel spoon and transferred to a polyethylene-lined box, which was sealed airtight.

Sterility

Testing of Extracts from Carica papaya Leaves and Root; The sterility of the extracts was tested by passing them through a Millipore filter (0.22 micrometer). Inoculation was performed by adding 2ml of sterile extracts into 10ml of sterile nutrient broth and 10ml of Sabouraud dextrose broth. The mixture was incubated at 37°C for 24 hours. The absence of turbidity or clarity in the broth after incubation indicated the sterility of the extracts [16].

Assay of Proteolytic Activity of Papain from Carica papaya Leaves, Roots, and Latex

Method Used was [17]. A 0.30ml sample of papain (prepared by dissolving a known weight of papain in 1.50ml of 5% acetic acid) was added to a 0.50ml milk solution (prepared by dissolving a known weight of milk powder in 100ml distilled water, resulting in a 1% (w/v) solution) warmed to 30°C in a water bath. The contents were thoroughly mixed, and the time taken for clotting (formation of lumps) to occur was recorded [17]. The experiment was repeated using different known amounts of papain solutions, resulting in a range of clotting times between 60 seconds and 420 seconds (7 minutes) for optimal results. The activity of the papain sample was calculated by plotting a graph of papain concentration (microlitre) (µg) against clotting time (seconds) and using that value in a formula to calculate the activity [17].

Modified Procedure [18] Papaya proteases from the peels and latex were investigated for their optimal pH and temperature, the effect of cysteine on the catalytic reaction, and their stability by using casein hydrolysis. The reaction mixture containing 0.10ml of enzyme solution, 0.30ml of buffer solution, and 0.10ml of activating agent (40mM cysteine - 20mM EDTA disodium salts) was incubated at a constant temperature for 5 minutes using an incubator. The reaction was initiated by adding 0.50ml of 1% (w/v) casein solution. After 10 minutes, 1.50ml of 5% cold trichloroacetic acid (TCA) was added to terminate the reaction. The supernatant was separated by centrifugation at 9000xg for 20 minutes using a centrifuge, and the absorbance was measured at 275 nm using a spectrophotometer. Additional experiments were conducted to study the effects of cysteine on enzyme catalysis, stability at different pH levels, and optimal temperature [18]. Effect of Cysteine: Reaction at 37°C in a water bath was performed using pH 8 and 100µl of 20mM ethylene diamine trichloroacetic acid (EDTA) disodium salt with various concentrations of cysteine instead of the normal activating agent [18].

Stability

The enzyme solution was incubated at a constant temperature of 37°C in 50mM phosphate buffer at pH 8 and 10 for 10 minutes. The incubated enzymes were then assayed for proteolytic activity [19]. Optimum Temperature: The optimum temperature of enzymatic activity was determined at various

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temperatures between 55°C, 60°C, and 65°C in a water bath at pH [19]. Optimum pH: The optimum pH of the reaction was performed at 37°C in a water bath using different buffers: 50mM phosphate buffer at pH 2, 7, and 11, and citrate buffer at pH 3 and 6. All assays were done in triplicate.

Statistical Analysis

This was done with the aid SPSS for windows: SPSS Inc., Chicago, Standard version 17.0 to determine difference between mean using Analysis of Variance (ANOVA). Data were reported as mean ± Standard error of mean [20].

RESULTS

Table 1 presents the results for the enzymatic activity of latex extraction from Carica papaya. The weights of the samples (Spl) are listed in grams (g) and milligrams (mg). The enzymatic activity of latex extraction from Carica papaya decreases as the sample weight increases.The weights of the samples increase proportionally with the increase in sample weight. I.e. for a sample weight of 1 gram, the weight of the sample (Spl) is 1000 ± 0.00 grams, and the enzymatic activity of the latex extraction (LX) is 151 ± 4.04 milligrams (mg). As the sample weight increases, the weight of the sample (Spl) also increases. For sample weights of 3 grams, 5 grams, 7 grams, and 10 grams, the weights of the samples are 3000 ± 0.00 mg, 5000 ± 0.00 mg, 7000 ± 0.00 mg, and 10000 ± 0.00 mg, respectively. The enzymatic activities of the latex extraction (LX) decrease gradually to 130 ± 4.04 mg, 109 ± 4.04 mg, 88 ± 4.04 mg, and 67 ± 4.04 mg, respectively.

Table 2 provides the results for the determination of enzyme activity from Carica papaya root and leaves using milk powder (casein) as a substrate. The enzyme activity of Carica papaya root and leaves increases as the sample volume increases.Using milk powder (casein) as a substrate enhances the enzyme activity of both root and leaves.For a sample volume of 0.3 ml, the values for extraction of root by grinding (ERG) and extraction of root by boiling (ERB) are 130 ± 4.04 nm and 67 ± 4.04 nm, respectively. The values for extraction of leaves by grinding (ELG) and extraction of leaves by boiling (ELB) are also 67 ± 4.04 nm. As the sample volume increases to 0.6 ml, the values for ERG and ERB increase to 151 ± 4.04 nm, while the values for ELG and ELB increase to 88 ± 4.04 nm. Further increasing the sample volume to 1.2 ml lead to higher values for ERG and ERB, which are 172 ± 4.04 nm, and higher values for ELG and ELB, which are 109 ± 4.04 nm.

Table 3 shows the proteolytic effect of root leaves and latex papain activity extract on cysteine at pH 8. The proteolytic activity of root papain on cysteine increases as the concentration of cysteine increases.Both boiling and grinding methods for root extraction result in increased proteolytic activity.As the concentration of cysteine increases from 0.1 ml to 0.3 ml, the values for extraction of root by boiling (RB) increase from 0.05 ± 0.01 nm to 0.13 ± 0.01 nm. Similarly, the values for extraction of root by grinding (RG) increase from 0.05 ± 0.01 nm to 0.08 ± 0.01 nm. The effect of leaves and latex papain activity on cysteine depends on its concentration.The proteolytic activity of leaves and latex papain on cysteine is influenced by the concentration of cysteine.For a cysteine concentration of 0.1 ml, the values for extraction of leaves by boiling (LB), extraction of leaves by grinding (LG), and extraction of latex (LX) are 0.05 ± 0.01 nm, 0.10 ± 0.01 nm, and 0.03 ± 0.01 nm, respectively. As the cysteine concentration increases to 0.3 ml, the values for LB, LG, and LX are 0.07 ± 0.01 nm, 0.10 ± 0.01 nm, and 0.06 ± 0.01 nm, respectively.

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Table 4 presents the effect of pH on the proteolytic activity of root, leaves and latex papain extracts using cysteine as the substrate. The proteolytic activity of root and leaves papain extracts on cysteine increases as the pH increases.The pH level affects the proteolytic activity of both root and leaves papain extracts.For the root papain extract, at pH 5, the values for extraction of root by boiling (RB) and extraction of root by grinding (RG) are 0.04 ± 0.01 nm and 0.07 ± 0.01 nm, respectively. As the pH increases to 7 and 9, the values for RB and RG also increase, reaching 0.08 ± 0.01 nm and 0.14 ± 0.01 nm at pH 9. The leaves papain extract, at pH 5; the values for extraction of leaves by boiling (LB) and extraction of leaves by grinding (LG) are 0.05 ± 0.01 nm and 0.10 ± 0.01 nm, respectively. As the pH increases to 7 and 9, the values for LB and LG also increase, reaching 0.08 ± 0.01 nm and 0.13 ± 0.01 nm at pH 9. The proteolytic activity of leaves and latex papain extracts on cysteine increases as the pH increases.Both leaves and latex papain extracts exhibit increased proteolytic activity at higher pH levels. For the leaves papain extract, at pH 5, the values for extraction of leaves by boiling (LB), extraction of leaves by grinding (LG), and extraction of latex (LX) are 0.08 ± 0.01 nm, 0.12 ± 0.01 nm, and 0.05 ± 0.01 nm, respectively. As the pH increases to 7 and 9, the values for LB, LG, and LX also increase, reaching 0.10 ± 0.01 nm, 0.14 ± 0.01 nm, and 0.08 ± 0.01 nm at pH 9. Similarly, for the latex papain extract, at pH 5, the values for LB, LG, and LX are 0.06 ± 0.01 nm, 0.10 ± 0.01 nm, and 0.03 ± 0.01 nm, respectively. As the pH increases to 7 and 9, the values for LB, LG, and LX increase as well, reaching 0.09 ± 0.01 nm, 0.12 ± 0.01 nm, and 0.06 ± 0.01 nm at pH 9.

Table 5 presents the effect of phosphate buffer (PO4) on the optimal temperature of root, leaves and latex papain activity. The phosphate buffer (PO4) has an impact on the optimal temperature for root papain activity.Proteolytic activity of root papain increases significantly at a temperature of 65°C.. At a temperature of 55°C, the values for extraction of root by boiling (RB) and extraction of root by grinding (RG) are 0.05 ± 0.01 nm and 0.06 ± 0.02 nm, respectively. Increasing the temperature to 60°C, the values for RB and RG remain relatively stable, with RB at 0.06 ± 0.02 nm and RG at 0.06 ± 0.02 nm. At a temperature of 65°C, there is a notable increase in proteolytic activity, with RB reaching 0.05 ± 0.01 nm and RG reaching 0.10 ± 0.00 nm. The effect of phosphate buffer (PO4) on the optimal temperature ofThe phosphate buffer (PO4) affects the optimal temperature for leaves and latex papain activity.Leaves and latex papain activities show a slight decrease at a temperature of 60°C.At a temperature of 55°C, the values for extraction of leaves by boiling (LB), extraction of leaves by grinding (LG), and extraction of latex (LX) are 0.11 ± 0.02 nm, 0.09 ± 0.02 nm, and 0.05 ± 0.01 nm, respectively. When the temperature is increased to 60°C, the values for LB, LG, and LX show a slight decrease, with LB at 0.09 ± 0.02 nm, LG at 0.08 ± 0.03 nm, and LX at 0.04 ± 0.00 nm. At a temperature of 65°C, there is a further decrease in proteolytic activity, with LB reaching 0.08 ± 0.01 nm, LG reaching 0.07 ± 0.01 nm, and LX reaching 0.03 ± 0.01 nm.

Table 6 displays the effect of phosphate buffer (PO4) on the optimal pH of root, Leaves and Latex papain activity. The phosphate buffer (PO4) affects the optimal pH for root papain activity.Root papain exhibits increased proteolytic activity at higher alkaline pH levels.The pH values considered are 2, 7, and 11. At pH 2, the values for extraction of root by boiling (RB) and

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extraction of root by grinding (RG) are 0.09 ± 0.01 nm and 0.13 ± 0.01 nm, respectively. At pH 7, both RB and RG show similar values, with RB at 0.12 ± 0.00 nm and RG at 0.13 ± 0.00 nm. When the pH is increased to 11, RB exhibits a significant increase in proteolytic activity, measuring 0.20 ± 0.07 nm, while RG remains relatively stable at 0.16 ± 0.04 nm. The phosphate buffer (PO4) influences the optimal pH for leaves and latex papain activity.Leaves and latex papain activities show enhanced proteolytic activity at higher alkaline pH levels.The pH values considered are 2, 7, and 11. At pH 2, both extraction of leaves by boiling (LB) and extraction of leaves by grinding (LG) exhibit similar values. LB shows a proteolytic activity of 0.11 ± 0.00 nm, while LG measures 0.11 ± 0.01 nm.

When the pH is set to 7, both LB and LG papain activities remain consistent, with values of 0.10 ± 0.00 nm and 0.11 ± 0.00 nm, respectively. At pH 11, there is an increase in proteolytic activity for both LB and LG. LB measures 0.21 ± 0.06 nm, while LG reaches 0.23 ± 0.03 nm. These values indicate enhanced papain activity at higher alkaline pH levels.

Table 7 presents the proteolytic effect of Citrate buffer on the optimal pH of Root, Leaves and Latex papain activity. The Citrate buffer affects the optimal pH for root papain activity.There is no significant change in proteolytic activity for root papain between pH 3 and 6.The pH values considered are 3 and 6. At pH 3, both extraction of root by boiling (RB) and extraction of root by grinding (RG) show similar proteolytic activities. RB exhibits a value of 0.12 ± 0.00 nm, while RG measures 0.14 ± 0.00 nm. When the pH is set to 6, both RB and RG papain activities remain consistent, with values of 0.13 ± 0.00 nm and 0.15 ± 0.00 nm, respectively. The Citrate buffer has an impact on the optimal pH for leaves and latex papain activity. There is no significant change in proteolytic activity for leaves and latex papain between pH 3 and 6.The pH levels examined are 3 and 6. At pH 3, the extraction of leaves by boiling (LB) exhibits a proteolytic activity of 0.16 ± 0.01 nm, while the extraction of leaves by grinding (LG) shows a value of 0.14 ± 0.00 nm. The extraction of latex (LX) demonstrates a proteolytic activity of 0.09 ± 0.00 nm. When the pH was adjusted to 6, both LB and LG papain activities remain relatively constant, with values of 0.14 ± 0.00 nm and 0.14 ± 0.00 nm, respectively. LX papain activity shows a slight increase to 0.12 ± 0.00 nm.

Fig 1a represents the Absorbance (nm) of Papain extracts against temperature (°C) for the extraction of root, leaves, and latex. The graph provides insights into the relationship between temperature and the absorbance of the papain extracts.In Fig 1b and Fig 1c we have similar graphical representations as Fig 1a, but with specific focus on the extraction of papain from root, leaves, and latex, respectively.By analysing the graphs, the temperature increases ($p<0.05$), as the absorbance of the papain extracts also increases. This suggests that higher temperatures positively influence the enzymatic activity of papain, resulting in higher absorbance readings. The data demonstrates that temperature plays a crucial role in the extraction process of papain from root, leaves, and latex.

In Fig 2a, this graph depicts the relationship between papain concentration (ml/mg) and time taken (seconds) for the extraction of papain from latex.Given: C = 2230.2 mg/ml (Concentration of papain in the latex) W = 1.8 mg (Sample weight) U = 6000 (Unit of activity). The activity per milligram (Activity/mg),Activity/mg = C * (1000 ÷ W) * (1000 ÷ 4) * (50 ÷ 7.5) * U. Thus Activity/mg = 2230.2 * (1000 ÷ 1.8) * (1000

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÷ 4) * (50 ÷ 7.5) * 6000 Activity/mg = 2230.2 * 556 * 250 * 6.667 * 6000= 124. Activity/mg per milligram is approximately ≈ 124. In Fig 2b, this graph represents the extraction of papain from leaves using boiling and grinding methods. Given: C = 8 mg/ml (Concentration of papain in the leaves) W = 1.8 mg (Sample weight) U = 6000 (Unit of activity). To calculate the activity per milligram (Activity/mg), we use the formula: Activity/mg = C * (1000 ÷ W) * (1000 ÷ 4) * (50 ÷ 7.5) * U. Plugging in the values:

Activity/mg = 8 * (1000 ÷ 1.8) * (1000 ÷ 4) * (50 ÷ 7.5) * 6000 Activity/mg = 8 * 556 * 250 * 6.667 * 6000 Activity/mg ≈ 448. Lastly, Fig 2c focuses on the extraction of papain from the root using boiling and grinding methods. Given: C = 4 mg/ml (Concentration of papain in the root) W = 1.8 mg (Sample weight) U = 6000 (Unit of activity). To calculate the activity per milligram (Activity/mg), we use the formula: Activity/mg = C * (1000 ÷ W) * (1000 ÷ 4) * (50 ÷ 7.5) * U. Plugging in the values:

Activity/mg = 4 * (1000 ÷ 1.8) * (1000 ÷ 4) * (50 ÷ 7.5) * 6000 Activity/mg = 4 * 556 * 250 * 6.667 * 6000 Activity/mg ≈ 222

Table1: Enzymatic activity for the extraction of latex

Weight of Spl (g)	Weight of Spl (mg)	LX
1	1000 ± 0.00a	151 ± 4.04e
3	3000 ± 0.00b	130 ± 4.04d
5	5000 ± 0.00c	109 ± 4.04c
7	7000 ± 0.00d	88 ± 4.04b
10	10000 ± 0.00e	67 ± 4.04a

* mean ± SEM, n= 3, Spl = sample, g = grams, mg = milligrams, LX = extraction of latex

Table 2: Determination of enzyme activity from Carica papaya Root and Leaves with milk powder (casein)

Volume (ml)	ERG (nm)	ERB (nm)	ELG (nm)	ELB (nm)
0.3	130 ± 4.04a	130 ± 4.04a	67 ± 4.04b	67 ± 4.04b
0.6	151 ± 4.04b	151 ± 4.04b	88 ± 4.04a	88 ± 4.04a
1.2	172 ± 4.04b	172 ± 4.04b	109 ± 4.04a	109 ± 4.04a

* mean ± SEM, n= 3, nm = nanometer, ml = millilitre, ERG = extraction of root by grinding ERB = extraction of root by boiling, ELG = extraction of leaves by grinding, ELB = extraction of leaves by boiling.

Table 3: Proteolytic effect of root, leaves and latex papain activity extract on cysteine at pH 8*

Cysteine concentrations (ml)	RB (nm)	RG (nm)	
0.1	0.05 ±0.01a	0.05 ± 0.01a	
0.2	0.10 ±0.01b	0.06 ± 0.01a	
0.3	0.13 ± 0.01b	0.08 ± 0.01a	
	LB (nm)	**LG (nm)**	**LX (nm)**
0.1	0.05 ± 0.01a	0.10 ± 0.01b	0.03 ± 0.01a
0.2	0.05 ± 0.01a	0.10 ± 0.01b	0.05 ± 0.01a
0.3	0.07 ± 0.01a	0.10 ± 0.01b	0.06 ± 0.01a

* mean ± SEM, n= 3, (PO4) = phosphate buffer, nm = nanometer, ml = millilitre, LB= extraction of leaves by boiling, LG= extraction of leaves by grinding, LX= extraction of latex

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Table 4: Effect of phosphate buffer (PO4)* on the stability of Root, Leaves and Latexpapain activity at pH 8 and 10

pH	RB (nm)	RG (nm)	
8	0.09 ± 0.01a	0.13 ± 0.02b	
10	0.08 ± 0.00a	0.06 ± 0.01a	
	LB (nm)	**LG (nm)**	**LX (nm)**
8	0.11 ± 0.01b	0.14 ± 0.03c	0.06 ± 0.01a
10	0.09 ± 0.01b	0.12 ± 0.01c	0.03 ± 0.01a

* mean ± SEM, n= 3, (PO4) = phosphate buffer, nm = nanometer, RB= extraction of root by boiling, RG= extraction of root by grinding, LB= extraction of leaves by boiling, LG= extraction of leaves by grinding, LX= extraction of latex

Table 5: Effect of phosphate buffer (PO4)* on the Optimum temperature of Root papain activity

Temperature (°C)	RB (nm)	RG (nm)	
55	0.05 ± 0.01a	0.06 ± 0.02a	
60	0.06 ± 0.02a	0.06 ± 0.02a	
65	0.05 ± 0.01a	0.10 ± 0.00b	
	LB (nm)	**LG (nm)**	**LX (nm)**
55	0.11 ± 0.02b	0.09 ± 0.02b	0.05 ± 0.01a
60	0.09 ± 0.02b	0.08 ± 0.03b	0.04 ± 0.00a
65	0.08 ±0 .01b	0.07 ± 0.01b	0.03 ± 0.01a

* mean ± SEM, n= 3, (PO4) = phosphate buffer, nm = nanometer, buffer, nm = nanometer, RB= extraction of root by boiling, RG= extraction of root by grinding, LB= extraction of leaves by boiling, LG= extraction of leaves by grinding, LX= extraction of latex

Table 6: Effect of phosphate buffer (PO4)* on Optimum pH of root papain activity at PH 2, 7, 11

pH	RB (nm)	RG (nm)	
2	0.09 ± 0.01a	0.13 ± 0.01b	
7	0.12 ± 0.00a	0.13 ± 0.00a	
11	0.20 ± 0.07b	0.16 ± 0.04a	
	LB (nm)	**LG (nm)**	**LX(nm)**
2	0.11 ± 0.00b	0.11 ± 0.01b	0.04 ± 0.00a
7	0.10 ± 0.00a	0.11 ± 0.00a	0.08 ± 0.00a
11	0.21 ± 0.06b	0.23 ± 0.03b	0.15 ± 0.05a

* mean ± SEM, n= 3, (PO4) = Phosphate buffer, nm = nanometer, RB= extraction of root by boiling, RG= extraction of root by grinding, LB= extraction of leaves by boiling, LG= extraction of leaves by grinding, LX= extraction of latex

Table 7: Proteolytic effect of Citrate buffer on Optimal pH of Root papain activity at PH 3 and 7*

pH	RB (nm)	RG (nm)	
3	0.12 ± 0.00a	0.14 ± 0.00a	
6	0.13 ± 0.00a	0.15 ± 0.00a	
pH	**LB (nm)**	**LG (nm)**	**LX (nm)**
3	0.16 ± 0.01b	0.14 ± 0.00b	0.09 ± 0.00a
6	0.14 ± 0.00a	0.14 ± 0.00a	0.12 ± 0.00a

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* mean ± SEM, n= 3, LB= extraction of leaves by boiling, RB= extraction of root by boiling, RG= extraction of root by grinding.LG= extraction of leaves by grinding, LX= extraction of latex

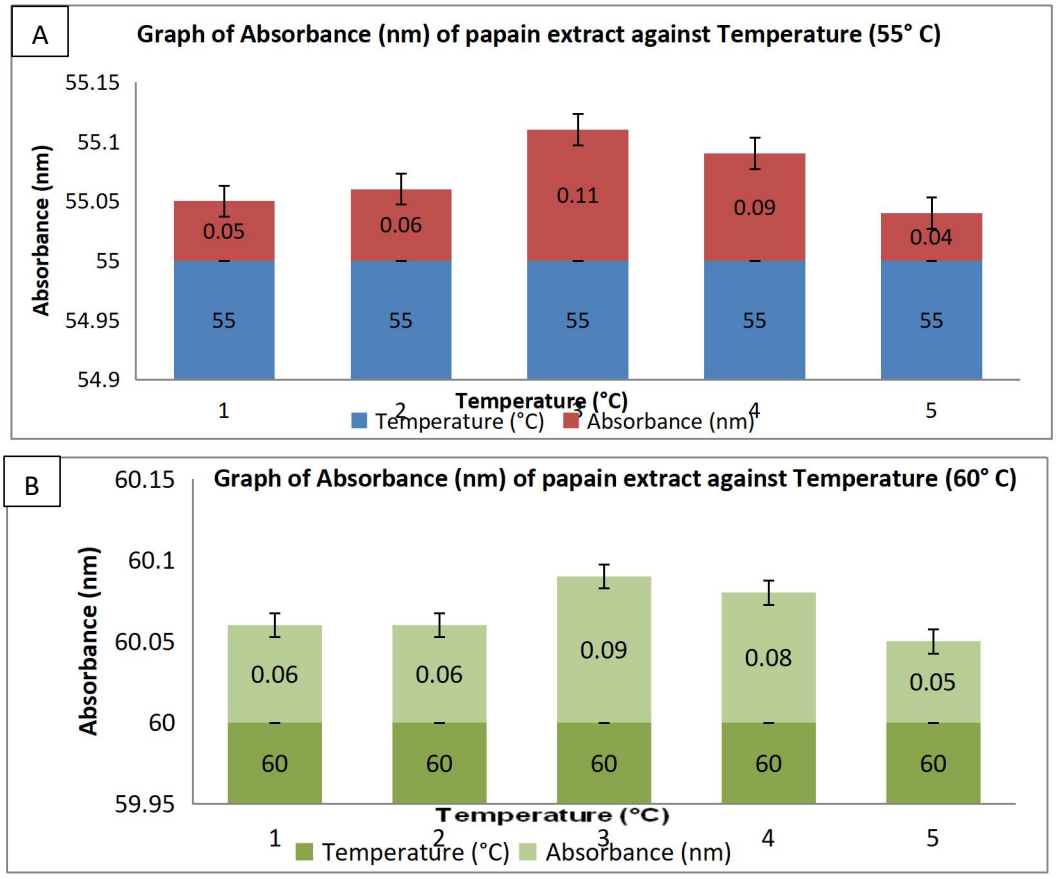

Figure1: a) Absorbance (nm) of Papain extracts. **b)**Absorbance (nm) of Papainagainst temperature (°C) for the extractionextracts against temperature (°C) forof root, leaves and latex the extraction of root, leavesand latex

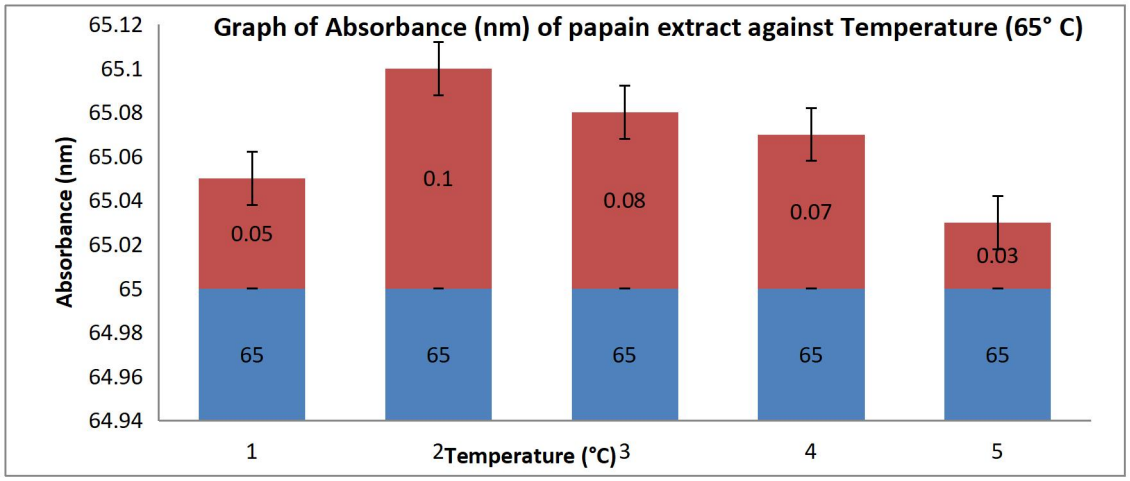

Figure1c:A graphical representation ofextraction of root, leaves and latex

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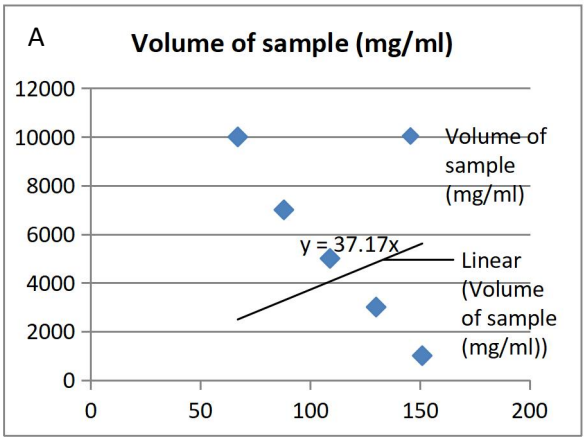

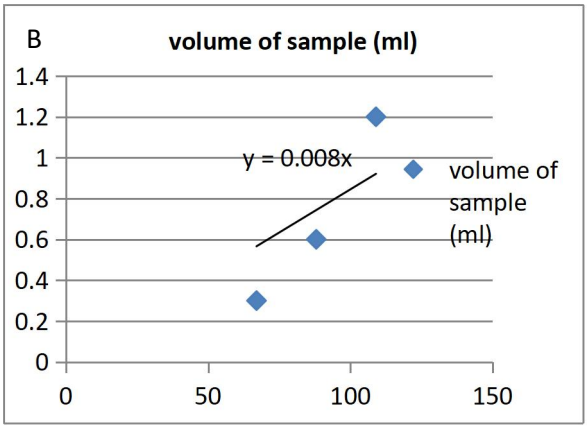

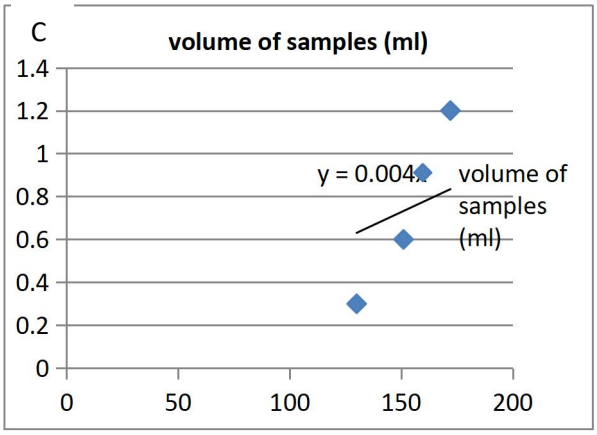

Figure 2: a) Papain concentration (ml/mg)Absorbance (nm) of Papain extractsagainst time taken (seconds)against temperature (°C) forthe extraction of Latex. **b)**Papain concentration (ml/mg)against time taken (seconds) for theextraction of Leaves by boiling and grinding. **c)** Concentration (ml) against time taken (seconds) for theextraction of Root by boilingand grinding.

DISCUSSION

The enzymatic activity of latex extraction from Carica papaya suggests a correlation between the amount of latex extracted and the enzymatic activity, with higher sample weights resulting in lower enzymatic activity. Previous studies [21-22], that identified enzymatic extracts from different parts of the Carica papaya plant, including the roots, leaves, and latex, confirming the presence of enzymes such as papain. These studies have also highlighted the diverse enzymatic activities exhibited by Carica papaya, indicating its potential for various applications in industries such as food, pharmaceuticals, and cosmetics.

The pH sensitivity observed in the enzymatic extracts of Carica papaya, with different optimal pH values for root papain, leaves papain, and latex papain, is consistent with previous research on plant-derived enzymes. Studies by [23-24]on other plant proteases have demonstrated varying pH optima, emphasizing the importance of maintaining specific pH conditions for optimal enzymatic activity. The increased proteolytic activity observed with both boiling and grinding methods for root extraction suggests the influence of cysteine concentration and the extraction method on the proteolytic activity of root papain. This finding aligns with previous studies on enzyme thermodynamics, such as those conducted by [24-26], which reported temperature-dependent patterns and highlighted the role of temperature in modulating enzyme activity.

The proteolytic activity of Carica papaya enzymatic extracts, including root papain, leaves papain, and latex papain, being influenced by Citrate buffer, highlights the importance of cysteine concentration in determining the proteolytic activity of these extracts. Studies by [27- 28] have documented

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the impact of Citrate buffer on pH sensitivity and stability of enzymes in various biological systems, confirming its role in modulating enzymatic activities. The identification of diverse enzymatic activities in Carica papaya, along with their pH and temperature sensitivities, suggests potential therapeutic applications. Previous literature by [29, 30]has demonstrated the therapeutic potential of proteolytic enzymes in wound healing, anti-inflammatory treatments, and cancer therapy. The current research findings support further exploration of Carica papaya enzymatic extracts for their medicinal applications.

The enzymatic extracts from Carica papaya hold promise for various industrial applications, as indicated by previous studies in industries such as food processing, detergent manufacturing, and leather processing [31, 32]. The current research contributes to the existing literature on industrial enzyme applications by providing insights into the enzymatic properties and optimal conditions for Carica papaya extracts. The development of extraction and purification techniques for Carica papaya enzymes, including root papain, leaves papain, and latex papain, builds upon previous studies on enzyme isolation and purification, employing methods such as precipitation, chromatography, and membrane filtration [33]. These findings expand the available techniques for future applications in various industries.

Figure 1a demonstrates that the increase in temperature ($p<0.05$) corresponds to an increase in the absorbance of papain extracts, indicating a positive correlation between temperature and enzymatic activity. This finding aligns with studies conducted by [34, 35] that have shown the temperature-dependent nature of enzymatic activity, emphasizing the importance of temperature control during the extraction process. In Figure 1b and c, the observed trends in the extraction of papain from root, leaves, and latex further support the positive correlation between temperature and the absorbance of papain extracts. These results are consistent with studies by [36] and [37] that have investigated the effects of temperature on enzyme activity in different plant sources, emphasizing the role of temperature in modulating enzyme activity.

Figure 2a-c provide insights into the enzymatic activity of papain extracted from different sources and the efficiency of the extraction methods employed. These calculations align with previous studies by [38, 397] that have used activity measurements to assess the efficiency and performance of enzyme extractions. Fig 2b and c demonstrate the extraction of papain from leaves and roots, respectively, using boiling and grinding methods. The calculated activity per milligram reflects the enzymatic activity of papain in these extracts. These findings are consistent with previous research by [40, 41] that have examined papain extraction methods from plant sources.

CONCLUSION

Tthe enzymatic activity of Carica papaya extracts, particularly the latex, root, and leaves, demonstrates the presence of various enzymes, including papain, with different enzymatic activities and sensitivities to pH, temperature, and buffer systems. The current research contributes to the existing literature on enzyme extraction, purification, and application methods. It expands our understanding of the enzymatic activity, extraction efficiency, and potential applications of Carica papaya enzymatic extracts. The study contributes to the scientific knowledge and lays the foundation for further investigations and advancements in

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the field of enzyme extraction and application. The findings underscore the potential of Carica papaya enzymatic extracts in fields such as medicine, food processing, detergent manufacturing, and leather processing.

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